DIABETIC RENAL–RETINAL SYNDROME

Diabetic Renal–Retinal Syndrome

21st Century Management Now

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Dedication

Diabetes can be a horrific affliction that erodes the spirit while inducing bodily disintegration. Coping with the inexorable downhill course that typifies the Diabetic Renal–Retinal Syndrome induces depression, mutes ambition, and destroys hope. Some diabetic individuals, however, evince an inner strength that permits endurance through sequential disasters that reduce less resourceful sufferers to homebound invalidism. Mildred (Barry) Friedman never complained or resorted to self pity through a course of type 1 diabetes plagued by stroke, vision loss, myocardial infarction, autonomic neuropathy, peripheral vascular disease, and adrenal insufficiency. Despite obligated retirement from teaching, Barry continued to be intellectually vital, writing medical columns for the American Association of Kidney Patients while serving as a volunteer patient advocate at University Hospital of Brooklyn. Barry died in September 1997, seventeen years after receiving a kidney transplant for diabetic nephropathy, in the midst of preparing her latest report for patients on the impact of diabetic vasculopathy. In respect for and recognition of a life force that inspired others to deal with the impossible, we dedicate this book to Barry.

E.A.F.
F.A.L., Jr.

Table of contents

Foreword – Inferences gleaned from twenty years of study of diabetic complications

ELI A. FRIEDMAN & FRANCIS A. L'ESPERANCE Jr.

Exactly twenty years has elapsed between the first Brooklyn conference exploring the Diabetic Renal–Retinal Syndrome and the proceedings contained in this volume, the fifth in the series held on 12–13 November 1979. The growing economic and resource burden imposed by complications of diabetes is evident from statistics compiled by the Health Care Finance Administration indicating that between the first and fifth conference the proportion of those with end-stage renal disease (ESRD) in the United States who had diabetes burgeoned from approximately 20% to 40%.

Concurrently, the estimated prevalence of diabetes in the general population increased between the first and current symposium from 2.3% to 5.9%. After the 1979 symposium, we observed that "few data are available from which to construct a natural history of diabetic nephropathy in maturity onset diabetes." Since then, a clearer picture of the microvascular and macrovascular complications of type 2 diabetes has emerged in the intervening 18 years. If there is a single overriding lesson to be learned from investigators conversant with diabetic complications it is that type 2 diabetes is not a benign disorder. Documented within reports that follow is the pragmatic inference that the severity of microvascular and macrovascular complications of diabetes are equivalent in type 1 and type 2 diabetes. Microalbuminuria, proteinuria, azotemia, and ESRD occur at the same incidence in both major diabetes types. Similarly, background and proliferative retinopathy are duration related in diabetes of either type. Neither renal pathologist nor ophthalmologist is able to distinguish diabetes type at any stage of deterioration leading to uremia and blindness. Gradually yielding to reality is the broadly held incorrect belief that individuals may have 'a touch of sugar' as a mild form of type 2 diabetes.

Permeating presentations at the fifth symposium is overriding optimism that the 'problem' of diabetic complications can be solved. Having learned that intensified metabolic regulation and reduction of hypertensive blood pressure slows progression of retinopathy and nephropathy in type 1 and type 2 diabetes transformed contemporary treatment regimens to include these components of care. The next plateau in interdicting diabetic complications – blocking molecular perturbations induced by hyperglycemia – is foreshadowed in the discussion of advanced glycated endproducts (AGEs). Studies in progress, to be reported within the next two years, will assess the value of aminoguanidine, a

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chemical shown to prevent diabetic nephropathy by blocking synthesis of AGEs, in the streptozotocin induced diabetic rat, in the proteinuric individual with type 1 and type 2 diabetes. Derivative studies of other AGE blockers herald intensifying recognition of the role of deranged molecular biological events in the pathogenesis of diabetic complications. Extrapolation from virtually total success applying aminoguanidine to arrest diabetic complications in rodent models is reason to anticipate that a reduction in diabetic complications is a truly attainable near-term objective.

Changes in presentation of diabetic renal–retinal syndrome

Variable	1979	1997
Incidence of diabetes	Few data. Estimated 'crude' annual incidence of 612 000 cases in the US in 1976[@]	798 000 new cases diagnosed in the US in 1996[#]
Prevalence of diabetes	2.3%	5.9% (diagnosed 10.3 million people, undiagnosed 5.4 million people)[#]
Annual cost of diabetes	Uncertain	Direct medical costs: $45 billion, indirect costs: $47 billion (disability, work loss, premature mortality)[#]
Prevalence of ESRD Due to Diabetes	20–25%	40–50%*
Blindness in RR syndrome	25–83%	5–8% (12 000 to 24 000 new cases of blindness due to diabetes annually)[#]
Hyperglycemia	Suspected in pathogenesis	Established as injurious
Cigarette Smoking	Not a risk factor	Established as injurious
Hypertension	Suspected as risk factor	Established as injurious
Pathogenesis of retinopathy	Unclear whether metabolic or vascular	Molecular explanation of sequence from hyperglycemia to cell injury via growth factors
Rheological perturbations	Suspected in retinopathy	No clear role in retinopathy or glomerulopathy
Fluorescein angiography	Proposed for routine application in eye care	Universally applied
Photocoagulation	Impedes not cures retinopathy	Stabilizes retinopathy for 20 or more years
Preazotemic nephropathy	First indication that in type 1 diabetes 'renal insufficiency can be postponed by antihypertensive treatment'	Consensus accepts value of universal antihypertensive therapy once microalbuminuria discovered. Angiotensin converting enzyme inhibitors first choice.
Streptozotocin-induced diabetes in the rat	Model proposed as valuable. Pathologic glomerular lesion reversible by islet transplants	Universally utilized for major advances including discovery of hemodynamic intraglomerular changes and role of mesangium.

Continued.

Variable	*1979*	*1997*
Peritoneal dialysis	Less than 20% alive after 2 years	About 60% of all diabetic patients alive at 2 years (higher mean age of subset)*
Hemodialysis	Approximately 50% alive after 2 years	About 60% of all diabetic patients alive at 2 years (higher mean age of subset)*
Cadaver donor kidney transplant	Patient survival about 50% at 3 years; graft function 35% at 3 years	Patient survival about 87% at 2 years; graft function 69% at 3 years*
Living donor kidney transplantation	Patient survival 75% at 3 years; graft function 68% at 3 years	Patient survival 90% at 2 years; graft function 82% at 3 years*
Islet transplants	Promising outcome (induced diabetes is curable) in rats	Sporadic clinical trials in patients, encapsulated islets under evaluation in China
Pancreas transplantation	Segmental grafts functioned 0 to 415 days in a series of 14 transplants in Lyon	World Pancreas Transplant Registry** indicates variable survival according to surgical technique; for simultaneous pancreas plus kidney with bladder drainage, 92% alive at 1 year with 79% pancreas graft function
Prognosis for vision retention	Uncertain. Ongoing trials of panretinal photocoagulation intercalated with vitrectomy promising	High probability of retaining at least ambulatory vision after laser treatment combined with reduction of hypertensive blood pressure.
Extent of rehabilitation	Dismal during dialytic therapy. Marginal after a kidney transplant	Poor during dialytic therapy. Substantive rehabilitation achieved in the majority of renal transplant recipients.
Key issues	Value of treating hypertension and striving for euglycemia. General survival. Wisdom of attempting organ replacement	Best strategy for blocking effects of noxious kinins and advanced glycosylated end-products (AGEs).

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1. Diabetes in the United States: epidemiology, scope, and impact

MAUREEN I. HARRIS

Editors' Comment:

Epidemiologists deduce, infer, postulate, and convincingly extrapolate from selected samples to the population at large. Once epidemiologists applied careful counting of incidence, prevalance, and mortality to diabetes, the extraordinary truth of its enormous impact on industrialized nations was apparent. The grim truth is that contracting diabetes means that life will be foreshortened by nearly a decade while its complication of blindness, renal failure, and limb amputation become major risks. In this report, Harris, who heads the National Institute of Health National Diabetes Data Group, recounts the statistic that more than 10 million Americans have diagnosed diabetes while an additional 5 million have undiagnosed diabetes. Harris points to the 'important new stage' in the epidemiology of diabetes in which discovery of the genes that cause diabetes and the molecular mechanisms underlying its complications provide the foundation for 'prevention of diabetes'. While gene extraction may not be practical either scientifically or economically for decades, understanding the molecular basis for perturbed metabolism induced by the diabetic genes(s) permits design of therapeutic intervention to interdict diabetic complications bypassing the need for often impossible to achieve normoglycemia.

Introduction

Epidemiologic data gathered from national and community-based studies during the past several decades have demonstrated clearly that we have greatly underestimated the scope and impact of diabetes in the United States. Diabetes has become a common disease with serious complications. About 10.3 million people are now known to have diabetes. An equally important issue is the large group of people with preclinical Type 2 diabetes, that is, patients who meet diagnostic criteria for diabetes but remain undiagnosed and untreated. Data suggest that the onset of Type 2 diabetes occurs approximately 10 years before clinical diagnosis is made and that the prevalence of undiagnosed diabetes in U.S. adults, based on fasting plasma glucose ≥ 126 mg/dl, is about 5.4 million. This high prevalence is similar to the situation with hypertension during the 1970's, in which undiagnosed and untreated hypertension was found to be very common and major national programs were initiated to detect cases and institute treatment. However, there is no concerted national effort to screen

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for and detect undiagnosed Type 2 diabetes in the United States. Diabetes causes substantial morbidity and premature mortality. It is now the leading cause of blindness, end-stage renal disease, and nontraumatic lower extremity amputations. Ischemic heart disease is 2–4 times more common in patients with Type 2 diabetes. Mortality is also 2–4 times higher and life expectancy in Type 2 diabetes patients is reduced by about 5–10 years.

Prevalence of diagnosed diabetes

Diabetes mellitus has been recognized clinically for centuries. However, intensive scientific study of the epidemiology of this disease has been conducted only within the past several decades. During this period, numerous research groups around the world have conducted investigations of the epidemiology of diabetes. Their findings show that diabetes is a growing problem worldwide. In the United States, the prevalence rate has increased dramatically. Data from national health surveys, in which diabetes was ascertained in representative samples of U.S. residents, indicate that in 1958 there were 1.5 million people who had been diagnosed with diabetes [1]. By 1997, we estimate that this had increased to 10.3 million people, representing a sevenfold increase in the prevalence of diabetes. About 90–95% of these patients have Type 2 diabetes.

The prevalence of diagnosed diabetes rises markedly with age (Figure 1). Rates range from 1.5% at age 45 years and younger to 6.5% at age 45 to 64 years and 10.5% at age 65 years and older [1]. Thus, in the Medicare population, one of every ten patients is known to have diabetes. In the middle aged group, one of every 15 is known to have diabetes. Diagnosed diabetes rates are similar for men and women even though it is widely believed that women have more diabetes than men. However, age-standardized prevalence in African Americans is 60% higher than in non-Hispanic whites and in Mexican Americans is 100% higher [2]. Native Americans can be even more severely affected, and the Pima Indians of southern Arizona have the highest prevalence of diabetes in the world. Many epidemiologic studies have established that prevalence of Type 2 diabetes can be vastly different among countries and among racial/ethnic groups. Such differences in diabetes prevalence led to epidemiologic investigations of risk factors for NIDDM, and numerous risk factors have been documented: total obesity, central obesity, abdominal fat deposition, duration of obesity, physical inactivity, dietary fat, family history of diabetes, genetic markers, hyperinsulinemia, and impaired glucose tolerance [3].

Preclinical Type 2 diabetes

An important issue is preclinical diabetes, that is, patients who meet diagnostic criteria for diabetes but who are undiagnosed [4]. Using the recently revised American Diabetes Association diagnostic criteria for diabetes [5], we calculate

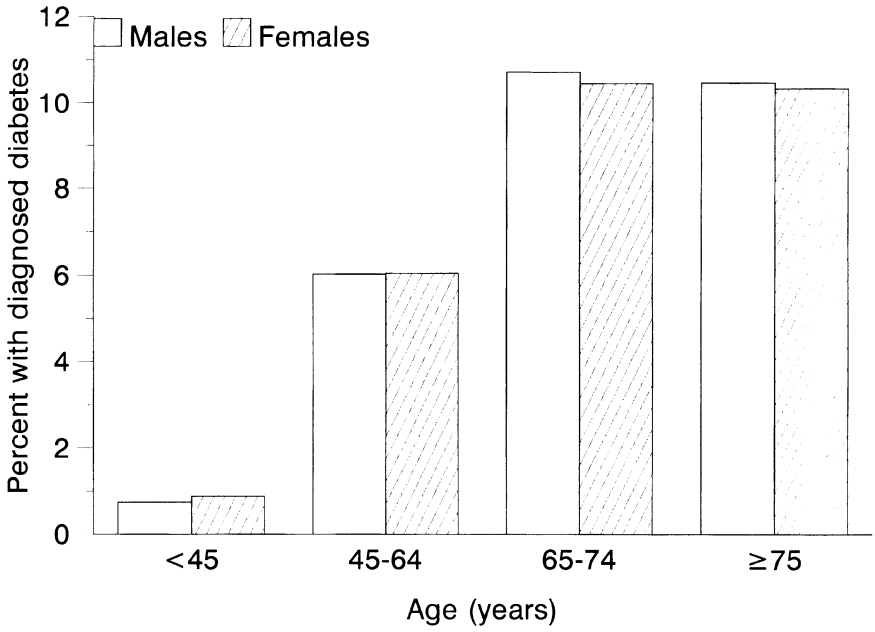

Fig. 1. Prevalence of diagnosed diabetes in men and women in the U.S. population, based on the 1992–94 National Health Interview surveys.

that the prevalence of undiagnosed diabetes based on fasting plasma glucose ≥126 mg/dl was 2.7% in a national survey of a representative sample of U.S. adults age 20 years or older [6].

Undiagnosed Type 2 diabetes is not a benign condition. Risk factors for micro- and macrovascular disease complications are very common and are almost as frequent as in diagnosed patients [7]. Retinopathy develops while the diabetes remains undiagnosed and untreated, and the prevalence of retinopathy is 21% at clinical diagnosis in Type 2 diabetes [6]. The presence of macrovascular disease in undiagnosed Type 2 diabetes is almost as high as in diagnosed cases and rates are twice those of nondiabetics. If efforts were made to identify the undiagnosed patients, effective treatment could be brought to bear on the risk factors for diabetic complications, including dietary and pharmacologic therapy and lifestyle and behavioral changes.

Complications of diabetes

Diabetes causes substantial morbidity and premature mortality. In the United States, it is the leading cause of blindness, end-stage renal disease, and nontraumatic lower extremity amputation. Each year, about one in 500 patients becomes blind, one in 300 patients develops renal failure, and one in 200 patients

has an amputation. Life expectancy for patients with these end-stage complications is only about 3–4 years.

Three complications of diabetes may lead to blindness: retinopathy, cataracts, and glaucoma. Decreasing visual acuity and increasing prevalence of blindness are strongly related to duration of diabetes. Based on data from the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), after 20 years of diabetes, almost 100% of patients with Type 1 diabetes, 80% of those with insulin-treated Type 2 diabetes, and 50% of Type 2 patients not treated with insulin have alterations in the small blood vessels in the retina characterizing diabetic retinopathy [8]. Approximately 40% of Type 1 patients and 5–10% of Type 2 patients have severe, vision-threatening proliferative retinopathy after 20 years of diabetes [8].

There are substantial racial differences in the prevalence of retinopathy in patients with diabetes. In a U.S. national survey of adults with Type 2 diabetes [6], we found that retinopathy was 46% more common in non-Hispanic blacks and 84% more common in Mexican Americans than in non-Hispanic whites. However, risk factors for retinopathy, including longer duration of diabetes and higher glycosylated hemoglobin levels, were also more common in the former two groups. After controlling for these factors in logistic regression, the risk of retinopathy was not elevated in non-Hispanic blacks but remained higher in Mexican Americans. The high rate in Mexican Americans versus non-Hispanic whites was also found in a community-based study in San Antonio, Texas [9], but not in the population of the San Luis Valley, Colorado [10].

The annual incidence of new cases of diabetic end stage renal disease (ESRD) based on the Medicare ESRD registry has increased steadily [11]. In 1982 there were about 5000 new cases of diabetic ESRD; in 1995 there were about 28,000 new cases, a 5.6-fold increase in only 13 years (Figure 2). The proportion of all ESRD due to diabetes has also increased during this period, from 22% in 1982 to 40% in 1995 [11]. Indeed, diabetes is now the leading cause of ESRD. Hypertension is the second leading cause (26%), glomerulonephritis is the third (11%), and a variety of other causes account for the remainder of new cases of ESRD. The incidence of ESRD per million U.S. population is similar for men and women, but there are marked differences among race/ethnic groups (Figure 3). Asian/Pacific Islanders have about twice the incidence of U.S. whites, but blacks and Native Americans are much more severely affected [11]. Treatment modalities for diabetic ESRD patients in 1995 are shown in Figure 4 and compared with modalities for all Medicare ESRD patients. About 18% of diabetic patients have functioning transplants, compared with 27% of all patients. Center dialysis is the most common treatment, being used for 68% of diabetic patients and 60% of all patients. Other forms of dialysis (home, continuous ambulatory peritoneal dialysis, other) comprise 14% and 13%, respectively [11].

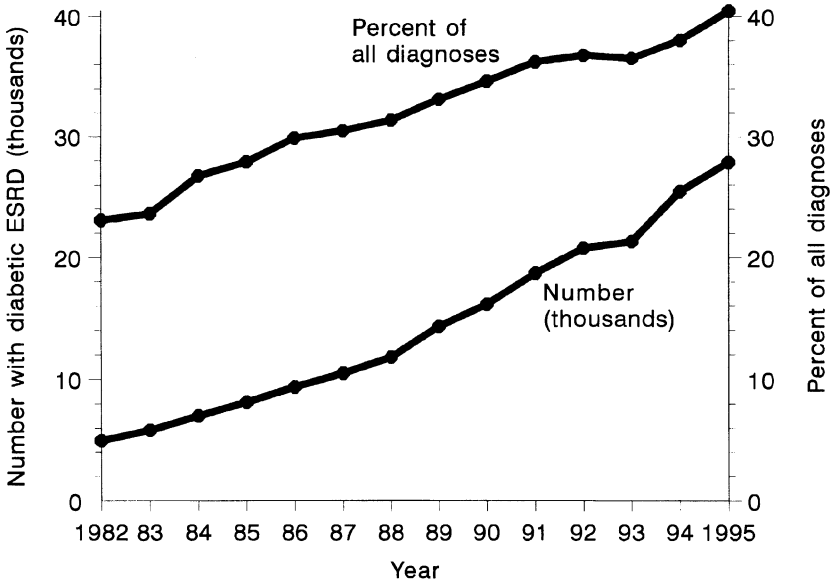

Fig. 2. Incidence of new cases of diabetic ESRD and proportion of all ESRD cases represented by diabetes, United States, 1982–95.

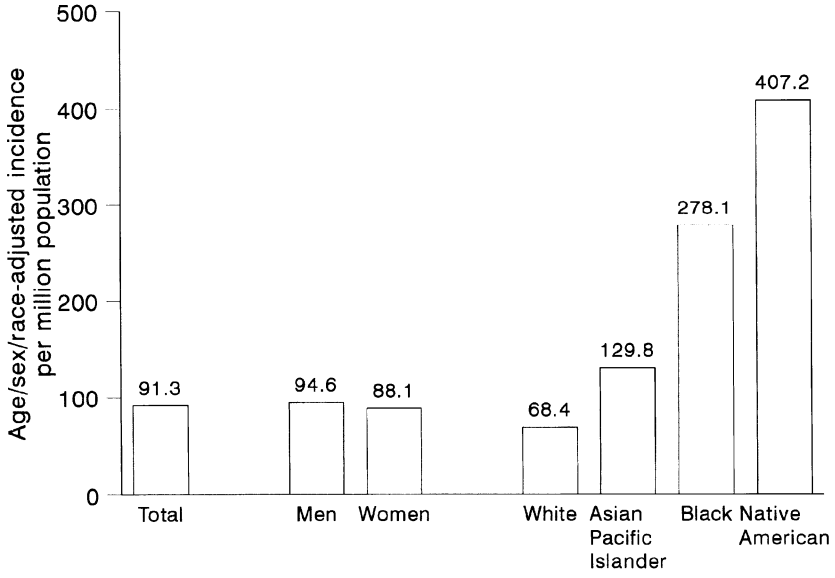

Fig. 3. Incidence of new cases of diabetic end-stage renal disease per million population by sex and race, U.S., 1995.

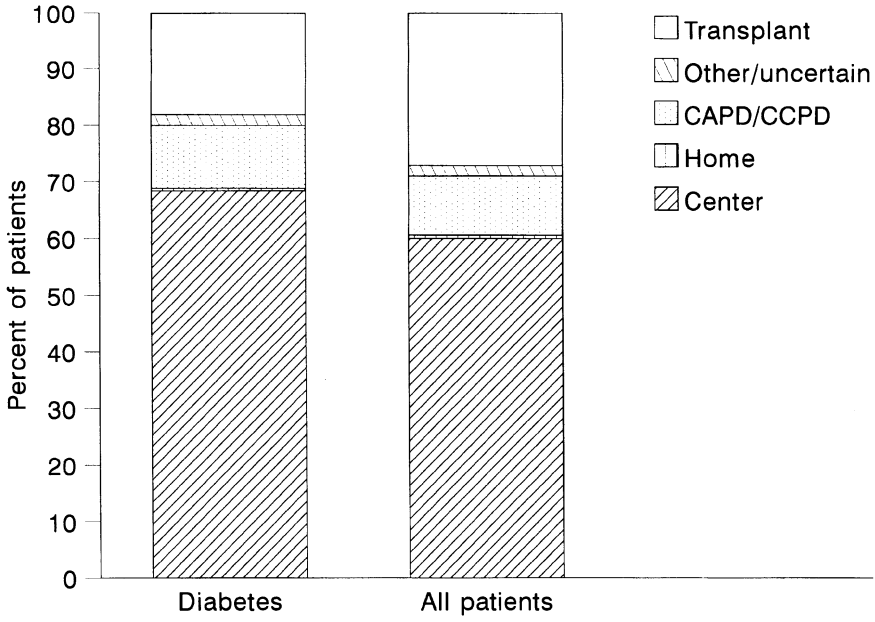

Fig. 4. Treatment modalities for diabetic ESRD patients compared to all ESRD patients, U.S., 1995.

Diabetes is also the leading cause of lower extremity amputations. Data from the U.S. National Hospital Discharge Survey were examined for all hospitalizations in which a lower extremity amputation occurred. For 51% of these, diabetes was listed on the hospital record [1]. Figure 5 shows the location of the amputation for diabetic patients: 40% were amputations of the toe, 15% were amputations of the foot or ankle, 25% were below the knee, and 20% were above the knee. Every year, one in every 150 diabetic patients has an amputation.

Neuropathy is also very common. For example, in the Rochester, Minnesota diabetic neuropathy study, 60–65% of diabetic patients had evidence of neuropathy although most of the neuropathy was subclinical [12]. The most common type was distal polyneuropathy, affecting about 45–55%. Carpal tunnel syndrome was found in 35% and autonomic neuropathy in 5–10%. There is also a substantial prevalence of cardiovascular disease in diabetes. Indeed, coronary heart disease (CHD) is the most common complication found in patients with Type 2 diabetes. Rates of CHD range from 30–50% and are about twice the rates found in nondiabetic patients [1]. Stroke is also twice as common in diabetic as in nondiabetic patients in the U.S. [1].

Mortality for patients with diabetes is often 2–4 times that of nondiabetic patients in the United States. In a 22-year follow-up of a national sample of

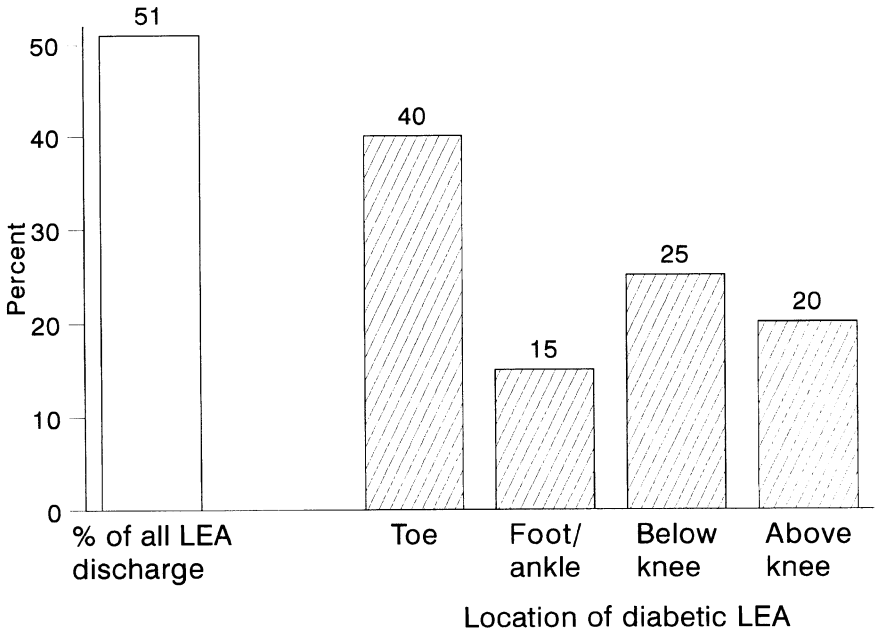

Fig. 5. Proportion of all nontraumatic lower extremity amputations (LEA) that were performed in diabetic patients and percentage distribution of the diabetic LEAs by location of the amputation, United States, 1989–92.

adults who were first studied in 1971–75, we found that diabetic patients comprised 5.4% of the cohort but accounted for 10.6% of deaths. A marked differential in mortality rates between diabetic and nondiabetic adults occurred in every age group (Figure 6), and the life expectancy of those with diabetes was about 5 years shorter than nondiabetic subjects. The most common cause of death was heart disease, particularly ischemic heart disease which was listed on 45% of diabetes death certificates.

Reducing diabetes complication rates

There is a large body of basic, clinical, and epidemiologic studies which demonstrate the strong relationship between the level and duration of hyperglycemia and the development and progression of diabetic retinopathy, nephropathy, and neuropathy. One such study, the Diabetes Control and Complications Trial (DCCT), clearly showed that diabetic microvascular complications can be substantially reduced in patients with diabetes [13]. In this trial, Type 1 diabetes patients who were intensively treated to lower their glycohemoglobin values showed decreases of 40–70% in microvascular disease of the eyes, nerves, and kidneys. Reduction of glycemia was accompanied by substantial decreases

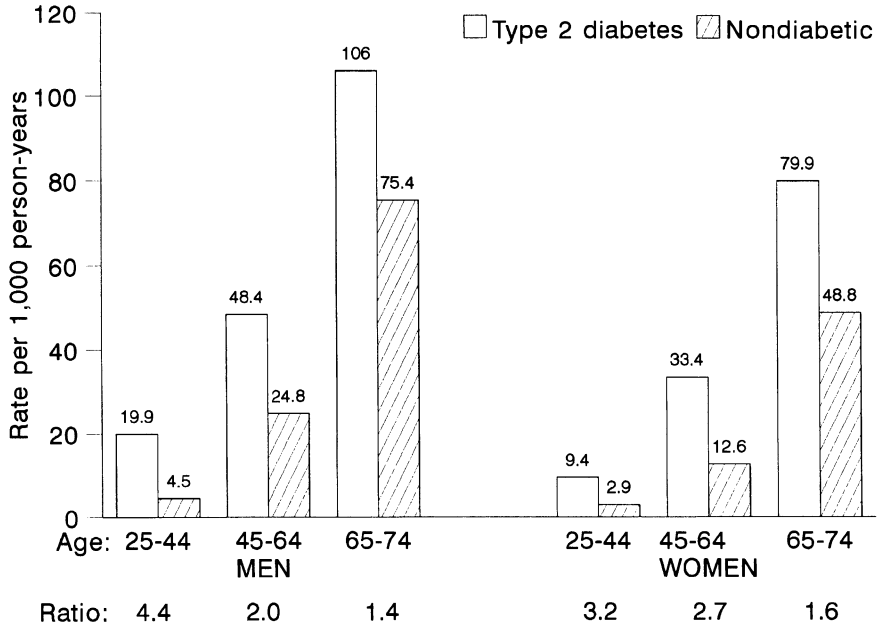

Fig. 6. Mortality rates in a representative cohort of diabetic and nondiabetic men and women age 25–74 years in 1971–75 who were followed for mortality through 1992–93.

in both the occurrence of new retinopathy in patients without retinopathy and decreases in the progression of retinopathy in patients who had retinopathy at the beginning of the study. Even though the DCCT was conducted in patients with Type 1 diabetes, the evidence from animal models, epidemiologic studies, and clinical trials strongly indicates that glycemic control will have the same beneficial effect in Type 2 diabetes as in Type 1 diabetes. For example, the incidence of retinopathy was virtually identical for Type 1 diabetes, insulin-treated Type 2 diabetes, and Type 2 diabetes not treated with insulin in patients in the WESDR study when they were stratified by their glycohemoglobin level [8]. A clinical trial of Type 2 diabetes patients in Japan showed that reductions in the incidence of microvascular disease could be achieved by intensive insulin therapy [14]. It appears that it does not matter what type of diabetes a patient has (Type 1 or Type 2 diabetes) or whether the patient is treated with insulin or not; the important factor in diabetic microvascular complications is level and duration of hyperglycemia. It is likely that reduction in the magnitude of diabetic complications will require use of the management concepts for tight glycemic control explicated in the DCCT.

Glycemic control is not optimal in the United States. Figure 7 shows mean fasting plasma glucose values in a number of community-based studies of Type 2 diabetes in non-Hispanic whites, Mexican Americans, Japanese Americans,

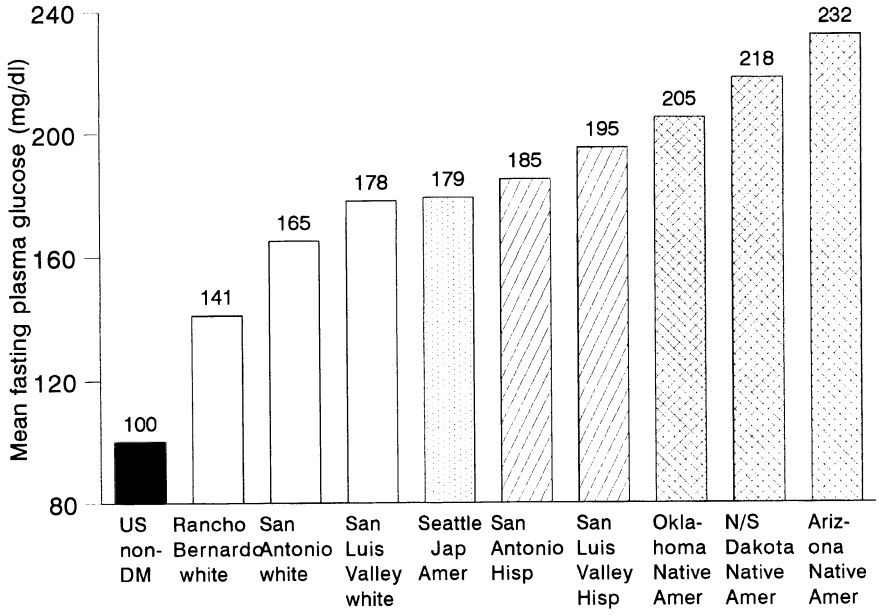

Fig. 7. Mean fasting plasma glucose in persons without diabetes in the United States and in diabetic patients in community-based studies.

and American Indians in the United States and compares these to data for a representative sample of the U.S. nondiabetic population [3]. It is readily apparent that blood glucose is substantially elevated in all groups of diabetic patients.

Risk factors for cardiovascular disease are also common in patients with Type 2 diabetes. Fifty percent are hypertensive, hypercholesterolemia is found in 36%, hypertriglyceridemia in 25%, extreme obesity in 36%, and 20% smoke cigarettes [3]. These are major reasons for the 2–4 fold higher rates of cardiovascular disease in these patients. However, a substantial degree of excess risk is still conferred by diabetes. For example, mortality rates were compared for people with and without diabetes who were in the screening stage of the Multiple Risk Factor Intervention Trial. Rates were two times higher in the diabetic patients, even after taking into account their higher rates of cardiovascular risk factors (high serum cholesterol, high systolic blood pressure, and cigarette smoking) [15]. The increased rates of coronary heart disease in diabetes may be due to high blood glucose, at least in part. One study found that CHD mortality in people with diabetes correlated directly with their HbA1c concentration [16]. Although the evidence on this topic is less conclusive than for microvascular complications of diabetes, hyperglycemia may be a more important factor in the development of CHD than previously thought.

Future directions for study of the empidemiology of diabetes

The epidemiology of diabetes has now moved into an important new stage: collaboration between epidemiologists and laboratory researchers to discover the genes that determine diabetes and the molecular mechanisms through which risk factors cause diabetes. The epidemiologic studies conducted during the past decade have also laid the foundation for implementing significant new programs for the prevention of diabetes [17–19].

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2. Stages of diabetic nephropathy

CARL ERIK MOGENSEN

Editors' Comment:

The key observation permitting understanding of the natural history of diabetic nephropathy was made by Keen who in 1963 noted the presence of previously undetectable amounts of urinary albumin early in the course of diabetes. Mogensen, Viberti, Parving and others developed the concept of microalbuminuria defined as a urinary albumin excretory rate of 20–200 μg/min and subsequently showed that in both type 1 and type 2 diabetes microalbuminuria was a silent marker for later clinical nephropathy. In this report, Mogensen ties together a large number of studies linking microalbuminuria in diabetes of both types to elevated blood pressure and poor metabolic control. Microalbuminuria predicts cardiovascular complications, increased morbidity and death in diabetics and also predicts death in the non-diabetic population. Given this 'handle' a broad attack on early nephropathy of diverse etiologies is now possible. For those with diabetes, microalbuminuria is reason to treat with an angiotensin enzyme inhibitor even in the absence of hypertension.

Introduction

An albumin excretion rate below the proteinuric level, but above the normal range, was introduced as a new concept in diabetic nephrology more than 3 decades ago [1–3] and later the condition was coined microalbuminuria. This abnormality, highly predictive of overt renal disease, seems neglected by some hypertensiologists and nephrologists [4]. The initial scope of the new sensitive albumin assays was to detect and possibly to treat early diabetic renal disease [3]. The same concept may apply to purely hypertensive renal damage in patients with essential hypertension [5] as is now being appreciated [6, 7]. Subsequently it has been shown that microalbuminuria in diabetes in general indicates incipient diabetic nephropathy or glomerulopathy [8], and that the same may be the case in other glomerulopathies. The consensus definition of microalbuminuria is an excretion rate in the range of 20–200 μg/min. Values below are designated normoalbuminuria and values above macroalbuminuria or overt proteinuria. Some authors propose a lower value because in population-based studies suggest a lower critical level [9].

Initially measurement procedures by radioimmunoassay were quite cumbersome, but this is now completely changed with immunoturbidimetry assays [10] and several reliable and simple bedside tests have been developed [11].

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Even quick quantitative tests with measurement of an albumin creatinine ratio [12] are now readily available.

Abnormal albuminuria in population studies

When measuring albumin excretion rates in healthy young individuals, rather consistent results were obtained from many laboratories. The mean excretion rate is around 5 μg/min but with a considerable variation. Excretion rates are about 30–40% higher during day time [13]. The excretion rate in young healthy individuals, however, very rarely exceeds 12–15 μg/min. In studies in the population, especially in elderly individuals, about 3–5% may exhibit microalbuminuria, the proportion obviously depending upon the precise definition employed [9]. These individuals with moderate elevation in albumin excretion usually exhibit other risk factors, mainly elevated blood pressure, but also signs of increased blood glucose, dyslipidaemia and obesity [13]. In some studies the abnormalities are related to insulin resistance including hyperinsulinemia [14]. Thus, it has been proposed that microalbuminuria may be part of the metabolic syndrome, show in evidence of early organ damage, predictive of advancing morbidity and increased mortality [15]. As stated earlier, the standard definition of microalbuminuria is 20–200 μg/min, but in population-based studies, it has been proposed to use an excretion rate higher than around 8–10 μg/min to define abnormal values [9]. Albuminuria is usually correlated to BP also in completely healthy young individuals, but this correlation can only be detected when 24 h ambulatory measurements of BP are performed [16]. The same is the case in normoalbuminuric Type 1-diabetic patients [17].

Type 1-diabetes, with special reference to microalbuminuria

Several studies have shown that microalbuminuria is strongly predictive of overt diabetic nephropathy when these individuals are followed without intervention for 8–15 years [18]. Overt nephropathy is eventually seen in about 25–30% of patients with diabetes [19]. Microalbuminuria usually progresses around 20%/year, correlated to both poor metabolic control and increase in blood pressure. The higher the excretion rate the higher the risk for further progression, even within the so-called normal range [20]. Microalbuminuria is also associated with structural damage when careful morphometric analysis is performed [8]. The biochemical mechanism behind diabetic renal disease is under intense investigation [21].

The main predictors, i.e. poor metabolic control and elevated blood pressure, are clearly modifiable. Good metabolic control prevents the occurrence of microalbuminuria and also slows down the progression [22]. Elevated blood pressure may not be an early abnormality, but in most patients blood pressure rises after the development of microalbuminuria [20]. Effective antihypertensive treatment, usually using ACE-inhibitors, is able to retard the progression,

or in some cases entirely prevent deterioration [23]. On the basis of these investigations, it is now proposed in several consensus documents to treat so-called normotensive IDDM patients with microalbuminuria with ACE-inhibitors or comparable agents, but obviously the best possible glycemic control should always be strived for [24]. All evidence suggests that the otherwise relentless progression to overt renal disease in this way is either considerably postponed or abolished. ACE-inhibition according to one large-scale two-year study [25] is also able to control progression of retinopathy, a highly significant finding [26].

The next stage of renal disease is overt nephropathy (UAE > 200 μg/min). GFR starts to decline, but the rate of decline is again related to both BP-level as well as glycemic control [19]. With optimal management, renal disease can be stable or only slowly progressive for many years [27].

Type 2-diabetes, with special reference to microalbuminuria

Microalbuminuria is also a strong risk factor for progression of renal disease in NIDDM patients [28], and it is also predictive of early mortality, as in the background population, and even more pronounced so. New studies document that optimized diabetes care is able to slow progression, and prevent progression in Japanese patients [29]. At the same time effective antihypertensive treatment, especially with ACE-inhibitors, is quite effective [30, 31]. Nevertheless an increasing number of Type 2-patients are enrolled in Renal Supportive programmes, perhaps because still older patients are now accepted for these regimens.

Type 2-diabetes is clearly associated with increased morbidity and mortality [32, 33]. Retrospective as well as prospective studies have demonstrated that in particular micro- and macroalbuminuria are associated with increased mortality. Several studies have demonstrated that microalbuminuria independently predicts mortality [34–45]. Even modestly increased urinary albumin excretion (in the upper range of normal) has been shown to be associated with increased mortality [41]. Microalbuminuria also predicts death in the general non-diabetic population [46, 47].

The excess mortality observed in NIDDM populations is mainly due to cardiovascular disease. Compared to the non-diabetic reference population, normoalbuminuric diabetic patients had a 2–3 times increased cardiovascular mortality in the study by Gall [41]. Death due to renal disease is by contrast not so common in most Caucasian Type 2-patients [36–39] in contrast to findings in non-Caucasian cohorts [48]. Microalbuminuria in Type 2-diabetes is associated with a clustering of risk factors favouring atherosclerosis, but underlying mechanisms for the accelerated atherogenesis in NIDDM patients remains unclear. Only a small proportion of the excessive risk of CHD can be explained by the impact of conventional cardiovascular risk factors. Measuring UAE and glycosylated haemoglobin helps to define the cardiovascular risk

profile for the individual NIDDM patient, since raised values of these variables indicate increased cardiovascular susceptibility. It is reasonable to assume that achievement of good glycemic control by diet or drug treatment and correction of other known modifiable risk factors, especially blood pressure, but also dyslipidaemia, obesity and smoking, would reduce the cardiovascular mortality in NIDDM patients, although any clear cut evidence from intervention trials is still lacking. However, reduction and stabilisation of microalbuminuria by improving glycemic control is likely to prevent advanced renal disease but may not protect against CVD [49].

The next stage after microalbuminuria is overt nephropathy, usually with a poor prognosis, especially with advanced disease. The rate of decline correlates strongly to BP-level and less significantly to HbA_{1C} [50]. Treatment with ACE-inhibition seems beneficial [30, 31, 50], as shown in new studies, also in patients with microalbuminuria [52].

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3. Mesangial expansion in diabetic nephropathy: functional and genetic considerations

MICHAEL MAUER & PAOLO FIORETTO

Editors' Comment:

By studying type 1 diabetic patients subjected to sequential renal biopsies five years apart, Mauer associated increasing urinary albumin excretion rate with expanding mesangial cell volume. This mesangial expansion is viewed as the most important lesion presaging clinical diabetic nephropathy. Mesangial volume is the structural measurement that is most closely (inversely) linked to glomerular filtration rate. Undetermined, however, is the question of causality between hypertension and glomerular morphologic lesions in type 1 diabetes. The authors caution that relationships between perturbed renal function and structural glomerular changes in type 2 diabetes are less completely understood and probably more complex. Structural heterogeneity noted in glomeruli in type 2 diabetes may relate to genetic variables not yet identified. Attributing an otherwise inexplicable range of severity of diabetic renal syndromes to genetic predetermination is consistent with the scanty evidence though still speculative. By offering an hypothesis, Mauer and Fioretto stimulate debate and needed derivative research.

Introduction

Diabetic nephropathy is the single most important cause of renal failure in the Western world and is consequent to the advanced progression of a constellation of renal lesions including widening of extracellular basement membranes, mesangial expansion, arteriolar hyalinosis, interstitial fibrosis and tubular atrophy, and global glomerular sclerosis [1]. This review argues that mesangial expansion is the most important lesion leading to clinical renal disease in patients with type I insulin-dependent diabetic mellitus (IDDM) but that the pathologic picture in type II non insulin-dependent diabetic mellitus (NIDDM) may be more complex. This review, in addition, presents some of the available and increasingly compelling evidence that the risk of diabetic nephropathy is strongly genetically determined. It is further argued that this genetic risk is reflected in *in vitro* differences in responses of non-renal cells, which can serve as markers for risk and models for pathogenetic studies.

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Diabetic nephropathy in IDDM

The basic lesions

Increase in glomerular basement membrane (GBM) width occurs early in IDDM [2] and is paralleled by widening of the tubular basement membrane (TBM) [3]. In the GBM this is associated with an increase in the density of 'novel chains' of type IV collagen in the lamina densa and a decrease in the density of 'classical' collagen chains ($\alpha 1$, $\alpha 2$ chains of type IV) in the lamina rara interna [4]. At more advanced stages of disease there is a decrease in the density of heparin sulfate proteoglycan charge sites in the lamina rara externa and interna of the GBM [5]. Increase in the fraction of glomerular volume which is mesangium (mesangial fractional volume or [Vv(Mes/glom)]) can also be measured within 3–5 years after onset of IDDM [2]. The increase in Vv(Mes/glom) is mainly due to expansion in the fraction of the glomerulus occupied by mesangial matrix [Vv(MM/glom)] although a lesser expansion of mesangial cell fractional volume [Vv(MC/glom)], also occurs [6]. The nature of the accumulating mesangial matrix material is not fully understood. The major component of MM, the classical chains of type IV collagen, decrease in density as the disease progresses [4], and the same appears to be true of type VI collagen (Moriya T, Mauer M, unpublished data). Quantitative immunohistochemical analyses of other MM molecules have not yet been done. Whatever its composition, the increase in the Vv(Mes/glom) eventually results in a decrease in relative peripheral GBM filtration surface [Sv(PGBM/glom)] [7]. Enlargement of glomerular volume (GV) can delay the decline of filtration surface per glomerulus (S/G) but, ultimately, as mesangial expansion progresses S/G declines [8]. Most patients developing diabetic renal disease have diffuse mesangial expansion but many also have nodular (Kimmelstiel-Wilson) lesions which appear to be consequent to the development of capillary microaneurisms [9].

Expansion of the fraction of renal cortex occupied by interstitium [Vv(Int/cortex)] occurs in most IDDM patients, even in relatively normal-appearing areas of renal cortex [areas without tubular atrophy, marked TBM thickening and reduplication, or global glomerular sclerosis (GS)] [10]. More severe interstitial expansion, however, occurs in areas of advanced tubular injury and glomerular scarring [10]. The molecular nature of this increase in interstitial extracellular matrix (ECM) has not been carefully studied, but it appears that, early on, there are increases in type I and III collagen while in the later stages of disease type IV collagen accumulates [11].

Hyalinosis of afferent and efferent arterioles, i.e. the replacement of smooth muscle cells by waxy, homogeneous, PAS positive material [1, 5], which appears to be consequent to an exudate of plasma proteins [12], is a characteristic diabetic renal lesion. Advanced arteriolar hyalinosis lesions, where one or more vessels on a renal biopsy are completely or nearly completely replaced by hyaline material, is associated with an increased percent of GS [13].

Although renal lesions in IDDM tend to develop in parallel, there are still considerable variations within a given patient in the severity of the individual lesions. Thus, for example, although highly statistically significant, the relationship of GBM thickness and Vv(Mes/glom) is not precise ($r = 0.56$, $p < 0.0005$) [5]. These structural interrelationships are present at both the earlier and the later stages of development of the renal lesions of diabetes with the exception that the relationship of Vv(Int/cortex) with glomerular parameters and with tubular basement membrane width are weak or not statistically significant at the earlier stages of the disease [3], and becomes significant only when large numbers of patients with more advanced nephropathology are included in these analyses [10, 14].

Renal structural functional relationships in IDDM

Vv(Mes/glom) is the structural measure which, on a cross-sectional analysis, is most closely (and inversely) related to glomerular filtration rate (GFR) [5], GBM [5] and TBM width [3] are also inversely related to GFR, but less precisely so. Vv(Int/cortex) is not correlated with GFR, unless more advanced cases of diabetic nephropathy are included [3, 10]. As noted above, the increase in Vv(Int/cortex) in these cases is in association with lesions of marked tubular basement membrane thickening and reduplication, tubular atrophy, and global glomerular sclerosis [10]. Thus, it is more likely that interstitial expansion in diabetes is, at least in part, consequent to advanced glomerular and tubular injury and interstitial fibrosis as a driving force for declining GFR may only become important at the late stages of diabetic nephropathy.

Increase in Vv(Mes/glom) is also the lesion most closely associated with increasing albumin excretion rate (AER) in IDDM [5]. This conclusion is based on sequential biopsies performed 5 years apart, in a group of long-standing IDDM patients whose albumin excretion rate was increasing over this time [15]. Several of the patients were in transition from normal to microalbuminuria, or from microalbuminuria to overt nephropathy. The single structural variable which correlated with the increase in AER over the 5 years of this study was the increase in the Vv(Mes/glom) over this same time. GBM width and Vv(Int/cortex) did not change significantly over this time [15]. These longitudinal studies strongly support the hypothesis that increase in Vv(Mes/glom) is causally related to changes in AER in IDDM patients.

It is difficult to sort out whether diabetic renal lesions are the cause or the consequence of elevated systemic blood pressure in IDDM patients. Our earlier studies suggested that rapid development of mesangial expansion can be occurring in patients with systemic blood pressures which are entirely normal and, in fact, are identical to those with patients with very slow development of mesangial expansion [16]. Thus, systemic hypertension is not a necessary precondition for rapid mesangial expansion. However, this does not answer

the question as to whether systemic hypertension can accelerate the rate at which the crucial lesions of diabetic nephropathy develop. Since hypertension is frequently the consequence of advanced glomerular and tubulointerstitial lesions, longitudinal studies with serial measures of blood pressure and renal structure are necessary in order to answer this question. Nonetheless, in cross-sectional studies, mesangial expansion was a stronger statistical predictor of GBM width, interstitial fibrosis, and glomerular sclerosis than was hypertension, whereas hypertension was more strongly related to arteriolar hyalinosis [16].

In summary, increase in Vv(Mes/glom), primarily due to an increase in Vv(MM/glom), is closely associated with all of the clinical manifestations of diabetic nephropathy including increasing AER, declining GFR, and rising systemic blood pressure. Although other structural variables are also related to these functional alterations, Vv(Mes/glom) is the strongest structural predictor of renal dysfunction in IDDM patients and, in longitudinal studies, the only correlate of increasing AER.

Diabetic nephropathy in NIDDM

Electron microscopic morphometric analysis of glomeruli in Japanese NIDDM patients revealed structural interrelationships and structural-functional relationships similar to those described for IDDM patients [17]. There was striking inverse correlation between Vv(Mes/glom) and Sv(PGBM). Further, Vv(Mes/glom) was closely and directly related to urinary protein excretion and inversely related creatinine clearance whereas relationships of these functional parameters to GBM thickness was weaker or nonexistent [17]. However, the picture of NIDDM patients is probably more complex than suggested by this study. Thus, Østerby et al. found that NIDDM patients tended to have less marked glomerular changes than IDDM patients with the same degree of proteinuria [18]. However, in this comparison, the IDDM patients had lower levels of GFR when compared to NIDDM patients with the same degree of proteinuria [18]. One possible explanation of these findings could be that by the much larger glomerular volumes in the NIDDM patients, results in preservation of filtration surface. However, the explanation for the proteinuria in these NIDDM patients was somewhat obscure. These authors noted that the NIDDM patients were more heterogeneous in their pathology, although this heterogeneity was diminished when patients with both proteinuria and retinopathy were considered. Nonetheless, these authors found a significant correlation between S/G and GFR, which ranged from 24–146 ml/min/1.73 m^2 in these patients [18]. In a study performed in NIDDM Pima Indians, Vv(Mes/glom) increased progressively from early diabetes, to long-term diabetes with normoalbuminuria, to microalbuminuria, and to clinical nephropathy [19]. There was no relationship between GFR and S/G in these various functional sub-

groups, but the range of GFRs in this patient population was not as great as in the Danish studies of Østerby et al. [18]. Global glomerular sclerosis was considered an important correlation of reduced GFR in this study of Pima Indian NIDDM patients [19].

The looser association between electron microscopic morphometric analysis of glomerular structure and renal function in NIDDM patients compared to IDDM patients noted in the above-mentioned studies may in part be explained by recent observations suggesting more complex patterns of renal injury in NIDDM patients with microalbuminuria [20]. Thirty-four Caucasian northern Italian NIDDM patients with microalbuminuria were biopsied for research purposes. Three categories of renal structure were discerned by light microscopic analysis. In category I [(CI), $n = 10$] renal structure by light microscopy was normal or near normal showing only mild mesangial expansion, tubulointerstitial changes, or arteriolar hyalinosis, in any combination. In category II [(CII), $n = 10$] patients had typical diabetic nephropathology with balanced severity of glomerular, tubulointerstitial, and arteriolar changes, more typical of what is seen in IDDM patients. Category III [(CIII), $n = 14$] patients had atypical patterns of renal injury. These patients had absent or only mild glomerular diabetic changes with disproportionately severe renal structural lesions including tubulointerstitial injury, advanced glomerular arteriolar hyalinosis, and global glomerular sclerosis exceeding 25%. That the lesions in CIII patients are of diabetic origin is suggested by the fact that hemoglobin A1C was more elevated in this group and in CII than in CI patients. However, CIII patients differed from CII patients in having higher body mass index and a lower incidence of proliferative retinopathy. Thus, the CII patients in this study may be similar to the NIDDM patients with retinopathy in the Danish study referred to above [18]. Findings in the CIII patients suggest that the kidney may react different to hyperglycemia in different subpopulations with NIDDM. This might reflect the heterogeneous nature of NIDDM *per se*. Alternately, lesions in CIII patients may be, at least in part, non-diabetic in origin.

Recent unpublished observations suggest that these patterns are also seen in proteinuric NIDDM patients, although a lower proportion of these patients are in CI compared to normo-or microalbuminuric patients; still, about 15% of NIDDM patients with proteinuria had minimal renal lesions (Fioretto P, Mauer M, Nosadini R, unpublished data). A substantial proportion of proteinuria NIDDM patients were in CIII, similar to that in microalbuminuric patients.

In summary, it appears that renal structural changes in NIDDM are more complex than in IDDM patients. Approximately one-third of the patients show atypical patterns of renal injury and these are related to greater body mass index and a paucity of advanced retinopathy findings. NIDDM patients with microalbuminuria or proteinuria may have minimal lesions. Further cross-sectional and longitudinal studies in NIDDM patients are required before the nature of these complexities can be better understood.

Genetic aspects of diabetic nephropathy

It is known that there is strong concordance of risk for or protection from diabetic nephropathy among IDDM sibling pairs [21]. Recent studies have shown that this is associated with concordance in the severity of glomerular lesions, particularly Vv(Mes/glom) and Vv(MM/glom). There is also concordance for the patterns of glomerular lesions. For example, if one member of an IDDM sibling pair has thick glomerular basement membrane relative to Vv(Mes/glom), the other member of the sibling pair is highly likely to have the same pattern (Fioretto P, Steffes M, Barbosa J, Rich S, Miller M, Mauer M, unpublished results). Thus, in IDDM patients, mesangial expansion (which is largely due to MM accumulation) may, in substantial measure, be under genetic control. It is tempting to speculate that the structural heterogeneity seen among NIDDM patients may be consequent to genetic variables. Perhaps the leaner NIDDM patients with retinopathy are more closely genetically parallel to IDDM patients, and this could explain the similarity in renal structure in CII NIDDM patients and IDDM patients.

Cellular studies in diabetic nephropathy

Skin fibroblasts cultured for several *in vitro* passages from long-standing IDDM patients with diabetic nephropathy behave differently from patients without nephropathy [22–25]. They have increased Na^+/H^+ antiporter activity [22–24] and increased rates of collagen synthesis [25]. These alterations also include quantitatively increased levels of mRNA for Na^+/H^+ antiporter and $\alpha 1$ [3] collagen chains in these diabetic nephropathy patients (Vats A, Mauer M, Fish A, Kim Y, unpublished data). Moreover, Na^+/H^+ antiporter activity of these cells are concordant in IDDM siblings who are concordant in renal structure (Trevisan R, Fioretto P, Mauer M, Nosadini R, unpublished data). Taken together, these studies are consistent with the concept that the genetic tendency to develop diabetic nephropathy in IDDM patients may be reflected in wide-spread differences in renal and non-renal cell responses to the diabetic state. This provides new directions for the development of improved markers of nephropathy risk and for new pathogenetic insights.

Acknowledgments

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4. Nephropathy in NIDDM – an update

EBERHARD RITZ & DANILO FLISER

Editors' Comment:

From long-term observational studies of large numbers of individuals we learned that the former broadly held belief that the probability of developing nephropathy in type 2 diabetes is less than in type 1 diabetes is incorrect. In Heidelberg, Germany, Ritz and his colleagues reported (as previously noted in Rochester, Minnesota by Humphrey et al.) that the rate of loss of glomerular filtration in type 2 individuals mirrors that noted in type 1. Nephrologists managing end stage renal disease are familiar with the relative importance of type 2 diabetes which accounts for 90–99% of newly treated dialysis patients in industrialized nations. Over the past five years, convincing reports show that the same therapeutic interventions (metabolid and blood pressure regulation) found to delay progression of nephropathy in type 1 patients are of value in type 2. Preliminary studies sustain the clinical impression that cigarette smoking is deleterious and actually hastens the onset of uremia in the type 2 patient. Pointing to cluster analysis in family studies, the authors suggest that genetic predetermination for nephropathy is coded separately from the risk of type 2 diabetes. The references collected in Tables 3 and 4 provide an excellent starting point for investigators pursuing the question of the role of genetic factors in diabetic nephropathy.

What is the magnitude of the problem?

In all Western countries, diabetic nephropathy has become a leading cause of endstage renal disease (ESRD) (Table 1) [1, 2]. This is true for the United States [3], Japan [4] and Europe [5]. While in the past it was felt that at least half of diabetic patients with ESRD suffer from insulin-dependent diabetes mellitus (IDDM), it has increasingly become apparent that the great majority

Table 1. Patients with diabetes mellitus admitted for renal replacement therapy (per million/year) (after ref. [2]).

	Total patients	Diabetes	NIDDM (% of all diab. pts.)
Lower Neckar/Germany	125	52	90
Cataluna/Spain	94	19	76
Lombardia/Italy	102	10	50
Shiga/Japan	161	36	99
USA (whites)	150	46	–

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of such patients indeed suffer from non-insulin-dependent diabetes mellitus (NIDDM) [6]. The discrepancy to previous reports may be due in part to misclassification of insulin-using NIDDM patients as IDDM, but the major cause is a true increase in NIDDM patients with ESRD [2]. The recent increase is particularly noticeable in such European countries where in the past the prevalence of endstage renal failure in particular with NIDDM tended to be low, e.g. Spain [7], Italy [8] and France [9]. In France, as late as 1992, the proportion of diabetic patients on dialysis was only 6%, whereas in 1995, 40% of new admissions for renal replacement therapy in the city of Strassburg suffered from NIDDM (T.P. Hannedouche, personal communication).

Is all renal failure in NIDDM due to Kimmelstiel Wilson's glomerulosclerosis?

NIDDM is frequent in the elderly. On the other hand, in the elderly renal disease is several 100-fold more common than in the young. It is obvious that, by coincidence, non-diabetic renal disease must be encountered in some NIDDM patients. The reported figures vary, but we found that approximately 25% of NIDDM patients taken on dialysis had some known chronic non-diabetic renal disease [10]. This may well be an underestimate, since our uncontrolled clinical observations suggest that ischemic nephropathy secondary to extensive atherosclerosis of the abdominal aorta is particularly frequent in NIDDM patients. Also a point of importance is the issue whether glomerulonephritis is more frequent in NIDDM (and by implication whether in NIDDM heavy major proteinuria is as equally reliable as an indicator of diabetic renal disease as in IDDM). Some renal biopsy series in NIDDM patients with heavy proteinuria indicated that 20% and more of patients suffered from glomerulonephritis [11, 12]. We did not find an excess frequency of glomerulonephritis either in an autopsy study [13] or in a renal biopsy study (unpublished), and this is in agreement with the results of two ongoing European multicentric trials. Nevertheless, in two independent studies of our group non-diabetic renal disease was found in 20% of NIDDM admitted for renal replacement therapy, e.g. analgesic nephropathy, polycystic kidney disease and urinary tract malformation. This is more than what is expected by chance and raises the interesting scientific issue whether superimposition of diabetes upon some primary renal disease increases the risk of development of and rate of progression of renal failure in non-diabetic renal disease [2].

Is the renal risk low in NIDDM?

It is only 1½ decades ago that authoritative reviews stated that the rate of loss of glomerular filtration rate (GFR) in NIDDM patients was fully accounted for by the age-related decrease in renal function [14]. This reflected the then widely held view that the renal prognosis in NIDDM was substantially better than in IDDM. It was argued that only a minor proportion of patients,

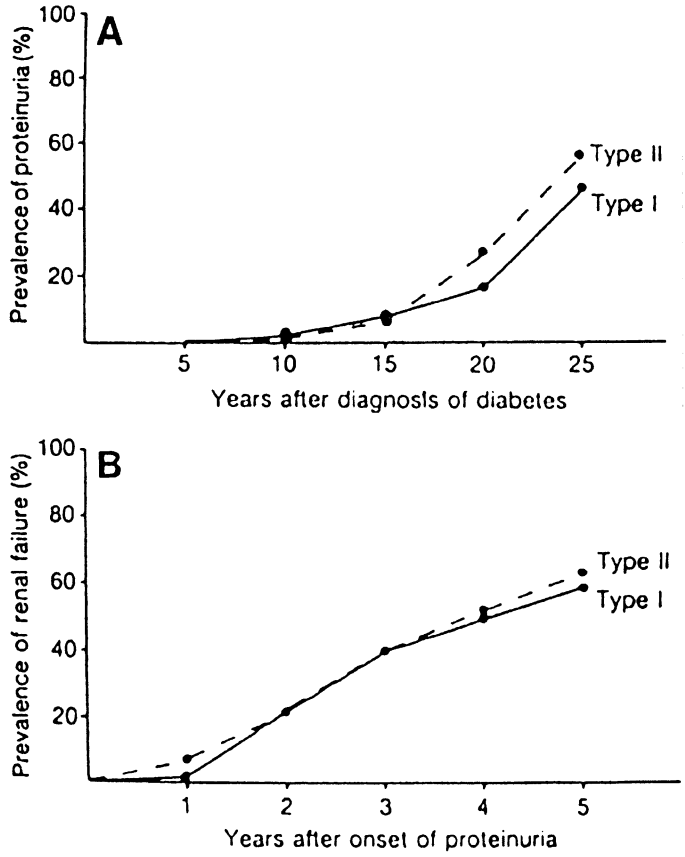

Fig. 1. Risk of proteinuria and renal failure in patients with type I (IDDM) and in patients with type II (NIDDM) diabetes mellitus (after ref. [16]).
A: cumulative prevalence of persisting proteinuria as a function of duration of diabetes in 292 IDDM and 464 NIDDM patients.
B: cumulative prevalence of renal failure (s-creatinine above 1.4 mg/dl) as a function of duration of diabetes in 48 proteinuric IDDM and 46 NIDDM patients.

approximately 5%, wound up in endstage renal failure and that the risk of ESRD was only slightly higher than in the background population [15]. This opinion led to the mistaken view that with respect to its renal sequelae NIDDM, particularly of the elderly, was a relatively benign condition (this largely accounts for the inertia in implementing effective measures of prevention for diabetic renal disease in elderly NIDDM). As shown in Figure 1, Hasslacher et al. [16] noted that the cumulative risk of proteinuria was comparable in NIDDM and IDDM patients as was the risk of renal failure in proteinuric IDDM and NIDDM patients, respectively. This conclusion is confirmed by

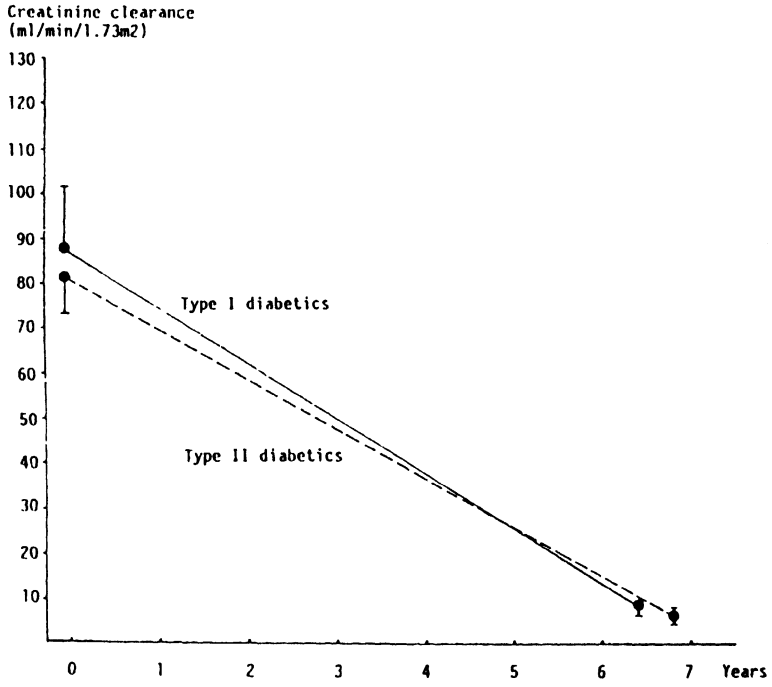

Fig. 2. Rate of loss of endogenous creatinine clearance in 16 patients with type I (IDDM) and in 16 patients with type II (NIDDM) diabetes mellitus during a 7 year observation period (after ref. [18]).

more recent findings [17]. Furthermore, the rate of loss of GFR in NIDDM patients with advanced nephropathy is quite comparable in NIDDM and IDDM, as documented by the study of Biesenbach (Figure 2) [18].

The question arises (i) why the previous misconception arose and (ii) why the incidence of endstage renal disease in NIDDM patients is so much on the rise. Although undoubtedly the frequency of diabetes in the population has dramatically increased as a result of increasing obesity and aging of the population, the major factor is the shift in balance of the competing risks of renal failure and cardiovascular death, respectively. In the Heidelberg clinic with effective antihypertensive intervention and coronary care the 5 year mortality in proteinuric NIDDM patients (the group at highest cardiovascular risk) decreased within 1 decade from 65% to 25% [2]. In other words, today many NIDDM patients live long enough to experience endstage renal disease.

Is the renal risk genetically determined?

Many authors noted clustering of diabetic nephropathy within families. This was particularly pronounced in the Pima Indians [19]. The information in

Table 2. Interaction between genetic risk (family history of cardiovascular accidents in first degree relatives) and glycemic control (after ref. [20]).

Group	Prevalence of microalbuminuria
No family history and HbA1 < 8% ($n = 12$)	0/12 (0%)
Either family history or HbA1 > 8% ($n = 52$)	1/52 (2%)
Family history and HbA1 > 8% ($n = 21$)	10/21 (48%)*

* Difference between risk groups $p < 0.0001$.

Caucasians is less complete, but we [20] and others noted that a history of hypertension and particularly of cardiovascular events in first degree relatives strikingly increased the renal risk in NIDDM in the proposites. In patients with newly diagnosed NIDDM, cardiovascular events in first degree relatives were the single strongest predictor of the presence of microalbuminuria. As shown in Table 2, the genetic risk strongly interacted with glycemia, the prevalence of microalbuminuria being particularly high when both a positive family history and poor glycemic control were present.

Recent advances in molecular biology raise the hope that the genes involved can be identified. The above observations would suggest that the renal risk is coded separately from the risk to develop NIDDM. In this context, positional analysis has led to first results implicating loci in the vinicity of MODY genes in families with NIDDM and nephropathy. Many studies assessed the role of so called candidate genes, mostly genes involved in the regulation of blood pressure. Marre et al. [21] made the initial observation that type I diabetic patients with a 287bp insertion in intron 16 of the ACE gene, had less frequently diabetic nephropathy. This proposition has been tested by a number of investigators [22, 23]. In NIDDM, there is no definite evidence for a relation between the D/I polymorphism and presence or absence of microalbuminuria or more advanced stages of nephropathy, at least in Caucasians, although in Japanese a significant association has been noted. In contrast, in NIDDM with established nephropathy, higher rates of albumin excretion and an accelerated decrease in GFR were noted in homozygotic carriers of the D-allele. Furthermore, an excess frequency of the D-allele was noted in NIDDM on maintenance hemodialysis [24]. This observation would also be consistent with a higher risk of progression to endstage renal failure. Table 3 summarizes the published literature on ACE gene polymorphism and Table 4 polymorphisms of some other candidate genes which have been examined in our laboratory [31].

Which factors accelerate progression of renal disease in NIDDM?

From the above it follows that the rate of progression is apparently determined by genetic factors. Beyond this, a number of factors have been identified which

Table 3. ACE polymorphism and diabetic nephropathy in IDDM and NIDDM – micro – or macroalbuminuria

authors	number of case/controls	frequency of D-allele	association with diabetes
IDDM			
Tarnow et al. [25]	−DN $n = 190$	0.55	no
	+DN $n = 198$	0.56	
Schmidt et al. [26]	−DN $n = 133$	0.62	no
	+DN $n = 114$	0.62	
Marre et al. [21]	−DN $n = 157$	0.53	yes
	+DN $n = 337$	0.60	
Overall	−DN $n = 480$	0.56	no
	+DN $n = 649$	0.59	($p = 0.122$)
NIDDM			
Schmidt et al. [26]	−DN $n = 208$	0.62	no
	+DN $n = 247$	0.62	
Dudley et al. [27]	−DN $n = 267$	0.54	no
	+DN $n = 163$	0.55	
Doi et al. [28]	−DN $n = 124$	0.32	yes
	+DN $n = 164$	0.44	
Mizuiri et al. [29]	−DN $n = 31$	0.47	no
	+DN $n = 80$	0.55	
Ohno et al. [30]	−DN $n = 53$	0.24	yes
	+DN $n = 79$	0.43	
Overall Caucasians	−DN $n = 475$	0.57	no
	+DN $n = 410$	0.59	($p = 0.419$)
Overall Japanese	−DN $n = 208$	0.32	yes
	+DN $n = 323$	0.46	($p = 0.0001$)

accelerate the rate of loss of GFR, so called progression promotors (Table 5). These include (i) blood pressure, (ii) albuminuria (independent of blood pressure; this explains, at least in part, the selective renal benefit of antihypertensive agents, which reduce proteinuria more than can be explained by lowering of blood pressure), (iii) glycemic control and (iv) smoking. The effects of dietary protein intake and hyperlipidemia are less well documented, at least in humans.

There is overwhelming evidence that a striking correlation exists between level of blood pressure and rate of progression. This has been found in patients with microalbuminuria, in patients with manifest (proteinuric) diabetic nephropathy and even in patients with preterminal renal failure [32–34]. It is of particular note that this relation holds true even for blood pressure values within the range of normotension according to WHO definition.

In the past there has been considerable doubt whether glycemic control is equally important in NIDDM as it is in IDDM [35]. Following the Kumamoto trial no more doubt is justified in this respect [36]. In this prospective controlled trial conventional treatment was compared with intensified insulin treatment in well over 1000 patients. Over a 6 year period the proportion of

Table 4. Assessment of role of further candidate genes in diabetic nephropathy (DN) of IDDM and NIDDM (after ref. [31]).

Candidate genes		Allele frequency	Association with DN
NO-synthase	IDDM	−DN a = 0.16; b = 0.84	no ($p = 0.83$)
	($n = 324$	+DN a = 0.17; b = 0.83	
	NIDDM	−DN a = 0.14; b = 0.86	no ($p = 0.31$)
	($n = 414$)	+DN a = 0.14; b = 0.86	
ANF	IDDM	−DN H1 = 0.02; H2 = 0.98	yes ($p = 0.041$)*
	($n = 360$)	+DN H1 = 0.05; H2 = 0.95	
	NIDDM	−DN H1 = 0.05; H2 = 0.95	no ($p = 0.35$)
	($n = 590$)	+DN H1 = 0.04; H2 = 0.96	
BR2	IDDM	−DN C = 0.91; T = 0.09	no ($p = 0.46$)
	($n = 446$)	+DN C = 0.93; T = 0.07	
	NIDDM	−DN C = 0.91; T = 0.09	no ($p = 0.47$)
	($n = 664$)	+DN C = 0.92; T = 0.08	
Paraoxonase	IDDM	−DN A = 0.73; B = 0.27	no ($p = 0.41$)
	($n = 272$)	+DN A = 0.68; B = 0.32	
	NIDDM	−DN A = 0.73; B = 0.27	no ($p = 0.89$)
	($n = 414$)	+DN A = 0.72; B = 0.28	

* Without Bonferoni correction; ANF – atrial natriuretic factor; BRS – bradykinin receptor (exon2)

Table 5. Progression promotors

Blood pressure
Albuminuria
Glycemic control
Smoking
Dietary intake of protein?
Hyperlipidemia?

patients who developed microalbuminuria was lower by factor of 3 [36]. Is near normoglycemia also a desirable goal in the NIDDM patient with proteinuria? In short-term observations, i.e. 18 month, of IDDM patients no change in the rate of loss of GFR was noted after institution of near normoglycemia with insulin pump treatment. Based on this observation it had been proposed that once the patient is proteinuric a 'point of no return' has been reached beyond which glomerulosclerosis progresses independent by ambient glucose concentrations [37]. This concept has led to considerable therapeutic nihilism. No formal studies are available in NIDDM, but more recent observational studies show that HbA_{1c} is an independent predictor of the rate of progression (H.H. Parving, personal communication).

Following the early report of Christiansen [38] a considerable body of evidence has accumulated that smoking is an independent predictor for the risk of a development of diabetic nephropathy and a factor promoting progression of nephropathy [39, 40]. A correlation has been documented between

smoking and the proportion of patients with, and the extent of, albuminuria in NIDDM [20, 40, 41]. Biesenbach found that the rate of loss of GFR was double in NIDDM patients who smoked [18].

What are accepted antihypertensive strategies in 1997?

Several recent consensus statements emphasized the importance of antihypertensive therapy in preventing development and progression of diabetic nephropathy [33, 42]. In diabetic (as in non-diabetic) glomerular disease, the kidney appears to be damaged by blood pressure values even within the range of normotension according to WHO or JNC criteria. Consequently, it has been recommended to treat even normotensive IDDM and NIDDM once microalbuminuria is present [33]. Although the evidence for a beneficial effect in NIDDM is more fragmentary than in IDDM, such advice seems reasonable, since the evolution of diabetic nephropathy is similar in NIDDM and IDDM.

There is general agreement that lowering blood pressure is important regardless of the agent used and that blood pressure values in the low normal range should be aimed at. The selection of antihypertensive agents is also important, however, since a 'renoprotective effect' has been shown for ACE inhibitors [43, 44] and, less uniformly, for calcium channel blockers (CCB) [45]. Prospective placebo-controlled trials in normotensive microalbuminuric NIDDM showed a diminished rate of progression of albuminuria with ACE inhibitors [46, 47]. In small studies with short follow-up, captopril was administered to normoalbuminuric, normotensive NIDDM and appeared to prevent the onset of microalbuminuria [48]. There is no doubt that in hypertensive microalbuminuric NIDDM ACE inhibitors diminish urine albumin excretion [46] or at least prevent a progressive rise in urine albumin excretion [49]. Based on available published evidence, ACE inhibitors are relatively safe despite the risks of renal failure from unrecognized renal artery stenosis and hyperkalemia. ACE inhibitors improve glucose uptake in peripheral tissue in NIDDM with beneficial effects of glycemic control [50], although at the expense of higher risk of hypoglycemia. ACE inhibitors reduce the measured loss of GFR [45] and in early diabetic nephropathy even an increase of GFR in early diabetic nephropathy was seen in one study [51]. In contrast to IDDM, no controlled prospective study has documented a benefit in NIDDM with renal failure, although the data of subgroup analysis in NIDDM patients of the AIPRI study are encouraging (Figure 3) [52]. Currently, two major trials in NIDDM with renal failure are underway to assess AT_1-receptor blockers. A metaanalysis showed that ACE inhibitors progressively lose their antiproteinuric advantage over other antihypertensive agents with progressive study durations by more intensive lowering of blood pressure [44]. Analysis of the captopril trial in IDDM showed that the event rate in the captopril and the placebo arms became identical once treated blood pressure values were below 95 mmHg mean arterial blood pressure (E. Lewis, personal communication).

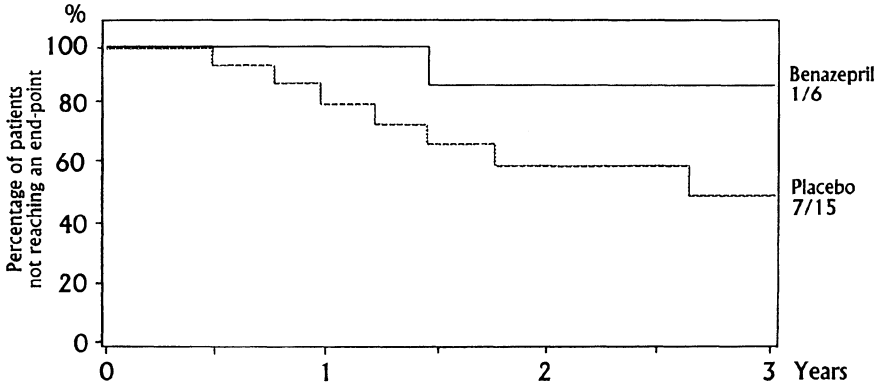

Fig. 3. Renal end-point (doubling of serum creatinine or renal death) in 21 NIDDM patients of the AIPRI study. Six patients were on the ACE inhibitor Benazepril and 15 patients on placebo (subgroup analysis of study [52]).

The effects of CCB are less consistent, presumably because of the heterogeneity of the compounds and greater variability of their renal effects. In several studies, CCB were compared with ACE inhibitors [53, 54]. In most, but not all, studies, ACE inhibitors were more beneficial with respect to urinary albumin excretion. A recent comparison of cilazapril and amlodipine in hypertensive NIDDM using chrom-51-EDTA clearance found similar efficacy with respect to the decline of GFR at blood pressure values below 140/85 mmHg [53]. Bakris et al. [45] found in NIDDM that lisinopril and non-dihydropyridine CCB (verapamil or diltiazem) were superior to atenolol in attenuating the rate of loss of creatinine clearance and in reducing proteinuria. It has been reasoned that the combination of CCB and ACE inhibitors might be superior to the monotherapies [55]. Bakris et al. [56] found that the combination of verapamil and lisinopril was more effective than the monotherapies in reducing proteinuria and the loss of GFR.

Since coronary heart disease is frequent in NIDDM, the issue of the J-curve phenomenon is relevant. It has been argued that aggresive lowering of diastolic blood pressure below 85 mmHg causes cardiac ischemia in patients with coronary heart disease. Fletcher et al. [57] commented that in patients with low diastolic pressures cardiac mortality is higher even in the placebo arm of trials suggesting that it is a marker of 'sick patients' at high risk of cardiac death rather than a cause of cardiac death. In the MDRD trial there was no evidence of the J-curve phenomenon. Although there is no statistical evidence for low blood pressure-induced critical cardiac ischemia, cautious lowering of blood pressure is appropriate, since cardiac ischemia may be provoked in some patients. Renoprotection is not the only goal of antihypertensive treatment in NIDDM; other endorgan diseases must also be considered. For instance,

Table 6. Renal function of the 9 NIDDM patients admitted to the renal unit Heidelberg for renal replacement therapy in the first half-year 1997 (after ref. [64]).

Creatinine clearance (ml/min)	10 (4–20)
Serum creatinine (mg/dl)	4.7 (2.0–7.7)
Serum urea (mg/dl)	137 (68–239)
Proteinuria (g/day)	7 (1.5–14.0)
Admission with vascular access	0/9 patients
Systolic blood pressure (mmHg)	180 (140–230)
Diastolic blood pressure (mmHg)	90 (70–130)
Number of antihypertensive agents	2–3
ACE inhibitor therapy	1/9 patients
Blood pressure self-measurement	6/9 patients
HbA_{1c} (%)	8.0 (4.4–11.7)
Serum cholesterol (mg/dl)	246 (109–373)
Serum triglycerides (mg/dl)	293 (148–634)
Treatment with statines	1/9 patients
Treatment with fibrates	0/9 patients
Opthalmological examination in the last 12 month	4/9 patients
Participation in a diabetes education program (ever)	1/9 patients
On insulin treatment	6/9 patients

Data as median and range

betablockers are grossly underused in uremic NIDDM with coronary heart disease [58].

To what extent are effective measures of prevention implemented?

Current quality of medical care for patients with NIDDM in general, and for proteinuric NIDDM patients in particular, is suboptimal [59]. ESRD associated with NIDDM, at least in the U.S., is mainly a disease of poor, elderly blacks and Hispanics who often had substandard medical care in the interval between diagnosis of diabetes and onset of ESRT. Friedman et al. assessed the impact of lacking health services on the course of diabetes in innercity residents. They found that among such patients newly admitted to the Kings County Hospital during the preceding year, fewer than one quarter had been examined by an ophthalmologist or podiatrist and only one third had any diabetic counseling [60]. Most patients with NIDDM are currently cared for by generalists. Poor adherence to treatment and screening guidelines has been reported by many authors [61] and this is not unique to the USA. In Germany similarly appalling figures have been reported [62] and criticised [63]. Despite the postulates of the San Vincent declaration, there is little evidence that state of the art treatment is provided to the majority of diabetic patients with renal disease. This is also reflected by our experience in the 9 NIDDM patients submitted for renal replacement therapy in the first 6 months of 1997 (Table 6)

[64]. This state of affairs is the more deplorable, since in NIDDM as in IDDM, end-stage renal failure is preventable, at least in principle.

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5. Clinical imperatives in diabetic nephropathy: the devastating impact of comorbidity

ELI A. FRIEDMAN

Editors' Comment:

Diabetes makes people sick because of its comorbid complications. Nephrologists and ophthalmologists caring for people with diabetes devote minimal time to metabolic regulation and maximal attention to the consequence of microvasculopathy in the kidney and eye. Fragmented medical management often results from tunnel vision in subspecialists, each handling a component of what should be a comprehensive medical regimen. The patient may be entrapped by conflicting advice, medications that interfere with the action of other drugs, and unneeded repeated expensive laboratory tests. Without a single coordinating physician diabetic patients too often receive fragmented and incomplete care delivered by an otherwise competent high powered team. By constructing a 'Life Plan' for each patient with periodic assessment of extrarenal comorbidity, the chances of overlooking an impediment to rehabilitation are reduced. The utility of ranking extrarenal diabetic complications numerically using a Co-Morbidity Index has been demonstrated in studies of hemodialysis and peritoneal dialysis patients and is proposed for kidney transplant recipients.

Introduction

Nephrologists in the United States (US), Japan, and industrialized Europe cope with the reality that in 1998, diabetes mellitus is the leading cause of end-stage renal disease (ESRD) surpassing glomerulonephritis and hypertension. European, Japanese, and North American registries of renal failure patients indicate that both the incidence and prevalence of ESRD attributed to diabetes has risen each year over the past decade. Underscoring this point, the United States Renal Data System (USRDS) in 1997 reported that of 257 266 US patients receiving either dialytic therapy or a kidney transplant in 1995, 80 667 had diabetes [1], a prevalence rate of 31.4%. Furthermore, during 1995, of 71 875 new (incident) cases of ESRD, 28 740 (40%) were listed as having diabetes (Figure 1).

Diabetes type in diabetic nephropathy

Diabetes in America is predominantly Type 2, fewer than 8% of diabetic Americans are insulinopenic, C-peptide negative persons who have Type 1

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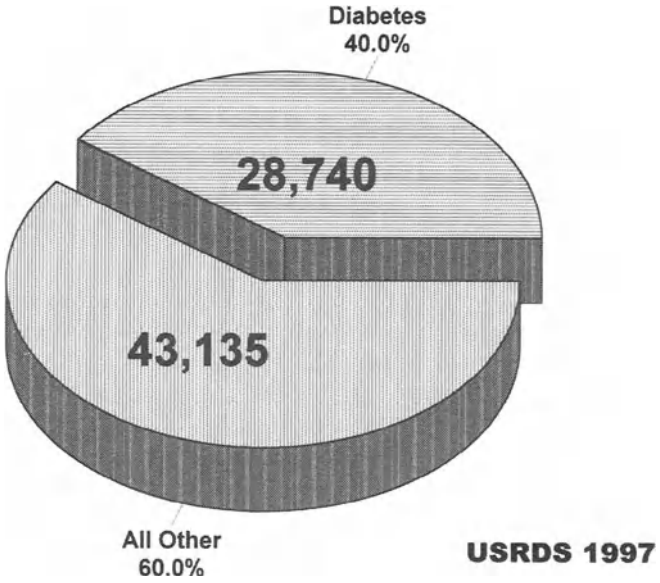

Fig. 1. Extracted from the 1997 report of the United States Renal Data System (USRDS) [1], the proportion of incident ESRD patients whose diagnoses was listed as diabetes was 40% in 1995. The USRDS does not distinguish between Type 1 and Type 2 diabetes in their reporting forms. Registries in Europe and Japan also list diabetes as the disease accounting for the greatest number of incident and prevalent ESRD cases.

diabetes. ESRD in diabetic persons reflects the demographics of diabetes *per se* [2] in that: (1) Incidence [3] is higher in women, blacks [4], Hispanics [5], and native Americans [6]. (2) Peak incidence of ESRD occurs from the 5th to the 7th decade. Consistent with these attack rates, is the reality that blacks over the age of 65 face a seven times greater risk of diabetes-related renal failure than do whites. In the United States, it is not surprising, therefore, that ESRD associated with diabetes is largely a disease of elderly blacks [7]. My sampling of hemodialysis units in New York, Chicago, Oklahoma City, San Antonio and Detroit, affirmed that from one-third to more than one-half of newly diagnosed inner city ESRD patients starting maintenance hemodialysis are diabetic black or Hispanic persons – predominantly women – over the age of 50. Within our Brooklyn city and state hospital ambulatory hemodialysis units in October 1997, 97% of prevalent patients had Type 2 diabetes.

Vasculopathic complications of diabetes including the onset and severity of hypertension are at least as severe in Type 2 as in Type 1 diabetes [8, 9]. In fact, recognition of the high prevalence of proteinuria and azotemia in carefully followed Type 2 diabetic subjects contradicts the common impression that Type 2 diabetes relatively infrequently induces nephropathy [10]. While there are differences between Type 1 and Type 2 diabetes in genetic predisposi-

tion [11] and racial expression, other aspects of the two disorders – particularly manifestations of nephropathy – are remarkably similar. Careful observation of the course of nephropathy in Type 1 and Type 2 diabetes indicates strong similarities in rate of renal functional deterioration [12] and onset of comorbid complications. Early nephromegaly, as well as both glomerular hyperfiltration and microalbuminuria, previously thought limited to Type 1 are now recognized as equally prevalent in Type 2 [13]. Not yet included in USRDS reports is any distinction between Type 1 and Type 2 diabetes in terms of dialysis morbidity and mortality or posttransplant patient and allograft survival.

Lack of precision in diabetes classification provokes confusing terms like 'insulin requiring' to explain treatment with insulin in persons thought to have resistant Type 2 diabetes. In fact, present criteria are unable to classify as many as one-half of diabetic persons as Type 1 or Type 2 diabetes [14, 15]. Consequently, literature reports of the outcome of ESRD therapy by diabetes type are few and imprecise.

Comorbid risk factors

At every stage of progressive renal insufficiency management of a diabetic person is more difficult than in an age and gender matched nondiabetic person because of extensive, often life threatening, extrarenal (comorbid) disease. Diabetic patients manifesting ESRD suffer a higher death rate than do nondiabetic ESRD patients due to greater attack rates for cardiac decompensation, stroke, sepsis and pulmonary disease (Figure 2). Concurrent extrarenal disease – especially blindness, limb amputations, and cardiac disease – limits and may preempt their rehabilitation. For most diabetic ESRD patients, the difference between rehabilitation and heartbreaking invalidism hinges on attaining a renal transplant (*vide infra*) along with effective comprehensive attention to comorbid conditions.

Illustrating this point, while establishing a hemodialysis vascular access in a nondiabetic person usually requires minor ambulatory surgery, equivalent surgery in a diabetic patient risks major morbidity from systemic infection, cardiac decomposition, or decubitus ulceration. Successive diabetic comorbid complications – seemingly unending – during dialytic therapy prompts a substantially higher rate of withdrawal from therapy (*de facto* suicide) [16] (Figure 3).

Listed in Table 1 are the major comorbid risks in diabetic patients that may worsen during the course of ESRD treatment. Coping with concomitant multisystem diabetic complications requires talents of a sophisticated team (Figure 4). Central to the team's effectiveness is the nurse educator who: teaches blood glucose testing, explains medications and dosing schedules, and serves as patient advocate, facilitating appointments and procedures. Diabetic retinopathy tops the list because of the hopelessness typically induced by blindness – with heart and lower limb disease – major concerns in overall patient care.

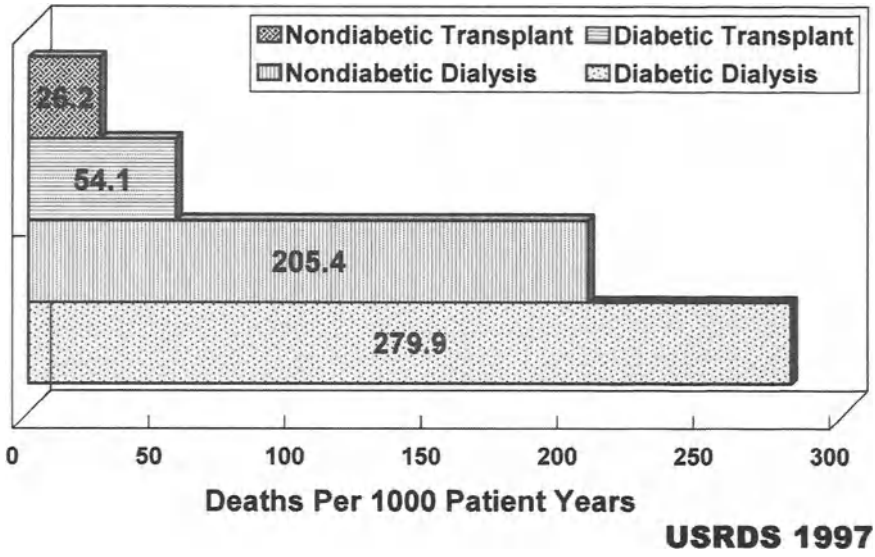

Fig. 2. While strong selection bias in assigning treatment modality obviously skews the data, it is still remarkable to note the superior survival of kidney transplant recipients over those ESRD patients treated by dialysis. For this analysis, survival of peritoneal and hemodialysis patients was grouped. Note that within both transplant and dialysis subsets, diabetic patients had greater mortality than did nondiabetic patients.

Blind limb amputees rarely are able to return to active employment or home responsibilities.

The omnipresent threat of blindness is documented in the histories of laser treatment and/or vitrectomy for retinopathy given by over 90% of diabetic individuals who begin maintenance dialysis or receive a renal allograft. Laser and/or vitreous surgery can be integrated as a component of comprehensive management [17]. Cardiologic evaluation, even in asymptomatic patients, including coronary angiography (if indicated), is essential to detect individuals for whom prophylactic coronary artery angioplasty or bypass surgery is likely to extend life [18]. Our podiatrist delivers routine foot care and initiates regular surveillance of patients at risk of major lower extremity disease thereby sharply reducing the chance of toe, foot, and limb amputations [19].

Autonomic neuropathy – expressed as gastropathy, cystopathy, and orthostatic hypotension – is a frequently overlooked, highly prevalent disorder [20] impeding life quality in the diabetic with ESRD. Diabetic cystopathy, though common, is frequently unrecognized and confused with worsening diabetic nephropathy and is sometimes interpreted as allograft rejection in diabetic kidney transplant recipients. We assessed bladder function in 22 diabetic patients who developed renal failure – 14 men and 8 women of mean age 38 years – finding that an air cystogram detected cystopathy in 8 (36%)

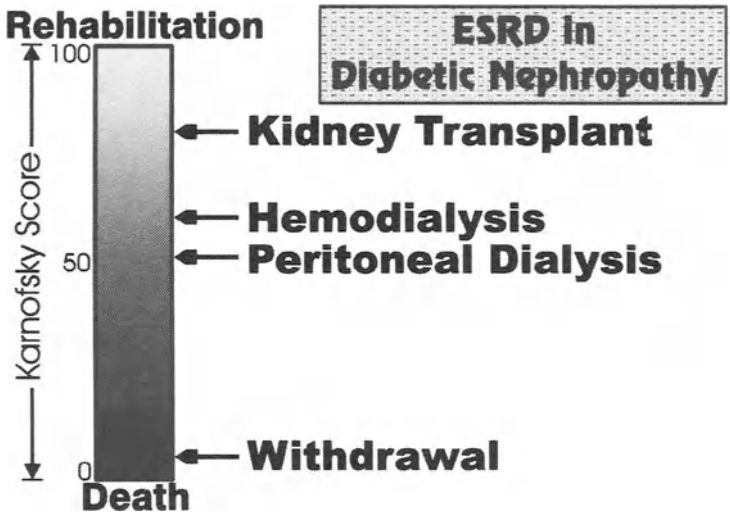

Fig. 3. Employing the easy-to-use Karnofsky Score [59], patient rehabilitation can be ranked from 0 to 100 where 100 equals perfect health and 0 equals death. A score below 70 indicates the need for substantive assistance in everyday activities while a score below 60 defines invalidism. Arrows reflect the author's bias as to the usual outcome for kidney transplant recipients, versus hemodialysis versus peritoneal dialysis treated diabetic ESRD patients.

Table 1. Diabetic complications which persist and/or progress during ESRD

1.	Retinopathy, glaucoma, cataracts.
2.	Coronary artery disease. Cardiomyopathy.
3.	Cerebrovascular disease
4.	Peripheral vascular disease: limb amputation.
5.	Motor neuropathy. Sensory neuropathy.
6.	Autonomic dysfunction: diarrhea, dysfunction, hypotension.
7.	Myopathy
8.	Depression

manifested as detrusor paralysis in 1 patient; severe malfunction in 5 patients (24%) [21]; and mild impairment in 1 patient. During initial evaluation for diabetic nephropathy, gastroparesis may be ignored though it afflicts one-quarter to one-half of azotemic diabetic persons [22]. Other expressions of autonomic neuropathy – obstipation and explosive nighttime diarrhea – often coexists with gastroparesis [23]. Obstipation responds to daily doses of cascara, while diarrhea is treated with psyllium seed dietary supplements one to three times daily plus loperamide [24] in repetitive 2 mg doses to a total dose of 18 mg daily.

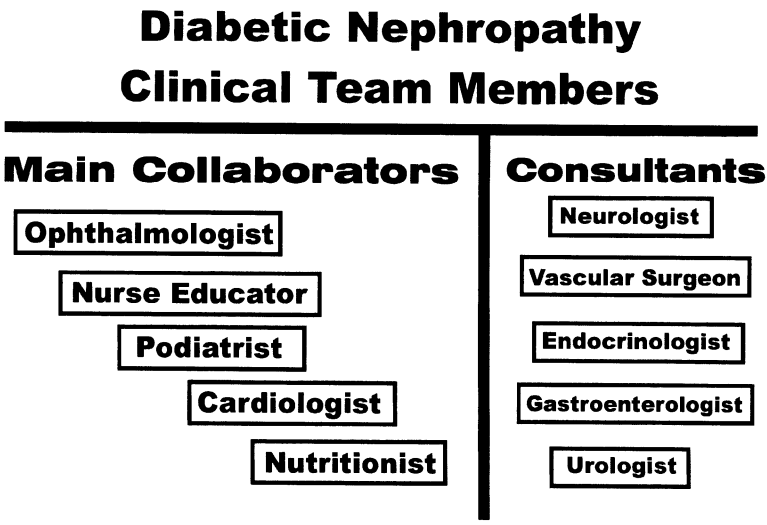

Fig. 4. Defining a Life Plan for each ESRD patient reduces stress imparted by indecision and confusion. Key collaborators are listed on the left while frequent consultants are listed on the right. Not included though perhaps equally important is 'The Patient' who should be an active participant in all decision making.

Pregnancy

Pregnancy in diabetes when complicated with kidney disease (proteinuria and/or azotemia) previously regarded as an unavoidable prelude to disaster – inordinate fetal loss and/or maternal morbidity and death – is now managed with a high probability of success. According to Gavin, Lyons, and Kitzmiller, detection of macroproteinuria, in the absence of urinary infection, in a Type 1 pregnancy will be followed by preeclampsia in 30–50%, though perinatal survival should exceed 95% [25]. Women whose serum creatinine concentration exceeds 2.0 mg/dl are counseled to avoid pregnancy though after a satisfactory renal transplant perinatal outcome is 'excellent' despite a superimposed preeclampsia rate of 20–40%. Miodovnik et al. followed 182 pregnant women with Type 1 diabetes, 46 of whom had overt nephropathy for a minimum of 3 years after delivery and concluded that pregnancy neither increases the risk of subsequent nephropathy nor accelerates progression of preexisting renal disease [26]. In an equally encouraging series from Finland, Kaaja et al. observed the course of 28 diabetic women for 7 years after delivery compared with 17 nulliparous controls matched for age, duration of diabetes, and severity of vasculopathy and concluded that "pregnancy does not seem to affect development or progression of diabetic nephropathy" [27].

Selecting uremia therapy

Depending on age, severity of co-morbid disorders, available local resources, and patient preference, the uremic diabetic patient may be managed according to different protocols. Absent strong family (social) support, diabetic ESRD patients chose to discontinue treatment, meaning passive suicide, more frequently than do nondiabetic patients [28]. Such a decision is understandable for blind, hemiparetic, bed-restricted limb amputees for whom life quality has been reduced to what is interpreted as unsatisfactory. On the other hand, attention to the total patient may restore a high quality of life that was unforeseen at the time of ESRD evaluation [29].

Evaluations in both Europe and the US discerned inadequate care, mainly absent attention to comorbidity in diabetic patients with symptomatic kidney disease. As inferred by Pommer et al. in German: "preterminal care in diabetic patients with ESRD" is deficient in amount and quality [30] with inadequate attention to control of hypertension, hyperlipidemia or ophthalmologic intervention [31]. In the United Kingdom, at variance with prior consensus, withdrawal from renal replacement therapy was not infrequent accounting for 17% of all deaths and was mainly due to comorbid medical problems (89%) especially diabetic vasculopathy [32]. Similarly, in a US study, Ifudu et al. found that because of excessive comorbidity: "Few elderly, diabetic hemodialysis patients conduct a substantive portion of their lives outside their homes" [33].

Hemodialysis

For the large majority – over 80% of diabetic persons who develop ESRD in the US – maintenance hemodialysis is the only renal replacement regimen that will be employed. Approximately 12% of diabetic persons with ESRD will be treated by peritoneal dialysis while the remaining 8% will receive a kidney transplant. To perform maintenance hemodialysis requires establishment of a vascular access to the circulation. Creation of what has become the standard access – an internal arteriovenous fistula in the wrist – is often more difficult in a diabetic than in a nondiabetic person because of advanced systemic atherosclerosis. For many diabetic patients with peripheral vascular calcification and/or atherosclerosis, creation of an access for hemodialysis necessitates resort to synthetic (Dacron) prosthetic vascular grafts. The typical hemodialysis regimen requires three weekly treatments lasting 4 to 5 hours each, during which, extracorporeal blood flow must be maintained at 300 to 500 ml/min. Motivated patients trained to perform self-hemodialysis at home gain the longest survival and best rehabilitation afforded by any dialytic therapy for diabetic ESRD. When given hemodialysis at a facility, however, diabetic patients fare less well, receiving significantly less dialysis than nondiabetic patients due, in part, to hypotension and reduced access blood flow [34]. Maintenance hemodialysis does not restore vigor to diabetic patients as docu-

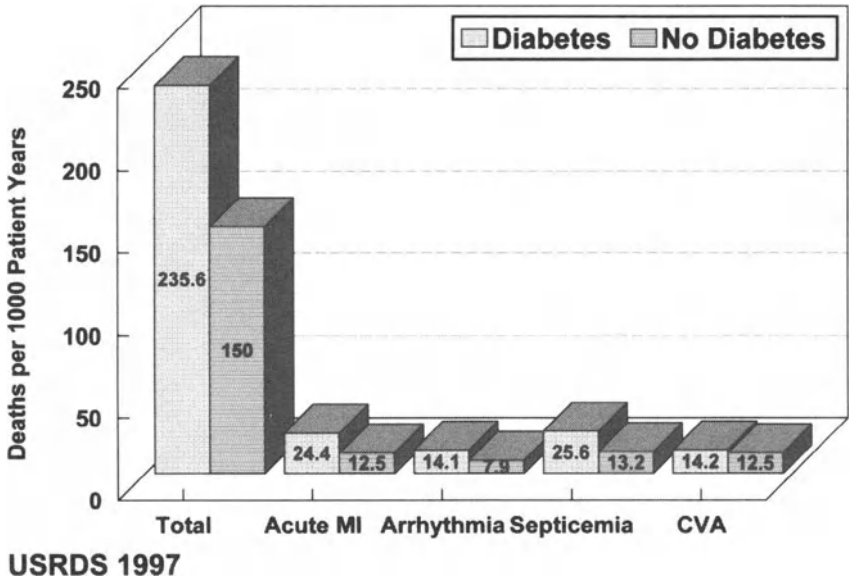

Fig. 5. Management of diabetic patients throughout the course of progressive diabetic nephropathy in actuality becomes the treatment of a series of worsening comorbid conditions. As shown in selected data from the most recent report of the United States Renal Data System (USRDS) [1], deaths during treatment for ESRD are greater in diabetic than in nondiabetic patients for myocardial infarction, septicemia, and cerebrovascular disease.

mented by Lowder et al., in 1986, who reported that of 232 diabetics on maintenance hemodialysis only seven were employed while 64.9% were unable to conduct routine daily activities without assistance [35]. Approximately 50% of diabetic patients begun on maintenance hemodialysis die within 2 years of their first dialysis. The impact of excessive comorbidity in diabetic hemodialysis patients is apparent in Figure 5 depicting selected USRDS death rates by diagnosis in hemodialysis patients treated from 1993 to 1995 [1]. Diabetic hemodialysis patients sustained more total, cardiac, septic, and cerebrovascular deaths than did nondiabetic patients.

Peritoneal dialysis

In the US, peritoneal dialysis sustains the life of about 12% of diabetic ESRD patients [1]. Continuous ambulatory peritoneal dialysis (CAPD) affords the advantages of freedom from a machine, performance at home, rapid training, minimal cardiovascular stress and avoidance of heparin [36]. Enthusiasts characterize CAPD as 'a first choice treatment' for diabetic ESRD patients [37]. Rubin et al. in a largely black diabetic population were less enthusiastic

[38]. Only 34% of their patients continued CAPD after 2 years, and at 3 years, only 18% remained on CAPD.

Objective comparisons of either mortality or comorbidity in hemodialysis versus peritoneal dialysis patients suffer from the limitations of starting with unequal cohorts reflecting selection bias. This problem is well illustrated in reports from the CANUSA study comparing survival and other variables in CAPD patients in the United States and Canada [39]. Overshadowing all results of the international comparison was the unexplained much higher relative risk of death (1.93%) in the US, perhaps a function of a two-fold greater acceptance rate for ESRD, including purportedly sicker (greater comorbidity) patients. Extracted data subsets from the USRDS Report for 1997 [1] show that diabetic patients in all subsets have a higher death risk on CAPD than on hemodialysis. Similarly, peritoneal dialysis patients in the US had a 14% greater risk of hospitalization than did patients undergoing hemodialysis [40]. By sharp contrast, Fenton et al. using data from the Canadian Organ Replacement Register for 11 970 patients who began ESRD therapy between 1990 and 1994 concluded that peritoneal dialysis "is not associated with increased mortality rates relative to hemodialysis" [41]. Weighing these conflicting reports, the decision to treat a diabetic patient with CAPD, therefore, must be individual-specific. Benefits of peritoneal dialysis including freedom from a machine and electrical outlets, and ease of travel stand against disadvantages of unremitting attention to fluid exchange, constant risk of peritonitis, and disappearing exchange surface.

Kidney transplantation

Sutherland et al. [42] correctly predicted that following a renal transplant, patient survival at one and two years would be equivalent in diabetic and nondiabetic recipients [43], though graft survival, in some large series, remains marginally lower in diabetic persons. At its best, as illustrated by a single center retrospective review of all kidney transplants performed between 1987 and 1993, there is no significant difference in actuarial 5-year patient or kidney graft survival between diabetic and nondiabetic recipients overall, or when analyzed by donor source. Furthermore, no difference in mean serum creatinine levels at 5 years was noted between diabetic and nondiabetic recipients [44]. Superiority of survival following a kidney transplant when compared to peritoneal and hemodialysis is shown in Figure 6 extracted from the 1997 report of the USRDS [1]. Fewer than five in 100 diabetic ESRD patients treated by dialysis will survive 10 years while cadaver donor and living donor kidney allograft recipients fare far better. Survival statistics following a renal transplant, compared with dialytic therapy, does not tell the whole story as rehabilitation is incomparably better. Enhanced life quality permitted by a kidney transplant is the reason to prefer this ESRD option for newly evaluated diabetic persons with ESRD under the age of 60. More than half of diabetic kidney transplant

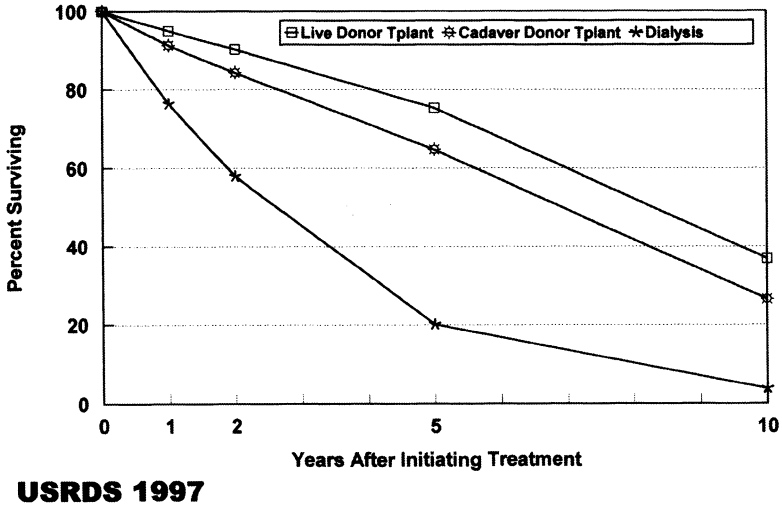

Fig. 6. Long-term observation of diabetic ESRD patients shown in selected data from the most recent report of the United States Renal Data System (USRDS) [1], depicts two key points: 1. Dialysis patients are nearly all dead within a decade. Renal transplant recipients have superior survival though more than one-half die within a decade whether treated with a living donor or cadaver donor kidney. The burden of advanced glycosylated end-product (AGE) toxicity in dialysis patients may explain the difference in outcome between modalities.

recipients in most series live for at least three years: many survivors return to occupational, school and home responsibilities.

Pancreas plus kidney transplantation

While regarded as investigational by some [45] and, even when successful, applicable to more than 9% of uremic diabetic patients who have Type 1 diabetes, pancreatic transplantation is growing in acceptability and technical success [46]. In one remarkable series, survival 1 year post-renal transplant, in 995 diabetic kidney recipients who also received a pancreas transplant renal allograft survival was a remarkable 84% [47]. World-wide results in simultaneous kidney–pancreas transplants show that more than 90% of recipients were alive at 1 year, more than 80% had functional kidney grafts, and more than 70% no longer required insulin [48].

Data thus far indicate that a functioning pancreas does not impede progression or recurrence of diabetic nephropathy. On the positive side, the course of diabetic neuropathy following combined pancreas and kidney transplantation varies from stabilization and, in some patients, improvement in diabetic motor neuropathy [49]. Also, in a comparison of renal transplant biopsies taken ≥ 2.5 years post-transplant, 92% of recipients of a combined pancreas and renal transplant, but only 35% of recipients with renal transplant alone

had normal glomerular basement membrane thickness [50]. Glomerular mesangial volume expansion in the renal transplant, another early sign of recurrent diabetic nephropathy, is also retarded by the presence of a functioning pancreatic transplant. Unfortunately, pancreas transplantation in patients with extensive extrarenal disease, has neither arrested nor reversed diabetic retinopathy, diabetic cardiomyopathy, or extensive peripheral vascular disease [51, 52]. Nevertheless, a functioning pancreas transplant does free patients with Type 1 diabetes from the dreaded daily sentence of balancing diet, excercise, and insulin dosage [53]. In 1997, consensus of clinical nephrologists is that the ESRD patient with Type 1 diabetes should consider a simultaneous kidney and pancreas transplant as at least a temporary cure of inexorable disease [54].

Patient survival during treatment of ESRD

Caution is appropriate when interpreting available studies of survival by ESRD modality in diabetic patients. All retrospective and prospective reports lack balanced (equivalent) treatment groups in terms of equalities in age, race, diabetes type, severity of complications, and degree of metabolic control. Even prospective studies of renal transplantation compared with peritoneal or hemodialysis might not overcome limitations imposed by patient and physician refusal to permit random assignment to one treatment over another. By current practice, younger patients with fewer complications are assigned to renal transplantation while residual older, sicker patients are treated by dialysis. Combined kidney/pancreas transplants are restricted to Type 1 diabetic patients younger than age 50 who have minimal cardiac disease.

Advancing age is a key variable in ESRD survival "irrespective of treatment modality and of primary renal disease" [55] as noted in reports by Brunner et al. who summarized the European Dialysis and Transplant Association (EDTA) Registry report. Illustrating the adverse effect of older age where comparative survivals 10 (58%) and 15 (52%) years after starting treatment in patients who were 10–14 years old when begun on ESRD therapy, compared to 28% and 16% of those who were 45–54 years old when starting ESRD therapy who were alive at 10 and 15 years. The same effect of increasing age was discerned in recipients of living related donor kidney transplants. In the early 1980s, kidney recipient survival was 92% at 5 years for patients younger than 15, 87% for the 15–44 year old cohort and 72% for those aged 45 or older.

At every age cohort, diabetes adds a severe restriction on life anticipation, imparting a threefold rise in risk of dying compared with either chronic glomerulonephritis or polycystic kidney disease. In the United Kingdom, diabetic and nondiabetic patients starting CAPD or hemodialysis in seven large renal units between 1983–1985 were monitored prospectively over 4 years. Of 610 new patients (median age 52 years, range 3–80 years) beginning CAPD and 329 patients (median age 48 years, range 5–77 years) starting hemodialysis,

patient survival estimates at 4 years were 74% for hemodialysis and 62% for CAPD [56]. Survival on CAPD and maintenance hemodialysis is lower in the US than in Europe. An explanation for diabetic dialysis patients' better survival in Europe is not evident, though the growing application of American practices of dialyzer reuse and shortened treatment hours have been incriminated as promoting fatal underdialysis [57]. A rebuttal to the allegation that US dialysis mortality is higher than that in Europe contends that because the US has approximately twice the incidence of ESRD acceptance than Europe, older and sicker patients in the US obviously will experience greater mortality [58].

Rehabilitation

Lessions gleaned from study of rehabilitation in the diabetic ESRD patient include: (i) Diabetic patients fare best when participating in their treatment regimen. (ii) A functioning renal transplant permits markedly superior rehabilitation than that attained by either peritoneal dialysis or hemodialysis. Unfortunately, strong bias when applying one ESRD regimen over another may prejudice the favorable view of kidney transplants to the extent that statistical corrections (Cox Proportional Hazards technique) cannot compensate for group differences. Obviously, comparisons in which mean age of transplant patients is a decade younger than that of CAPD or hemodialysis groups are preordained to discern better functional status in the younger group. In the US, for example, patients over the age of 69 years who comprised 27% of all dialysis patients in 1979 increased by 450% between 1974 and 1981, will make up 60% of all dialysis patients by the year 2010. An ageing dialysis population compared with a stable of transplant recipients has a declining rate of employment and increasingly prevalent comorbid complications.

Unless patient cohorts (hemodialysis versus CAPD versus renal transplantation versus combined kidney and pancreas transplantation) are shown to have equivalent comorbidity at the time of comparison, conclusions about the effect of modality on outcome are liable to be erroneous. To quantify the course of diabetic patients over the course of ESRD treatment we periodically inventory the type and severity of common co-morbid problems. Numerical ranking of this inventory constitutes a comorbid index (Table 2). We utilize the Karnofsky scoring system [59] to assess patient well being [60]. Applying the Karnofsky score to 2481 dialysis patients irrespective of location or type of dialysis, Gutman et al. in the 1970s found that diabetic patients achieved very poor rehabilitation; only 23% of diabetic patients (versus 60% of nondiabetic patients) were capable of physical activity beyond caring for themselves [61]. Confirming and extending this conclusion a decade later, Lowder et al. reported abysmal rehabilitation in diabetic hemodialysis patients. More recent confirmation of this point was afforded by Ifudu et al. who documented pervasive failed rehabilitation in multicenter studies of diabetic and nondiabetic [62], and elderly inner-city [33] hemodialysis patients. An inescapable conclusion of

Table 2. Variables in morbidity in diabetic kidney transplant recipients the co-morbidity index

1.	Persistent angina or myocardial infarction.
2.	Other cardiovascular problems, hypertension, congestive heart failure, cardiomyopathy.
3.	Respiratory disease.
4.	Autonomic neuropathy (gastroparesis, obstipation, diarrhea, cystopathy, orthostatic hypotension.
5.	Neurologic problems, cerebrovascular accident or stroke residual.
6.	Musculoskeletal disorders, including all varieties of renal bone disease.
7.	Infections including AIDS but excluding vascular access-site or peritonitis.
8.	Hepatitis, hepatic insufficiency, enzymatic pancreatic insufficiency.
9.	Hematologic problems other than anemia.
10.	Spinal abnormalities, lower back problems or arthritis.
11.	Vision impairment (minor to severe – decreased acuity to blindness) loss.
12.	Limb amputation (minor to severe – finger to lower extremity).
13.	Mental or emotional illness (neurosis, depression, psychosis).

To obtain a numerical Co-Morbidity Index for an individual patient, rate each variable from 0 to 3 (0 = absent, 1 = mild – of minor import to patient's life, 2 = moderate, 3 = severe). By proportional hazard analysis, the relative significance of each variable can be isolated from the other 12.

studies to date is that maintenance hemodialysis – in many instances – does not permit return to life's responsibilities for diabetic individuals. The authors' assessment of the relative values of options in ESRD therapy when applied to diabetic patients is given in Table 3 and Figure 3.

Pre-ESRD intervention

Like the proverbial iceberg, approximately 7/8ths of the course of progressive loss of kidney function in diabetes is invisible by routine laboratory screening tests (Figure 7). It is during these 'silent years' that proper medical care may proffer greatest benefit. Establishment of a single physician responsible for guiding the diabetic patient with a renal syndrome is essential for coping with the complicated and potentially enervating interaction with multiple medical disciplines as renal function declines and comorbidity becomes more severe.

Every diabetic individual should have annual screening for microalbuminuria and a spot urine albumin: creatinine ratio should be calculated to identify those diabetics at risk for nephropathy, retinopathy, and cardiovascular disease as recommended by the National Kidney Foundation [63]. Upon discovery of microalbuminuria in Type 1 diabetes, treatment with an angiotensin-converting enzyme (ACE) inhibitor should be started whether or not the individual is hypertensive [64]. The same advice holds for Type 2 individuals as shown by Ravid et al.'s double-blinded randomized control study of 94 normotensive Type 2 diabetic individuals with microalbuminuria treated with enalapril for 7 years that showed stabilized renal function with an "absolute risk reduction of 42% for nephropathy" [65].

Table 3. Comparison of ESRD options for diabetic patients

FACTOR	PERITONEAL DIALYSIS	HEMODIALYSIS	KIDNEY TRANSPLANT
Extensive extrarenal disease	No limitation	No limitation except for hypotension	Excluded in cardiovascular Insufficiency
Geriatric patients	No limitation	No limitation	Arbitrary exclusion as determined by program
Complete rehabilitation	Rare, if ever	Very few individuals	Common so long as graft functions
Death rate	Much higher than for nondiabetics	Much higher than for nondiabetics	About the same as nondiabetics
First year survival	About 75%	About 75%	> 90%
Survival to second decade	Almost never	Fewer than 5%	About 1 in 5
Progression of complications	Usual and unremitting. Hyperglycemia and hyperlipidemia accentuated.	Usual and unremitting. May benefit from metabolic control.	Interdicted by functioning pancreas + kidney. Partially ameliorated by correction of azotemia.
Special advantage	Can be self-performed. Avoids swings in solute and intravascular volume level.	Can be self-performed. Efficient extraction of solute and water in hours.	Cures uremia. Freedom to travel.
Disadvantage	Peritonitis. Hyperinsulenemia, hyperglycemia, hyperlipidemia. Long hours of treatment. More days hospitalized than either hemodialysis or transplant.	Blood access a hazard for clotting, hemorrhage and infection. Cyclical hypotension, weakness. Aluminium toxicity, amyloidosis.	Cosmetic disfigurement, hypertension, personal expense for cytotoxic drugs. Induced malignancy. HIV transmission.
Patient acceptance	Variable, usual compliance with passive tolerance for regimen.	Variable, often noncompliant with dietary, metabolic, or antihypertensive component of regimen.	Enthusiastic during periods of good renal allograft function. Exalted when pancreas proffers euglycemia.
Bias in comparison	Delivered as first choice by enthusiasts though emerging evidence indicates substantially higher mortality than for hemodialysis.	Treatment by default. Often complicated by inattention to progressive cardiac and peripheral vascular disease.	All kidney transplant programs preselect those patients with fewest complications. Exclusion of those older than 45 for pancreas + kidney simultaneous grafting obviously favorably predjudices outcome.
Relative cost	Most expensive over long run	Less expensive than kidney transplant in first year, subsequent years more expensive	Pancreas + kidney engraftment most expensive uremia therapy for diabetic. After first year, kidney transplant – alone – lowest cost option.

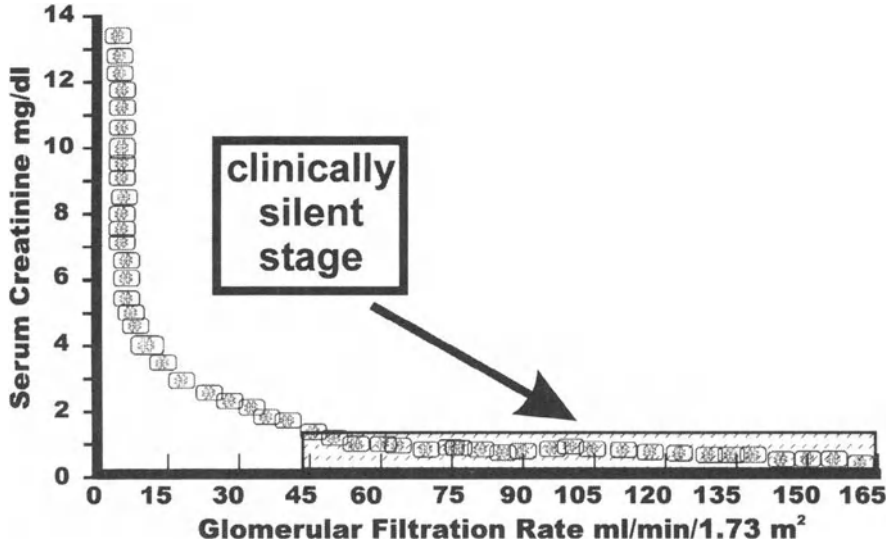

Fig. 7. Throughout the course of diabetes, hyperglycemia is modifying protein composition producing advanced glycosylated end-products (AGEs) causing macro and microvasculopathy. Diabetic nephropathy remains a clinically silent disease until about three-fourths of renal function is lost as judged by glomerular filtration rate. It follows from this reality that measures to protect against tissue and organ damage must be instituted before expression of signs and symptoms.

Other signs have been proposed for risk factors of renal deterioration. For example, a prolonged QT interval in a standard 12-lead electrocardiogram was a predictor of an increased risk of death in 85 proteinuric Type 1 patients followed for 5–13 years [66]. For the present, however, microalbuminuria and hypertension are the most reliable indicators of impending renal failure.

As reviewed by Nathan [67], both nephropathy and retinopathy are delayed in onset by a regimen termed intensive therapy of diabetes which consists of striving for euglycemia (tight control), dietary protein restriction, and blood pressure reduction. Sustained euglycemia reduces enlarged kidney size typical of early hyperfiltration [68]. Striving for euglycemia reduces the cumulative incidence and overall risk for development of microalbuminuria as well as clinical albuminuria (defined as ≥ 208 μg/min), a derivative finding in the Diabetes Control and Complications Trial (DCCT) [69]. Another strategy retarding diabetic microvasculopathy – in trial in Spain and Russia – is a reduction in erythrocyte stiffness, a hemorrheological alteration universally noted in diabetes, by administration of pentoxifylline [70, 71] in both Type 1 and Type 2 diabetic persons.

Hypertension

Hypertension is a major confounding factor in the genesis and progression of nephropathy. In hypertensive subjects with Type 2 diabetes > 10 years, 36%

had impaired renal function defined as a glomerular filtration rate < 80 ml/min/1.73 m^2 or a serum creatinine concentration > 1.4 mg/dl and 75% had microalbuminuria or clinical proteinuria [72]. Control of hypertension [73] – increasingly by angiotensin-converting enzyme inhibition [74] – and hyperglycemia [75] are the main components of contemporary treatment. It is now clear that use of an ACE inhibitor proffers unique benefit to halting progression of both microalbuminuria and proteinuria or in diabetic patients [64]. Studies with beta-blockers, calcium antagonists, diuretics, and AGE inhibitors in hypertensive diabetics with microalbuminuria have all shown significant reduction in urinary albumin excretion rates. When applied as monotherapy for 12 weeks in 31 diabetic patients with established microalbuminuria, captopril and indapamide were equivalent in blood pressure reduction and decrease of proteinuria [76]. Depending on the choice of calcium antagonist, sodium intake may modulate reduction of albumin excretion; diltiazem which decreased proteinuria in patients fed a diet containing 50 mEq/day of sodium was ineffective in reducing proteinuria when sodium intake was increased to 250 mEq/day while nifedipine decreased proteinuria independent of dietary sodium intake [77]. Treatment with captopril, an ACE inhibitor administered for 18 months to 24 NIDDM patients with proteinuria > 500 mg/day reduced proteinuria and prevented decrease in GFR compared with 18 NIDDM treated with 'conventional' antihypertensive drugs [78]. Recently, the renal protective effects of Ace inhibitors have been extended to reducing proteinuria, limiting GFR decline, and preventing ESRD in proteinuric, nondiabetic renal disorders except for polycystic kidney disease [79].

Smoking cigarettes increases systolic blood pressure and proteinuria in both micro- and macroalbuminuric Type 1 diabetic patients and should be counted as a risk for faster progression of diabetic nephropathy [80]. Wang, Lau, and Chalmers conducted a meta-analysis of the effects of intensive blood glucose control on the development of late complications of IDDM concluding that "Long-term intensive blood glucose control significantly reduces the risk of diabetic retinopathy and nephropathy progression" [81]. Dietary protein restriction, previously thought to be beneficial in retarding loss of renal function [82] was inefficacious in a prospective multicenter trial in nondiabetic patients in Italy. Furthermore, in Type 2 diabetes, dietary protein intake does not correlate with the degree of proteinuria [83]. By contrast, in a meta-analysis of five randomized, controlled or time-controlled with nonrandomized crossover design studies comprising 108 patients with Type 1 diabetes with a mean follow-up of 9 to 35 months, "a low-protein diet significantly slowed the increase in urinary albumin level or the decline in glomerular filtration rate or creatinine clearance" [84]. By consensus, most nephrologists now advise limitation in dietary protein in the belief that the rate of deterioration of renal function will be slowed.

Gaining acceptance of any dietary regimen by a diabetic patient is a substantive challenge. This chore is compounded by the absence of consensus as to

exactly what the diabetic diet should comprise. A European study group devised reasonable recommendations [85] that have been updated [86]. Considering the need to normalize body mass, limit saturated fatty acids, sucrose, sodium, and protein, while increasing foods rich in tocopherols, carotenoids, vitamin C, and flavenoids, compliance is contingent on the belief by health care providers that the necessary time and instruction are worthwhile. Few nephrologists have either the time or interest in dietary management to recruit patient obeisance to a confusing and expensive diet. What actually happens is that fragmentary components of a nutritional plan are suggested and followed to a minimal extent. The solution to this problem awaits simplification of nutritional instructions, first to physicians, then to the renal team, and ultimately to the patient.

Once renal failure has developed, the value of strict metabolic control is speculative and unsubstantiated. Nevertheless, it is circumspect to anticipate that all of the benefits to native kidneys of blood pressure and blood glucose control should be conferred on a renal transplant, retarding the recurrence of diabetic nephropathy in the kidney allograft. Anemia in azotemic diabetic patients adds to comorbidity and is responsive to treatment with recombinant erythropoietin. Slowed progression of renal insufficiency [87] and clearing of macular edema [88] have been noted in small series of azotemic patients treated with erythropoietin. Concern over an increase in severity of hypertension as red cell mass increases is based in the finding that ambulatory maintenance hemodialysis patients evince such a change [89].

Management of the myriad micro- and macrovascular comorbid diabetic complications manifested as azotemia increases, is contingent on an orderly approach that can be termed a Life Plan. Preparing for ESRD therapy for a diabetic individual whose kidneys are failing requires appreciation of the patient's family, social, and economic circumstances. Home hemodialysis, for example, is unworkable for a blind diabetic who lives alone. Deciding upon a kidney transplant requires knowledge of the patient's family structure, including its willingness to participate by donating a kidney. Without premeditation, the diabetic ESRD patient is subjected to repetitive, inconclusive studies instead of implementation of urgently required treatment (such as panretinal photocoagulation or arterial bypass surgery). A Life Plan may elect 'no treatment' when life extension is unacceptable. A blind, hemiparetic diabetic patient experiencing daily angina and nocturnal diarrhea, who is scheduled for bilateral lower limb amputation may rationally elect to discontinue medical care despite his family's plea to start maintenance dialysis. Remembering that azotemic diabetic patients typically are depressed, however, a rational decision to die must be distinguished from temporary despair over a transient setback. We have noted that profoundly despondent diabetics on occasion respond to visits by rehabilitated dialysis patients or transplant recipients by reversing their decision to die. On the other hand, it is counterproductive to coerce acceptance of dialysis or a kidney transplant, when life has minimal (or even negative) value. Diabetic patients forced into uremia therapy by family or the health

care team are often noncompliant to dietary and drug regimens, thereby expressing behavior which culminates in passive suicide.

Future directions of therapy

Perturbed micro- and macrovascular function is strongly implicated in the cellular and molecular abnormalities of vascular endothelium in diabetes [90]. Hyperglycemia is increasingly linked to the pathogenesis of nephropathy, retinopathy, and atherosclerosis in individuals with long-duration diabetes [91]. The metabolid pathway between a high ambient glucose concentration and end organ damage in diabetes is under intensive investigation. Three candidate mechanisms are [92]: (1) activation of the aldose–reductase pathway leading to toxic accumulation of sorbitol in nerves; (2) accelerated nonenzymatic glycosylation with deposition of advanced glycosylated endproducts [93]; (3) activation of isoform(s) of protein kinase C in vascular tissue initiating a cascade of events culminating in diabetic complications [94]. PKC activity is increased in renal glomeruli, retina, aorta, and heart of diabetic animals, probably because of increased synthesis *de novo* of diacylglycerol (DAG), a major endogenous activator of PKC [95].

Aging in humans is associated with the Maillard reaction in which protein alteration results from a nonenzymatic reaction between ambient glucose and primary amino groups on proteins to form glycated residues called Amadori products. Amadori products are transformed to stable covalent adducts called advanced glycosylation endproducts (AGEs) by a series of dehydration and fragmentation reactions. Diabetes accelerates synthesis and tissue deposition of AGEs, a perturbation hypothesized to contribute to the pathogenesis of comorbid complications [96]. AGEs are bound to a cell surface receptor (RAGE) inducing expression of vascular cell adhesion molecule-1 (VCAM-1), an endothelial cell surface cell–cell recognition protein that can prime diabetic vasculature for enhanced interaction with circulating monocytes thereby initiating vascular injury [97].

Linkage between hyperglycemia, hyperlipidemia, nitric oxide activity, and atherosclerosis in the pathogenesis of diabetic complications is highly probable. Azotemic diabetic individuals manifest elevation in the plasma level of apoprotein B (ApoB), very low density lipoprotein (VLDL) and low density lipoprotein (LDL). Circulating high levels of AGEs react directly with plasma lipoproteins preventing their recognition by tissue LDL receptors significantly increasing the level of AGE-modified LDL in the plasma of diabetic or nondiabetic uremic patients compared with normal controls, possibly contributing to the accelerated atherosclerosis that is typical of diabetes and uremia [98]. LDL modified *in vitro* by AGE-peptides to the level present in azotemic diabetic patients markedly impaired LDL clearance kinetics when injected into transgenic mice expressing the human LDL receptor indicating that AGE modification of LDL receptors promotes elevated LDL levels in azotemic

diabetic patients. Studies in rodents suggest that AGEs exert their toxicity by impairing nitric oxide-mediated vital processes including neurotransmission [99], wound healing [100] and blood flow in small vessels [101]. Thus, AGEs by blocking the synthesis of nitric oxide, almost certainly interfere with maintenance of normal physiologic processes such as autoregulation of blood flow [102]. Those actions of nitric oxide that may be pertinent to nephrologists have been reviewed [103]. Prevention of AGE formation is an attractive means of preempting diabetic microvascular complications because it bypasses the necessity of having to establish euglycemia, an often unattainable goal. Aminoguanidine is similar to α-hydrazinohistidine, a compound known to reduce diabetes-induced vascular leakage, while having opposite effects on histamine levels [104]. By blocking non-enzymatic glycosylation [105] aminoguanidine reduces measured AGE levels in the streptozotocin-induced diabetic rat. In rats made diabetic aminoguanidine: (i) Treatment with aminoguanidine 25 mg/kg prevented development of cataracts in rats. (ii) Prevented AGE accumulation (measured by tissue fluorescence) in glomeruli and renal tubules and lessened glomerular basement membrane thickening. (iii) Reduced severity of experimental diabetic retinopathy as judged by a decrease in the number of acellular capillaries by 50% and complete prevention of arteriolar deposition of PAS-positive material and microthrombus formation after 26 weeks of induced diabetes in spontaneous hypertensive rats [106]. (iv) Prevented development of the 'stiff myocardium' that is a main component of diabetic cardiomyopathy; in a dose of 7.35 mmol/kg/dl for 4 months [107]. (v) Prevented diabetes-induced 24% impairment in maximal endothelium-dependent relaxation to acetylcholine for phenylephrine precontracted aortas [108]. The strategic potential of blocking AGE formation to impede development of diabetic complications has been reviewed [109, 110]. An attractive aspect of this approach to impeding diabetic complications is the elimination of the necessity for euglycemia [111].

AGEs in uremia

An elevated serum level of AGEs in renal insufficiency is proportional to the degree of reduced GFR. Renal clearance of AGE-peptides is 0.72 ± 0.23 ml/min for normal subjects and 0.61 ± 0.2 ml for diabetics with normal glomerular filtration (p value NS) [112]. Diabetic uremic patients accumulate advanced glycosylated end-products in 'toxic' amounts that are not decreased to normal by hemodialysis or peritoneal dialysis [113] but fall sharply, to within the normal range, within 8 hours of restoration of half-normal glomerular filtration by renal transplantation [114]. From the forgoing, it has been inferred that the higher mortality of peritoneal and hemodialysis treated diabetic patients compared with those given a renal transplant may relate – in part – to AGE toxicity. To test this hypothesis, a multicenter trial of aminoguanidine in diabetic hemodialysis patients was initiated in 1996.

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6. Dialysis in diabetic patients: three decades of experience, from 1964 to 1997

M. M. AVRAM

Editors' Comment:

When Avram first reported in 1966 that diabetic uremic patients might benefit from repetitive hemodialyses, no one was prepared for the extent of diabetic nephropathy that was unmasked. Over the ensuing three decades, every estimate of how many ESRD patients would have diabetes has been surpassed. The upward slope of both the incidence and prevalence curves for diabetic ESRD patients has not yet leveled off. Searching for markers of morbidity and mortality in the expanding number of diabetic ESRD patients, Avram's group added protein-energy malnutrition to recognized risk factors including hypertension and poor metabolic control. A comprehensive management strategy for diabetic kidney patients is proposed employing early treatment with erythropoietin, cardiovascular protection by diet, exercise and/or drugs, maintaining normal body weight, and blood pressure control. Remembering the early trials of dialytic therapy in diabetes when first-year survival was less than 25%, current prospects for a 75% survival in newly started hemodialysis patients indicates remarkable progress. Still, rehabilitation presently is far from satisfactory and further modifications in diabetic management to reduce comorbidity — including assessing the value of aminoguanidine to block micro and macrovascular complications are under active clinical trial.

Introduction

Diabetes Mellitus has become the leading cause of end-stage renal disease (ESRD) worldwide. According to several registries, a total of about 500 000 patients are now receiving renal replacement therapy. In nearly 100 000 patients ESRD is believed to be caused by diabetes (both Type I and Type II) [1]. It must be noted that these numbers are underestimates since no precise figures about prevalence of ESRD are available from several countries e.g., India, China and Indonesia [1]. According to the 1997 report of the United States Renal Data System, of 257 266 patients receiving renal replacement therapy in 1995, 80 667 were diabetic with a prevalence rate of 31.4%. In 1995, there were 68 870 new cases of ESRD; 27 851 were diagnosed as diabetes related [2]. Patients with diabetic nephropathy constitute the fastest growing group of ESRD patients: from 1979 to 1989 this group increased more than 10-fold while the total ESRD population increased by 3.21-fold [3]. Not only

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are the numbers of diabetics increasing among ESRD population, but the mortality of diabetic patients is higher than non-diabetic patients. Marcelli et al. reported survival of all diabetic patients on dialysis to be 86.5% at 1 year, 52% at 3 years and 34% at 5 years [4]. Increase in the number of diabetic patients is one of the most important contributory factors for the high mortality rate in US dialysis patients. Therefore, it is highly relevant to study predictors of mortality in diabetes related ESRD patients, so as to positively impact the outcome of ESRD patients overall.

In 1966, we reported for the first time that hemodialysis can prolong the life of patients suffering from diabetic uremia [5]. Since then we have updated our experience on diabetic dialysis patients from time to time [6–10]. We have compiled an extensive database for our hemodialysis (HD) and peritoneal dialysis (PD) patients attending the Long Island College Hospital. We have conducted retrospective and prospective analyses of our experience with diabetic ESRD patients over the past 20 years to examine whether data that are routinely collected during dialytic therapy might serve as biochemical and nutritional markers for excess mortality in this patient population. In this review, we have examined the survival of diabetic hemodialysis and peritoneal dialysis patients compared with non-diabetics, predictive markers for longevity and contribution of lipid abnormalities to the mortality in diabetic dialysis patients.

First dialysis treatment of diabetic patients

In 1964, one of the nurses on our nephrology floor at the Long Island College Hospital asked us to treat her husband who was suffering from insulin dependent diabetes mellitus (IDDM) and became uremic. In those days, it was absolutely contraindicated to treat diabetics with hemodialysis since it appeared very unlikely that they might benefit from the experience, even for a short period of time. We attempted to treat this patient, monitoring blood glucose and insulin with great care while on dialysis. Surprisingly, the patient regained much vigor and zest for life, to the point where he was able to leave the hospital and return to work. We reported the experience in 1966, which is the first report in the literature about the treatment of diabetic renal failure with hemodialysis [5].

Predictors of mortality in diabetic and non-diabetic dialysis patients

Despite significant progress in the delivery of renal replacement therapy, mortality rates among ESRD patients in the United States remain unacceptably high [2]. Co-morbid factors such as advanced age, malnutrition, infection, cardiovascular disease and malignancy, and technical factors such as adequacy of dialysis, vascular access complications, and peritoneal membrane characteristics may all contribute to this dismal survival among HD and PD patients. Several

predictors of survival on renal replacement therapy have been described in short term and cross-sectional studies. These include demographic characteristics such as age, race, gender, and diabetic status; nutritional status as assessed by albumin, prealbumin, creatinine, and cholesterol levels; and dialytic factors such as the dose of dialysis [11–15].

Malnutrition is highly prevalent in dialysis patients [16, 17]. It is now well established that protein-energy malnutrition is a strong predictor of morbidity and mortality in HD and PD patients [18]. Diabetic patients tolerate renal failure less well than non-diabetic, and malnutrition can develop more rapidly in these patients.

In an earlier study, we analyzed the survival of 221 HD patients followed for 5 years [19]. The patients were divided into three groups by outcome: group I died ≤ 12 months after enrollment ($n = 42$); group II died 13–58 months after enrollment ($n = 59$); group III survived > 24 months before follow-up was censored ($n = 77$). Using group III as reference, a model was constructed by multinomial logistic regression that predicts the likelihood of group I (vs. III) and group II (vs. III). Diabetes was associated with a 2.6-fold increase in early death (group I, died ≤ 12 months) risk ($p = 0.04$) and a less significant increase in late risk (group II, died within 13–58 months) (odds ratio 1.57, $p = 0.265$). Four year trends in nutritional markers (1987–1991) in stable patients (21 non-diabetic and 7 diabetic) indicated that both albumin and creatinine fell significantly over 4 years (1987 vs. 1991). However the comparison between 1987 and 1991 were not significant for the diabetic subgroup because of smaller sample size.

In a subsequent analysis [20], non-diabetic patients had significantly better survival than diabetic patients (45.6% vs. 24.3%, $p = 0.045$ by log rank test) followed for 5 years. We compared various nutritional markers by diabetic status adjusted for age, gender, race, and prior months on dialysis. Non-diabetics had higher serum creatinine (13.5 vs. 11.6 mg/dL, $p = 0.001$) and lower levels of non-albumin protein (2.6 vs. 2.8 g/dL, $p = 0.03$). Diabetics had significantly lower serum levels of prealbumin (24.5 vs. 27.5 mg/dL, $p = 0.018$) than non-diabetics. Patients were stratified by diabetic status and outcome into 4 groups: Non-diabetic alive, diabetic alive, non-diabetic dead and diabetic dead. Compared with surviving non-diabetics, both groups that died during the study (diabetics and non-diabetics) had significantly lower serum albumin. Both groups of diabetics (survivors and dead) had significantly lower serum creatinine, but non-diabetics that died had similar levels to those that survived. Diabetics that died during the study had significantly higher levels of non-albumin protein than non-diabetic survivors. Non-diabetics that died had significantly lower cholesterol than non-diabetic survivors. All groups had lower prealbumin levels than non-diabetic survivors (Table 1). These findings suggest several differences between diabetic and non-diabetic dialysis patients which may impact on mortality. Diabetics appear to be at increased risk of malnutrition, which should be recognized and addressed as early as possible.

Table 1. Nutritional markers in diabetic and non-diabetic HD patients by survival status (1987–1992)

	Non-diabetic ($n = 134$)		Diabetic ($n = 87$)	
	Survived	Died	Survived	Died
Albumin (g/dl)	3.97 ± 0.36	3.67 ± 0.49*	3.83 ± 0.39	3.74 ± 0.47*
Creatinine (mg/dL)	13.75 ± 4.74	13.41 ± 5.22	11.68 ± 4.08*	11.55 ± 4.53*
Non-albumin protein (g/dL)	2.50 ± 0.57	2.8 ± 0.55	2.67 ± 0.43	2.87 ± 0.68*
Cholesterol (mg/dL)	193 ± 46	165 ± 45*	183 ± 55	162 ± 48*
Prealbumin (mg/dL)	28.22 ± 5.10	22.51 ± 7.59*	24.69 ± 6.67*	22.82 ± 7.56*

$p < 0.05$, compared to non-diabetic survived by Tukey's HSD test.

The finding of higher non-albumin protein in diabetics, and its relation to mortality risk, deserves further investigation.

When 250 HD patients were prospectively followed for 7 years, independent predictors of patient survival as determined by Cox's proportional hazards model were age, diabetes, serum albumin and serum cholesterol. Adjusting for other variables in the model, diabetes was associated with almost 2-fold increase in mortality risk compared with non-diabetic patients [12]. Among 140 PD patients, age, diabetes, serum albumin, serum creatinine were significant independent predictors of mortality risk. Adjusting for other predictors, diabetes was associated with 1.5-fold increase in mortality compared to non-diabetics. Diabetes was an important and independent risk factor for long-term survival in both HD and PD patients.

In a retrospective analysis of very long-term survivors on HD up to 30 years, demographic and biochemical indices reflecting nutritional status predicted prolonged survival. Younger age, male gender, black race, and non-diabetic status were significantly associated with long-term (10–15 years) and very long-term survival (15–30 years) on HD. The percentage of diabetics in very long-term, long-term and average (< 5 years) HD survivors were 17, 28 and 55 respectively. Observed and expected ($p < 0.006$) survival (after adjustments were made for age, albumin, creatinine and cholesterol) were significantly lower among diabetics than non-diabetics. PD patients who survived more than 10 years were also younger at the beginning of dialysis compared to average survivors (< 5 years) (50.4 vs. 61.8 years, $p = 0.01$). There were 21% diabetics among long-term survivors vs. 55% among average survivors ($p = 0.001$). Expected survival among diabetics in PD was significantly lower than non-diabetics after adjusting for age, albumin, creatinine and cholesterol ($p < 0.002$) [21].

Most recently we have analyzed survival of 383 HD patients enrolled from 1987 and followed up to October, 1997. Mean age was 59.8 ± 15.4 (SD) years, 44% were male. The racial composition was 56% black, 28% white and 16% Hispanic. Forty six percent of patients were diabetic. Patient demographics by

Table 2. Demographics of HD patients by diabetic status

	Non diabetic ($n = 208$)	Diabetic ($n = 172$)	p
Age at enrollment (year, mean ± SD)	57.80 ± 17.4	62.30 ± 12.1	0.01
Race (%)			
White	32	22	0.02
Black	56	57	
Hispanic	12	21	
Gender (%)			
Male	50	38	0.02
Female	50	62	
Months on dialysis	32.40 ± 50	14.60 ± 25.2	0.01

Table 3. Nutritional markers in HD patients by diabetic status

	Non-diabetic ($n = 164$)	Diabetic ($n = 105$)	p
Albumin (g/dL)	3.37	3.45	0.33
Creatinine (mg/dL)	11.2	9.76	0.01
Cholesterol (mg/dL)	202	206	0.5
Prealbumin (mg/dL)	27.82	24.16	0.01

Mean, adjusted for age, gender, race, and months on dialysis.

diabetic status is shown in Table 2. Diabetic patients were older than non-diabetic patients (62 vs. 58 years, $p = 0.01$). There was a significant difference in racial composition between non-diabetic and diabetic patients. Among non-diabetic patients, percentage of white was higher and percentage of Hispanic was lower compared with diabetic patients. At enrollment, diabetic patients had been on dialysis for fewer months than non-diabetic patients (14.6 vs. 32.4 months, $p = 0.01$). Enrollment levels of nutritional markers such as serum albumin ($p = 0.02$), creatinine ($p = 0.01$), prealbumin ($p = 0.01$) of diabetic patients were significantly lower than non-diabetic patients. Even when adjusted for age, gender, race and months on dialysis, serum creatinine ($p = 0.01$) and prealbumin ($p = 0.01$) of diabetic patients were still significantly lower than non-diabetics (Table 3). The serum levels of albumin and prealbumin reflect the size and function of the visceral protein pool. Serum creatinine is a marker of somatic protein content. The finding that visceral and somatic protein level is significantly lower in diabetic HD patients compared to non-diabetic patients indicates that diabetic patients are more malnourished compared to non-diabetic counterparts. This is one of the most important causes of higher mortality rate in diabetics compared to non-diabetics. Independent predictors of mortality as analyzed by Cox's proportional hazards model is shown in Table 4. Diabetic patients had 1.54-fold mortality risk ($p = 0.05$) compared to

Table 4. Predictors of mortality in hemodialysis ($n = 228$)

	Relative risk	p
Age (years)	1.04	0.001
Albumin (g/dL)	0.51	0.0178
Creatinine (mg/dL)	0.97	0.18
Cholesterol (mg/dL)	0.99	0.164
Diabetes (yes vs. no)	1.54	0.05
Months on dialysis	1.01	0.022

Cox' proportional hazards analysis adjusting for age, months on dialysis, diabetes, and nutritional markers.

Table 5. Predictors of mortality in hemodialysis

	Non-diabetic ($n = 179$)		Diabetic ($n = 152$)	
	Relative risk	p	Relative risk	p
Age (years)	1.05	0.001	1.04	0.001
Race				
Black vs. white	1.25	0.318	0.29	0.001
Hispanic vs. white	0.88	0.746	0.30	0.001
Months on dialysis	1.01	0.051	1.01	0.002

Cox's proportional hazards analysis adjusting for age, race, prior months on dialysis.

non-diabetics. As expected, age was a significant predictor of mortality in this model. Enrollment serum albumin was also a significant predictor of mortality. Independent predictors of survival as analyzed by Cox's proportional hazards model for non-diabetics and diabetics are shown in Table 5. For diabetic patients, age, race, and months on dialysis were independent predictors of mortality. Risk of death for white diabetic hemodialysis patients was significantly higher compared to black ($p = 0.001$) and Hispanic patients ($p = 0.001$). But in non-diabetic patients race was not an independent predictor of mortality. It has been reported that diabetic white Americans 40 years of age and older with ESRD have higher mortality rates than diabetic African-Americans, Native Americans, Asian Americans and Mexican Americans [22–24]. Despite several hypotheses, this shorter survival in white Americans has not been fully explained. Type I diabetes related ESRD is more frequent in white Americans and type II diabetes related ESRD is more common in African Americans, Native Americans and Mexican Americans. Recently it has been reported that ethnicity is a predictor of survival in Type I but not in Type II diabetes. Although there is a survival advantage on dialysis of African Americans and Mexican Americans over non-Hispanic whites, among Type II patients this minority survival advantage disappears [25]. Other predictors independently associated with survival of diabetic dialysis patients were age, high self-reported

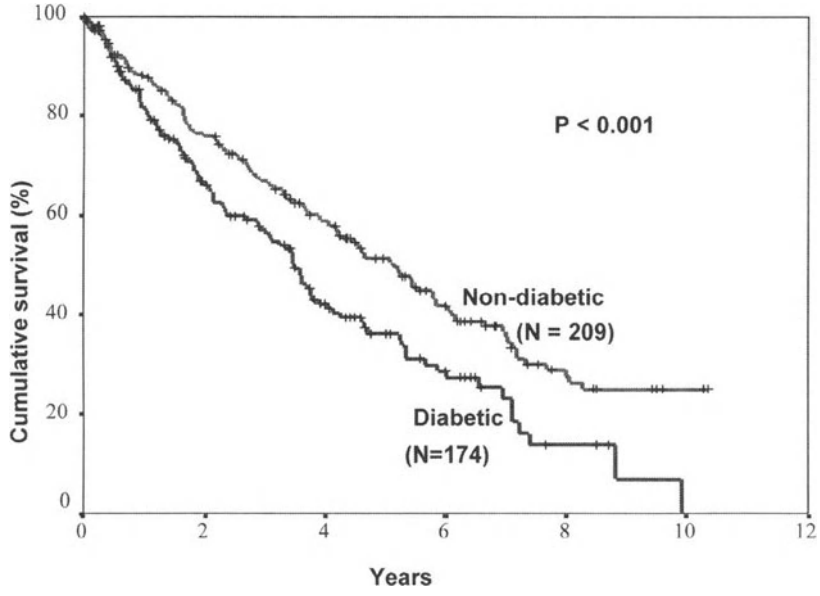

Fig. 1. Observed survival in non-diabetic and diabetic hemodialysis patients.

physical disability, coronary artery disease, lower extremity amputation and average blood glucose level prior to ESRD [25]. Another study found a diminished effect of age, with the 1- to 45-year age group and the 46- to 60-year age groups having almost identical rates of survival [26]. Observed 10 years survival of our diabetic HD patients as measured by Kaplan Meier method is shown in Figure 1. Survival of diabetics was significantly ($p < 0.001$) lower than that of non-diabetic patients.

We enrolled 320 PD patients (196 non-diabetic and 124 diabetic) from 1985 and followed them up to 1997. Mean age was 53.0 ± 15.6 (SD) years. Similar to HD patients, diabetic PD patients were older than non-diabetic patients (56 vs. 51 years, $p = 0.01$). There was a significant difference in racial composition between diabetic and non-diabetic PD patients. Percentage of Hispanics was higher in diabetics compared with non-diabetics (25% vs. 12%) (Table 6). Only enrollment serum creatinine ($p = 0.01$) was lower in diabetic compared with non-diabetic patients. Even after adjusting for age, gender, race and months on dialysis, serum creatinine remained significantly lower in diabetics compared to non-diabetics (Table 7). The observed survival of diabetic PD patients up to 12 years was significantly ($p < 0.0001$) lower compared to non-diabetics (Figure 2). The risk of death increased over time. Zimmerman et al. reported that survival of PD patients 55 years of age or older was significantly different between diabetic and non-diabetic patients [27]. The lower creatinine level in diabetics, similar to diabetic HD patients suggest that diabetics had poorer

Table 6. Demographics of PD patients by diabetic status

	Non-diabetic ($n = 148$)	Diabetic ($n = 96$)	p
Age at enrollment (year, mean ± SD)	51.1 ± 17.2	56.1 ± 12.1	0.01
Race (%)			
White	27	20	0.03
Black	61	55	
Hispanic	12	25	
Gender (%)			
Male	50	50	0.99
Female	50	50	
Months on dialysis	8.3 ± 15.2	4.6 ± 6.6	0.01

Table 7. Nutritional markers in PD patients by diabetic status

	Non-diabetic ($n = 123$)	Diabetic ($n = 80$)	p
Albumin (g/dL)	3.87	3.82	0.42
Creatinine (mg/dL)	13.95	11.61	0.001
Cholesterol (mg/dL)	181	172	0.22
Prealbumin (mg/dL)	34.21	31.79	0.38

Mean, adjusted for age, gender, race, and months on dialysis.

nutritional status than that of non-diabetics. Poor nutritional status may contribute to the higher mortality in diabetics. Prevalence of malnutrition in diabetic CAPD patients has been reported by several workers [28, 29]. In one study, 91% of diabetics and 76% of non-diabetics showed some degree of malnutrition. Diabetic patients had significantly higher malnutrition ($p = 0.02$) [28].

Table 8 shows Cox's proportional hazards models for a subset of patients enrolled between 1991 and 1994 for diabetes and several biochemical markers [12]. Diabetes, which is a mortality risk predictor, loses discrimination after biochemical markers are considered. Interestingly, this is similar to the findings of Lowrie and Lew [14, 30]. This suggests that biochemical markers reflect an aspect of mortality risk that is characteristic of diabetes, e.g., reduced somatic protein. Excess mortality among diabetic patients with ESRD has been associated with differences in nutrition and the intensity of dialysis [15, 30].

Lipid abnormalities in diabetic dialysis patients

Diabetic patients undergoing renal replacement therapy have a high cardiovascular mortality. Diabetes and dialysis are risk factors for atherosclerosis, coronary heart disease, stroke and peripheral vascular disease. Disorders of lipid

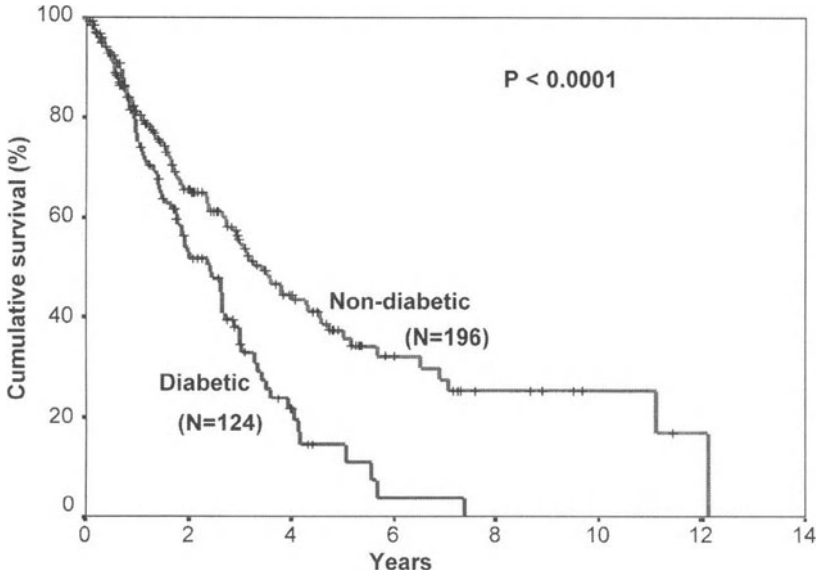

Fig. 2. Observed survival in non-diabetic and diabetic peritoneal dialysis patients.

Table 8. Interaction of diabetes and nutritional markers: effects of patient mortality in hemodialysis

Model	Relative risk	95% CI	*p*
Diabetes	1.64	1.33–3.54	0.015
Prealbumin	0.91	0.87–0.95	0.001
Diabetes	2.22	1.15–3.64	0.001
Apo B	0.98	0.97–1.00	0.07
Prealbumin	0.93	0.88–0.98	0.001
Diabetes	1.62	0.87–3.03	0.13
Apo B	0.98	0.96–0.99	0.009
Creatinine	0.86	0.78–0.96	0.005
Prealbumin	0.99	0.92–1.05	0.654

Cox' proportional hazards analysis.

metabolism are believed to play an important role in the pathogenesis of atherosclerotic cardiovascular disease in patients with chronic renal failure. Dyslipidemia may contribute to high mortality of diabetic dialysis patients. Baseline serum lipids (on admission) is the strongest predictor of myocardial infarction or sudden death in diabetic HD patients followed up to 36 months as reported by Koch et al. [31, 32]. We conducted a lipid survey among HD and PD patient population and evaluated data relevant to the lipid and diabetic status of these groups. Interestingly, longer duration of HD in non-diabetic

Table 9. Lipid profile of HD and PD patients by diabetic status

	Hemodialysis		Peritoneal dialysis	
	Non-diabetic (43)	Diabetic ($n = 12$)	Non-diabetic ($n = 32$)	Diabetic ($n = 8$)
Total cholesterol (mg/dL)	161 ± 51	180 ± 44	208 ± 56	211 ± 57
HDL-C (mg/dL)	37 ± 12	36 ± 11	47 ± 14	29 ± 4*
TC/HDL-C	4.9 ± 2.7	5.3 ± 2.1	5.2 ± 2.5	7.2 ± 1.7
Apo-A-1 (mg/dL)	87 ± 19	88 ± 16	107 ± 22	93 ± 8
ApoB (mg/dL)	65 ± 28	69 ± 21	88 ± 37	104 ± 30
ApoA-1/ApoB	1.7 ± 1.0	1.4 ± 0.5	1.4 ± 0.7	0.9 ± 0.2

*$p < 0.05$ vs. non-diabetic PD.

Table 10. Longitudinal apolipoprotein changes in non-diabetic and diabetic HD patients

	Non-diabetic		Diabetic	
	1988	1990	1988	1990
Apo A1 (mg/dL)	87 ± 2	85 ± 2	91 ± 4	87 ± 4
Apo B (mg/dL)	70 ± 5	63 ± 4*	71 ± 5	67 ± 4
Apo A1/Apo B	1.43 ± 0.10	1.61 ± 0.14*	1.40 ± 0.10	1.41 ± 0.11

*$p < 0.05$ vs. 1988 non-diabetic.

patients was associated with lower cholesterol, but not HDL, which may reflect diminished cardiac risk and mortality in the long-term survivors. Diabetic patients showed lower HDL but no cholesterol change with longer duration on HD [9]. We reported lipid and apolipoprotein values over 12–14 months in 55 HD and 40 PD patients [33]. In cross sectional study, there were no statistically significant difference in lipid parameters between non-diabetic and diabetic HD patients whereas in PD patients, diabetics had significantly lower HDL-C compared to non-diabetics (Table 9). Our longitudinal data showed that lipids, apolipoproteins and risk ratios remain stable over time on HD and PD. In fact, the anti-atherogenic index, apoA-1/apoB, improved in HD patients, especially in non-diabetics (Table 10). In summary, with years of observation, lipid profile improved slightly in non-diabetic patients. Surviving diabetic dialysis patients had long-term unchanged, persistently deranged lipid profiles.

Concluding remarks

Renal disease attributed to diabetes is a worldwide problem which contributes to the high mortality in dialysis patients. Diabetic patients should not be excluded from uremia therapy regimens. Clues to improving survival are: improving nutritional status, controlling blood sugar, reducing atherogenic

lipids and lipoproteins by diet, exercise or drugs, blood pressure control and maintaining normal body weight. There is a reason for optimism because of extensive early application of erythropoietin, introduction of aminoguanidine, and the availability of safe, profoundly effective anti-hypertensive drugs.

Acknowledgements

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7. Hemodialysis in patients with diabetes mellitus

ANNE MARIE MILES

Editors' Comment:

Miles focuses on currently possible improvements in the hemodialysis regimen as a means of reducing morbidity and mortality. Noting that diabetic hemodialysis patients have a 20% greater rate of intradialytic hypotension, and that recurrent intradialytic hypotension results in underdialysis, this complication is analyzed in detail. Potential management strategies are proposed including a lower ultrafiltration rate with increased duration of hemodialysis treatments. Proposed corrective measures such as limiting meals before or during hemodialysis, omitting antihypertensive medications on the morning of hemodialysis, and leg toning exercises to improve venous return are refreshing in their simplicity and avoidance of expense. An apparently higher rate of vascular access thrombosis is attributed to a greater use of synthetic grafts in diabetic hemodialysis patients. But, resort to a synthetic graft over a primary arteriovenous fistula is often a decision forced by inadequate or damaged vessels at the time of access creation. No easy remedy for effective vascular access in diabetic hemodialysis patients is evident.

Introduction

Because diabetes mellitus is now the commonest cause of renal failure in the incident and prevalent end stage renal disease (ESRD) population in America [1] (Figure 1, 2), nephrologists today are confronted with the multi-system complications of diabetic dialysis patients with increasing frequency. It is estimated that 80–90% of diabetics in the ESRD program are Type II diabetics, in keeping with the overwhelming predominance of Type II diabetes in the general population. In the 1970's, 2-year mortality for diabetics in hemodialysis programs was distressingly high, ranging between 60 to 75% [2–4]. With advances in the management of coronary artery disease and critical care medicine, improvements in the area of vascular access, and a tendency for earlier initiation of dialytic therapy in diabetics (at creatinine clearances of 10–15 ml/min), the subsequent 2 decades have seen a progressive decline in the mortality rates of diabetics on dialysis, and the 1 year mortality rate now stands at 27%. Long term mortality rates in diabetic patients are still 2–3 times higher than that in non-diabetic dialysis patients however. Cardiovascular and cerebrovascular disease, and sepsis account for 61% of deaths in diabetic

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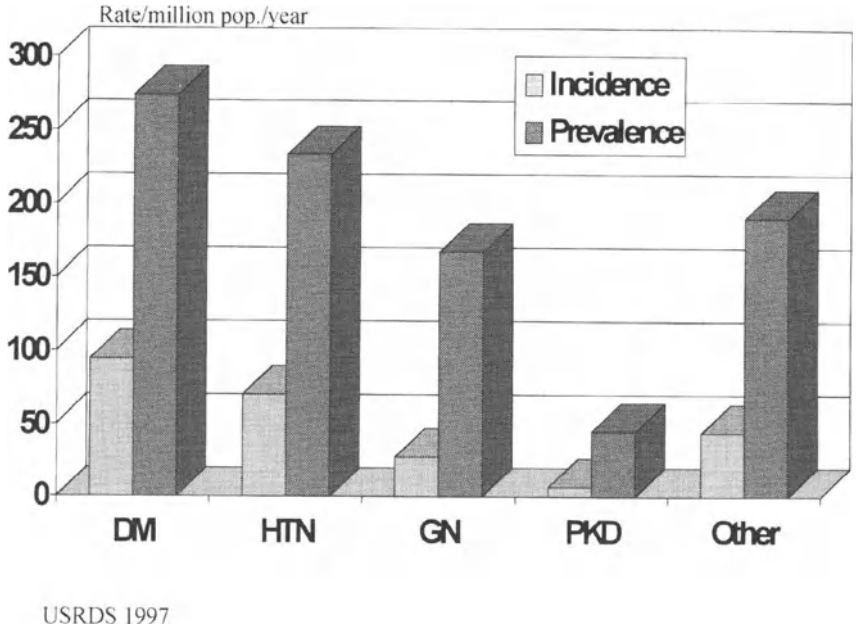

Fig. 1. Treated ESRD incidence and prevalence by primary diagnosis (1993–95). Diabetes mellitus (DM), hypertension (HTN), chronic glomerulonephritis (GN), polycystic kidney disease (PKD).

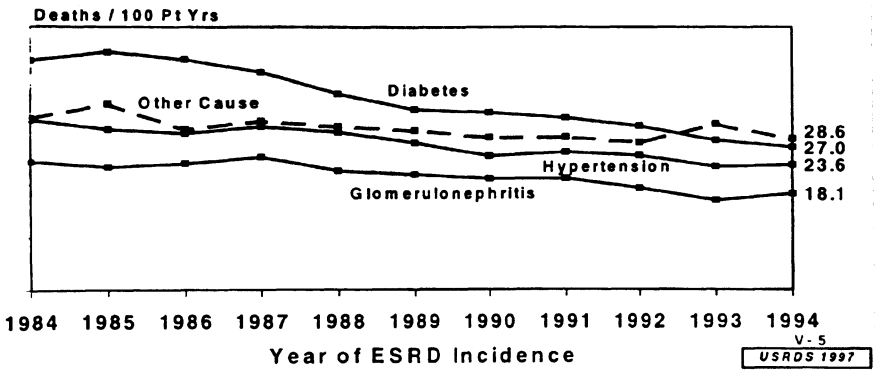

Fig. 2. Adjusted 1 year death rates for dialysis patients by diagnosis and year of incidence, 1984–1994.

dialysis patients [1]. Diabetic patients on hemodialysis require aggressive, preemptive, multidisciplinary management to ameliorate the devastating complications related to peripheral vascular, cardiovascular and cerebrovascular disease, retinopathy and other organ system complications [5]. Diabetics are

also at particular risk of a number of hemodialysis-related complications which contribute to morbidity and mortality, and this review will address these complications with particular emphasis on those relating to vascular access.

Hemodialysis-related complications in diabetic dialysis patients

Hypotension

Diabetics have a 20% increase in episodes of hypotension during hemodialysis when compared to non-diabetics [6]. Intradialytic hypotension in diabetic hemodialysis patients is multifactorial, often associated with nausea and vomiting, and sometimes occurs in the face of obvious clinical volume overload and edema. Recurrent intradialytic hypotension contributes to the specter of underdialysis and increased mortality in diabetics [7], by reducing clearance through reductions in blood flow rate or early termination of dialysis. Major contributors to dialysis-associated hypotension in diabetics include the following:

1. Reduced myocardial ejection fraction due to atherosclerotic coronary artery disease is a major contributor to intradialytic hypotension in diabetic patients [8], and hypotensive episodes may be accompanied by angina pectoris, and result in, or be a consequence of myocardial infarction.
2. Diastolic dysfunction related to diabetic cardiomyopathy with resultant decreased left ventricular compliance and filling [9], may also contribute to reduced cardiac output and intradialytic hypotension in diabetics.
3. Autonomic neuropathy (diabetic $\pm$ uremic), is also frequently present in diabetic dialysis patients, and results in abolition of the reflex increase in heart rate and increased peripheral vascular resistance that usually occur to prevent hypotension before interstitial fluid is mobilized into the intravascular compartment.
4. Anemia also predisposes to intradialytic hypotension by reducing blood viscosity and peripheral vascular resistance and impairing the ability to maintain blood volume during ultrafiltration [10]. Anemia may precipitate dialysis-related angina pectoris, and a recent fall in hematocrit should always be sought in the diabetic patient who develops chest pain on dialysis.
5. Hypoalbuminemia, resulting from nephrotic syndrome or malnutrition, produces low colloid oncotic pressure that reduces the plasma refilling rate and also contributes to hypotension [7].
6. The theory of thermal amplification proposed by Gotch et al. [11] may provide another contributory factor to dialysis-associated hypotension: the slight build-up of core heat during hemodialysis (42), in association with a reduction in heat loss caused by cutaneous vasoconstriction in response to hypovolemia early in dialysis, results in reflex vasodilation of the cutaneous blood vessels near the end of dialysis and sudden hypotension.

Approaches to managing dialysis-related hypotension are listed in Table 1. In some cases, recurrent hypotension on hemodialysis may be severe enough

Table 1. Managing intradialytic hypotension in diabetics

Use bicarbonate dialysate
High sodium (140–145 mmol/L) dialysate with linear sodium modeling
Reduce the rate of ultrafiltration ± increase dialysis time
Sequential ultrafiltration
Prime dialysis circuit with hypertonic albumin
Maintain hematocrit at or above 30 vol% with erythropoietin
No antihypertensive medications on morning of dialysis
Restrict meals immediately before or during hemodialysis
Leg toning exercises to improve venous return
Decrease dialysate temperature (particularly near end of dialysis)
Medications: α-agonists eg: midodrine

to require change to another modality of treatment. In patients with inoperable coronary artery disease and dialysis-precipitated angina, maintaining the hematocrit level above 30%, nasal oxygen during dialysis, and topical or sublingual nitroglycerin given just prior to dialysis are useful in preventing attacks of intradialytic angina.

Hypertension

Hypertension is more common in diabetic than nondiabetic hemodialysis patients and is a major contributor to their death from cardiovascular disease. Fifty percent of hemodialyzed diabetics require antihypertensive medications compared with 27.7% of nondiabetics [6]. Although hypertension is largely volume dependent in most diabetics, improves as a hemodialysis session proceeds, and ameliorates or disappears as dry weight is attained, some patients continue to require antihypertensive medications after initiation of hemodialysis, and some may, in addition, experience progressive elevation in blood pressure during hemodialysis sessions or at the end of treatment. Exacerbated hypertension during dialysis may be due to acute activation of the renin-angiotensin system by reduction in intravascular volume produced by ultrafiltration. Use of angiotensin-converting enzyme inhibitors as part of an antihypertensive regimen, or at the start of, or during dialysis, usually controls this problem. Calcium channel blockers and central vasodilators such as clonidine are recommended for treatment of hypertension in diabetic dialysis patients. Rarely, in those with recalcitrant hypertension, is it necessary to add minoxidil for blood pressure control. Unless indicated for cardiac reasons, β-Blockers should be avoided in diabetics because they exacerbate hypertriglyceridemia, worsen glucose control, and may mask symptoms of severe hypoglycemia.

High interdialytic weight gain

Diabetics gain 30–50% more weight in interdialytic periods than nondiabetics, and in an early study, weight gain did not correlate with glycemic control, age,

Table 2. Vascular access in hospitalized elderly diabetic hemodialysis patients at Kings County Hospital (1987–1991). (Reprinted with permission from Miles AMV, Hong JH, Sumrani N et al. J KAMA 1996; 2: 25–28)

	Diabetic no. (%)	Non-diabetic no. (%)
Access type		
Graft	39 (98)	43 (90)
Fistula	1 (2)	5 (10)
Access location		
Wrist	0 (0)	5 (10)
Forearm	2 (5)	4 (8)
Upper arm	38 (95)	39 (82)

duration of ESRD, continuing urine output, dry weight, or duration of diabetes [6]. Correlation between degree of hyperglycemia and amount of interdialytic weight gain has been reported recently however [12]. In many patients, non-compliance with sodium and water restrictions contribute to large increments in weight (> 5–10 lb) between dialysis sessions. High intracellular sodium content in diabetic patients, producing increased thirst, is one proposed mechanism for excessive weight gain between dialyses [13].

Volume overload worsens hypertension and contributes to cardiovascular morbidity and mortality in diabetics. Attempting to ultrafilter excess interdialytic fluid gain often results in painful muscle cramps and hypotensive episodes. Intensive dietary counseling, improved glycemic control, use of sodium modelling, sequential ultrafiltration, and increasing dialysis time to enable slower ultrafiltration may ameliorate the problem.

Vascular access in diabetic dialysis patients

As in the rest of the hemodialysis population, vascular access is the Achilles heel of the diabetic patient. Advanced peripheral vascular disease related to medial arterial calcinosis and older age, combined with destruction of veins due to previous intravenous cannulation or injections often preclude creation of an endogenous Brescia–Cimino fistula in diabetic patients, or result in maturation failure of the fistula if created. Hence, most diabetic patients on hemodialysis have a synthetic, usually polytetrafluoroethylene (PTFE), graft. In a review of diabetic hemodialysis patients above 65 years of age hospitalized for failed vascular access between January 1, 1987 and December 31, 1991 at Kings County Hospital, Brooklyn, New York, only 1 of 40 (2%) of diabetic patients had a fistula, compared to 5 of 48 (10%) of non-diabetic hemodialysis patients in the same age group hospitalized during the same time period for failed vascular access (Table 2). Ninety five percent of diabetics had an upper arm arteriovenous graft.

Table 3. Morbidity in elderly diabetics following vascular access placement at Kings County Hospital 1987–1991. (Reprinted with permission from Miles AMV, Hong JH, Sumrani N et al. J KAMA 1996; 2: 25–28)

	Diabetic number (Total)	Non-diabetic number (Total)
Wound infection (post-op)	4 (40)	0 (48)*
Steal syndrome		
Upper arm graft	3 (38)	0 (39)*
Forearm/wrist	1 (2)	0 (9)
Thrombosis within 6 months		
Upper arm graft	17 (38)	13 (39)
Forearm graft	2 (2)	3 (4)
Wrist	0 (0)	1 (5)

* $p < 0.05$.

Access thrombosis in diabetic hemodialysis patients

Thrombosis of arteriovenous accesses is most often due to venous outflow stenosis occurring just distal to the venous anastomosis where the jet of blood shunted across the access impinges on the vessel wall and produces intimal hyperplasia [14]. Coexistent infection of the access may be present in the absence of local signs of infection and may result in overwhelming sepsis if not diagnosed early. Whether diabetic patients have an increased propensity to thrombose their vascular accesses is controversial. In vitro evidence indicates that diabetic platelets tend to aggregate more easily [15] and to produce higher levels of thromboxane B_2 [16], in addition, increased levels of von Willebrand factor have been found in diabetic sera [11]. The 1991 report of the USRDS, implicated diabetes as a risk factor for arteriovenous access thrombosis. Diabetic hemodialysis patients underwent 0.42 hospitalizations per years at risk for vascular access complications compared to 0.35 hospitalizations per year at risk for non-diabetics [17]. In the review of vascular access in elderly hemodialysis patients conducted at our institution (Table 3), thrombosis within the 6 month period following a hospitalization for vascular access failure was not significantly higher in diabetic compared to non-diabetic patients, however. National statistics in 1993 indicated that diabetic hemodialysis patients had more frequent and more prolonged hospitalizations than non-diabetics [18]; at our institution however, similar lengths of stay in hospital for problems related to vascular access in diabetic and non-diabetic dialysis patients have been documented [19].

Between 8–10% of the $50 000 Medicare expenditure per year for each diabetic ESRD patient relates to the maintenance of vascular access [1], and the cost of vascular access is the same for all patient groups, diabetic and non-diabetic. The $10–12 000 greater cost of treating diabetic patients documented

in the 1997 USRDS report, is related to under-representation of diabetics in the most cost-effective modality of renal replacement therapy: renal transplantation, and not to increased costs relating to vascular access. Additional evidence refuting diabetes as a specific risk factor in access thrombosis comes from a recent analysis of 784 incident hemodialysis patients enrolled in the USRDS Case Mix Adequacy Study in 1990 (a systematic sample of patients from 523 hemodialysis units across the United States) [20]. Of 245 hemodialysis patients with an endogenous fistula, 71 (29%) were diabetic, while of 539 patients with a graft, 219 (41%) were diabetic. Hence 3 times as many grafts as fistulae were placed in diabetic patients. One year survival rates for arteriovenous fistulae versus grafts were 70% versus 47% in this study, and though initial evaluation suggested an increased tendency for access failure in diabetics, on Cox's proportional hazard analysis, diabetes was not an independent predictor of access failure, the only such factor being peripheral vascular disease. It is likely, therefore, that the greater number of synthetic grafts placed in diabetic patients, with their associated higher failure rate, accounts for the apparent predisposition of diabetic patients to access thrombosis in earlier reports.

Vascular steal syndromes in diabetic hemodialysis patients

The low pressure run-off system created by an arterio-venous access, particularly a brachiocephalic access or a side to side radiocephalic fistula, may short-circuit blood from the palmar arch and ulnar arteries and result in a steal syndrome. Progressive ischemic pain leading to dry gangrene of one or more fingers may develop days to weeks after placement of the access and is more frequently seen in diabetic patients who often have pre-existing, severe medial arterial calcinosis of the ulnar and digital arteries [21]. Non-healing wounds of the fingers may also be a manifestation of vascular steal [22], and in cases of clinical uncertainty, digital pressures of < 50 mmHg on non-invasive vascular studies, and arteriography help to confirm the diagnosis. In severe cases of arterial steal syndromes, the onset may be more acute, within hours of creation of the access, and signs of acute arterial insufficiency such as pallor and pulselessness may be seen. Ligation of the distal limb of the radial artery in a side to side radiocephalic fistula or ligation or removal of a brachiocephalic access is necessary to correct the syndrome. Amputation of one or more digits and even below elbow amputation may sometimes be necessary.

Ischemic monomelic neuropathy

The term ischemic monomelic neuropathy was coined in 1983 by Wilbourn [23]. It is a complication of vascular access seen almost exclusively in diabetic patients [24], and refers to the development of acute pain, weakness and paralysis of the muscles of the forearm and hand, often with sensory loss, developing immediately after placement of an arteriovenous access usually in

the brachiocephalic or antecubital location. The condition results from diversion of the blood supply to the nerves of the forearm and hand; the acute ischemic insult being severe enough to damage nerve fibers, but insufficient to produce necrosis of other tissues. The propensity of development of the complication with accesses in these locations relates to the fact that the brachial artery constitutes the sole arterial inflow to the forearm and hand, and in the absence of collateral vessels about the elbow, diversion of all or most of brachial arterial blood through a fistula or graft results in distal ischemia. Nerve conduction studies are helpful in diagnosing the syndrome, and early access removal or ligation is necessary to prevent permanent paralysis of the hand.

Conclusion

Patients with diabetes mellitus comprise the largest and highest risk group of hemodialysis patients today. Though significant improvements in short term mortality rates have been seen over the past two decades, macrovascular disease limits long term survival. Dialysis-related complications of hypotension and excessive interdialytic weight gain are frequent and often difficult to manage in diabetic hemodialysis patients. Apparently higher access thrombosis rates in diabetics in prior reports are likely related to greater use of synthetic grafts rather than endogenous fistulae in diabetics; but primary maturation failure of Brescia–Cimino fistulae, and ischemic, neurologic and infectious complications related to vascular access are more frequently seen in diabetic than non-diabetic hemodialysis patients. Nephrologists must meet the challenge of managing diabetic dialysis patients by a adopting a combined approach of patient education, ensuring preemptive multidisciplinary management of cardiovascular and peripheral vascular disease and retinopathy, securing adequate vascular access, treating hypertension, and minimizing intradialytic complications.

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8. Continuous ambulatory peritoneal dialysis in 224 diabetics with end stage renal disease: evidence of improved survival over the past 10 years

PLOUMIS S. PASADAKIS & DIMITRIOS G. OREOPOULOS

Editors' Comment:

Dating back to its earliest application in diabetic ESRD patients, Oreopoulos has championed peritoneal dialysis. In the present update of an experience with 224 diabetic patients, peritonitis, the technique limiting complication of peritoneal dialysis, was reduced to about one episode in 16–19 months, an impressive accomplishment. Survival increased to a remarkable 93% at one year and 70% after three years. By five years, however, two-thirds of patients were dead, the consequence of unrelenting comorbidity. Assigning a place for peritoneal dialysis as opposed to hemodialysis as a first choice regimen for diabetic patients with ESRD is more a matter of bias and personal experience than of objective data analysis. In Canada, 52% of diabetic patients were receiving peritoneal dialysis three months after initiation of dialytic therapy while in the United States fewer than 20% are similarly treated. Why? Enthusiasts like Oreopoulos have advanced acceptance of peritoneal dialysis by demonstrating that it can be performed with high patient acceptance, lower cost than hemodialysis, and superior patient survival. Lacking the commitment and expertise of physicians dedicated to peritoneal dialysis, nephrologists are unlikely to gain results that meet the standard of excellence exemplified by the present report.

Introduction

The proportion of diabetics among patients requiring RRT continues to increase and diabetes mellitus became the leading cause of ESRD worldwide. Between 1984 and 1992 the fraction of new patients starting RRT whose ESRD was due to diabetes increased from 11% to 17% in Europe [1] and 27 to 36% in US [2]. Data from 1997 United States Renal Data System (USRDS) annual report presented an incidence of 37.4% for diabetes on ESRD treated patients between 1991–1995 [3].

Twenty years ago renal replacement therapy (RRT) would provide a limited benefit to most patients with end stage renal disease (ESRD), due to diabetic nephropathy. However, during this interval the overall survival of the diabetic patients on hemodialyis (HD), peritoneal dialysis (PD) and transplantation

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Table 1. CAPD diabetics

Diabetics	*n* (%)	Males (%)	Females (%)	Duration* (months)	Range
Type I, juvenile type	87 (38.84)	48 (55.17)	39 (44.83)	23.01 ± 14.7	1.5 ± 62
Type II, adult type	137 (61.16)	81 (59.12)	56 (40.88)	25.81 ± 16.9	1 ± 74
Total patients	224 (100.00)	129 (57.59)	95 (42,41)	24.69 ± 16.0	1 ± 74

* Duration (mean ± SD).

(Tx) have improved dramatically. Although continuous ambulatory peritoneal dialysis (CAPD) offers some advantages to the diabetic patient with ESRD [4, 5], it is not clear how to select the most appropriate renal replacement treatment for diabetics, especially those with multiple organ involvement.

USRDS registry reported a similar mortality for diabetics in HD, PD and renal transplantation for the age groups of 20–44 and 45–54, but a higher mortality in older patients. The reverse was observed among Canadian patients [6]. During RRT the main cause of death is cardiovascular diseases, which accounts for about 55% of deaths in both diabetics and non diabetics, a percentage that has not changed during the past decade.

This report describes our experience with 224 diabetic patients with end-stage renal disease treated in the years 1990–1996 with continuous ambulatory peritoneal dialysis at the Western Division of Toronto Hospital (TTH), Toronto.

Patients

Between 1990 and 1996, we trained for CAPD 224 diabetic patients with ESRD – 129 males (57.6%) and 95 females (42.4%). Their mean age was 57.4 years for males and 56.3 years for females, while the mean duration of CAPD was 24.7 ± 16 months (mean ± SD) (range 1–74 months).

In addition to the classic definitions, we assigned type of diabetes using as criteria: (a) the patient's age at first diagnosis of diabetes and (b) the need for insulin for glycemic control. Thus patients under 40 years of age who were using insulin from the initiation of treatment were classified as type I (juvenile type, insulin dependent diabetes mellitus, IDDM) while the rest were classified as type II (adult type, non-insulin diabetes mellitus, NIDDM). Patients under 40 years, who were treated with diet or/and oral hypoglycemic agents, were considered to be type II diabetics. According to these definitions, 87 patients (38.8%) were classified as type I diabetics (mean age 43.3 ± 11 years, (mean ± SD), mean duration on CAPD 23 ± 14.7 months); and 137 patients (61.2%) were type II diabetic patients (mean age 65.6 ± 7.9 years, mean duration on CAPD 25.8 ± 16.9 months) (Tables 1 and (ii). The mean age at the time of diagnosis was 19.8 ± 10.8 years for type I diabetic patients and 52.6 ± 11 years

Table 2. Ages of CAPD diabetics by sex and type of diabetes

	Males		Females		All the patients	
	Age*	Range	Age	Range	Age*	Range
Type I, juvenile type	45.1 ± 10.7	25–65	41.0 ± 11.0	27–68	43.3 ± 11.0	25–68
Type II, adult type	64.7 ± 8.3	47–86	67.0 ± 7.1	52–83	65.6 ± 7.9	47–83
Total patients	57.4 ± 13.2	25–86	56.3 ± 15.6	27–83	56.9 ± 14.3	25–83

* Age in years (mean ± SD).

Table 3. Kidney diseases that were diagnosed in diabetic patients

Diagnosis	Patients (*n*)	%*
Diabetic glomerulosclerosis	26	11.6
Other glomerulopathies	9	4.0
Focal segmental glomerulosclerosis	4	1.7
Membranous glomerulonephritis	2	0.9
Proliferative glomerulonephritis	2	0.9
IgA – nephropathy	1	0.4
Hypertensive nephropathy	2	0.9
Polycystic kidney disease	2	0.9
Nephrolithiasis	3	1.3
Interstitial nephritis	3	1.3
Multiple myeloma	3	1.3
NSAIDs nephrotoxicity	1	0.4
Renal-cell carcinoma	2	0.9
Renal papillary necrosis	1	0.4

* Proportion of total 224 patients.

for type II. The interval between the date of diagnosis and the initiation of peritoneal dialysis was 23.4 ± 8.3 and 13.1 ± 7.3 years respectively.

Renal biopsy was performed in 35 patients, 26 of whom had diabetic glomerulosclerosis, and 9 who had various other glomerular diseases (focal segmental glomerulosclerosis (FSGS) (4), membranous (2), proliferative GN (2), IgA nephropathy (1)). Seventeen other patients had various other renal diseases in addition to diabetes – hypertensive nephropathy (2), polycystic kidney (2), nephrolithiasis (3), interstitial nephritis (3), multiple myeloma (3), NSAIDS nephrotoxicity (1), renal-cell carcinoma (2), and renal papillary necrosis (1), (Table 3).

Prevalence and incidence of ESRD

According to their age at the beginning of peritoneal dialysis, these patients were divided into four age groups: 20–44 years, 45–64 years, 65–74 years, and older than 75 years old patients (75+). Table 4 shows the percent of each

Table 4. CAPD group ages, by type of diabetes and sex

Characteristics	*n*	%	Type of diabetes		Gender	
			Type I	Type II	Males	Females
Age groups						
Age 20–44	47	28.5	47	0	21	26
Age 45–64	100	60.6	37	63	66	34
Age 65–74	60	36.4	3	57	33	27
Age 75+	17	10.3	0	17	9	8
Total	224	100.0	87	137	129	95

Table 5. Prevalence (P) counts by age and type of diabetes at end of the years 1991–1996

	1991	1992	1993	1994	1995	1996
Age groups						
Age 20–44	10	20	28	26	23	23
Age 45–64	23	38	59	62	52	49
Age 65–74	14	24	26	30	31	28
Age 75+	6	8	8	6	6	8
Total	53	90	121	124	112	108
Type of diabetes						
Type I	14	31	51	49	44	40
Type II	39	59	70	75	68	68

Table 6. Incidence counts by age and type of diabetes during the years 1991–1996

	1991	1992	1993	1994	1995	1996
Age groups						
Age 20–44	6	12	10	5	5	5
Age 45–64	15	18	24	11	12	9
Age 65–74	10	10	9	10	8	8
Age 75+	4	2	2	1	3	3
Total	35	42	45	27	28	25
Type of Diabetes						
Type I	8	18	24	8	11	9
Type II	27	24	21	19	17	16

group in the total population. The 45–64 group made up 60.6% while only 10.3% of the patients were older than 75 years (Table 5).

Between 1991 and 1993 there was an increase both in ESRD prevalence and incidence (Table 6) but thereafter there was an unexplained decline.

Table 7. Proportion of patients with pre-dialysis co-morbidity factors, by type of diabetes

Factors	Total (*n*)	%	Type I (*n*)	%*	Type II (*n*)	%*
Arterial hypertension	175	78.1	67	77.1	108	78.8
Cardiovascular disease						
Angina	43	19.0	16	18.3	27	19.7
Myocardial Infarction	37	16.5	12	13.7	25	18.2
NQWMI	11	4.9	4	4.5	7	5.1
LV**- Dysfunction	10	4.5	6	6.8	4	2.9
CABG or angioplasty	17	7.5	6	6.8	11	8.0
Congestive heart failure	40	17.8	9	10.3	31	22.6
Acute pulmonary edema	20	8.9	7	8.0	13	9.4
Cerebrovascular disease	28	12.5	10	11.5	18	13.1
Peripheral vascular disease	40	17.8	13	14.9	27	19.7
COPD***	18	8.0	8	9.1	10	7.3
Smoking	40	17.8	15	17.2	25	18.2

* Ppercentage of patients among the same group (Type I and Type II).
** LV = left ventricular.
*** COPD = chronic obstructive pulmonary disease.

Predialysis co-morbidity

Preexisting arterial hypertension was present in 175 patients (78.1%) – (67 patients of type I diabetes (77%) and 108 patients of type II diabetes (78.8%)). The mean duration of hypertension was longer in type II (12.9 ± 10.7 years vs. 6.4 ± 5.6 years in type I patients) (mean $\pm$ SD, $p = 0.004$).

History of previous well-documented cardiac disease (angina, mural myocardial infarction, non-Q-wave ('silent') myocardial infarction and left ventricular dysfunction) is shown in Table 7. Coronary artery bypass surgery (CABG) or angioplasty was observed in 17 patients (7.5%). Symptomatic congestive heart failure and episodes of acute pulmonary edema were described in 40 (17.8%) and 20 (8.9%) patients respectively. Evidence of cerebrovascular disease was observed in 28 (12.5%) while peripheral vascular disease was observed in 40 (17.8%) (Table 7).

Chronic obstructive pulmonary disease (COPD) was present in 18 (8%) patients (8 type I patients (9.1%) and 10 type II patients (7.3%)) while 40 patients were chronic smokers.

Regarding diabetic neuropathy, there was evidence of gastroparesis in 10 type I patients (11.5%) and 4 type II diabetic patients (2.9%), three patients had a history of atonic-neurogenic bladder and one patient has malabsorption syndrome. Peripheral neuropathy was confirmed by electrophysiological studies in 25 patients (11%), (16 of type I (18.3%) and 9 patients of type II (6.5%)).

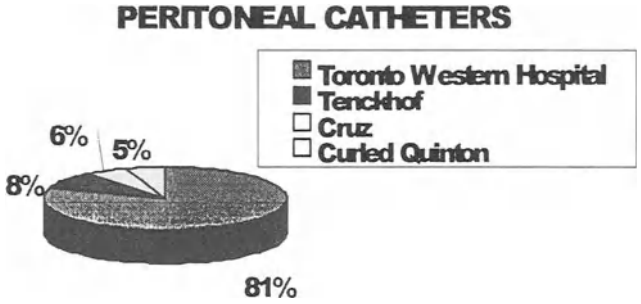

Fig. 1. Indwelling peritoneal catheters used in 224 diabetic patients on CAPD in Toronto Western Hospital, during the period 1990–1996.

Visual disorders – blindness

Diabetic retinopathy was present in 53 type I patients (61%) and in 38 type II patients (28%). Photocoagulation therapy (Laser) was performed in 21 patients of type I (24%) and 14 patients of type II (10.2%).

Twelve type I patients (13.8%) and three type II patients (2.2%) were totally blind while 5 type I patients (5.7%) and 4 type II patients (2.9%) had unilateral blindness. Impaired visual acuity (poor vision) was present in 9 patients, 4 type I and 6 of type II.

Vitrectomy was performed in 18 patients – 13 of type I and 5 type II. History of unilateral cataract extraction was present in 11 right eyes and 9 left eyes of type I patients and 17 right eyes and 16 left eyes of type II diabetics. Glaucoma was present in 11 patients (6 of type I and 5 of type II).

Other ophthalmologic complications were retinal detachment, recorded in 9 patients (6 type I patients and 3 type II), vitreous hemorrhage in 2 type I patients, corneal ulcer and chronic keratitis sicca in two type I patients.

Technique of CAPD and laboratory methods

Chronic peritoneal dialysis was started after surgical implantation of an indwelling chronic peritoneal catheter. Toronto Western Hospital catheters (TWH) were implanted in 182 (81.2%) of these patients. Tenckhoff catheters were implanted in 19 (8.4%) patients, Cruz catheters in 13 (5.8%) and curled Quinton catheters in 12 (5.3%) (Figure 1).

The dialysis system used initially was mainly the double bag system in 70%, the UV-flash® system in 26% (mainly those with visual impairment) and the old spike system in 4% (Figure 2).

Eighty six percent of the patients were dialysed with four 2L exchanges per day using dialysis solution with a D-glucose monohydrate concentration of 1.36, 2.27, and 3.86 g/dl. Eleven percent of the patients were using a larger

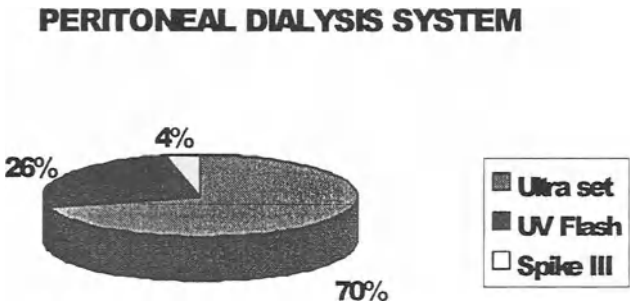

Fig. 2. Peritoneal dialysis system used in 224 diabetic patients on CAPD in Toronto Western Hospital, during the period 1990–1996.

exchange volume (10% 2.5 L and 1% 3 L) while 3% of the patients were using smaller volume of 1.5 L.

Continuous ambulatory peritoneal dialysis (CAPD) was the main modality in 211 patients, 94%); 11 patients (4%) were on nocturnal intermittent peritoneal dialysis (NIPD) and 5 patients (2%) were on continuous cyclic peritoneal dialysis (CCPD). During training, the patients were taught about the signs and symptoms of peritonitis and shown how to add antibiotics and insulin to the dialysis bag.

After completion of training the patients were seen monthly for routine blood tests and evaluation of home blood sugar control. Glycosylated hemoglobin (HbA1c), serum lipids profile and PTH levels were measured every 3 months. Skeletal surveys including measurements of bone mineral density (BMD) and nerve-conduction velocities were conducted every 12 months, during the early phase of dialysis treatment.

Routinely residual renal function and dialysate volumes were measured whenever there was any change in patient's clinical or biochemical status. The computer program ADEQUEST® was used to estimate the peritoneal dialysis adequacy and nutritional indices. A dietician, who is a permanent member of our team, regularly reviewed all these patients.

If hypertension persisted after the patient had reached 'dry weight', defined as being edema-free with no cardiac failure, we added antihypertensive medication. Calcium, vitamin D supplements and phosphate binders were used in doses appropriate to maintain the serum calcium and phosphate levels in normal values. Also all patients received a multivitamin preparation daily.

Erythropoietin (rHu-EPO) was given to 48% of the 224 patients in order to maintain the levels of Hct between 30–33% – 4000 units twice a week for 35% of the patients and 4000 units once a week for 13%.

Blood sugar was controlled by the intraperitoneal administration (IP) of insulin in 71 type I (81.6%) and 77 type II patients (56.2%). Subcutaneous (SC) administration was used as exclusive route in 6 patients of type I (6.8%)

and 10 patients of type II (7.3%). In a small number of patients, the combination of IP and SC administration was used to maintain blood sugar levels of 140 mg/dl (8 mmol/L) during fasting, and less than 200 mg/dl (11 mmol/L) one hour after meals. Also there were 16 patients of type II (11.7%) who received oral hypoglycemic agents, 30 type II patients (21.9%) achieved good glycemic control by antidiabetic diet only.

Table 8 shows the biochemical profile of the CAPD patients. Patients were divided into 3 groups according to the duration of CAPD: group I – 62 patients for 3–12 months on CAPD, group II – 139 diabetic patients on CAPD for 13–48 months; and finally group III – 23 patients who were dialysed for more than 48 months. Table 8 shows the mean initial (beginning) and last (end) available values in these three groups. As it is shown, most of these variables were maintained either in normal range or at acceptable levels during the study period.

Renal osteodystrophy

Microradioscopy of hand bones was performed in 54 patients (24% of the diabetic patients) – 25 type I and 29 type II patients, revealed evidence of subperiosteal resorption of hyperparathyroidism in 9 patients (16.6%) (4 type I and 5 type II diabetics). Also 7 patients (13%) showed findings of intracortical metacarpal resorption suggesting high bone turnover.

Evidence of tissue and vascular calcifications was sought in routine metabolic skeletal survey. Soft tissue calcifications were found in only 2 patients, while a large number of patients had arterial calcifications. The areas involved are shown in Table 9.

In the first 2 months of peritoneal dialysis, measurements of bone mineral mass (mg/cm^2), combined cortical thickness (mm), and bone mineral density (mg/cm^3), were evaluated in these 54 patients (25 type I and 29 type II). Table 10 shows the mean values of these parameters. All were within the lower end of normal limits.

In 18 patients (7 of type I and 11 of type II) comparison of the initial and subsequent values of all three parameters after a duration of 15.3 ± 1.4 months revealed no statistically significant change. Bone mineral mass changed from 573.34 ± 44.3 mg/cm^2 to 574.16 ± 43.6 mg/cm^2, cortical thickness from 5.97 ± 0.36 mm to 5.91 ± 0.36 and bone mineral density changed from 948.34 ± 34.7 mg/cm^3 to 967.89 ± 49.9 mg/cm^3. Serum levels of PTH, ionized Ca++, P and Mg also were measured routinely during the study and shown in Table 13.

Peripheral vascular disease – amputations

Forty patients had a history of peripheral vascular disease. In this group there were 16 below knee amputations (BKA) and 2 above knee amputations (AKA).

Table 8. The effect of CAPD on biochemical control of the diabetic patients

Variable	Normal range	DURATION OF CAPD (number of diabetic patients)					
		3–12 months (patients $n = 62$)		13–48 months (patients $n = 139$)		>48 months (patients $n = 23$)	
		Beginning	End	Beginning	End	Beginning	End
Hb	120–160 g/L	99.9 ± 13.3	99.7 ± 12.7	104.9 ± 18.6	105.9 ± 17.3	95.4 ± 15.8	102.7 ± 13.8
Hct	0.33–0.47	0.299 ± 0.04	0.298 ± 0.04	0.314 ± 0.05	0.307 ± 0.05	0.289 ± 0.05	0.310 ± 0.04
MCV	80–95 fL	87.6 ± 6.2	88.7 ± 6.2	88.8 ± 6.1	88.9 ± 7.5	87.6 ± 5.3	90.3 ± 6.8
Ferritin	6–186 μg/L	214.8 ± 267.0	209.6 ± 267.3	161.9 ± 175.0	132.5 ± 144.6	178.9 ± 218.5	149.5 ± 125.4
Iron saturation	0.25–0.50	0.50 ± 1.5	0.49 ± 1.5	0.81 ± 2.6	0.24 ± 0.1	0.19 ± 0.1	0.21 ± 0.07
Iron Total	9–32 μmol/L	11.4 ± 4.2	11.2 ± 4.0	12.4 ± 6.1	11.2 ± 5.8	15.0 ± 4.5	11.6 ± 4.7
Transferrin	2.0–4.0 μg/L	1.9 ± 0.4	1.9 ± 0.4	2.1 ± 0.5	2.0 ± 0.05	2.5 ± 0.5	2.2 ± 0.5
Glucose	<8 mmol/L	9.7 ± 3.9	9.8 ± 3.9	9.7 ± 3.4	9.3 ± 3.2	9.6 ± 4.2	9.7 ± 3.7
HbA_{1C}	<0.09	0.078 ± 0.016	0.077 ± 0.016	0.084 ± 0.019	0.087 ± 0.017	0.082 ± 0.013	0.081 ± 0.015
Na^+	135–145 mEq/L	136.6 ± 3.4	136.6 ± 3.3	136.5 ± 3.9	135.7 ± 3.7	135.9 ± 4.0	134.8 ± 3.7
K^+	3.2–5.0 mEq/L	3.9 ± 0.7	3.9 ± 0.7	3.8 ± 0.6	3.8 ± 0.6	4.0 ± 0.8	5.4 ± 1.7
Chloride	96–106 mEq/L	99.3 ± 5.1	99.4 ± 5.0	98.7 ± 4.4	97.5 ± 4.3	97.5 ± 4.7	93.6 ± 10.8
Bicarbonate	22–28 mEq/L	27.8 ± 10.6	27.8 ± 10.6	26.0 ± 3.0	25.9 ± 3.4	24.8 ± 2.5	26.3 ± 3.4
Urea	3–7 mmol/L	20.4 ± 7.3	19.9 ± 6.9	19.9 ± 7.0	18.0 ± 6.4	20.0 ± 5.5	20.6 ± 5.3
Creatinine	70–120 μmol/L	630.6 ± 216.1	628.2 ± 210.1	659.1 ± 194.0	702.3 ± 234.3	743.3 ± 214.1	790.0 ± 199.2
Total protein	65–80 g/L	69.7 ± 10.5	69.6 ± 10.4	70.8 ± 7.2	70.6 ± 9.3	72.3 ± 7.4	72.5 ± 8.5
Albumin	38–50 g/L	37.8 ± 4.9	37.8 ± 4.9	37.8 ± 4.8	38.0 ± 5.4	38.9 ± 2.7	39.1 ± 3.0
Ionized Ca^{++}	1.12–1.32 mmol/L	1.22 ± 0.23	1.23 ± 0.23	1.22 ± 0.13	1.22 ± 0.12	1.20 ± 0.08	1.23 ± 0.11
Phosphate	0.8–1.4 mmol/L	1.52 ± 0.48	1.49 ± 0.47	1.46 ± 0.41	1.54 ± 0.49	1.53 ± 0.36	1.68 ± 0.35
ALP	<110 U/L	95.64 ± 51.4	95.1 ± 51.5	94.3 ± 75.2	92.1 ± 67.2	85.8 ± 24.9	86.3 ± 36.2
PTH	1.3–7.6 pmol/mL	17.4 ± 17.0	17.4 ± 17.1	13.0 ± 13.3	18.4 ± 24.6	9.2 ± 9.0	18.6 ± 18.0
Mg	0.7–1.1 mmol/L	1.09 ± 0.23	1.12 ± 0.3	1.20 ± 0.21	1.21 ± 0.183	1.29 ± 0.28	1.40 ± 0.27
Aluminum	<560	364.0 ± 333.9	364.0 ± 333.9	358.0 ± 244.3	349.7 ± 199.6	374.9 ± 624.5	417.78 ± 255.4
AST (SGOT)	<35 U/L	21.1 ± 19.9	21.3 ± 20.4	27.8 ± 53.7	20.0 ± 11.6	19.5 ± 12.1	19.1 ± 6.8
ALT (SGPT)	<40 U/L	20.5 ± 23.5	19.9 ± 23.6	28.0 ± 26.0	29.1 ± 24.2	21.0 ± 22.8	20.0 ± 22.8
TSH	0.1–5.8 mU/ml	3.0 ± 1.7	2.8 ± 1.7	5.7 ± 2.3	2.4 ± 1.5	2.7 ± 1.1	2.8 ± 2.1
Total cholesterol	<5.2 mmol/L	5.6 ± 1.3	5.6 ± 1.3	5.9 ± 1.6	5.6 ± 1.6	6.1 ± 1.68	6.0 ± 1.8
LDL-cholesterol		3.3–0.9	3.2 ± 0.9	3.3 ± 1.4	3.2 ± 1.3	3.6 ± 1.6	2.9 ± 0.7
HDL-cholesterol		0.95–0.34	1.01 ± 0.4	1.15 ± 0.7	2.10 ± 0.9	0.97 ± 0.33	1.41 ± 0.47
Triglycerides	<1.8 mmol/L	2.8 ± 1.7	2.8 ± 1.7	3.2 ± 2.2	3.1 ± 2.3	3.6 ± 2.4	2.6 ± 1.7

Table 9. Radiological findings of bone – arterial and tissue calcifications among 54 diabetic patients on CAPD

Radiological findings	Patients (*n*)	%
Evidence of subperiosteal resorption of HPPT	9	16.6
Intracorical resortion in the metacarpals	7	12.9
Osteosclerosis	2	3.7
Soft tissue calcifications	2	3.7
Arterial calcifications of:		
small vessels of wrist and hands	21	38.8
small vessels of ankles and feet	25	46.3
pelvic femoral areas	20	37.0
abdominal aorta	21	38.8

Seven of 16 BKA were performed before the initiation of CAPD treatment and nine during the dialysis therapy. Of the two AKA, one was performed in the predialysis period in one type I patient and the other was done in one type II patient after PD initiation. Additional 23 amputations were performed because of progressive vascular disease and the presence of gangrene in smaller areas: 20 toes, 1 foot and 1 arm and 1 finger (index) (Table 11).

Residual renal function

This analysis was performed using the data of 159 diabetic patients (71% of the total). Urine volume and residual renal clearance were measured at initiation of CAPD and at 12-month intervals. There was a stable decline in 24 hour urine volume from 785.4 ± 616 ml (mean $\pm$ SD) at the initiation of PD therapy to 157.0 ± 87 ml at 48 months; the creatinine clearance decreased from 5.34 ± 2.9 ml/min to 0.9 ± 0.3 ml/min over the same period. Although at the start type I diabetics had higher values than did type II diabetics, their values decreased at a similar rate during the 48-month period (Figures 3, 4).

Peritonitis rate – exit site infections

Over a total 5531 patient months of follow-up, there were 316 episodes of peritonitis – 107 in type I and 219 in type II patients. These episodes represent an overall incidence of one episode of peritonitis every 17 patient-months. The rate was slightly lower in type I (1 episode/18.7 patient-months) than in the type II diabetic population (1 episode/16.1 patient-months). The frequency of Gram (+) and Gram (−) organisms was 65% and 18.1% respectively; in 18 episodes cultures failed to reveal any organism (5.5%) (Table 12).

Also, there were 123 episodes of exit-site infections – 43 in type I patients and 80 in type II. The calculated rate of exit-site infections was 1 episode every 22.2 patient months. The causative organisms isolated from the cultures are

Table 10. Metabolic skeletal survey in 54 CAPD diabetics at the beginning of dialysis

	All the patients	Type I ($n = 25$)	Type II ($n = 29$)	Males ($n = 34$)	Females ($n = 20$)
M-BMM (mg/cm^2)	591.3 ± 179	623.2 ± 173	563.8 ± 182	636.9 ± 173	513.8 ± 165
C-CCT (mm)	5.8 ± 1.3	6.0 ± 1.3	5.8 ± 1.4	6.2 ± 1.3	5.3 ± 0.9
D-BMD (mg/cm^3)	979.8 ± 225	1002.78 ± 266	960.0 ± 184	999.9 ± 232	945.5 ± 212

Normal ranges (D = M/C)	*Males*	*Females*
M-BMM = bone mineral mass (mg/cm^2)	*600–1000*	*510–910*
C-CCT = combined cortical thickness (mm)	*5.6–8.7*	*4.8–7.2*
D-MBD = bone mineral density (mg/cm^3)	*950–1400*	*950–1400*

* Values in mean ± SD.

Table 11. Amputations among CAPD diabetic patients

	Total amputations	Type I (*n*)		Type II (*n*)	
		Predialysis	After PD start	Predialysis	After PD start
Lower limbs					
Toes	20	4	11		5
Foot	1	1			
Leg BKA*	16	4	5	3	4
Leg AKA**	2	1			1
Upper limbs					
Finger	1		1		
Arm	1	1			

* BKA = below knee amputation.
** AKA = above knee amputation.

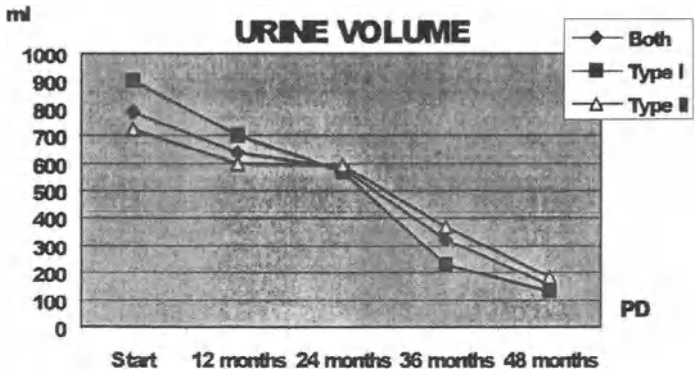

Fig. 3. Changes in mean values of 24-hour urine volume (ml) of 159 diabetic patients during the study period.

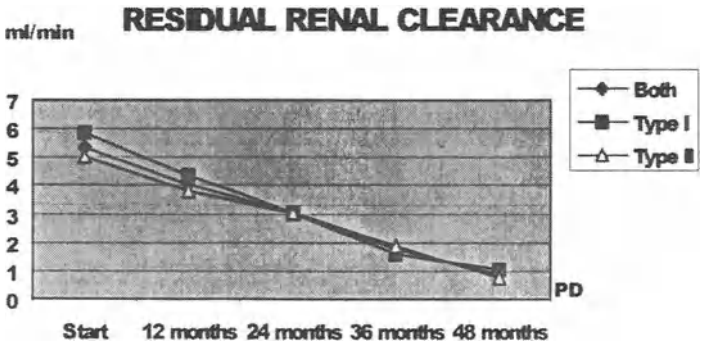

Fig. 4. Changes in mean values of residual renal creatinine clearance (ml/min) of 159 diabetic patients during the study period.

Table 12. Frequency of organisms isolated from dialysate cultures of CAPD diabetic patients with peritonitis

Organism		Total (*n*)	(%)	Type I	Type II
Gram positive bacteria					
Staphylococcus epidermidis		85	26.1	28	57
Staphylococcus aureus		93	28.5	37	56
Streptococcus a-Hemolytic		25	7.7	8	17
Streptococcus faecalis (enterococcus)		7	2.1	1	6
Streptococcus non-Hemolytic		1	0.3		1
Streptococcus viridans		1	0.3		1
	Total	212	65.0	74	138
Gram negative bacteria					
Pseudomonas aeruginosa		11	3.4		11
Klebsiella		9	2.8	5	4
Escherihia coli		8	2.5	6	2
Serratia species		7	2.1	2	5
Proteus species		6	1.8	4	2
Acinetobacter species		6	1.8	2	4
Coliforms		6	1.8	2	4
Enterobacter cloacae		3	0.9		3
Citrobacter species		1	0.3		1
Xanthomonas maltophilia		1	0.3		1
Neisseria species		1	0.3		1
	Total	59	18.1	21	38
Mycobacterium Tuberculosis		3	0.9		3
Yeasts (*Candida* species)		12	3.7	2	10
Other species		22	6.7	4	18
No growth		18	5.5	6	12
	Total	55	16.9	12	43

shown in Table 13. *Staphylococcus aureus* was the predominant organism for approximately one-half of the episodes (54.5%).

Peritoneal catheters were removed from 52 patients (23.2%) and replaced by a new catheter. Peritonitis was responsible for 34 catheter replacements (65.3%); the remaining causes were catheter obstruction in 10 (19.2%), abdominal operation in 4 (7.7%), tunnel abscess in 3 (5.8%) and accidental cuff extraction in one (1.9%).

Adequacy of peritoneal dialysis

During the study period peritoneal equilibration tests of adequacy were performed in 111 patients with the same computer program (ADEQUEST)®. The patients' permeability characteristics were divided into four groups: high trans-

Table 13. Frequency of organisms isolated in cultures from CAPD diabetic patients with exit-site infections

Organism		Total (*n*)	(%)	Type I	Type II
Gram positive bacteria					
Staphylococcus epidermidis		31	25.2	17	14
Staphylococcus aureus		67	54.5	18	49
Streptococcus a-Hemolytic		4	3.3	2	2
	Total	102	82.9	37	65
Gram negative bacteria					
Pseudomonas aeruginosa		4	3.3		4
Klebsiella		2	1.6	1	1
Escherihia coli		6	4.9	4	2
Serratia species		2	1.6	1	1
Proteus species		3	2.4		3
Citrobacter species		2	1.6		2
Morganella morganii		1	0.8		1
	Total	20	16.3	6	14
Yeasts (*Candida* species)		1	0.3		1

porters (H), high average transporters (HA), low average (LA) and low transporters (L). The distribution of these permeability properties were H (21%), HA (48%), LA (29%) and L (2%). The mean initial values ($\pm$ SD) of total, residual and dialysate weekly Kt/V urea (L/week/V) were 2.39 ± 0.56, 0.51 ± 0.41 and 1.98 ± 0.42 and the corresponding values of normalized weekly creatinine clearance (L/week/1.73m^2) were 76.2 ± 21.5, 29.5 ± 19.5, and 52.6 ± 8.4, (Table 14). The normalized protein catabolic rate (NPCR) for all patients was 0.929 ± 0.28 g/kg/24 h. There was not observed any statistically significant difference between the initial adequacy indices of type I and type II diabetic patients (Table 14).

To evaluate the changes in peritoneal dialysis adequacy with time, we analyzed the results of two consecutive tests of 22 diabetic patients. The mean interval between the two tests was 16.6 ± 7 months. During this period the number of anuric patients was increased from 2 to 5 patients, the 24-hour urine volume decreased from 577.8 ± 596 ml/day to 290.3 ± 411 ml/day ($p = 0.069$) and the residual renal creatinine clearance decreased from 2.9 ± 2.8 ml/min to 1.86 ± 1.9 ml/min ($p = 0.162$). These changes were due to a decrease in residual weekly Kt/V urea values – from 0.61 ± 0.66 to 0.34 ± 0.42 and weekly normalized creatinine clearance (from 29.3 ± 28 to 17.3 ± 19 L/week/1.73 m^2). However, these changes had been compensated by an appropriate increase in the peritoneal dialysis and therefore there was no statistical change in the total dialysis dose over that interval (Figures 5, 6). Thus, dialysate weekly Kt/V urea had been increased significantly (from 1.84 ± 0.37 to 2.12 ± 0.34 ($p = 0.007$))

Table 14. Results of analysis* of dialysis adequacy from 111 CAPD diabetic patients (type I, $n = 41$; type II, $n = 70$)

Measurement	All patients	Type I	Type II	p
Weekly Kt/V urea (L/week/V)				
Total	2.39 ± 0.56	2.42 ± 0.56	2.37 ± 0.57	$p = 0.62$
Residual	0.51 ± 0.41	0.49 ± 0.35	0.52 ± 0.44	$p = 0.71$
Dialysate	1.98 ± 0.42	2.09 ± 0.48	1.93 ± 0.37	$p = 0.09$
Weekly creatinine clearance (L/week/1.73 m^2)				
Total	76.2 ± 21.5	76.5 ± 25.0	76.0 ± 19.3	$p = 0.98$
Residual	29.5 ± 19.5	31.5 ± 21.5	28.2 ± 18.3	$p = 0.44$
Dialysate	52.6 ± 8.4	53.6 ± 10.3	51.9 ± 7.0	$p = 0.36$
NPCR (gr/Kg/H)	0.929 ± 0.28	0.953 ± 0.30	0.917 ± 0.3	$p = 0.49$

* ADEQUEST (mean $\pm$ SD).

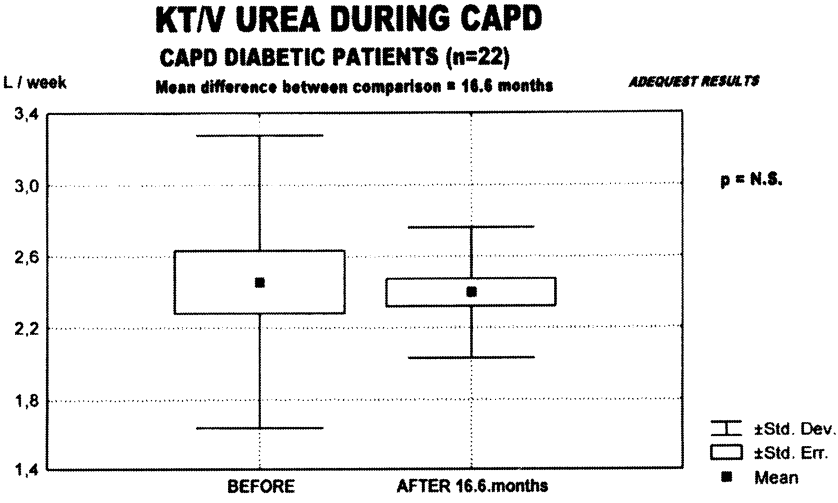

Fig. 5. Kt/V urea of 22 CAPD diabetics with mean interval between comparison 16.6 months. There is no statistically significant difference (p = N.S.).

and the similar change was also observed in dialysate creatinine clearance (from 48.8 ± 5.6 to 55.2 ± 9.7 ($p = 0.012$).

Outcome

Thirty-three patients (14.7%) received a renal transplant, 23 patients of type I diabetes (26.4%) and 10 patients of type II (7.3%). The mean interval from the initiation of dialysis to transplantation was 25 ± 12.1 months (22 ± 10.3 months in type I and 32 ± 13.2 months in type II).

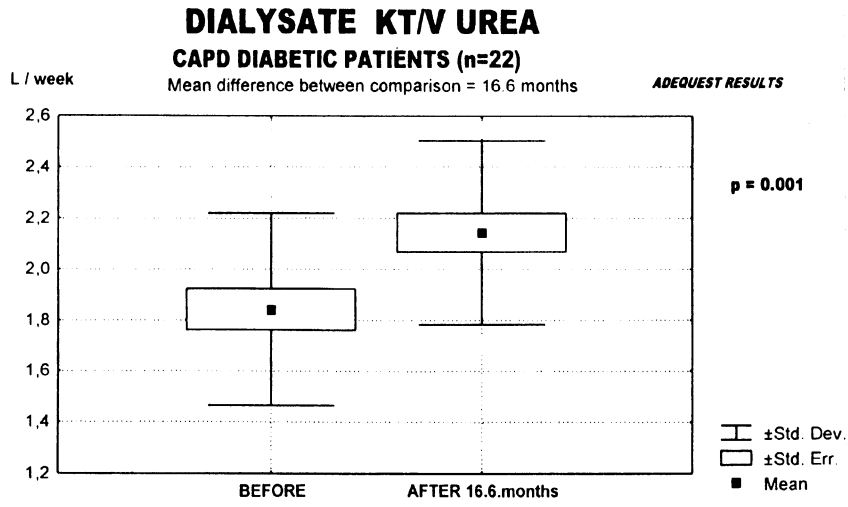

Fig. 6. Dialysate Kt/V urea of 22 CAPD diabetics with mean interval between comparison 16.6 months. There is statistically significant difference ($p = 0.007$).

Of the 224 patients, 49 (15 type I and 34 type II) were transferred to hemodialysis. The main cause for transfer was peritonitis in 32 patients (66.7%). Recurrent bacterial peritonitis was the cause in 18 patients, while 11 peritonitis episodes were due to yeast and 3 episodes to M. tuberculosis. Loss of ultrafiltration was the cause of transfer in 5 patients (10.4%) while 2 suffered from underdialysis. Table 15 shows the various causes of CAPD treatment 'drop out'.

Patient survival was calculated by Kaplan-Meier product-limit method from the time patients started dialysis therapy until they died. Patients who received a transplant or switched to hemodialysis were ruled 'lost to follow-up'. Cumulative proportion survival at 1st, 3rd and 5th year of CAPD treatment for all diabetic patients was 93%, 70% and 33% respectively, (Figure 7). Type I patients had better survival rates than type II diabetic patients, the former had survival rates at 1st, 2nd, 3rd, 4th, and 5th year of 95%, 84%, 76%, 56%, 33% versus 92%, 79%, 66%, 47%, 33% for type II patients (Figure 8).

During the study period, 65 patients died (20 patients of type I and 45 patients of type II). Death in 37% was due to cardiac causes (cardiac arrest, myocardial ischemia and infarction as well as other causes of cardiac failure). In 17% of deaths the cause was uncertain, in 12% the cause was septicemia while cerebrovascular accident was the cause in 9% (Table 16). In 4 patients (6% of all deaths) treatment was withdrawn at the patient's request or on the suggestion of the treatment team.

Technique survival was calculated from the time the patients started the CAPD treatment until the date they switched to hemodialysis. Patients who received a transplant or died were considered to be lost to follow-up at the

Table 15. Etiology of transfer of CAPD diabetic patients to hemodialysis

Etiology	Total (*n*)	(%)	Type I	Type II
Peritonitis				
Recurrent bacterial	18	37.5	7	11
Yeast	11	22.9	2	9
TBC	3	6.3		3
	32	66.7	9	23
Underdialysis	2	4.2	1	1
UF failure	5	10.4	3	2
Abdominal surgery	2	4.2		2
Recurrent pancreatitis	1	2.1	1	
Renal cell carcinoma	1	2.1		1
Cerebrovascular disease	2	4.2	1	1
Non-compliance	1	2.1		1
Failure to cope	1	2.1		1
Patients choice	1	2.1		1
Depression	1	2.1		1
	17	35.4	6	11

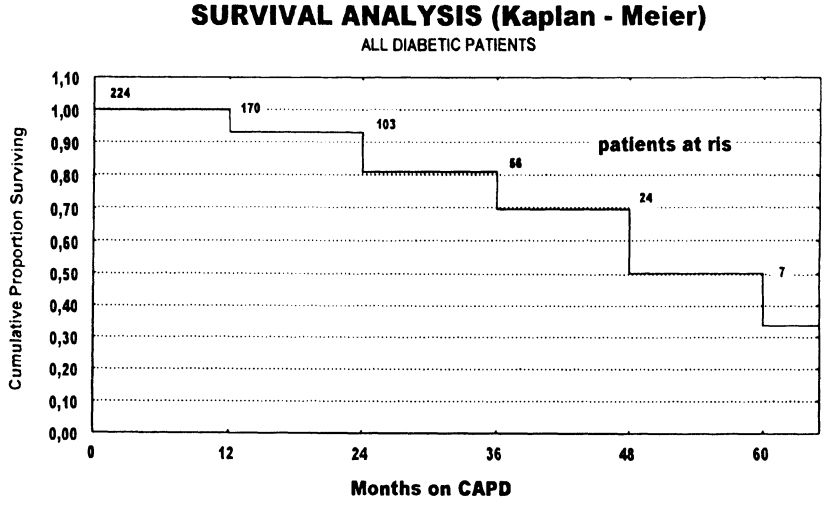

Fig. 7. Kaplan–Meier survival analysis of all diabetic patients of the study. End event is the date of death.

time the event occurred. Technique survival rates for the 1st, 3rd and 5th year of CAPD treatment period were 93%, 72% and 44% respectively as shown (Figure 9). Type I patients had slightly better technique survival than type II diabetic patients, (Figure 10).

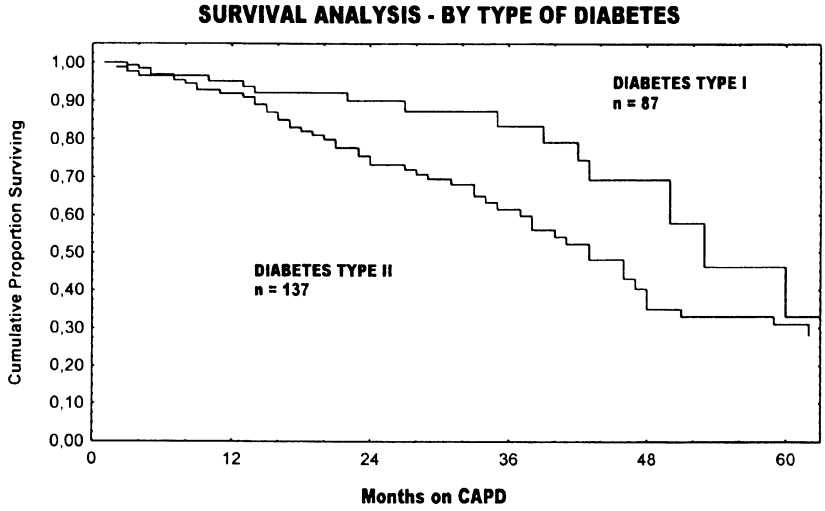

Fig. 8. Kaplan–Meier survival analysis by type of diabetes of CAPD patients. End event is the date of death. As it is shown the cumulative proportion surviving was better for type I diabetics than type II diabetic patients.

Table 16. Causes of death of CAPD diabetic patients

Etiology	Total (*n*)	(%)
Cardiovascular		
Cardiac arrest	11	17
Myocardial ischemia and MI	9	14
Other causes of cardiac failure	4	6
Uncertain cause/other unspecify	11	17
Septicemia	8	12
Cerebrovascular accident	6	9
Patient refused further treatment	4	6
Malignant disease	4	6
Therapy ceased for other reason	4	6
Respiratory arrest/failure	4	6
Total	65	100

Discussion

Management of renal failure in a diabetic patient with progressive renal insufficiency is more difficult than in age- and gender-matched nondiabetics. The toll of comorbid conditions, especially blindness, limb amputations and cardiac disease, limits or preempts rehabilitation [7]. In diabetics with ESRD continuous ambulatory peritoneal dialysis offers several advantages such as good control of blood sugar by intraperitoneal insulin, which eliminates the need for multiple subcutaneous injections, the elimination of the need for

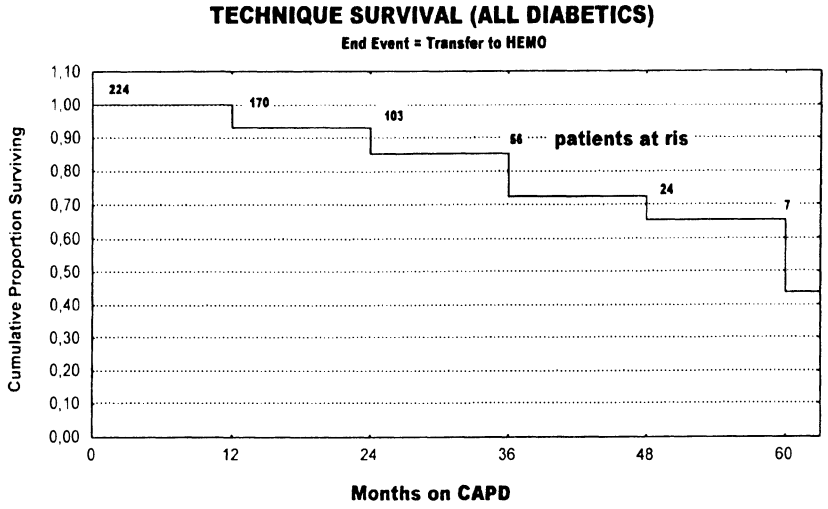

Fig. 9. Technique survival was calculated from the time the patients started the CAPD treatment until the date that they switched to other form of dialysis.

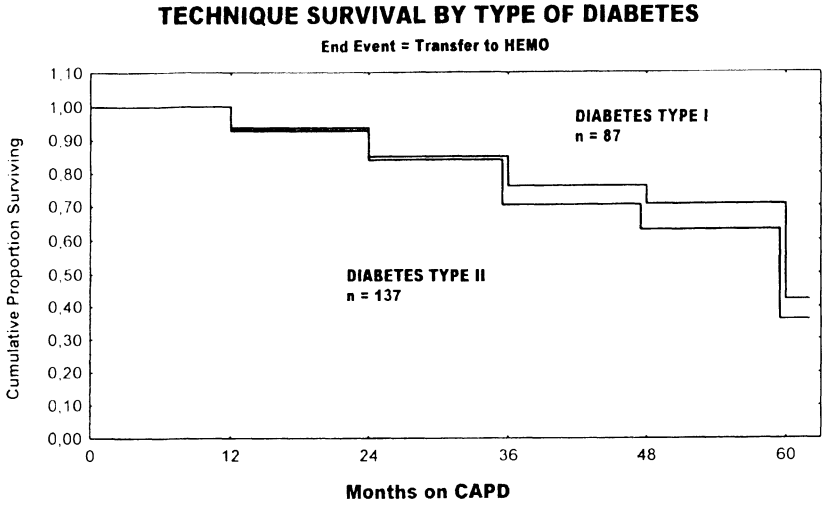

Fig. 10. Comparison of technique survival curves between type I and type II diabetic patients. Type I diabetics had a higher technique survival rate than type II patients.

vascular access and heparinization, and a steady state control of uremia, and stable cardiovascular status without rapid fluid shifts. Thus CAPD which can be easily taught as a home dialysis treatment that allows flexibility and enables patients to enjoy most of their activities has become increasingly popular for diabetics with ESRD.

However some of the shortcomings of peritoneal dialysis are peritonitis, the complications of exit-site infection, and peritoneal protein loss with the accompanying malnutrition. Also long-term studies of CAPD in patients with diabetic nephropathy have demonstrated that the micro- and macrovascular complications continue to progress after its initiation, leading to ongoing problems with cardiovascular disease, malnutrition, autonomic neuropathy, retinopathy and peripheral vascular disease [8–10].

In Canada peritoneal dialysis is used more extensively among diabetics than non-diabetics. According to a recent annual report of Canadian Organ Replacement Register (1996), the percentage of diabetics versus non-diabetics at 3 months and 1 year after dialysis initiation was 52% vs. 37% and 39% vs. 30% respectively ($p < 0.05$) [6].

Most of the diabetic patients in our study (61%) had type II disease (NIDDM) similar to a distribution reported from 1997 United States Renal Data System (USRDS) annual report of 59%. There is some confusion concerning the criteria that should be used to classify the type of diabetes. In Sweden as many as 14% of patients originally diagnosed as NIDDM progressed to IDDM, whereas 10% of newly diagnosed diabetics could not be classified [7]. To address this problem in this study – in addition to the classic clinical definition depending upon the propensity to diabetic ketoacidosis and hyperglycemic-hyperosmolar coma, we assigned the type of diabetes using two additional criteria: (a) the patient's age on the date of first diagnosis of diabetes and (b) the need for the use of insulin for glycemic control at that time. Patients under 40 years of age who had used insulin from the initiation of the treatment were classified as type I (juvenile type, IDDM) while the rest of the patients were classified as type II (adult type, NIDDM). Classification of the two diabetic types is important, as outcomes of these two diabetic types on PD appear to be quite different [11]. Type II diabetes had a higher prevalence than type I patients in our study.

The mean age of the CAPD diabetic population showed an increase of approximately 7 years over that of previously published studies from our hospital, from 49.8 years at the interval 1978–1981 [12] to 56.9 years of this study. This is in accordance with the increase observed in the age of all dialysis patients over the past 10 years. Fifty-eight years has been reported in 1994 as the mean age of new registered dialysis patients for 1994 in Canada [6]. Also when our patients were divided into their various age groups, approximately 72% of the patients were 45–74 years old and 45% were in the 45–64 age group. The latter group had a higher incidence during the study. Recently published USRDS data suggests that diabetes predominates in the age group 20–64-years, whereas hypertension is the largest cause of ESRD in the over 65-year group [3].

Although recent studies of cohort groups followed longitudinally indicate that in both types of diabetes the risk of developing nephropathy is approximately equal [13], we observed a marked difference in the interval between

first diagnosis of diabetes mellitus and initiation of peritoneal dialysis (23.4 years for type I diabetics and 13.7 years for type II diabetics).

As is well known, diabetics are at a higher risk of developing other concurrent illnesses than the general population and, among ESRD patients, comorbidity is more common in diabetics than non-diabetics. Thus arterial hypertension, angina, myocardial infarction, left ventricular dysfunction, cerebrovascular accidents, and peripheral vascular diseases are significantly more frequent among diabetics than non-diabetics [6]. Except for left ventricular dysfunction, the proportion of patients with predialysis comorbidity factors was slightly higher in type II patients than type I. This may be due to the older age of the former because vasculopathic complications of diabetes are at least as severe in NIDDM as in IDDM [14, 15]. The main causes of death in both groups were cardiac arrest, myocardial ischemia and infarction, as well as other causes of cardiac failure (37%). Recent Canadian registry data show that the respective percentages for cardiac deaths were 44% of deaths among diabetics aged 0–44 years versus 28% among non-diabetics [6].

Because of the impact of pre-existing cardiac events on patients' mortality, Friedman [7] has proposed that all uremic diabetics should undergo periodic cardiac evaluation and all diabetics being considered for organ transplant surgery and who can exercise to a maximum, should have a stress thallium scintigraphic study. If this is not possible, a dipyridamol thallium study should be performed. Positive stress tests are indications for coronary catheterization to guide subsequent angioplasty or coronary artery bypass surgery (CABG). Following these principles, we performed CABG or angioplasty on 17 patients in our group.

At least 15% of the diabetic patients will have a foot ulcer at some time, of these 15% to 20% will undergo lower limb amputation [16]. In the present study, 8 type I patients (9.2%) and 10 type II patients, (7.2%) underwent leg amputations. Eight of these had the operation before beginning peritoneal dialysis while the remaining 10 patients had the amputation after CAPD initiation. In our previous report [17] the percentage of patients with amputations was slightly lower (6.2%) which may be due to the shorter mean duration of CAPD in that study (17 months versus 24.7 months in this study). In diabetic patients amputation is the end product of coincident peripheral vascular disease, autonomic and somatosensory neuropathy and arterial hypotension. Since peritoneal dialysis does not seem to prevent microvascular disease, one might expect to encounter this complication more frequently in such patients after long term CAPD. In our study population, we did not observe such deterioration and it seems that the main determinant of the appearance of such complications during CAPD is the severity of diabetic vasculopathy at initiation of peritoneal dialysis. As has been emphasized earlier, the identification of those patients at risk, intensive patient education and a multidisciplinary approach to foot care can decrease the amputation rate significantly [18]. In

this study we observed peripheral vascular disease in 18% of these patients while 11% had peripheral neuropathy.

Autonomic dysfunction with severe orthostatic hypotension, and bladder and bowel dysfunction, present a real challenge to those treating diabetics; but a most frustrating complication is gastroparesis and the consequent malnutrition. We observed gastroparesis in few of our diabetics, but only one patient was reported to have malabsorption syndrome.

Diabetic retinopathy exceeds heart and lower limb disease as the major concern in overall patient care. It had been reported that, with careful medical observation and selection, approximately 100% of diabetic individuals with ESRD have undergone laser treatment for retinopathy with or without vitrectomy [7]. In our study, 36 patients (16%) received photocoagulation, while vitrectomy was performed in 18 (8%) of our diabetic patients. Most type II diabetics have irreversible visual lesions before starting dialysis, especially during the terminal phase of renal failure, when hypertension tends to be severe. By the time most of them reach dialysis, ocular lesions are too far advanced to expect any useful recovery. However, patients who started peritoneal dialysis with normal visual function tend to preserve their visual acuity during the treatment as was experienced in our previous report [17].

The initial fears that intraperitoneal administration of insulin in diabetics on CAPD would increase the frequency of peritonitis have been dissipated by experience of many centers that found the incidence to be similar to that of nondiabetics [17, 19–21]. Furthermore the overall incidence of peritonitis has been decreasing gradually over the past few years. For the period 1977–1984 the incidence at our center [17] was 1 episode every 12.4 or 9.9 patient-months respectively for type I and type II diabetics; in this study, the estimated overall incidence was 1 episode every 17 patient-months or 0.70 peritonitis episodes per patient-year. Again the incidence of peritonitis was slightly higher in type II diabetics (1/16.1 patient-months or 0.74 episodes per patient-year) than in type I patients (1 episode/18.7 patient-months or 0.64 episodes per patient-year). In a multicenter study, after analyzing the data from 10 years' experience with CAPD in diabetics, Viglino et al. found the incidence of peritonitis to be 0.95 per patient-year in diabetics and 0.65 and non-diabetics [9]. During the last 3 years of follow-up, these authors found that the incidence of peritonitis in diabetics was 0.67 and 0.77 episodes per patient-year respectively in patients using or not using intraperitoneal insulin.

The bacteria isolated from the peritonitis episodes in the present study were from contamination from common bacteriological species and once again most of them were thought to be due to skin as reported by other investigators [9, 20, 22].

Peritonitis was the most common complication and the major cause of hospitalization. Recurrent episodes of peritonitis were the leading cause in 66% of the permanent transfers to hemodialysis. Also peritonitis was the main cause

(65%) of catheter replacement, while septicemia was responsible for 12% of deaths in this study.

The preferred route of insulin in most of these patients was the intraperitoneal because it seems that it gives better blood glucose control as assessed by measurements of glycemic excursions and glycosylated hemoglobin (HbA1L) [23, 24]. We saw no alteration in the patients' lipid profile related to its route of administration.

Regarding control of hypertension, a relative euvolemic state was maintained by the continuous ultrafiltration and the peritoneal sodium removal [25, 26]. In our study there was a satisfactory arterial pressure control in diabetic patients and its reduction was well related with the reduction in body weight.

A recent multicenter prospective cohort study (CANUSA) [27] showed that decreased values of Kt/V urea and weekly creatinine clearance were associated with an increased relative risk of death. Total weekly (peritoneal and renal) Kt/V urea of 2.1 and a weekly creatinine clearance (CCr) of 70 L/1.73 m^2 BSA were associated with a 78% expected, 2 year survival rate. Also others have reported that underdialysis increases mortality in peritoneal dialysis patients with ischemic cardiac disease or left ventricular disfunction [28, 29]. More recent schedules for continuous peritoneal dialysis in patients with residual renal function recommend targets of 1.9–2.0 for to the Kt/V urea, or 60–70 L per 1.73 m^2 body surface area of the weekly creatinine clearance [30].

During the study period we performed peritoneal equilibration tests of adequacy in 111 patients with the same computer program (ADEQUEST)®. Among these patients the mean calculated values of total weekly Kt/V urea and of normalized creatinine clearance were 2.39 L/week/V and 76.2 L/week/1.73 m^2 respectively – levels that exceeded recent recommendations. Also comparing these parameters in 22 CAPD diabetic patients after a mean interval of 16.6 months, we found no statistically significant changes even though there was an increase in the proportion of anuric patients and also a decline in 24-hour urine volume and in residual renal creatinine clearance. This means that the dose we delivered by dialysis has been appropriately adjusted during that time interval of CAPD therapy.

Previously reported 2 year patients' survival rates from this center were 78% and 47% for type I and type II diabetic patients respectively [17]. The data from the present study shows an improved probability of survival for all patients after 12, 24, 36, 48 and 60 months at 93%, 81%, 70%, 50% and 33% respectively. More type I than type II diabetic patients survived after 12, 24, 36 and 48 months of CAPD – 95%, 84%, 76%, 56% versus 92%, 79%, 66%, 47%. The difference in survival between these two diabetic types may be due to the fact that type II patients are older and usually have severe atherosclerotic heart disease and other medical problems [17]. These values are comparable to the observed survival rates in similar studies of CAPD patients. Giovanni et al. reported a survival rate of 36% after 60 months in a study of 139 CAPD patients in which 21% were diabetics [31], while the calculated survival rates

for PD and HD populations were similar. Schaubel and Fenton reported that survival rate of diabetics on CAPD was 49% and 33% for 3rd and 5th year respectively [6]. Also, Rottembourg et al. [32] reported that the actuarial survival of 92% at 1 year and 50% at 4 years was mainly influenced by age; they concluded that peritoneal dialysis is the technique of first choice in young diabetics and the preferential technique for home dialysis. Age and cardiac problems as the important determinants in the survival of diabetics have been proposed by Coronel et al. [33] who reported survival rates of 90%, 80% and 62% in 12, 24, and 36 months respectively. Although older diabetics were found to have a lower risk of death on CAPD that on HD [34], data from USRDS suggested that older diabetic patients with ESRD will survive longer if they receive HD versus PD [35]. The findings of the latter retrospective study also suggested that younger type I diabetic patients, who receive peritoneal dialysis, have a longer survival than their counterparts on hemodialysis.

Regarding the measured difference in survival rates between type I and type II diabetic patients, Churchill et al. recently reported a similar difference between the 2 year survival probabilities of 83.3% and 65.5% for diabetic type I and type II diabetic patients respectively [36]. They suggested that this difference would be attributed to the older age, the worse functional status, the more frequent presence of cardiovascular disease at dialysis initiation and the lower residual renal function, of type II diabetic patients, findings that were also observed in our diabetic population.

During the period of the study 65 patients died (20 patients of type I and 45 patients of type II). Cardiac arrest, myocardial ischemia and infarction as well as other causes of cardiac failure were the main causes of death (37%). In a small number of patients (4) treatment was withdrawn at the patient's request or on the suggestion of the treatment team.

Technique survival rates for the 1st, 2nd and 5th year of CAPD treatment period were 93%, 85% and 44% respectively. Comparing the technique survival rates between type I and type II diabetic patients shows that the former had a better survival. Similarly, Churchill et al. reported [36] that the 2 year technique survival for those without diabetes, type I and type II patients were 75.8%, 74% and 71.6% respectively, while death was an additional censored observation in their study. Our results were also comparable to those recently reported by Canadian registry, with technique survival rates of 60% and 42% for the 2nd and 5th year respectively. Tables 17 and 18 indicate patient and technique survival data from this study in comparison with data from other studies previously reported (6, 11, 17, 33, 36).

This study was designed to analyze retrospectively the experience during 1990–1996 with a large diabetic population (224) undergoing CAPD in our center. Although these ESRD diabetics who entered CAPD modality were 7 years older and had more comorbidity factors than those we reported previously, their life expectancy was longer. This improvement might be due to lower rates of peritonitis and a more effective dose of dialysis received by

Table 17. Survival probabilities of diabetics

Reference	Diabetes	Patients (*n*)	Years on CAPD				
			1st	2nd	3rd	4th	5th
This study	All diabetics	224	93%	81%	70%	50%	22%
	Type I vs. type II	87 vs. 137	95% vs. 92%	84% vs. 79%	76% vs. 66%	56% vs. 47%	33% vs. 30%
Ramesh Khanna [17]	Type I vs. type II	41 vs. 15	87% vs. 80%	78% vs. 47%	47·5% vs. 47%	39%–	
Shaubel and Fenton [6]	All diabetics	2958	82%	62%	49%	44%	33%
Churchill et al. [36]	Type I vs. type II	99 vs. 104	94% vs. 86.6%	83.5% vs. 65.5%			
Zimmerman S [11]	Type I vs. type II	263	92% vs. 74%			57% vs. 5%	
Coronel et al. [33]	All diabetics	41	90%	80%	62%	62%	

Table 18. Technique survival of diabetics on CAPD

Reference	Diabetes	Patients (*n*)	Years on CAPD				
			1st	2nd	3rd	4th	5th
This study	All diabetics	224	93%	85%	72%	66%	44%
	All diabetics*	224	87%	69%	51%	33%	14%
	Type I vs. type II	87 vs. 137	95% vs. 92%	84% vs. 79%	76% vs. 66%	56% vs. 47%	
Ramesh Khanna [17]	Type I vs. type II	41 vs. 15	78% vs. 74%	60% vs. 38%	38% vs. 38%	24%–	
Schaubel and Fenton [6]		2958	84%	70%	60%	50%	40%
Churchill et al. [36]*	Type I vs. type II	99 vs. 104	85% vs. 76%	74% vs. 71.6%			

* End events are transfer to hemo and deaths.

diabetics treated more recently. Also the higher technique survival rates demonstrate that, in the treatment of diabetics with end-stage renal disease CAPD now is more effective than ever before as chronic renal replacement therapy. The results of this study support the assertion that the peritoneal membrane maintains its ability to achieve adequate blood purification for a long time. As long as we can maintain acceptable survival with adequate delivery of dialysis, CAPD should be considered an excellent form of renal replacement for the appropriate diabetic ESRD patient.

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9. The natural history and classification of diabetic retinopathy[1]

FRANCIS A. L'ESPERANCE Jr

Editors' Comment:

Vision loss due to retinopathy is the diabetic complication most likely to cause gloom and despair while transforming a functioning individual into an invalid. Recognized as duration related, diabetic retinopathy has had many classifications that attempted to make sense of diverse ophthalmoscopic findings and a variable clinical course. Updated herein is the VAHEX classification originally devised in 1981. The acronym stands for the stages of nonproliferative diabetic nephropathy (V, venous dilatation, A; microaneurysm formation; H hemorrhagic activity, E, retinal edema; X exudate formation). Proliferative diabetic retinopathy is graded according to the extent of vitreoretinal neovascularization, glial proliferation, and vitreoretinal traction. By comparing patient retinal photographs against a set of reference photographs, the category and severity of retinopathy can be assigned for investigational and prognostic purposes. Universal application of reproducible criteria for classifying the diabetic eye facilitates collaborative trials of therapeutic regimens such as panretinal photocoagulation, aspirin and antiplatelet drugs. Ultimately, the ability to categorize and sort eye disorders in diabetics should promote an explanation for a still obscure pathogenesis leading to definitive therapy less injurious than laser photocoagulation.

Introduction

The natural history and course of diabetic retinopathy is not always predictable, is variable within limits, can progress or regress in relatively short intervals, but generally is progressive and therefore can be fairly accurately classified from several viewpoints. The classification of a disease provides a basis for discussion of the natural history of the disease and the evaluation of treatment. Unfortunately, diabetic retinopathy does not lend itself easily to classification. The lesions of diabetic retinopathy are multiple, and the clinical course is quite variable. The salient features of diabetic retinopathy may be enumerated, but one or another feature may predominate the fundus picture at a given time, and individual components may progress or regress independently. Above all, the pathogenesis of diabetic retinopathy remains obscure so that absolute scientific validity is lacking in any classification [1].

[1] This Chapter was modified and edited from Chapter 5, appearing in: L'Esperance FA Jr, James WA Jr. *Diabetic Retinopathy: Clinical Evaluation and Management*. St. Louis, C.V. Mosby Co., 1981.

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It is not surprising that many classifications for diabetic retinopathy have been proposed, but none is universally accepted. Some classifications appear too simple to be adequate, others too complex to be readily useful. No single classification merits discussion to the exclusion of all others. However, a review of several historically representative classifications does add some perspective to current thinking concerning the clinical progression of the disease. Furthermore, the very lack of a single, acceptable classification has stimulated efforts, more recently, to devise grading systems. These systems will hopefully provide more accurate assessment of the natural course of retinopathy and the effects of treatment.

Clinical classifications

Historical classifications

Hirschberg [2] presented the first comprehensive classification of diabetic retinopathy. He distinguished, basically, between exudative and hemorrhagic retinopathy and divided retinopathy into three types.

Type I (retinitis centralis punctata diabetica). Hirschberg characterized this type as an inflammation of the central part of the retina. Small, bright, shiny masses could be seen within the retinal tissue around the posterior pole. These masses could coalesce into bands or semicircles (Figure 1). Small retinal hemorrhages in the form of blood islets or dabs could also be seen. Larger retinal hemorrhages or hemorrhages into the vitreous did not occur in this type of retinopathy. Changes in the larger retinal blood vessels also did not occur, and Hirschberg attributed this type of retinopathy to disease of tiny retinal vessels. Hirschberg distinguished Type I retinopathy from albuminuric retinopathy, which could produce gross changes in the larger retinal vessels as well as typical star-shaped exudates. He also distinguished Type I retinopathy from senile macular changes.

Hirschberg noted that Type I retinopathy tended to occur in patients aged 45 to 65 with mild diabetes of many years' duration. Quite often the diabetes remained undiagnosed until visual symptoms appeared. Visual symptoms tended to be of gradually increasing severity and were almost always bilateral.

Type II (retinitis hemorrhagica diabetica). Retinal hemorrhages predominated the fundus picture in this type of retinopathy, followed by other inflammatory changes and abnormalities. The hemorrhages were often small or dotlike. However, larger 2 to 3 mm hemorrhages could occur, and these often produced severe visual loss. The larger hemorrhages could also break into the vitreous, forming cloudy, mobile masses. In one patient with Type II retinopathy, Hirschberg described a tentlike 'preretinal connective tissue structure' protruding above the optic nerve. In another patient he described hemorrhaging 'clumps' along the retinal surface. Hirschberg noted that Type II retinopathy, like Type I, tended to be bilateral, but that one eye often showed more severe

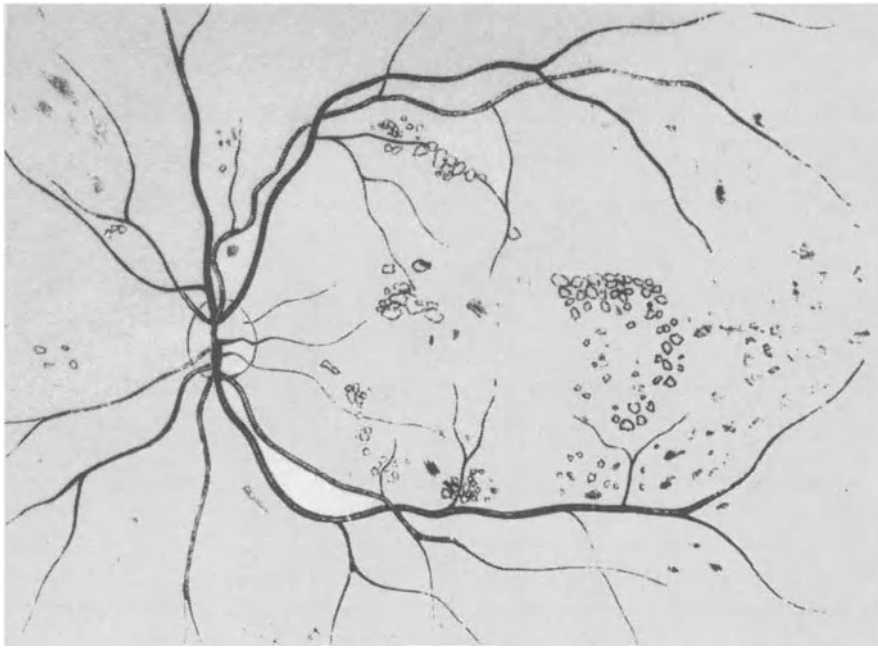

Fig. 1. Michaelsen's sketch of Type I retinopathy noted by Hirschberg in a patient whose urine showed sugar but no albumin. (From Hirschberg J. Centralblatt. Augenheilk 1891; 15: 18)

hemorrhagic activity than the other eye. Patients with Type II retinopathy could develop venous occlusions or hemorrhagic glaucoma, which indicated a particularly poor visual prognosis.

Type III. This type consisted of rare forms of retinal inflammation and degeneration, but Hirschberg was unsure that these were related to diabetes.

Hirschberg's classification did not include proliferative retinopathy specifically, although he described apparent proliferative changes under his Type II retinopathy. Prior to Hirschberg's report, proliferative changes had been described by Leber [3] (1875), Manz [4] (1876), MacKenzie [5] (1879), and Nettleship [6] (1888).

Very little was added to Hirschberg's classification for almost half a century, as debate ensued about whether or not retinopathy represented a disease entity specific to diabetes. Raia [7] (1922) noted that proliferative changes could occur as a primary form of retinopathy and not necessarily as the result of a previous hemorrhage. In 1934, based upon study of 1052 diabetics, Wagener, Dry, and Wilder [8] elaborated upon Hirschberg's classification. They distinguished between hard and soft exudates; venous changes were emphasized, and proliferative retinopathy was noted as a specific category. This classification presented a theory of the natural evolution of diabetic retinopathy, progressing

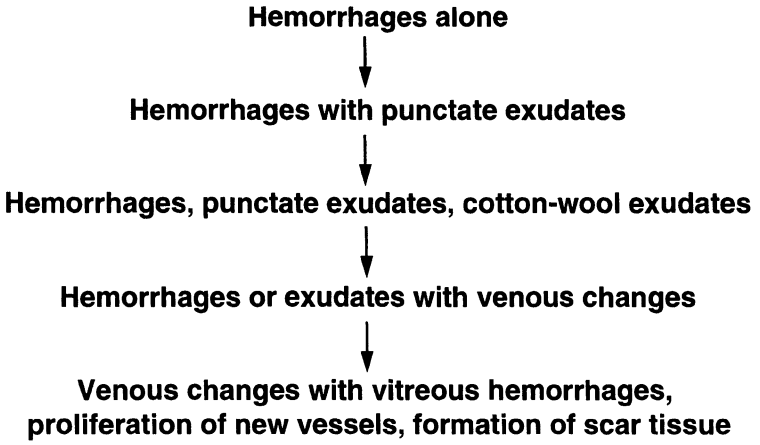

Fig. 2. Classification and progress of diabetic retinopathy. (Compiled from data presented in Wagener HP, Dry TJS, Wilder, RM. N Engl J Med 1934; 211: 1131.)

through five distinct phases (Figure 2). Each phase represented a more severe form of retinopathy than the previous phase. Cotton-wool exudates were presumed to arise from more advanced vascular injury than punctate exudates. Venous changes then occurred, consisting of nodular dilations, constrictions, and sheathing, followed by proliferative retinopathy.

In 1943, Ballantyne and Loewenstein [9] presented their classic paper in which microaneurysms were rediscovered. This work led Ballantyne [10] to a classification that emphasized microaneurysms as distinctive and early lesions of diabetic retinopathy (Figure 3). Ballantyne noted that microaneurysms represented a breakdown within the capillary circulation, followed by what today might be termed intraretinal microvascular abnormalities. This, to Ballantyne, indicated a chronic condition of stasis and anoxia on the venous side of the retinal circulation, followed by grosser changes in the larger retinal veins and, finally, by increasingly severe forms of proliferative retinopathy.

The classifications of Wagner, Dry, and Wilder and Ballantyne indicated that venous changes tend to occur after background retinopathy is relatively advanced. Other classifications noted that venous changes may occur earlier in the course of retinopathy [11, 12] or even as the first observable sign of retinopathy [13] Scott [14, 15] has formulated an intricate classification which indicates that venous changes occur early and independently from other background changes (Figure 4). Burditt, Caird, and Draper [16] noted that venous changes tend to regress spontaneously and occasionally evolve into background retinopathy but never into proliferative retinopathy (Figure 5).

The variable clinical course and obscure pathogenesis make it difficult to classify retinopathy in terms of the progression of its clinical features. Some clinical classifications have thus become less and less complex, especially for

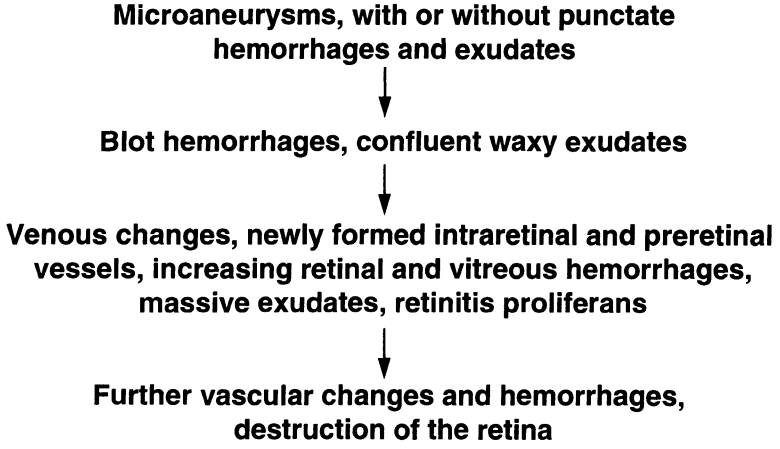

Fig. 3. Progression of diabetic retinopathy. (Compiled from data presented in Ballantyne AJ. Trans Ophthalmol Soc. UK 1946; 66: 503.)

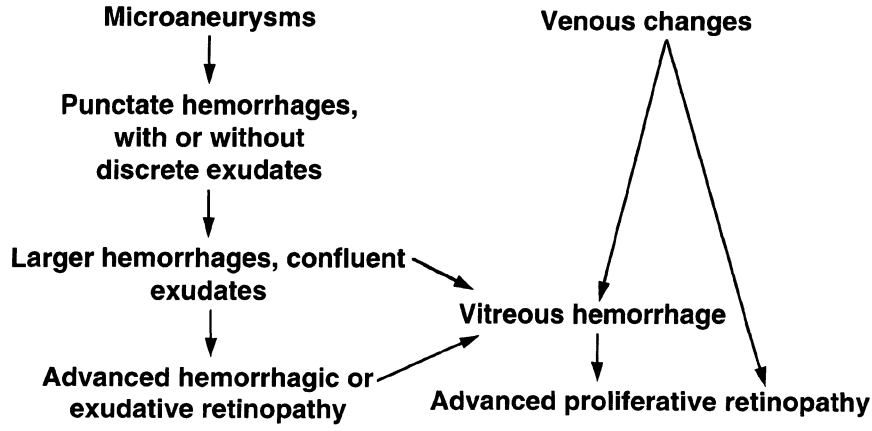

Fig. 4. Clinical classification of diabetic retinopathy. (From Scott G I. Proc R Soc Med 1951; 44: 743, and Br J Ophthalmol 1953; 37: 705.)

teaching purposes, in order to facilitate understanding of the basic features of retinopathy. Scuderi [17] has proposed a classification based upon three forms of retinopathy, subdivided in terms of severity:

I. Background retinopathy
   A. Early
      1. Microaneurysms, hemorrhages
      2. Punctate retinitis
   B. Advanced

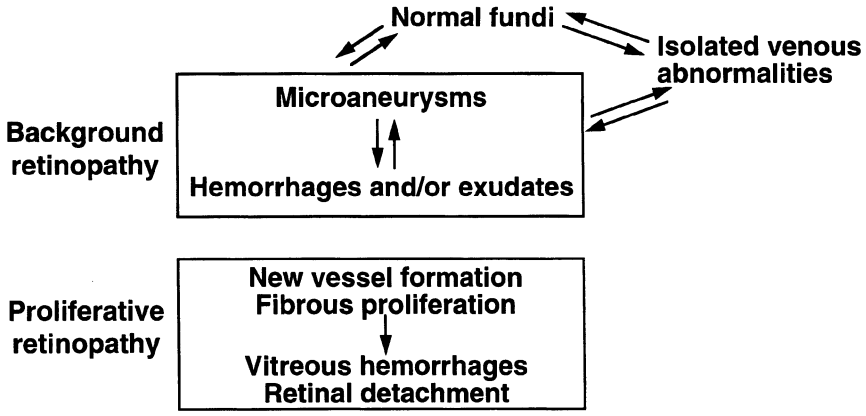

Fig. 5. Classification of diabetic retinopathy. (From Burditt AF, Caird FI, Draper GJ. Q J Med 1968; 37: 303.)

1. Hemorrhagic
2. Exudative

C. Severe
1. Retinal and preretinal hemorrhages, venous changes
2. Vitreous hemorrhage
3. Venous thrombosis

II. Proliferative retinopathy

III. Mixed retinopathy
A. Diabetic and arteriosclerotic
B. Diabetic and renal

Fabrykant and Gelfand [18] have proposed an even simpler classification, based upon presumably reversible versus irreversible lesions:

Reversible
Class 1: Venous changes, microaneurysms
Class 2: Microaneurysms, hemorrhages, exudates

Irreversible
Class 3: Above, plus neovascularization
Class 4: Above, plus fibrotic bands and membranes

Beetham [19] has noted that the most useful classification divides retinopathy into only two types, proliferative and nonproliferative. This classification would probably be agreed upon most universally among clinicians today. Beetham's observation is a sober reminder that although Hirschberg did not specifically recognize proliferative retinopathy, the classification of diabetic retinopathy has progressed little over the past century.

Biochemical classification

Beaumont and Hollows [20] have proposed a classification correlated with biochemical abnormalities present in diabetes. This classification emphasizes the potential role of medical treatment for diabetic retinopathy, in conjunction with specific recommendations for pituitary ablation and, in selected cases, photocoagulation.

Type I: *Diffuse capillary retinopathy*
Defect: Abnormal growth hormone
Insulin insensitivity
Treatment: Improve diabetic control
Chemical suppression growth hormone
Pituitary ablation

Type II: *Lipid retinopathy*
Defect: Impaired lipolysis
Treatment: Medical, restore normal lipid metabolism

Type III: *Obstructive retinopathy*
Defect: Obstructive vascular disease
Altered blood rheology
Treatment: Lower intraocular pressure
Medical, improve fibrinolysis

Type IV: *Minimal retinopathy*
Defect: Minimal
Treatment: Reassurance

Type I (diffuse capillary retinopathy). This type of retinopathy is seen in younger insulin-dependent diabetics who generally exhibit poor control of their diabetes. There is widespread occlusion and dilation of the capillaries of the central retina, with profuse fluorescein leakage. Proliferative retinopathy develops rapidly, with extensive bleeding and subsequent blindness.

Biochemical abnormalities include high growth hormone levels, a high hypoglycemic growth hormone response, striking insensitivity to insulin, and high levels of fasting nonesterified fatty acids. Exudate formation is minimal, despite extensive disruption of the blood–retinal barrier, because of increased lipolysis from the high growth hormone levels.

Treatment includes rigorous control of diabetes, with multiple insulin injections if necessary. Suppression of growth hormone, with a resultant increase in insulin sensitivity, can be accomplished chemically with medroxyprogesterone or by pituitary ablation. Photocoagulation may be employed as an ancillary measure to slow the progression of the retinopathy.

Type II (lipid retinopathy). This type of retinopathy is generally the opposite of Type I. It occurs in diabetics after age 40 who are typically overweight and may be insulin dependent. The retinopathy is characterized by the slow accumu-

lation of hard exudates around the posterior pole. Microaneurysms and hemorrhages are few; capillary disruption is minimal, and fluorescein studies show only mild or localized leakage. Neovascularization rarely occurs.

Biochemical abnormalities include a suspected defect in lipolysis. There may be hyperlipoproteinemia and high triglyceridemia. The hypoglycemic growth hormone response is low, and there are low fasting nonesterified fatty acids. Blood viscosity is normal.

Treatment consists of diet, weight reduction, and clofibrate. Fenfluramine may be used to stimulate lipolysis. Pituitary ablation would only aggravate the defect in lipolysis and is contraindicated. Photocoagulation is not usually indicated.

Type III (*obstructive retinopathy*). This type comprises the largest group of patients, who are generally over age 30, may require insulin, and are often hypertensive. The retinal circulation shows both arterial and venous signs of obstructive disease, including variations in vessel caliber, arteriovenous crossing defects, cotton-wool spots, venous distension, and flame-shaped or larger retinal hemorrhages. There is disruption in the capillary circulation (although less than in Type I), with numerous microaneurysms, microinfarcts, areas of capillary closure, and diffuse fluorescein leakage. Proliferative retinopathy may develop as the underlying obstructive vascular disease becomes more severe. This type of retinopathy is generally slowly progressive, but some patients do enter a more rapidly progressive phase.

Biochemical abnormalities are varied, including increased blood viscosity, elevated sedimentation rate, hyperlipoproteinemia, defective fibrinolysis, elevated fibrinogen, and presence of cryofibrinogen and macroglobulins. There is no definite pattern to the fasting nonesterified fatty acid levels or to the hypoglycemic growth hormone response.

Treatment consists of attempting to relieve the vascular obstructive disease by lowering the intraocular pressure. Photocoagulation may be indicated, and hypertension, if present, should be controlled. Since the biochemical abnormalities are variable, drug therapy is less specific for this type of retinopathy, but phenformin and ethylestrenol may be given in an effort to stimulate fibrinolysis.

Type IV (*minimal retinopathy*). This type of retinopathy is seen in long-duration, insulin-dependent diabetics who maintain rigid control of their diabetes. Occasional microaneurysms, dot hemorrhages, small exudates, and slight fluorescein leakage can be seen. There are no larger hemorrhages or cottonwool exudates. These patients are insulin sensitive and do not show significant biochemical abnormalities. The only treatment necessary is encouragement and reassurance.

The classification of Beaumont and Hollows, as with practically any classification of diabetic retinopathy, has not gone undisputed [21]. It is unclear to what extent observed biochemical abnormalities in diabetics might influence retinopathy, especially the variable list of biochemical changes associated with their Type III retinopathy. The efficacy of drug treatment on established

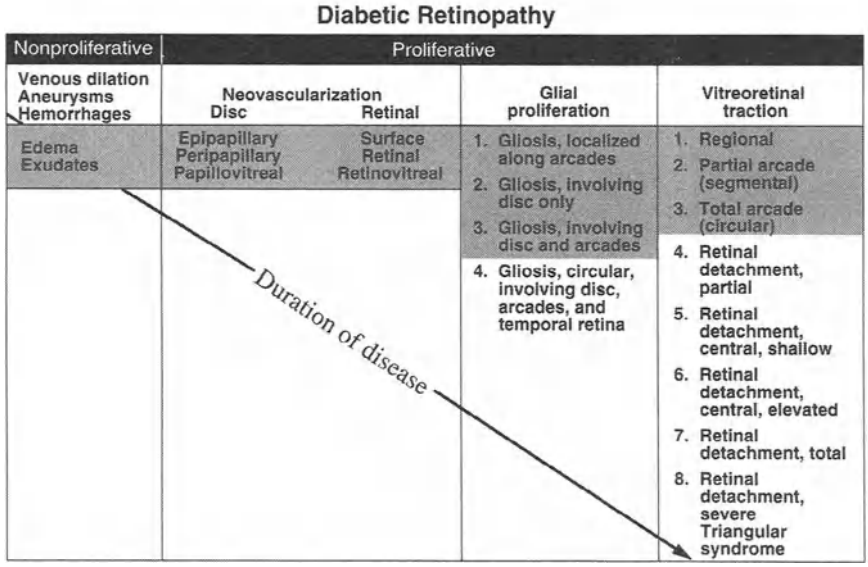

Fig. 6. Classification of nonpoliferative and proliferative retinopathy. The VAHEX acronym is derived from the features of nonproliferative retinopathy. Those features of nonproliferative and proliferative retinopathy that may be considered for photocoagulation treatment are listed within the shaded area. If glial proliferation or vitreoretinal traction have progressed beyond Grade 3, vitrectomy or retinal surgery may be required if photocoagulation causes further vitreous shrinkage and traction. Severe grades of glial proliferation and vitreoretinal traction may constitute contraindications to photocoagulation treatment.

retinopathy remains inconclusive. Nevertheless, this classification does divide retinopathy into clearly defined types, each with specifically recommended methods of treatment. This creates opportunities for clinical trials that deserve further investigation.

VAHEX classification

A classification of diabetic retinopathy, devised by the author, that embodies both the ophthalmoscopic appearance and dynamic factors affecting the ultimate prognosis for each eye with diabetic retinopathy is presented in Figure 6. [22–27] This classification is useful for determining which patient might benefit from photocoagulation treatment and/or vitreo-retinal surgery.

Nonproliferative diabetic retinopathy

Diabetic retinopathy can be divided basically into two categories: the nonproliferative phase and the proliferative phase. The nonproliferative phase consists of a continuum involving venous dilation, microaneurysm formation, the appearance of small intraretinal hemorrhages, the formation of patches of

edema, and the later formation of exudates, particularly in the macular and paramacular regions. These changes may progress to involve most of the posterior pole, resulting in markedly decreased visual acuity.

The nonproliferative portion of the classification is designed so that the appearance of one of these abnormalities signals a particular stage in the evolution of the retinopathy + process. It is assumed, for purposes of simplification and with clinical justification in the majority of cases, that one disease category or stage leads to the next and has arisen from a previous stage. Probably the first change in diabetic retinopathy that can be visualized is a slight dilation of the veins; this stage is followed, in most cases, by scattered microaneurysm formation. Microaneurysms eventually lead to intraretinal hemorrhages that may or may not occur from the microaneurysm sites. As the retinopathy progresses, breakdown of the vessel wall permits serum transport into the surrounding retinal tissue, and edema is produced. When the retinal tissue is affected directly by the edematous process, cellular death and serum transport derangement through the retina can be anticipated, and exudates may begin to accumulate in various parts of the posterior pole. Therefore, with few exceptions, the progression, although gradual, from venous dilation to exudate formation in the nonproliferative phase of diabetic retinopathy can be classified by the observation and documentation of each stage of the pathologic process.

The stages of nonproliferative diabetic retinopathy can be remembered most easily by the acronym VAHEX (V, venous dilation; A, microaneurysm formation; H, hemorrhagic activity; E, retinal edema; X, exudate formation) [28]. These five stages are then classified according to a one to four severity rating. As each earlier stage of nonproliferative diabetic retinopathy increases in severity, the next more pathologic component appears, and the classification then shifts into that category. Therefore, classifications three and four usually involve only the production of the end phase exudates. Exudate formation can be quite extensive; there can be intraretinal hemorrhages and edema throughout the posterior regions of the fundus. However, there may be no sign of neovascularization or glial tissue proliferation (Figure 7A).

Proliferative diabetic retinopathy

Proliferative diabetic retinopathy is a large classification that includes the production or proliferation of new tissue, supportive or neovascular in nature, in the chorioretinal area secondary to injury by the diabetic process. The amount and type of neovascular and glial tissue proliferation, as well as the relationship between the vitreous and the retinal structures, must be evaluated. If these three components of the proliferative stage of diabetic retinopathy (vitreoretinal neovascularization, glial proliferation, and vitreoretinal traction) can be accurately classified, the condition of the eye can be determined more precisely on a pathophysiologic basis. This classification provides a more accurate assessment of the desirability for photocoagulation or vitreo-retinal

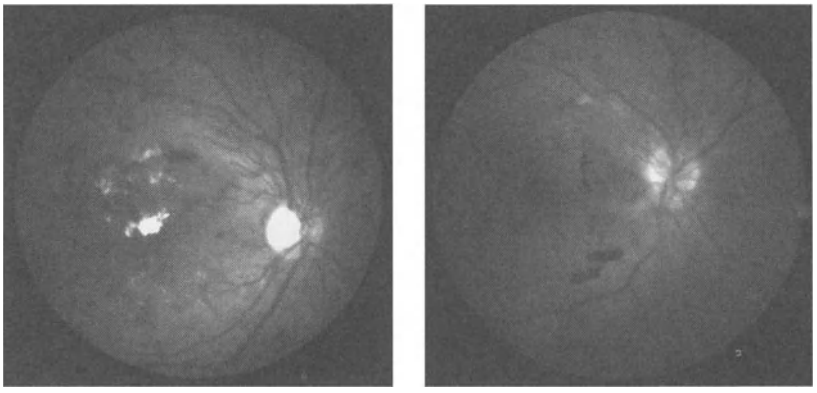

Fig. 7A. Significantly advanced nonproliferative retinopathy, which shows salient features of VAHEX classification: venous changes, microaneurysms, retinal hemorrhages, edema, and exudates. *B*, Proliferative diabetic retinopathy with large neovascular frond extending off optic disc.

surgical therapy and offers a more exact prognosis for a particular eye (Figure 7B).

Neovascularization

Neovascularization must first be categorized as one of the five types seen in diabetic retinopathy: epipapillary, peripapillary, papillovitreal, retinovitreal, or preretinal (surface).

Epipapillary neovascularization

Ophthalmoscopic appearance. Epipapillary neovascularization can be defined as new blood vessel formation extending from the central optic disc area a short distance into the vitreous. These vessels take the form of wispy or fibrillar channels of blood that may be interspersed with more closely associated tufts or berrylike clumps of vascular tissue. This neovascularization usually carries a small volume of blood and is usually not supported by extensive glial proliferation during its early stages. At this time the new vessels are confined principally to the optic nerve area; there is little tendency for them to proliferate along the vascular arcades (Figure 8).

Epipapillary neovascularization usually begins with minute budding from one of the smaller vessels on the optic nerve; this budding later develops into a network of delicate vessels that can be seen only with the magnification afforded by the direct ophthalmoscope or by biomicroscopy with the precorneal or corneal lens. More mature and later phases of epipapillary neovascularization suggest that the neovascularization is adherent to the posterior hyaloid for support. Occasionally, proliferation can extend along the vascular arcades. Neovascularization of this type tends to grow rather slowly unless it is aggra-

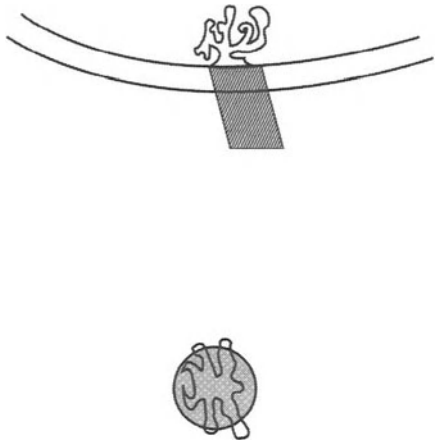

Fig. 8. Epipapillary neovascularization, characterized by wispy, threadlike neovascular growths from the optic nerve into the vitreous.

vated by hypertensive episodes or other systemic problems. Ultimately, it can extend far into the vitreous, accompanied by marked glial proliferation around the vessels, and convert to the more widespread papillovitreal type of neovascularization.

Peripapillary neovascularization

Ophthalmoscopic appearance. Peripapillary neovascularization can be described as neovascularization extending centrifugally from the optic nerve in a slightly elevated fashion over the surrounding retina, vascular arcades, and occasionally the macular region. The neovascularization extends from the rim of the optic nerve to the peripapillary region and forms delicate arcades and webs of vessels with multiple arteriolar feeder and venular drainage channels (Figure 9).

The peripapillary distribution of vessels is much like the mesenteric vessel distribution, and the progress of the neovascularization is by small endothelial tuft formation in a radial and circumferential manner. Neovascularization can originate in any quadrant of the optic disc; in early neovascularization, however, the spread of the disease appears to be toward the temporal vascular arcades. Extensive peripapillary neovascularization can extend 3 to 4 disc diameters over the retinal surface; resembling a shallow saucer, its end curls gently toward the vitreous. The border of peripapillary neovascularization is scalloped like the arborization on a leaf; radiating channels extend from the optic disc to peripheral circumferential channels. Bleeding into the vitreous usually occurs from the distal vascular tufts along the arching channels, but decompensation may occur suddenly and massively from the more well-developed central neovascular patches. The feeder vessels originate from the optic nerve region, although collateral circulation may be established later from the major vascular

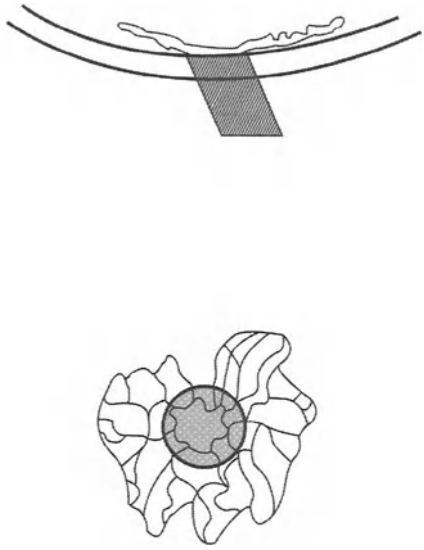

Fig. 9. Peripapillary neovascularization, which is growing in saucerlike configuration from the optic nerve.

arcades. Little glial tissue appears between the vascular elements at first, but eventually glial proliferation becomes extensive and is closely interdigitated between the vascular elements.

Papillovitreal neovascularization

Ophthalmoscopic appearance. Papillovitreal neovascularization can be defined as the massive amount of combined neovascular and glial tissue that extends from the head of the optic nerve far into the vitreous body. It may represent the final developmental of epipapillary or possibly peripapillary vascularization and usually has well developed vascular channels initially. As the papilovitreal frond matures, the glial component is most prominent and becomes closely interdigitated with the vascular channels, tend to regress to some degree. The neovascular proliferation extends into the vitreous by one of three processes. The most common process of proliferation is the twining of neovascular buds along the posterior hyaloid face, which acts as a trellis work. The next most common proliferation along the residue of fibrin from a previous intravitreal hemorrhage. The least common proliferation process is the gradual budding and extension of the vascular channels into the vitreous from a well-established neovascular stalk that has become supported by glial tissue. These papillovitreal stalks are not likely to proliferate a great distance into the vitreous because of their lack of vertical supportive structures.

Three basic types of papillovitreal neovascularization exist: columnar, arcuate, and confluent. The columnar papillovitreal neovascularization extends

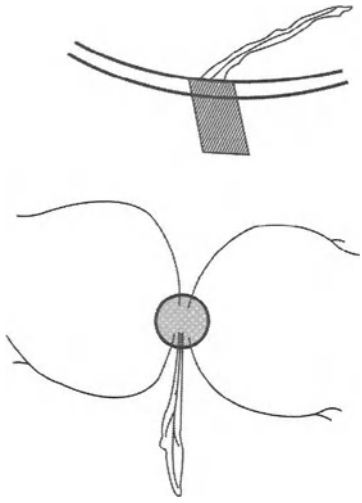

Fig. 10. Columnar papillovitreal neovascularization extends in a characteristically narrow and direct fashion from the optic nerve.

along the posterior hyaloid in a closely gathered group of vessels and glial tissue proliferation. This extension of neovascular tissue is neither confined by nor closely associated with any of the vascular arcades and appears elongated without much lateral extension. One or more feeder vessels extend from the optic nerve into the frond, and two to six collector vessels then return the blood to the optic nerve region. Detection of the feeder and collector vessels is easily accomplished, and photocoagulation is usually successful (Figure 10).

The arcuate type of papillovitreal neovascularization originally extends along the vascular arcades, more frequently to the temporal side, and initially begins with a somewhat higher proportion of glial tissue than does the columnar type. As the arcuate papillovitreal frond matures, a greater degree of lateral and intravitreal extension appears, as well as neovascular proliferation along the distal end of the supportive vascular arcade. In some cases, this type of neovascularization connects horizontally with the opposite arcade to form an interdigitation of neovascular and proliferative tissue, resulting in a larger interhemispheric shunting complex. Growth during the initial stages of formation is rapid throughout the central body of the frond, but during the late, mature stages it is confined to endothelial budding along the distal aspect and border of the large frond. During the early stages of development, bleeding can occur from any of the vessels and may be disastrous. The later stages are marked by smaller hemorrhagic episodes, which usually emanate from the small endothelial buds that form along the outer margins of the body of the frond. Because of the marked involvement of the posterior hyaloid face by the proliferating glial and vascular tissue, contracture of the frond, the posterior

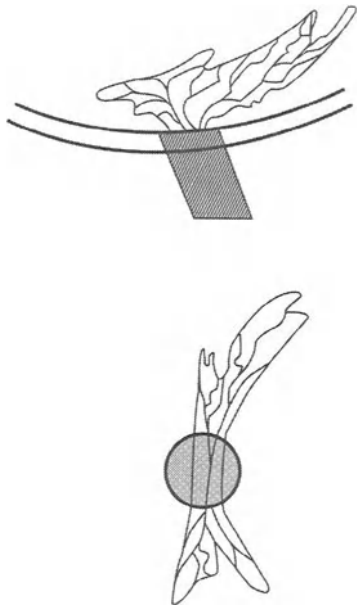

Fig. 11. Arcuate papillovitreal neovascularization extending off the optic nerve and along the vascular arcades.

hyaloid, or any of the components of the vitreous body will cause great strain on the retina in the posterior pole in the peripapillary region. It is common to find the end stage of this type of neovascularization represented by a central, tentlike retinal detachment with marked striae radiating from the central frond to the more peripheral parts of the retina (Figure 11).

The confluent type of papillovitreal neovascularization can be described as neovascular glial tissue that has extended from the optic nerve into the vitreous body without lateral extension or any close association with the vascular arcades. This neovascularization can take the form of either a shallow saucer or a partial, deeper, cuplike configuration and can extend to any size. The feeder vessels are multiple, and without the aid of fluorescein angiography they are difficult to differentiate from the collector vessels, which also may be multiple. Growth may be extremely rapid with this type of neovascularization because of its multidimensional vascularity. Growth is usually oriented toward the nasal side, but large fronds have been seen extending directly over the macular area in a canopy fashion, completely obscuring the central vision. Bleeding into the vitreous is usually from the distal portion of the frond; occasionally, however, massive vitreal hemorrhages occur from the proximal feeder or collector vessels during a period of extreme hypertension (Figure 12).

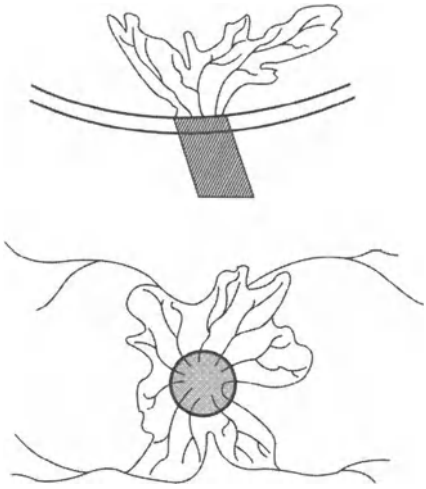

Fig. 12. Confluent papillovitreal neovascularization, which extends in a cuplike or funnel configuration off the optic nerve.

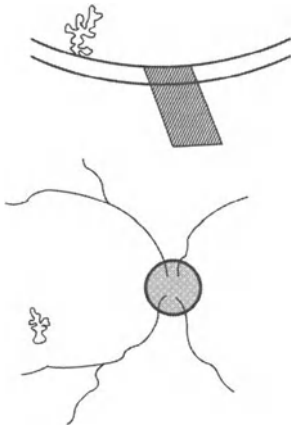

Fig. 13. Retinovitreal neovascularization, extending from the retinal surface into the vitreous by means of adherent vitreous or fibrin strands.

Retinovitreal neovascularization

Ophthalmoscopic appearance. Retinovitreal neovascularization may be defined as neovascular tissue extending from the retina toward the central vitreous cavity from any portion of the retina, with the exception of the optic nerve region (Figure 13). Proliferation usually occurs from a larger vessel – in most cases, one of the veins in the vascular arcade. Extension into the vitreous occurs along the latticework provided by an adherent vitreoretinal strand or along

fibrin produced by a previous vitreal hemorrhage. Retinovitreal neovascularization can occur from any portion of the vascular tree but usually is found distal to the first bifurcation of the large arcades. It can be arteriolar or venular in distribution, usually growing slowly along the vitreal surface in a well-entrenched, deliberate manner. The retinovitreal frond is primarily vascular during its early stages; it slowly becomes entwined with a large amount of glial proliferation and grows on this proliferation farther into the vitreous body. These fronds usually do not bleed because of increases in blood pressure; instead, they bleed because of the shrinkage of the vitreous body and the inevitable stress on the vitreoretinal strand to which the retinovitreal neovascularization is intimately adherent. This type of neovascularization is usually associated with a highly detached posterior hyaloid from which stringy vitreoretinal strands are adherent to the vitreal portion of the neovascularization. In contrast to this mound like neovascular tuft configuration, retinovitreal neovascularization may occasionally proliferate along the very shallowly detached posterior hyaloid, particularly in the distal arcade areas, and appear as a flat, tablelike area of neovascularization in that particular portion of the fundus.

Preretinal neovascularization

Ophthalmoscopic appearance. Preretinal neovascularization may be defined as that type of neovascular tissue that extends onto or a short distance into the internal limiting membrane; it is usually derived from the retinal vessels apart from the vascular tissues on the optic nerve (Figure 14). These areas of neovascularization may be fan shaped or threadlike. They may be extremely tortuous, or they may form clusters or vinelike configurations. The neovascularization may be intermeshed with small or large amounts of glial tissue, depending on the maturity of the vascular process. This type of neovascularization can be found anywhere in the posterior pole, although there seems to be a predilection for the distal ends of the vascular arcades, the macular and paramacular areas, and the area between the arteriole and venule in the main vascular arcades. In many cases the vitreous face or posterior hyaloid is intimately approximated to the neovascularization and sometimes provides a latticework on which the neovascularization converts from the surface variety to the retinovitreal or peripapillary type. In these cases, evaluation of the vitreous and the state and position of the posterior hyaloid before photocoagulation and/or vitreo-retinal surgery is most important.

Glial proliferation

Glial tissue may proliferate alone or in combination with neovascular elements. Glial proliferation may occur on the optic disc and along the vascular arcades, particularly the temporal vascular arcade, and may not be involved with neovascularization. The proliferation may grow along the posterior hyaloid from the region of the optic disc and vascular arcades without evidence of

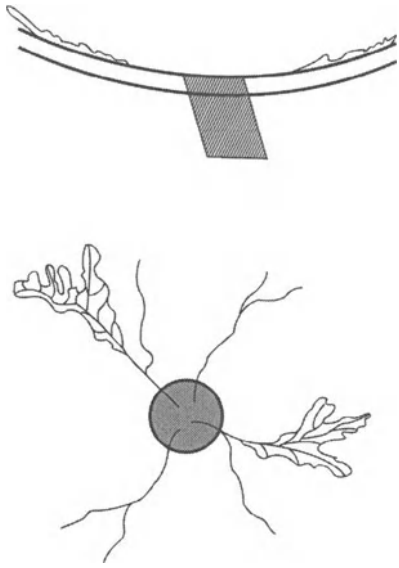

Fig. 14. Preretinal neovascularization, which extends along the internal limiting membrane, is especially prone to occur at the distal end of the vascular arcades.

neovascularization. Not uncommonly, glial tissue will proliferate from the optic disc over the macular region in a canopy fashion along the surface of a shallowly detached posterior hyaloid. In many cases this canopy effect causes a loss of vision, while the underlying retina remains relatively normal.

Glial tissue may also be intertwined with epipapillary or papillovitreal neovascularization. As the neovascular frond matures, the glial tissue component increases, and the percentage of neovascularization decreases. Older intravitreal fronds can be heavily gliotic and have little neovascularization in or on the surface of the glial tissue. In all cases the severity of the glial proliferation should be categorized in grades 1 to 4, which need only reflect the relative quantity of glial tissue seen by ophthalmoscopic examination. Glial proliferation on the retinal surface is graded from 1 to 4 as follows:

Grade 1: patchy gliosis in the posterior retina or along the midportion of the vascular arcades, not involving the optic disc (Figure 15)
Grade 2: gliosis involving the optic disc area only (Figure 16)
Grade 3: gliosis of the arcade region and the optic disc (Figure 17)
Grade 4: a circular band of gliosis involving the optic disc, vascular arcades, and temporal interarcade retinal area (Figure 18)

Vitreoretinal traction

The condition of the vitreous body must be analyzed if one is to make an accurate evaluation of diabetic retinopathy. Knowing the state of the vitreous

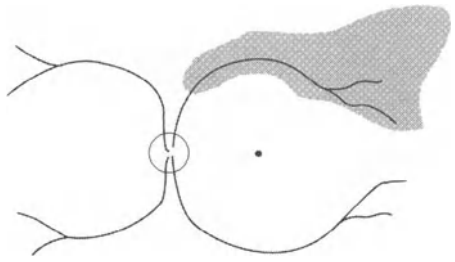

Fig. 15. Grade 1 glial proliferation showing gliosis at the distal aspect of the superior temporal vascular arcade, the area where gliosis is most commonly found initially.

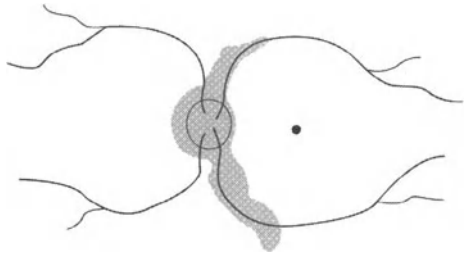

Fig. 16. Grade 2 glial proliferation. Gliosis involves optic nerve head and the adjacent portions of the superior and inferior temporal vascular arcades.

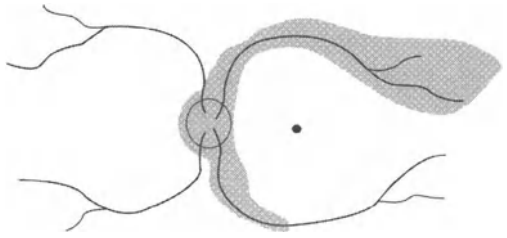

Fig. 17. Grade 3 glial proliferation. Gliosis of the optic nerve region extends along the superior temporal vascular arcade to the most distal area of that arcade.

and the relationship of the posterior hyaloid to the retina is most significant for determining the type of photocoagulation treatment or vitreo-retinal surgery that should be employed as well as the ultimate prognosis for a particular eye.

If a portion of the posterior retinal area has become organized with glial proliferation or neovascularization and is undergoing traction toward the central vitreous, the vitreoretinal traction is designated as *Grade 1*. This category denotes traction of the vitreous on the retinal structures in sectional or regional areas but not along any well-organized path or zone (Figure 19).

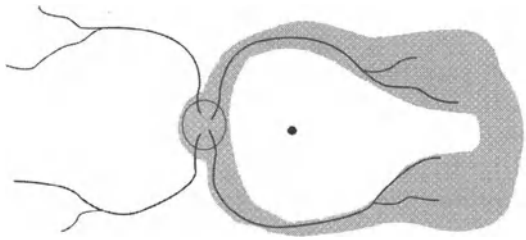

Fig. 18. Grade 4 glial proliferation. There is complete circular involvement of the superior and inferior temporal vascular arcades as well as the interarcade area temporal to the macular region.

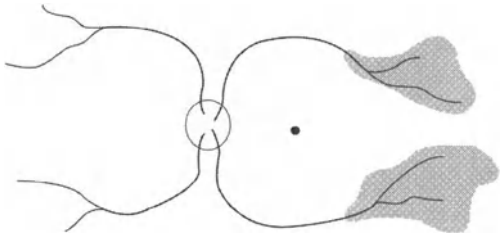

Fig. 19. Grade 1 vitreoretinal traction with vitreous traction on the retina in the most common regions, the distal aspects of the superior and inferior temporal vascular arcades.

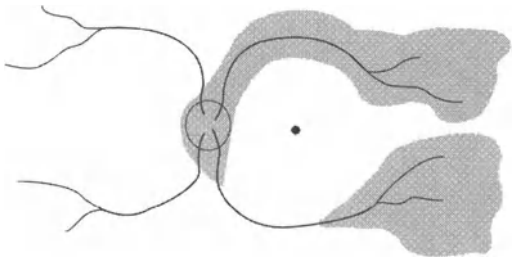

Fig. 20. Grade 2 vitreoretinal traction with vitreous traction along the entire course of the temporal vascular arcades and the optic nerve region.

Grade 2 indicates that the vitreoretinal adhesion has extended along one of the major vascular arcades, usually the temporal arcade, and that traction is exerted on the retina in that particular area. The traction is not generalized but is concentrated in one geographic or segmental zone of the posterior fundus (Figure 20).

Grade 3 indicates that more than one of the segmental sections of the posterior retina, usually the vascular arcades, is involved with more severe vitreous contraction. In these cases the inferior and superior temporal vascular arcades are usually under considerable traction by vitreoretinal adhesions, and the traction may form a circular, ringlike area of retinal tenting (Figure 21).

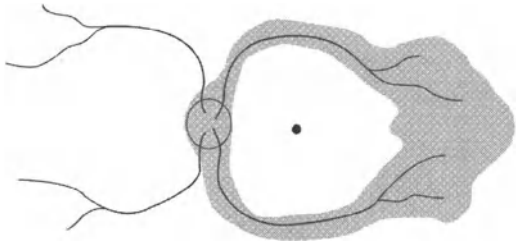

Fig. 21. Grade 3 vitreoretinal traction with vitreous adhesions and traction in a circular fashion, involving temporal vascular arcades, optic nerve region, and interarcade horizontal raphe area.

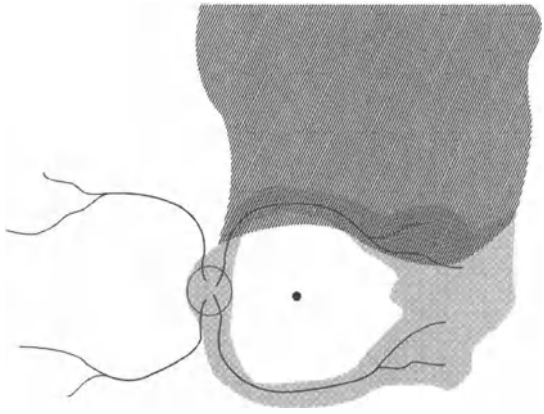

Fig. 22. Grade 4 vitreoretinal traction indicates advanced vitreoretinal traction in a circular fashion around the temporal vascular arcades associated with segmental localized traction retinal detachments. Early diabetic traction retinal detachments are usually located in the superotemporal quadrant but can occur in any region of the posterior pole.

Grade 4 indicates that the contraction of the vitreous has been sufficiently severe to pull the sensory retina from its attachment to the pigment epithelium, thereby causing a traction retinal detachment. These traction detachments are usually small at first but can proceed to total detachment of the retina (Figure 22).

Grade 5 denotes that stage of vitreoretinal contraction where the entire central portion of the retina is moderately detached by a shallowly detached posterior hyaloid (Figure 23).

Grade 6 indicates a moderately elevated detachment of the central retina by a highly detached cone-shaped posterior hyaloid (Figure 24).

Grade 7 indicates a markedly elevated detached retina by traction from a highly detached posterior hyaloid adherent to the vitreous base region (Figure 25).

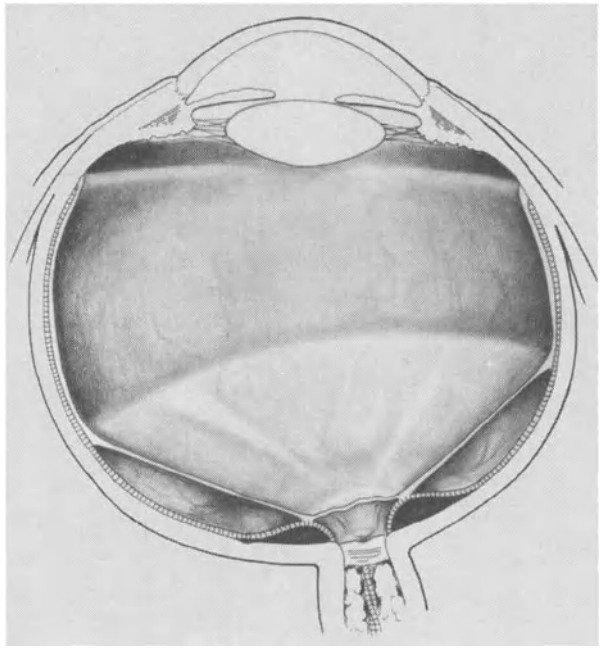

Fig. 23. Grade 5 vitreoretinal traction indicates a shallow, circular, centrally located retinal detachment around the region of the optic nerve and posterior pole caused by traction from a shallowly detached posterior hyaloid membrane.

Grade 8 indicates a vitreoretinal configuration where the retina is pulled forward into the retrolenticular space by a highly detached posterior hyaloid – the so-called triangular syndrome (Figure 26).

General considerations

This classification of diabetic retinopathy attempts to categorize the various phases of diabetic retinopathy as an evolving process starting with early venous dilation and ending with gross glial proliferation, neovascularization, marked vitreoretinal traction, and total retinal detachment. The classification is purely ophthalmoscopic and does not involve the use of fluorescein angiography or any of the electrophysiologic studies. Fluorescein angiography has been found to add to the diagnostic and therapeutic information gathered before photocoagulation but does not change the overall classification or prognosis significantly; electrophysiologic tests are nonspecific and require elaborate equipment that is not readily available to all ophthalmologists.

The total designation and classification of an eye with diabetic retinopathy is accomplished by determining whether the condition can be categorized as nonproliferative or proliferative. If it is nonproliferative, one simply selects the

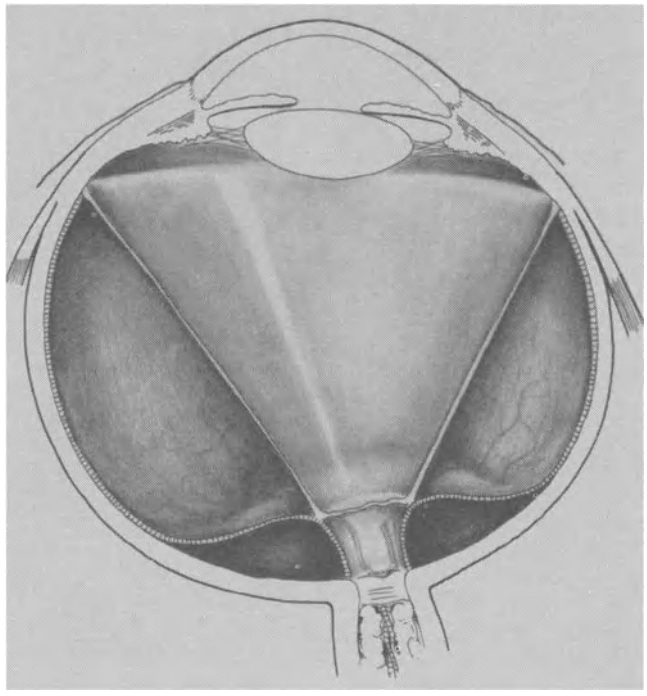

Fig. 24. Grade 6 vitreoretinal traction indicates a centrally located retinal detachment caused by traction from a highly detached posterior hyaloid membrane extending to the region of the ora serrata.

most advanced stage of involvement and a severity gradation from one to four; for example, one might classify the condition as X2.

If the proliferative phase is observed during examination of the diabetic eye, the nonproliferative classification is made, and the three proliferative components are judged individually. The predominant form of neovascularization is determined first, and the appropriate representative letter (E, epipapillary; P, peripapillary; V, papillovitreal; R, retinovitreal; and S, surface neovascularization) and severity (number from 1 to (iv) are noted. The remaining categories of glial proliferation (G) and retinovitreal traction (T) are then judged for severity (for example, G4 and T5) and noted. The categories are separated by hyphens. The entire classification would then read X2-E2-G4-T5. In essence, these four categories serve to allow the examining ophthalmologist (or any other ophthalmologist) to have a distinct impression of the pathophysiologic nature of that particular eye with diabetic retinopathy.

In general, photocoagulation, especially with the argon laser, can be considered if the nonproliferative diabetic retinopathy process has progressed to the level of edema or exudates especially with a concurrent visual acuity decrease

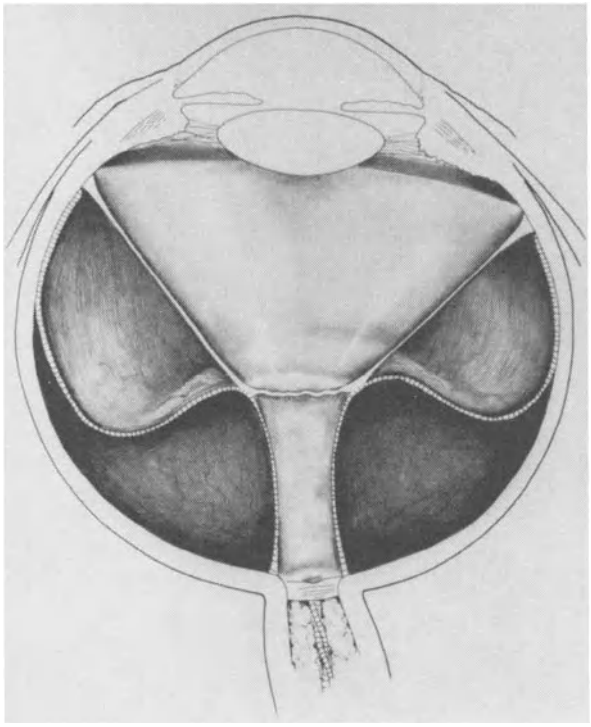

Fig. 25. Grade 7 vitreoretinal traction indicates a more extensive and more highly elevated traction retinal detachment caused by traction exerted by a highly detached posterior hyaloid membrane.

or involvement of the foveal region. An eye with Grade 3 or 4, and usually Grade 2, vitreoretinal traction should not be treated with photocoagulation unless vitrectomy or retinal surgery can be employed immediately if vitreous shrinkage occurs. Similarly, eyes with Grades 3 and 4 glial proliferation should not be considered for photocoagulation but should be evaluated for vitreoretinal surgery. In these cases the small amount of heat generated by the photocoagulation energy passing through the ocular media could be sufficient to cause further shrinkage of the vitreous body and subsequent retinal detachment.

Diabetic maculopathy

Diabetic maculopathy consists of macular involvement from one or more components of retinopathy, with resultant loss of macular function and visual acuity. Kohner [29] has classified diabetic maculopathy as an intermediate

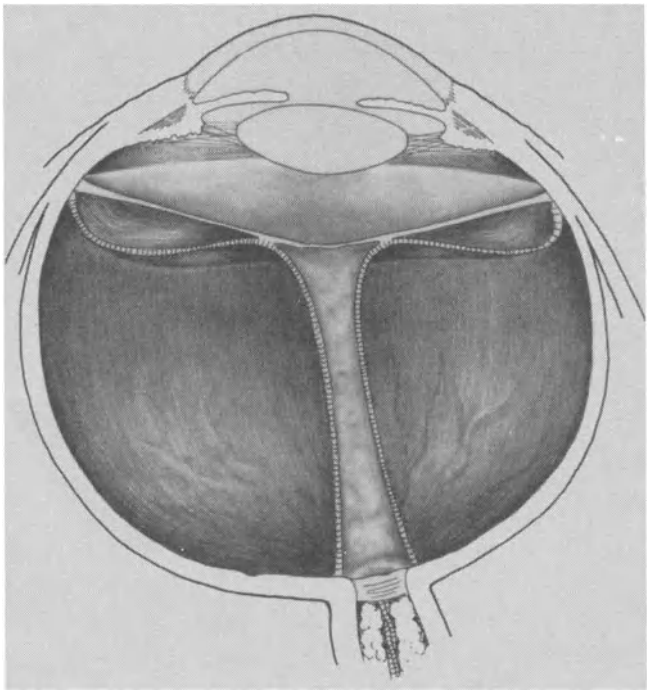

Fig. 26. Grade 8 vitreoretinal traction indicates a highly and totally detached retina caused by excessive traction from a highly detached posterior hyaloid and cyclitic-like membrane causing the retina to be positioned in the retrolenticular space. This produces a characteristic ultrasonographic echo resembling a triangle – the so-called triangular syndrome.

stage in the evolution of retinopathy that occurs after early background retinopathy, but prior to the development of proliferative retinopathy (Figure 27).

In Kohner's series, 75% of the cases of maculopathy were observed in patients with onset of diabetes after age 40. Kohner recognized three clinical types of maculopathy. The most common type, Type 1, consisted of exudate rings with frequently associated microvascular lesions in the center of the rings. Capillary perfusion was good, and fluorescein studies showed only focal leakage. Visual prognosis for this type of maculopathy remained good as long as the exudate rings or microvascular lesions did not encroach upon the central macula. Type II maculopathy showed larger exudate plaques, rather than rings, with increasing retinal hemorrhages. Capillary loss could be seen in both the central and peripheral retina. Visual prognosis for Type II maculopathy was worse than for Type I because of more frequent central macular edema and because of the later development of proliferative retinopathy in some patients. Type III maculopathy consisted of severe central macular ischemia and edema, but few or no exudates. Visual prognosis for this type of maculopathy was the

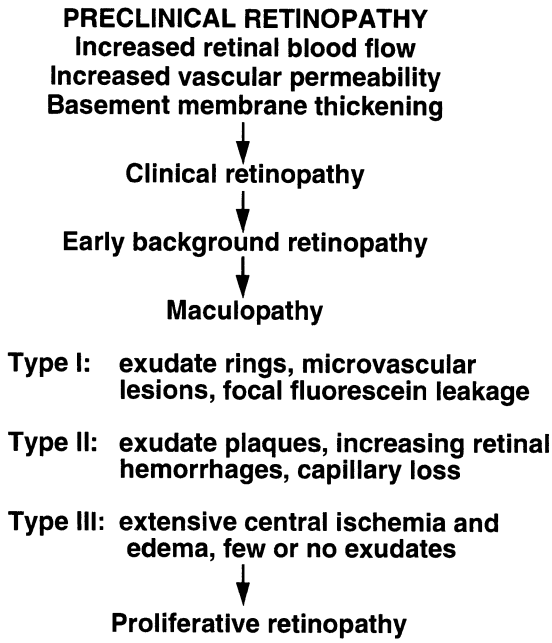

Fig. 27. Clinical evolution of diabetic retinopathy with classification of diabetic maculopathy (Compiled from data presented in Kohner EM. Int Ophthalmol Clin 1978; 18(4): 1.)

worst of the three types, due to the combination of both central ischemia and exudates and the frequent development of proliferative retinopathy.

Following is Sigelman's [30] classification of diabetic maculopathy:

Stage I: *Background maculopathy*
Sparse microaneurysms
Variable but small amount of exudates and hemorrhages
Early ischemic foci
Macular anatomic structure relatively intact

Stage II: *Focal leakage*
Focal beds of leaking microaneurysms
Variable accumulation of serous and lipid exudates
Larger ischemic foci
Early distortion of macular anatomic structure

Stage III: *Diffuse leakage*
Diffuse beds of leaking microaneurysms
Marked accumulation of serous and lipid exudates
Large ischemic foci
Severe distortion of macular anatomic structure
Formation of microcysts

Stage IV: *Cystoid degeneration*
Diffuse beds of leaking microaneurysms
Marked accumulation of serous and lipid exudates
Large ischemic foci
Permanent damage of macular anatomic structure
Formation of irreversible macrocysts

Two simultaneous processes cause maculopathy to progress: increasing capillary obliteration and ischemia and increasing leakage from microaneurysms. Eventually, normal macular architecture is permanently damaged, with loss of Muller fibers and the formation of microcysts and macrocysts. Photocoagulation treatment, with a reasonably good prognosis, is indicated when maculopathy has progressed to Stage II (focal leakage). Stage III maculopathy (diffuse leakage) may be treated by photocoagulation, but with a guarded prognosis. Stage IV maculopathy involves permanent macular damage and is untreatable.

Grading systems

The need for a standard, acceptable classification of diabetic retinopathy became apparent when it was realized that results of the treatment of diabetic retinopathy reevaluating treatment have not been accurate enough to know whether the course of retinopathy has been altered by treatment, since an accepted classification of the natural history of retinopathy is lacking. Therefore, several classifications have been proposed that record the progress of retinopathy objectively and that assess changes in retinopathy by some quantitative method of grading. Ideally, such a classification should include retinopathy of all degrees of severity so that patients are not grouped into categories too broad for meaningful study. Results of grading methods should also be reproducible in the hands of different observers if the grading system is to be used in large-scale clinical studies [31].

In most grading systems, fundus photography is the standard method of recording retinopathy. Fluorescein angiography is also useful, particularly in the study of nonproliferative retinopathy and in assessing microvascular changes of early proliferative retinopathy. However, not all lesions can be successfully photographed, especially in eyes with hazy media. Standard photographic fields may also miss significant areas of pathology outside these fields. Therefore, some grading systems supplement these methods with ophthalmoscopic examination (including indirect ophthalmoscopic and slit-lamp fundus examinations), fundus drawings, and detailed written descriptions. Visual acuity may be useful in comparing results among different collaborative studies but can be misleading in indicating the progress of retinopathy in an individual patient [32].

Methods of grading most commonly used compare patient photographs against a set of standard reference photographs. By making this comparison,

the observer then determines, for an individual patient, the category or degree of severity of any retinopathy present. This type of system is not directly quantitative and does contain sources of error if results are compared among different observers [33]. Parr and Spears [34] have devised a system of directly counting lesions to grade retinopathy. This system, too, contains sources of observer error, and the only lesions counted were microaneurysms, hemorrhages, and exudates, which limits the types of retinopathy available for study with this system. Grading systems thus have limitations of their own, both in methods of recording retinopathy and in assessing severity or progression of retinopathy. Since there is no accepted classification for diabetic retinopathy, there is no standard agreement as to which features of retinopathy should be included for grading. Despite these limitations, grading systems represent a significant attempt to evaluate the results of treatment and to document the natural history of diabetic retinopathy.

One of the first grading systems was proposed by Lee, McMeel, Schepens, and Field [35]. Retinopathy was recorded by indirect ophthalmoscopic examination and by detailed fundus drawings. A fundus diagram was used that divided the retina into 12 sectors of 30 degrees each, with the apices starting at the macula and extending out to the equator. Specific types of pathology were recorded according to a conventional color code. The components of retinopathy chosen for study in this system are noted in Table 1. Each component was graded in degrees of severity from zero to five, according to the extent of retinal area involved, with the exception of venous dilation, which was graded according to changes in the arteriovenous ratio.

This system provided detailed and statistically reproducible recordings of the retinopathy present in 400 patients. The use of the indirect ophthalmoscope allowed both peripheral retinal and macular changes to be recorded. Such detailed fundus drawings, however, would be laborious for any large scale collaborative study.

The Hammersmith grading system

This system was the first to use fundus photography for the recording and grading of retinopathy [36]. Twenty color photographs serve as standards to define four degrees of severity (A to D) for each of five components of retinopathy. Patient color photographs are obtained in nine predetermined photographic fields around and including the optic disc. Patient photographs are then graded for each component of retinopathy, by comparison with four degrees of severity in the standard photographs. Thus, patient grade three would be worse than standard grade B but better than standard grade C.

Components of retinopathy in this system are graded as follows: microaneurysms and hemorrhages are directly counted; venous irregularities are graded by changes in vessel caliber; exudates, new vessels, and retinitis proliferans are graded by extent of retinal area involved. Among different observers this system

Table 1. Features of retinopathy graded by detailed fundus drawings*

Type of lesion	0 Not present	1 Mild	2 Moderate	3 Advanced	4 Far advanced	5 End stage
1. Angiopathy (A) Venous dilation, estimated by A/V ratio and associated changes	A/V = <1/1.5; normal retinal vessels	A/V = 1/1.5 but <1/2; uniform vessel caliber	A/V = 1/2 but <1/2.5; tortuosity and slight variation in caliber	A/V = 1/2.5 but <1/3; marked tortuosity and variations in caliber in less than 1/2 of vascular tree	A/V = 1/3 or over; marked tortuosity and variations in caliber in 1/2 or more of vascular tree	
Microaneurysms and hemorrhages (retinal edema and preretinal hemorrhage included), estimated by area of fundus involved	Not present	<1/12 of fundus area and pinpoint lesions	1/12 to <2/12 often with larger intraretinal hemorrhages	2/12 to <3/12 intraretinal and occasionally preretinal hemorrhages	3/12 or over (if over, indicate how much); intraretinal and preretinal hemorrhages	
Neovascularization, estimated by area of fundus involved	Not present	Not present	<1/12 of fundus area	1/12 to <2/12	2/12 or over (if over 3/12, indicate how much)	
2. Exudates (E), soft and hard, estimated by area of fundus involved	Not present	<1/12 of fundus area	1/12 to <2/12	2/12 to <3/12	3/12 or over (if over 4/12, indicate how much)	
3. Proliferative retinopathy (P), angiopathic (Pa), and nonvascular (Pn), estimated by area of fundus involved and the extent of the arc formed around the macula	Not present	<1/12 of fundus area or <45° arc around macula	1/12 to <2/12 or 45- to <90° arc	2/12 to <3/12 or 90- to <180° arc	3/12 or over, 180° arc or over (if over 4/12 or over 225°, indicate how much)	
4. Vitreous hemorrhage (V), estimated by area of fundus observed	Not present	<2/12 of fundus	2/12 to <4/12	4/12 to <8/12	8/12 or over	

* From Lee P, McMeel JW, Schepens CL, Field RA. Am J Ophthalmol 1996; 62: 207.

produced over 90% agreement in the grading of all components of retinopathy recorded, with the exception of venous irregularity, which produced considerably less agreement. This system has received further trials in various countries [37].

The O'Hare grading system

This system was devised by a committee prior to a symposium on the treatment of diabetic retinopathy [38]. The purpose of this system was to see if such a classification, by retrospective study among different groups reporting at the symposium, would yield any valid data concerning the treatment of retinopathy. It was never intended that this classification would be universally accepted. However, this effort was the first major step in the United States to develop a more generally acceptable grading system.

This system divides retinopathy into background retinopathy and proliferative retinopathy, the latter being further subdivided into vitreous hemorrhage, new vessel formation, and fibrous proliferation. The O'Hare classification follows [38]:

B All degrees of background retinopathy
H_0 No vitreous hemorrhage
H_1 Vitreous hemorrhage present, but retina can be seen well enough to be classified
H_2 Vitreous hemorrhage so extensive that the retina cannot be classified
N_1 Four or fewer discrete patches (on retina) disc areas of new vessels
N_2 Greater than four discrete patches (on retina) or greater than four disc areas of new vessels
F_0 No fibrous proliferation extending into the vitreous cavity (although flat retinal fibrous proliferation may be present)
F_1 Fibrous proliferation extending into the vitreous cavity, but involving four or fewer discrete patches (on retina) and four or fewer disc areas
F_2 Fibrous proliferation extending into the vitreous and involving greater than four discrete patches (on retina) or greater than four disc areas

Background retinopathy is not subdivided, which makes the system somewhat insensitive. Clinical studies, however, have produced meaningful data using this system [39, 40], although one of these studies did subdivide background retinopathy and used fluorescein angiography to refine the grading system [39].

The Airlie grading system

This system was formulated by a committee and represents a joint effort from both the United States and Great Britain to find comprehensive, generally accepted classification and grading system for diabetic retinopathy [41]. In a sense, this system combines some of the best features of the three grading systems discussed previously.

The Airlie system proposes more extensive recording methods and patient evaluation than any other system. These include fundus photography, fluorescein angiography, ophthalmoscopy (both direct and indirect), slitlamp biomicroscopy of the retina, fundus diagrams, and, where indicated, detailed written descriptions. The list of retinopathy components selected for grading is more comprehensive than that of any other system (Table 2). Components amenable to photographic assessment are graded by reference to one standard color photograph: grade 0, component absent; grade 1, component less severe than standard photograph; grade 2, component more severe than standard photograph. Other components are graded by reference to standard written descriptions.

Several questions were raised at the time that the Airlie system was formulated. It remained uncertain whether all components listed in this system could be accurately graded by different observers [31]. Macular edema in particular has proved a difficult feature to grade [33], as have venous abnormalities [35]. The use of only one standard photograph might result in loss of sensitivity

Table 2. The Airlie classification*†

Lesion	Grade 0	Grade 1	Grade 2
Nonproliferative			
Hemorrhage and/or microaneurysms	None	Present but not as severe as standard photograph	As severe or worse than standard photograph
Hard exudates	None	Present but not as severe as standard photograph	As severe or worse than standard photograph
Soft exudates	None	1 to 4 in entire fundus	5 or more in entire fundus
Venous abnormalities	None	Present but not as severe as standard photograph	As severe or worse than standard photograph
Intraretinal microvascular abnormalities	None	Present but not as severe as standard photograph	As severe or worse than standard photograph
Retinal edema (at macula)	None	Covers less than 1 disc area	Covers 1 disc area or more
Fluorescein			
Arteriovenous phase (15 to 25 s)	Normal	Intraretinal microvascular abnormalities and microaneurysms less than standard	Abnormalities as severe as or worse than standard
Late phase (3 to 5 min)	No dye	Dye leakage less than standard photograph	Dye leakage equal to or worse than standard photograph

Continued

Table 2. (Continued.)

Lesion	Grade 0	Grade 1	Grade 2
Proliferative			
Neovascularization within 1 disc diameter of disc	None	Present but not as severe as standard photograph	As severe or worse than standard photograph
Neovascularization areas other than disc	None	1 to 4 discrete patches and 4 or less disc areas of new vessels	5 or more discrete patches or more than 4 disc areas of new vessels
Fibrous proliferation within 1 disc diameter of disc	None	Present but not as severe as standard photograph	As severe or worse than standard photograph
Fibrous proliferation areas other than disc	None	1 to 4 discrete patches and 4 or less disc areas	5 or more discrete patches or more than 4 disc areas
Plane of proliferation	Within 1/4 disc diameter of surface of attached retina	Anterior to retina's normal position by 1/4 to 2 disc diameters	Anterior to retina's normal position by 2 disc diameters or more
Retinal elevation	None	4 or less disc areas in extent	More than 4 disc areas in extent
Vitreous hemorrhage			
Preretinal hemorrhage	None	Present but not as severe as standard photograph	As severe or worse than standard photograph
Vittreous hemorrhage	None	1a – present but good quality photos possible; 1b – too much for photo, but can see well enough to classify ophthalmoscopically	Too much to allow ophthalmoscopic classification

found in the Hammersmith system [31]. Eyes with more advanced proliferative changes cannot always be accurately graded usually due to a poor fundus view because of vitreous hemorrhage or extensive fibrous tissue formation [42].

These and other questions are currently being tested in collaborative clinical studies. The answers will hopefully improve our understanding and classification of diabetic retinopathy.

Acknowledgements

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10. Long-term visual outcome of diabetic patients treated with pan-retinal photocoagulation

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& ELI A. FRIEDMAN

Editors' Comment:

Between the first and the present conferences on the Diabetic Renal–Retinal Syndrome, no advance in therapy has been more important to patient rehabilitation than the application of panretinal photocoagulation. As recounted in this retrospective assessment of a single ophthalmologist's private practice, 96% of long-term survivors (mean follow-up 20 years) of panretinal photocoagulation retained visual function in at least one eye that was sufficient to ambulate thereby facilitating self-sufficiency. Although 27% of treated subjects had an adverse consequence — most often a loss of 4 or more Snellen lines of vision — 62% scored 20/40 or better at most recent examination. While sample bias (predominantly white middle class subjects) is reason for caution in extrapolating these results to the general population, the favorable outcome supports the broad use of laser photocoagulation as an antiblindness technique. Every diabetic person referred for a renal evaluation must be seen by a collaborating ophthalmologist early and repeatedly as a component of state-of-the-art comprehensive management. Burning the retina to preserve sight though counter-intuitive is nevertheless an essential intervention once neovascularization is discovered. Every laser surgeon looks forward to replacing the retinal injury imparted by photocoagulation with more subtle and specific interventive measures based on humoral or biochemical lock and key correction of whatever is found to cause diabetic retinopathy. The good news delivered in this report is that despite an absence of clarity in understanding why diabetic retinopathy occurs, an effective treatment is in hand. Ensuring universal availability of laser photocoagulation to all who might benefit is a vital challenge in health care delivery.

Introduction

Pan-retinal photocoagulation has been the mainstay of treatment for proliferative diabetic retinopathy for over 20 years. While the long-term success of panretinal photocoagulation has been reported in the literature, patient follow-up beyond 10 years is lacking. This is the first report to analyze the outcome of patients with proliferative diabetic retinopathy treated and followed in a single-practice setting for 15 or more years.

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Diabetic retinopathy accounts for 7.9% of blindness in adults [1], and is the leading cause of acquired blindness among working-age Americans [2]. An estimated 175,000 person-years-sight (the number of sighted persons × the number of years during which sight remains) at a cost-savings to Medicare/ Medicaid of one-half billion dollars [3, 4] could be preserved with timely screening and early or initial treatment of diabetic eyes. This estimate, however, does not include any additional costs of retreatment over 15 years since such long-term data (i.e., recurrence of disease, frequency and cost of retreatment) is limited [4].

The beneficial effects of retinal photocoagulation in the course of diabetic retinopathy, first elucidated by Meyer-Schwickerath [5] in 1956 employing a xenon-arc photocoagulator, ultimately led to the development of the argon laser in 1968 and its first clinical application in diabetic patients by L'Esperance [6] at Columbia-Presbyterian Medical Center. That panretinal photocoagulation successfully ameliorates proliferative diabetic retinopathy was demonstrated in the Diabetic Retinopathy Study [7] (DRS) launched by the National Eye Institute in 1970 and concluded in 1979 [8, 11].

Concensus thinking recognizes that panretinal photocoagulation preserves vision in diabetic retinopathy employing either a xenon-arc instrument or an argon blue-green laser [8]. Long-term visual outcome after pan-retinal photocoagulation, while not assessed by the DRS, has been reported up to 15 years following initial treatment [9, 10].

The purpose of this report is to reconstruct the modified (i.e., treatment, follow-up, and retreatment as needed) long-term course of treated diabetic retinopathy. By retrospective analysis of a single ophthalmologic practice, we correlated long-term visual outcome as well as diabetologic and renal markers in patients followed for 15 years to 27 years (mean follow-up period 21 years) after xenon-arc, ruby laser or argon laser photocoagulation for diabetic retinopathy.

Methods

Study design and selection of patients

A single referral ophthalmologic practice in New York City was analyzed retrospectively by review of consecutively numbered patient charts (1-3219, spanning January 1969 to January 1976) to select the first consecutive 50 patients (100 eyes, $n = 100$) who met the following parameters: (i) diagnosis of diabetes established by the referring internist; (ii) recorded follow-up, within the single practice, of ≥ 15 years; (iii) presence of DRS high-risk characteristics [11] (level ≥ 65) established by fundus photography and fluorescein angiography; (iv) bilateral diabetic retinopathy; (v) treatment with xenon-arc, ruby laser or argon laser pan-retinal photocoagulation in one or both eyes; (vi) post-photo-coagulation treatment follow-up ≥ 15 years. Exclusion criteria included:

(i) other retinal vascular disease, i.e., sickle cell retinopathy, sarcoidosis, venous occlusive disease; (ii) other (non-diabetic) ocular diseases, i.e., glaucoma, uveitis, age-related macular degeneration.

Selected subjects were characterized by age, gender, race, type and duration of diabetes, renal function, initial and final visual acuities, initial and final DRS high-risk factors, onset and duration of regression, recurrence, type of photocoagulation treatment, vitrectomy, presence of cataracts and performance of cataract surgery, and development of rubeosis irides.

Ophthalmologic evaluation

Subjects were evaluated initially and periodically in a single outpatient private practice office setting. Visual acuity (Snellen) measurement, slit-lamp exam, intra-ocular pressure (applanation), fundus exam and fundus photography were performed at each encounter. Fluorescein angiography was performed at baseline and subsequently when indicated by suspected recurrence of neovascularization, loss of acuity unexplained by media opacification, or the development of macular exudation. Each patient had documented high-risk characteristics (≥ 3 out of 4 DRS high-risk factors) in each eye before administering panretinal photocoagulation. Duration of follow-up was tabulated in years, according to most recent visit.

Visual outcome

Visual acuity was compared at baseline and most recent follow-up. Measured acuity was grouped into one of five levels: ≥ 0.50 (20/40); 0.40–0.20 (20/50–20/100); 0.10–0.02 [20/200-counting fingers (CF)]; < 0.02 [hand motion (HM)/light perception (LP)]; no light perception (NLP) – at baseline and most recent follow-up. Change in Snellen lines of acuity was grouped into one of four levels: $\leq$ one line, 1; $>$ one line but $<$ three lines, 2; $>$ three lines but $\leq$ four lines, 3; $>$ four lines, 4; a plus or minus sign was then used to denote loss or gain.

Neovascularization

Regression of high-risk characteristics was tabulated by initial and final scores of high-risk factors. High-risk characteristics noted were defined as the presence of 3 or more of the following high-risk factors: neovascularization of the disc, neovascularization elsewhere, extent of neovascularization, and vitreous hemorrhage [11]. Duration of regression was tabulated in years, according to most recent visit. Recurrence of neovascularization if present was tabulated as elapsed time, in years, since completion of the last photocoagulation treatment. Onset of regression of neovascularization was tabulated as occurring within or beyond 3 months after photocoagulation.

Photocoagulation

Patients were treated with a high-pressure xenon-arc incandescent source photocoagulator(Zeiss; Oberkochen, Germany) and/or an argon blue-green laser photocoagulator (Coherent, Inc., Palo Alto, CA) employing burn widths of 250 to 1000 microns with sufficient energy and duration to produce an intense coagulation. In two patients, initial photocoagulation was performed with a ruby laser (Optics Technology, Inc., Palo Alto, CA) 250 to 500 coagulations were performed at each session, some (6 of 100 eyes, 6%) requiring retrobulbar anesthesia (2% lidocaine). Photocoagulation treatment was delivered in 3 to 6 divided sessions (each consisting of 150 to 750 coagulations, 7 to 14 days apart) within 3 months, and with augmentation (1 to 3 additional sessions) as necessary over a course of 3 months to 1 year until regression of neovascularization was documented. The total number of burns per patient ranged from 1500 to 2500. Augmentation treatment, provided in instances of recurrence or late regression of neovascularization, employed the argon blue-green laser. The total course of photocoagulation treatments ranged from 6 weeks to 1 year.

Diabetes

Each patient was characterized as having type I (insulin-dependent) or II (non-insulin dependent) diabetes by written communication from their internist. Duration of known diabetes, in years, was tabulated according to the patient's most recent visit.

Renal parameters

Available limited laboratory reports detailing each patient's renal function were assessed either at baseline or during the course of follow-up by written communication with the patient's internist and/or nephrologist. Renal function was classified according to the most recent visit as being grossly normal, partially impaired (azotemic), dialysis-dependent or replaced by a kidney transplant.

Statistical analysis

Since the sample size was small, only exact statistics were used to test associations. The probability of recurrence of neovascularization was assessed by the log rank statistic and graphed with Kaplan–Meier curves. Contingency tables were assessed using the Fisher–Freeman–Halton test. The non-parametric Kruskal–Wallis test for unordered categories and the Jonckheere–Terpstra test for ordered groupings were used for comparisons among several groups. The kappa statistic was used to assess concordance in vision between baseline and follow-up; in general a kappa < 0.4 is considered to represent poor agreement.

*Table 1.*Subject parameters

Age, mean ($n = 50$) in years	63
Gender ($n = 50$):	no.
Male	24
Female	26
Type DM ($n = 50$):	no.
Type I	28
Type II	22
Duration DM, mean ($n = 50$) in years	43
Duration of F/U, mean ($n = 50$) in years	21
Race ($n = 50$):	no.
White	49
B lack	1
Baseline HRFs, mean ($n = 50$)	3.4

DM = diabetes mellitus; F/U = follow-up; HRFs = high risk factors.

Finally, exact logistic regression analysis was used to test for association between age, gender, type of diabetes, onset of regression, and adverse outcomes. Computations were done using standard computer programs such as SPSS Exact, StatXact, and LogXact.

Results

Patient parameters. (Table 1)

The selected cohort of 50 patients began treatment between 1969 and 1976, and were followed through December, 1996. The group consisted of 24 men and 26 women, aged 38 to 96 years (mean age = 63 ± 33 years). There were 49 whites, 1 black, 30 type I and 20 type II diabetics. Duration of follow-up ranged from 15 to 27 years (mean follow-up = 21 years ± 6 years). Duration of diagnosed diabetes ranged from 21 to 61 years (mean duration of diabetes = 43 years ± 18 years), subdivided as type I, 45 ± 16 years and type II 43 ± 10 years. 5 patients had died at most recent follow-up.

Visual outcome (Tables 2–5, Figure 1)

Initial baseline Snellen chart visual acuity (VA) ranged from 20/20 to LP (mean VA = 20/30) while final visual acuity ranged from 20/20 to NLP (mean VA = 20/60). At baseline, 71 eyes (71%) had VA ≥ 20/40, 13 eyes (13%) had VA 20/50 ≥ 20/100, 12 eyes (12%) had VA 20/200 ≤ CF, and 4 (4%) eyes had VA < CF (HM to LP). At most recent follow-up after a mean of 21 ± 6 years, 43 (43%) eyes had VA ≥ 20/40, 19 (19%) eyes had VA 20/50 ≥ 20 100, 14 (14%) eyes had VA 20/200 ≥ CF, and 24 eyes (24%) had VA < CF, 13 (13%) of which had NLP, the remaining 13 eyes (13%) had HM to LP (Table 3).

Table 3. Levels of VA subdivided

Visual acuity (VA)	OD ($n = 50$) # of eyes	OS ($n = 50$) # of eyes
Baseline VA ≥ 0.50	36 (36)	34 (34)
Baseline VA 0.50 – 0.20	7 (7)	7 (7)
Baseline VA 0.10–0.02	5 (5)	7 (7)
Baseline VA < 0.02	2 (2)	2 (2)
Final VA ≥ 0.50	21 (21)	22 (22)
Final VA 0.50–0.20	13 (13)	6 (6)
Final VA 0.10–0.02	8 (8)	6 (6)
Final VA < 0.02	5 (5)	6 (6)
Final VA NLP	3 (3)	10 (10)

OD = right eye; OS = left eye.

Table 2. VA and mean Δ in VA

Visual acuity (VA)	OD ($n = 50$)	OS ($n = 50$)
Mean VA		
Baseline	0.67 (20/30)	0.65 (20/30)
Final	0.37 (20/60)	0.35 (20/60)
Mean Δ in VA (lines)	−2.0	−1.7

OD = right eye; OS = left eye.

Mean VA at baseline was identical in both eyes: 20/30 for right eyes and 20/30 for left eyes. At most recent follow-up, mean VA was 20/60 for right eyes and 20/30 for left eyes ($p < 0.61$). Mean change in VA (Table 2) as measured by the Snellen chart was a loss of 1.7 lines for right eyes, and a loss of 2.0 lines for left eyes, over a mean time period of 21 ± 6 years, ($p < 0.35$).

Functional vision, defined as the best corrected vision in either eye per patient was, at baseline: 43 patients (86%) scored 20/40 or better, 6 patients (12%) scored between 20/50 and 20/100, and 1 patient (2%) scored between 20/200 and 20/400. At most recent follow-up: 31 patients (62%) scored 20/40 or better, 12 patients (24%) were seeing 20/50 to 20/100, 5 patients (10%) scored 20/200 to CF, and 2 patients (4%) scored less than CF. Retention of at least navigational or ambulatory vision was observed in 48 patients (96%) at most recent follow-up (Table 4, Figure 1). Baseline functional vision correlated positively but weakly with final functional vision (kappa = 0.334, $p < 0.002$, 95% CI = 0.06–0.61).

Neovascularization

At baseline, the mean extent of neovascularization was 3.3 out of 4 high-risk factors (see Methods section, *neovascularization*) for right eyes, and 3.4 out of

Table 4. Cross-comparison of baseline and final levels of visual function

Visual function ($n = 50$)	Final ≥20/40	Final 20/50–20/100	Final 20/200–4/200	Final	<4/200
Baseline ≥20/40	29 (58)	10 (20)	4 (8)	0	
Baseline 20/50–20/100	2 (4)	2 (2)	1 (2)	1 (2)	
Baseline 20/200–4/200	0	0	0	1 (2)	

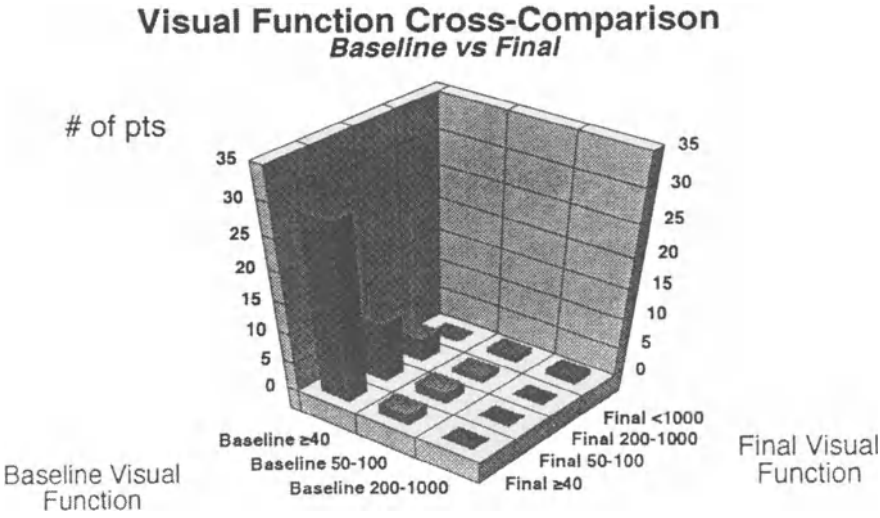

Fig. 1. Visual parameters.

4 for left eyes. At most recent follow-up, the mean extent of neovascularization was 0.7 high-risk factors for right eyes, and 1.0 for left eyes ($p < 0.65$).

Photocoagulation (Tables 5–6, Figures 2–6)

48 eyes (48%) of subjects were treated with argon blue-green laser with a mean follow-up of 19 years ± 6 years, 19 eyes (19%) were treated with xenon-arc photocoagulation instrument with a mean follow-up of 21 years ± 5 years, 29 eyes (29%) were treated with both the argon blue-green laser and the xenon-arc photocoagulator with a mean follow-up of 20 years ± 6 years, 2 eyes (2%) were treated with ruby red laser and argon blue-green laser with a mean follow-up of 27 years, and 2 eyes were treated with xenon-arc instrument and ruby red laser with a mean follow-up of 17 years. Regression of proliferative retinopathy was documented within 3 months in 46 eyes (46%) and beyond 3 months in 54 eyes (54%). The duration of regression ranged from 0 to 26 years, with a mean duration of 17 years ± 7 years. Recurrence of neovascular-

Table 5. Final levels of VA by subject parameters and PRP response

Characteristic ($n = 100$)	Final VA $\geq 20/40$	Final VA 20/50–20/100	Final VA 20/200–4/200	Final VA $< 4/200$
Age (yrs)	58.8	67.7	66.6	66.8
Sex:				
Male (eyes)	21 (21)	4 (4)	5 (5)	18 (18)
Female (eyes)	22 (22)	15 (15)	9 (9)	6 (6)
VA, baseline (eyes):				
$\geq 20/40$	37 (37)	16 (16)	7 (7)	10 (10)
20/50–20/100	5 (5)	1 (1)	3 (3)	5 (5)
20/200–4/200	1 (1)	3 (3)	3 (3)	5 (5)
$< 4/200$	0 (0)	0 (0)	1 (1)	3 (3)
Early reg (eyes)	23 (23)	8 (8)	6 (6)	7 (7)
Late reg (eyes)	20 (20)	12 (12)	7 (7)	17 (17)
Recurrence (eyes)	0 (0)	0 (0)	3 (3)	9 (9)
Duration DM (yrs)	43.3	41.3	47.9	42.7
Duration F/U (yrs)	22	20.2	18.3	20.3

reg = regression; yrs = years; PRP = pan-retinal photocoagulation.

Table 6. Laser modality and response rate

PRP mode	Early reg # of eyes	Late reg # of eyes (R + +NR)	F/U years	Recur (R) # of eyes	Augm tx # of eyes late reg NR	Augm tx # of eyes late reg R	Avg # years since PRP in R eyes
Argon	29	19	19	4	16	3	9.3
Argon/xenon	4	25	20	6	19	6	7.5
Xenon	12	7	21	2	5	2	2
Argon/ruby	0	2	27	0	2	0	–
Xenon/ruby	1	1	17	0	0	1	–

reg = regression; R = recurrence; NR = non-recurrence; F/U = follow-up; augm tx = augmentation treatment; PRP = pan-retinal photocoagulation.

ization following regression was documented in 12 eyes (12%), all of which were affected by adverse consequences (see below). Augmentation photocoagulation treatment – applied in 1 to 5 additional sessions per eye (within 1 year and as long as 15 years after regression was first documented) – was performed in 12 eyes (12%) with recurrence of neovascularization and in 42 (42%) late regressing eyes.

A protective effect against visual deterioration was observed when both eyes showed early regression ($p < 0.03$, Figure 2). An associated finding was the low probability of recurrence of neovascularization when both eyes demonstrated early regression ($p < 0.02$, Figure 3). Quite noteworthy was the inverse relationship between age and the likelihood of early regression when both eyes demonstrated early regression ($p < 0.028$, Figure 4). There was a suggestion that recurrence of neovascularization was less apt to occur in women than men,

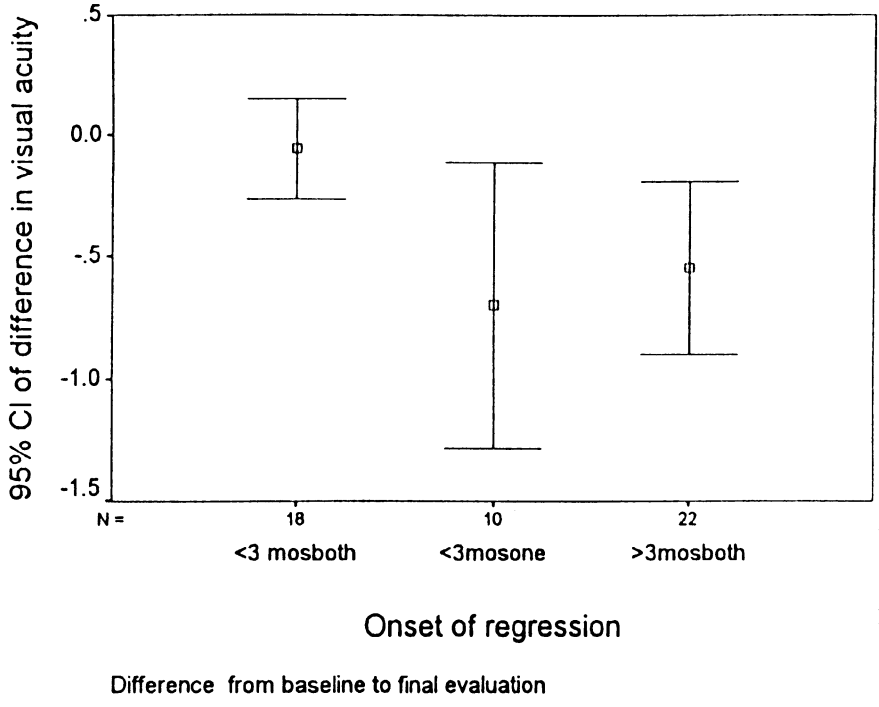

Fig. 2. Difference in visual acuity by regression onset.

but not significantly ($p < 0.15$, Figure 5). There was no significant difference in the frequency of recurrence of neovascularization by photocoagulation modality ($p < 0.69$).

Adverse consequences (Table 7, Figure 6)

Adverse consequences of the treatment regimen were defined as loss of 4 or more Snellen lines of vision and/or progression to NLP. 27 eyes (27%) in 23 patients (15 men, 8 women) were identified as having adverse consequences. 3 (6%) patients died – 2 men, 1 woman; 1 type I, and 2 type II. 5 patients (10%) were affected by adverse consequences bilaterally, 1 of whom (2%) had deteriorated to NLP bilaterally. Type I diabetes was identified in 13 patients, and type II in 10 patients. 13 of the 27 eyes (48%) deteriorated to NLP; the range of acuity in the remaining 14 eyes was between 20/100 and LP. Poor visual function was explained by macular ischemia in 9 of 27 eyes (33%), persistence of hemorrhaging in 5 of 27 eyes (19%), tractional retinal detachment in 12 of 27 eyes (45%), and endophthalmitis in 1 eye (3%). Of the 5 eyes with persistent hemorrhaging, 3 eyes (11%) were noted to have florid characteristics despite treatment, and vitrectomy was deferred (due to systemic medical condi-

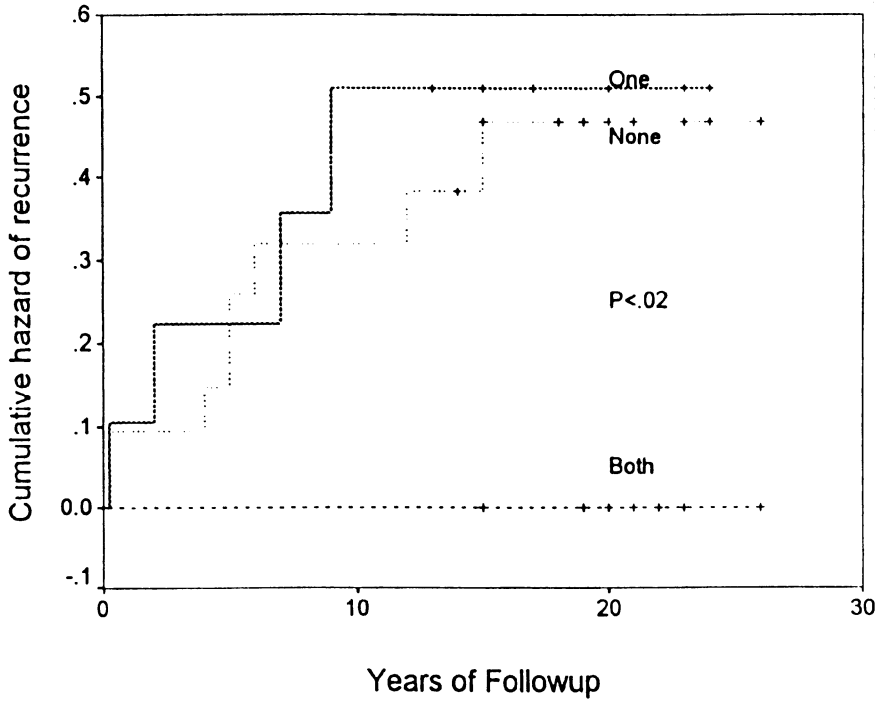

Fig. 3. Hazard of recurrence by regression onset within 3 months.

tion) in 2 (8%) eyes; of the 12 eyes with tractional detachment, scleral buckling was indicated in only 2 eyes (8%) and deferred in the remainder due to excessive fibrovascular proliferation.

Neovascularization began regressing after a lag of 3 months in 15 of 27 eyes (56%), and recurred in 12 of 27 eyes (45%). None of the eyes regressing within 3 months of initial photocoagulation were affected by adverse consequences. Consequently, late regression was identified as a risk factor for the development of adverse consequences (relative risk = 7.4, $p < 0.0001$). Furthermore, recurrence of neovascularization was identified as an additional risk factor for the development of adverse consequences compared to the late regression group without recurrence ($12/12 = 100\%$ vs. $15/42 = 36\%$, relative risk = 1.93, $p < 0.001$). Late regression was the only independent predictor (OR = 9.5; $p < 0.02$; 95% CI = 1.4–119.0) in a logistic regression model which considered age, type of diabetes mellitus, gender, or photocoagulation modality.

Rubeosis

Rubeosis irides was identified in 12 eyes (12%) during the course of routine follow-up; 11 of 12 (92%) were affected by adverse consequences. 1 rubeotic eye had no light perception at baseline.

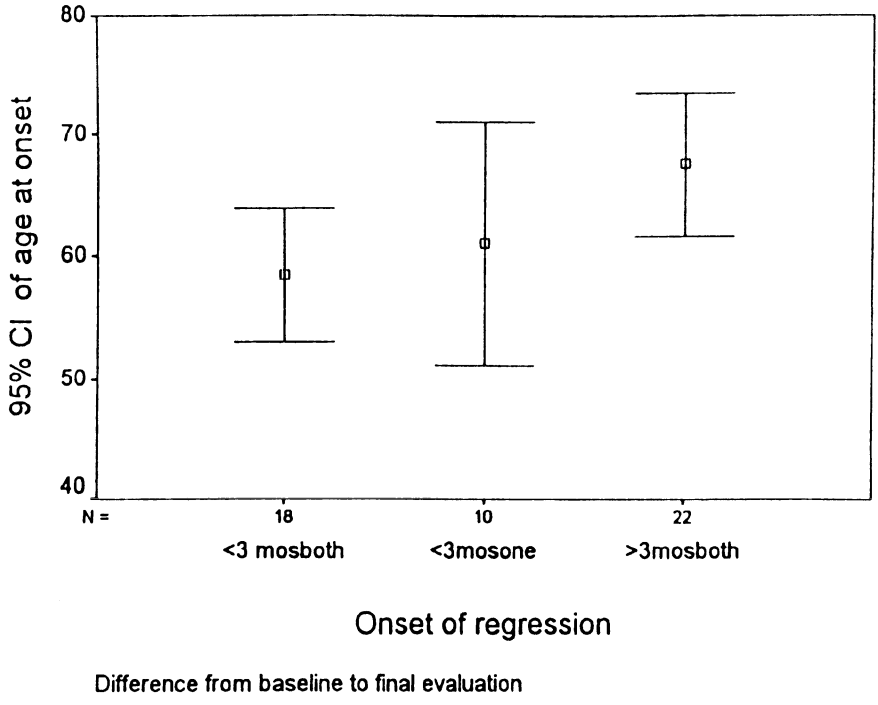

Fig. 4. Difference in age by regression onset.

Ocular surgery (Table 8)

Vitrectomy – for florid proliferative retinopathy

Vitrectomy was performed in 14 eyes (14%) after or during the course of photocoagulation. Of these, 7 (50%) subsequently developed adverse consequences. Visual outcome after vitrectomy ranged between 20/30 to 20/200 in 8 eyes (57%), and between 20/300 to NLP in 6 eyes (43%). Only 3 eyes (21%) subjected to vitrectomy still had florid characteristics at most recent follow-up. Seven of 11 eyes (64%) were ultimately noted to have rubeosis, 2 of which had also undergone intracapsular cataract extraction. Vitrectomy was bilateral in 3 subjects, and was performed only once per eye in all 14 eyes (although one eye had a secondary washout).

Cataract extraction

Lensectomy for cataracts was performed in 42 eyes (42%), 28 of which (28%) were extracapsular with implant, and 14 eyes (14%) had intracapsular extraction without implant. Of those 28 eyes with extracapsular surgery, none developed rubeosis. By contrast of 14 eyes with intracapsular surgery, 5 eyes (36%) developed rubeosis. Final Snellen visual acuity in the subset of the 28 eyes with

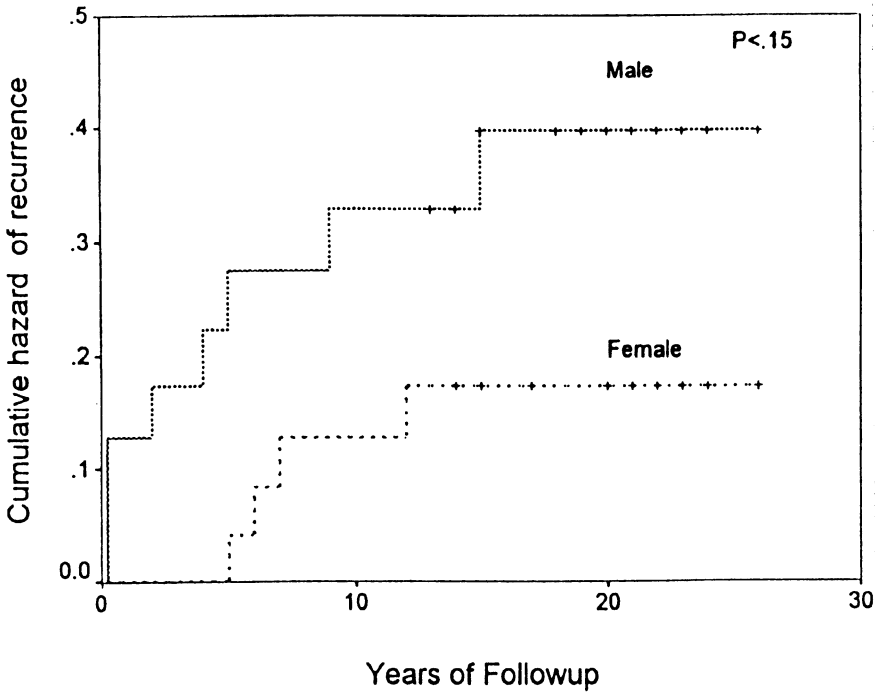

Fig. 5. Hazard of recurrence by gender.

Table 7. Adverse consequences by subject parameters

Characteristic ($n = 27$ eyes)	Final VA $0 \geq 4/200$	Final VA $< 4/200 \geq$ LP	Final VA NLP
Age (yrs)	65.4	70.3	65.0
Sex:			
Male (eyes)	5 (19)	4 (15)	8 (30)
Female (eyes)	4 (15)	3 (11)	3 (11)
VA, baseline (eyes):			
$\geq 20/40$	6 (22)	3 (11)	4 (15)
20/50–20/100	3 (11)	4 (15)	2 (7)
20/200–4/200	0 (0)	0 (0)	5 (19)
$< 4/200$	0	0	0
Early reg (eyes)	3 (11)	0	6 (22)
Late reg (eyes)	6 (22)	7 (26)	5 (19)
Recurrence (eyes)	3 (11)	1 (4)	8 (30)
Duration DM (yrs)	40.3	33.0	39.6
Duration F/U (yrs)	17	19.4	18.5

reg = regression; yrs = years; PRP = pan-retinal photocoagulation.

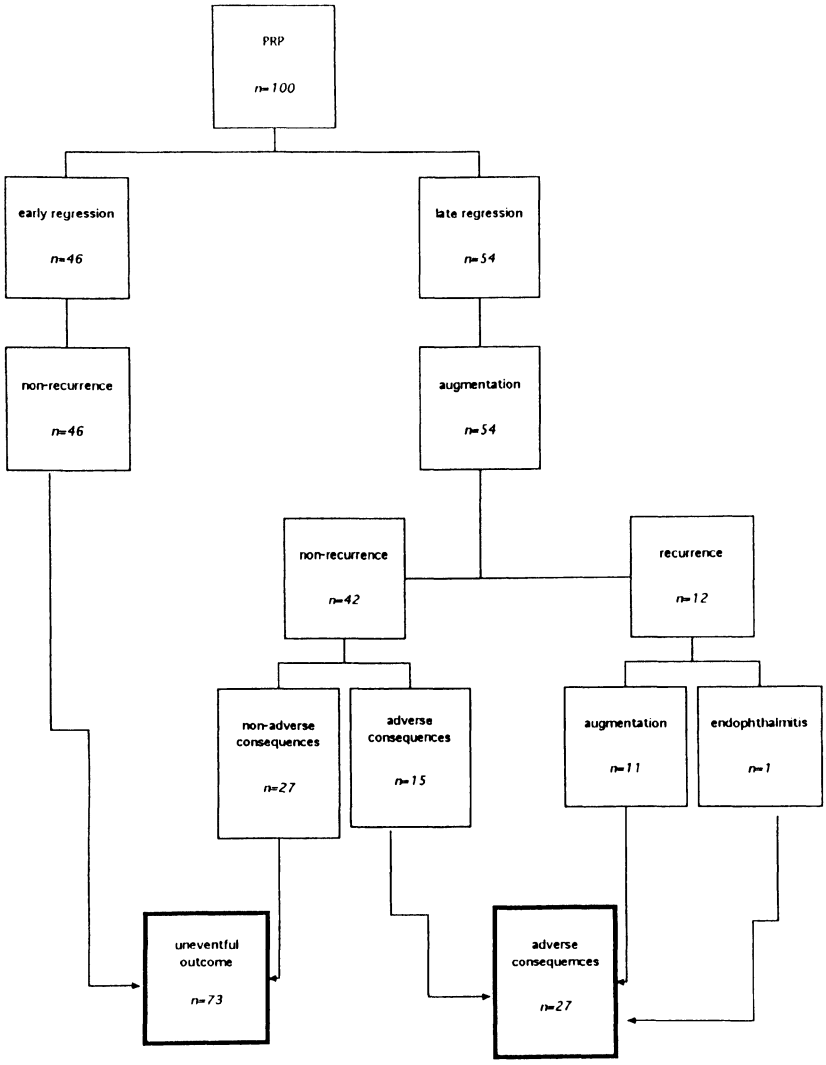

Fig. 6. Treatment algorithm.

a lens implant was $20/40 \pm 4$ lines, while in those without implant, acuity was $20/50 \pm 4$ lines.

Scleral buckling

Scleral buckling, as described by Schepens [12], was applied to 2 eyes (2%) because of rhegmatogenous/tractional retinal detachment. Neither eye regained visual acuity beyond 20/200.

Table 8. Ocular surgery performed

Ocular surgery	OD # eyes	OS # eyes
Vitrectomy	7	8
Cataract surgery	24	19
Scleral buckle	1	1

OD = right eye; OS = left eye.

Table 9. Visual and surgical outcome by renal status

Renal status ($n = 26$ eyes)	Final VA ≥20/40	Final VA 20/50–20/100	Final VA 20/400–4/200	Final VA <4/200	Adv con	Cat surg	Vtrcmy
Full	4 (15)	0 (0)	1 (4)	1 (4)	1 (4)	3 (12)	0 (0)
Partial	3 (12)	3 (12)	0 (0)	2 (8)	2 (8)	4 (15)	3 (12)
CRF:							
HD	3 (12)	0 (0)	3 (12)	2 (8)	5 (19)	3 (12)	0 (0)
TPLANT	2 (8)	1 (4)	1 (4)	0 (0)	1 (4)	3 (12)	2 (8)
Total	12 (46)	4 (15)	5 (19)	5 (19)	9 (35)	13 (50)	5 (19)

Fisher's Exact Test: (full renal function, $n = 6$, overt renal disease, $n = 20$)
Adv con – 8/20 vs 1/6, $p < 0.07$
Cat surg – 10/20 vs 3/6, $p < 0.99$
Vtrcmy – 5/20 vs 0/6, $p < 0.30$
Adv con = adverse consequences; Cat surg = cataract surgery; Vtrcmy = vitrectomy; CRF = chronic renal failure; HD = hemodialysis; TPLANT = renal transplant.

Renal parameters (Table 9)

Renal status correlated inversely with visual outcome and ocular surgery performed based on limited data collated from 13 patients (26 eyes). 9 eyes (35%) suffered adverse consequences, compared to 27% in the total study population. 13 eyes (50%) required cataract surgery, compared to 43% in the total study population. 7 eyes (28%) required vitrectomy, compared to 15% in the total study population. Within the known renal status group of those with overt renal disease, 8 of 20 eyes (40%) suffered adverse consequences, preferentially affecting patients in chronic renal failure ($p < 0.07$); 10 of 20 (50%) eyes required cataract surgery ($p < 0.99$); and 5 of 20 (25%) required vitrectomy ($p < 0.30$).

Discussion

Our findings delineate the remarkable long-term success of photocoagulation in patients identified with high-risk characteristics of proliferative diabetic retinopathy. While the study is a retrospective analysis, the consecutive selection of patients meeting defined criteria in a single large volume practice setting minimized methodological differences in patient selection. Parameters derived

from this report more closely resemble the actual outcome observed in a private office clinical practice setting in that eyes were not excluded from treatment by methods employed in double-blind randomized analyses such as the DRS. On the other hand, the higher proportion of type I and white patients in the present report is atypical of the USA population which has higher diabetes attack rates in African Americans and Hispanics than in whites.

Blankenship's [9] report in 1991 recounted the outcome at 15 years in patients who had been enrolled in the DRS, analyzing 51 eyes in the 51 patients reported, excluding eyes which had been randomized to no treatment, and not documenting subject parameters (i.e., age, diabetes type and duration). The number of eyes progressing to severe vision loss defined as $\leq 5/200$ in our study was 24 (24%), more than double the figure (10%) reported by Blankenship [9], and more than double the figure (10%) reported by Favard et al. [10] after 16 years, but slightly higher than the figure of 17.5% reported in the DRS [8], which involved only 6 years of follow-up. These differences in outcome may reflect: (a) the smaller number of eyes (51 and 40, respectively) followed for 15 or more years; (b) differences in statistical methods; (c) inclusion of patients in our study who died, but nevertheless had 15 or more years of follow-up; (d) inclusion of older subjects (mean age = 63) who were more likely to regress late and have a worse visual outcome than younger patients. Furthermore, observed differences in visual outcome may be attributed to the presence of less severe disease in surviving patients. Consequently, healthier patients would have a better visual outcome, a conclusion also reached by Blankenship [9].

Analysis of our data delineate potentially treatable and untreatable complications of diabetic retinopathy. Notably, 9% or one-third the number of eyes suffering adverse consequences, developed macular ischemia that is not currently reversible. Secondly, persistence of neovascularization and tractional retinal detachment, accounting for two-thirds of eyes affected by adverse consequences, would probably have benefited from early vitrectomy given the synergistic deleterious effect of the vitreous on initiation, persistence, and progression of fibrovascular proliferation [13, 14, 15]. Although prior reports document a favorable outcome of diabetic eyes treated with early pan-retinal photocoagulation as well as the benefits of augmentation treatment [16, 17, 18], persistence or subsequent development of microscopic neovascular foci that remain subclinical until a catastrophic event occurs, may become evident only after many years of follow-up [19] (Table 5 – 17% of eyes with late regression of neovascularization progressed to VA $< 4/200$ compared to 7% in eyes with early regression). This reality further underscores the need for aggressive mangement as suggested by Favard et al. [10], though its long-term effects remain to be substantiated.

Extrapolation of outcome data from a private practice which is predominantly white and middle-class to the larger population of diabetic patients may be inappropriate. Poverty, overcrowded clinics, and absence of social support

may detract from potential benefit of laser photocoagulation in black and hispanic inner city diabetic patients.

What is particularly noteworthy is that 96% of our patients had a long-term level of visual function in at least one eye sufficient to ambulate permitting relative self-sufficiency. Such quality of life issues are even more compelling as treatment of underlying systemic pathophysiology continues to advance, consequently improving survival. Early vitrectomy, better glycemic control, more efficient dialysis methodology, advances in renal and pancreatic transplantation, and a better understanding of microvascular pathophysiology offer patients an even brighter prospect of enjoying the highest quality of life.

In summary, we present the longest follow-up series after photocoagulation for diabetic retinopathy yet reported, permitting extrapolation to treatment planning projections. Our findings describe realistic visual outcome which may be applied to the private practice setting.

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Dedication

This manuscript is dedicated to Mrs. Mildred Barry Friedman, whose courage, wisdom, and compassion were incessantly imparted to family, friends, medical staff, and especially to fellow patients. Barry will be terribly missed by us all.

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11. A new hypothesis on mechanisms of retinal vascular permeability in diabetes

THOMAS W. GARDNER, ERICH LIETH, DAVID A. ANTONETTI & ALISTAIR J. BARBER

Editors' Comment:

Increased microvascular permeability, a characteristic abnormality in diabetes, is thought to result in microalbuminuria. In this report, Gardner and associates provide evidence that retinal hemorrhages, lipid exudate deposition, and macular edema result from aberrant production of vasoactive agents. Candidate vasoactive factors include histamine and vascular endothelial growth factor/vascular permeability factor (VEGF/VPF). Either or both of these substances may be released in excessive amounts after diabetes induced damage to glial cells that support the metabolism of neurons and blood vessels. VEGF/VPF is indicated as having substantive involvement in development of proliferative diabetic retinopathy. Both histamine and VEGF/VPF increase permeability to sodium fluorescein after injection into the vitreous cavity of normal rats. The authors theorize that vasoactive agents may regulate permeability by modifying cellular tight junction proteins of which ZO-1 has been shown to be regulated by histamine. Further, VEGF reduces the amount of occludin, another seven junctional proteins that spans plasma membranes serving as a transmembrane protein permitting cell-to-cell interaction through tight junctions. Diabetic retinopathy may be viewed as a consequence of disturbed cellular metabolism producing vasoactive factors by neurons and/or glia that impact on tight junction proteins. Treatments testing this hypothesis have been designed: a randomized trial of the histamine H_1 antagonist, astemizole, to determine whether blocking the effect of histamine will reduce macular edema has begun. As specific antagonists are synthesized, other studios to determine the benefit of interrupting the action of vasodilators on tight junction proteins can be anticipated.

Introduction

The diabetic renal–retinal syndrome is characterized by functional and structural changes in the kidney and retina, respectively. Functional changes include increased permeability of the renal and retinal microcirculations, as exhibited clinically by proteinuria and macular edema, respectively. Common structural lesions include basement membrane thickening in both circulations. These similarities have long raised suspicions of homologous pathogenic features, but no specific common mechanism has yet been discovered. In this paper we consider how diabetes alters the structure and function of endothelial cell tight

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junction proteins by releasing vasoactive agents from the retina. Understanding the cellular and molecular mechanisms of vascular leakage in the retina may provide insights into the generalized mechanism of edema formation in diabetes.

Tissue edema in diabetes

Increased microvascular permeability has long been recognized as a characteristic feature of diabetes. This increased permeability is manifested in the kidneys first as microalbuminuria and may progress to overt proteinuria. The retina initially has small hemorrhages and lipid exudate deposition and may progress to clinically significant macular edema. Microalbuminuria and macular edema occur in 25–30% of patients with diabetes [1, 2] and often develop concurrently, which suggests possible common mechanisms. What cellular and molecular event(s) could lead to increased permeability, and impairment of both renal and visual function? Before considering specific diabetes related permeability changes, it should be born in mind that abnormal vascular permeability is a common response of many tissues to a variety of insults. For example: trauma, allergic reactions, tumors, and infections all induce edema, and in fact, the classic signs of inflammation – edema (*tumor*), erythema (*rubor*) and warmth (*calor*) – all represent increased tissue permeability and blood flow. Diabetic nephropathy and retinopathy both exhibit increased permeability and blood flow as early manifestations of their respective tissue damage [3, 4]. The changes in tissue permeability associated with diabetes have been attributed directly to increased blood glucose [5]. However, recent evidence reveals an exciting novel possibility – that diabetes causes aberrent production of vasoactive agents which act on the respective circulations to increase permeability and blood flow (Figure 1).

What then are potential alterations in the retina which might increase retinal vascular permeability? It is important to remember that the retina is normally transparent, so ophthalmoscopic and fluorescein angiographic examination of the retina reveals only the vascular elements. The retina is an integral part of the central nervous system, and is comprised of neurons (photoreceptors, horizontal cells, amacrine cells, bipolar cells, and ganglion cells), and glia (astrocytes and Muller cells). The neurons sense light, and generate and transmit electrical impulses to the brain. The glial cells support the metabolism of both neurons and blood vessels. Specifically, glial cells regulate metabolism of neuronal transmitters, such as glutamate, maintain ionic balance, particularly of sodium and potassium ions, and store glycogen [6]. In addition, astrocytes develop [7] and maintain the integrity of the blood–retinal barrier. Visual function depends on normal interactions between the neuronal, glial and vascular elements of the retina. Disruption of any of these elements may alter the others. It follows that neuronal injury must cause visual impairment, and that visual impairment must involve neuronal injury. Thus, although the ophthalmoscopic examination reveals vascular cell pathology in diabetes, it does not

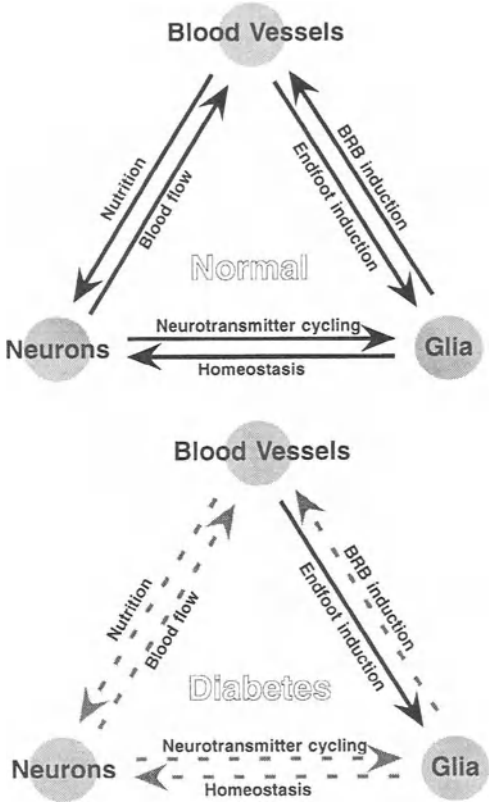

Fig. 1. Interactions between blood vessels, neurons and glial cells in the normal retina (A). These interactions are impaired in diabetes (B).

exclude the possibility of pathological changes to other elements of the retina. Indeed, it is likely that all these elements are affected.

Expression of vasoactive factors by the retina

Just as other tissues elaborate vasoactive factors in response to injury by increasing the permeability of their vascular systems [8], the retina probably reacts in a similar fashion. Histamine and vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) are commonly released in response to trauma, infections, tumors and allergic reactions [9]. There is substantial evidence that both of these potent factors are produced in the retina in diabetes and may contribute to the vascular lesions.

Histamine has long been known to be present in the retina of various mammalian species including humans [10, 11] although the specific source of

this histamine was not identified. Clearly mast cells are not found in the retina [10] although they reside in the choroid. Histamine H_1 receptors are present in the retina and on retinal blood vessels [12]. Hollis et al. observed that histamine metabolism was increased in experimental diabetes. First, the activity of histidine decarboxylase (HDC), the enzyme which synthesizes histamine from histidine, is significantly increased in retinas after just three weeks of experimental diabetes [13]. Insulin treatment reversed the increase in HDC activity, as did a specific inhibitor of histidine decarboxylase, α-hydrazino–histidine. We have observed recently increased HDC mRNA expression in diabetic rat retina by *in situ* hybridization [unpublished data]. The HDC mRNA is expressed by neurons of the inner and outer nuclear layers and to a lesser degree by ganglion cells. Therefore, histamine production by the neural retina may be one mechanism by which the retina compensates for altered metabolism. At this point it is not clear how or why diabetes increases HDC expression and activity.

In addition, Hollis et al. demonstrated that histamine receptors contribute to retinal vascular permeability in experimental diabetes. Administration of an H_1 receptor antagonist diphenhydramine (Benadryl) or astemizole (Hismanal) reduced blood–retinal barrier (BRB) permeability to fluorescein isothiocyanate conjugated to bovine serum albumin (FITC-BSA) [14–16]. An H_2 receptor antagonist ranitidine (Zantac) likewise partially normalized BRB permeability in the presence of uncontrolled diabetes. In addition, histamine receptor antagonists significantly reduced retinal capillary basement membrane thickening in experimental diabetes [17]. Thus, histamine may participate in the disturbed retinal vascular autoregulation in diabetes and contribute to macular edema, as proposed by Bresnick [18].

In addition to histamine, VEGF/VPF has been identified as a major element in the development of proliferative diabetic retinopathy. Aiello et al. observed an increase in VEGF/VPF content in the ocular fluid of patients with proliferative retinopathies [19]. VEGF/VPF is rapidly broken down by serum proteases, so the production of VEGF/VPF probably arises from the ocular tissues. Indeed, several investigators have identified increased VEGF/VPF protein and mRNA expression in the retinas of humans with diabetic retinopathy [20–22] and in rats with experimental diabetes. VEGF/VPF increases after as little as 6 weeks of diabetes in the Goto-Kakizaki rat, a model of Type II diabetes [23]. Murata et al. [20] have shown VEGF/VPF expression in rats after 6 months by immunohistochemistry with co-localization for albumin permeation. Notably, the VEGF/VPF was expressed by neurons and glial cells as well as by endothelial cells in diabetes. While VEGF/VPF expression is clearly regulated by hypoxia, its production increases prior to the onset of significant capillary closure. This strongly suggests that VEGF/VPF is expressed in response to the metabolic state of diabetes. Aiello and colleagues have also demonstrated recently that both VEGF/VPF and histamine, when injected into the vitreous cavity of normal rats, increase permeability to sodium fluorescein

[24]. A specific inhibitor of protein kinase C (LY333531) inhibits this permeability change. Taken together, these observations strongly suggest that both histamine and VEGF are products of the retinal parenchyma in diabetes and may alter retinal vascular function. In order to understand why diabetes should cause the retinal parenchyma to induce changes in vascular permeability we must first examine more closely the specialized interactions between the parechymal elements of the retina and its vasculature.

Structure of the blood–retinal barrier

The blood retinal barrier (BRB) is analogous to the blood–brain barrier. The vascular systems of these two neural tissues share common neuroectodermal embryologic origins, and are structurally and functionally similar [25, 26]. They both provide for selective amino acid and glucose uptake, fluid movement, and protect neurons and glia from circulating antigens. The retinal endothelial cells are characterized by an absence of fenestrae, a paucity of vesicles, and well organized tight junctions as shown by electron microscopy. In comparison vessels from tissues that lack a barrier, such as skin, lack these features. The blood–brain barrier is induced by glial cells processes wrapped around brain blood vessels and which secrete solube *trans*-acting factor(s) [27]. *In vitro* models of the blood–brain barrier have shown that astrocytes increase expression of tight junction proteins and reduce permeability [28].

In the retina, Muller cells and astrocytes perform an analogous function to astrocytes in the brain. We have demonstrated recently that rat brain astrocytes induce increased expression of the tight junction protein ZO-1 and increase transendothelial electrical resistance [29]. If glial cells are responsible for maintaining normal barrier function then it follows that glial cell dysfunction is likely to impair BRB integrity and/or contribute to neuronal dysfunction. Indeed, glial cells accumulate glycogen in diabetes [], which suggests that glial metabolism is impaired. Lorenzi [30] and Lieth [31] have recently observed evidence of glial reactivity in humans and in rat models of diabetic retinopathy, as assessed by increased expression of glial fibrillary acidic protein (GFAP) in the Muller cells of diabetic subjects and rats with diabetes. In humans, these findings are present in eyes with less than 10 years of diabetes and with nonproliferative retinopathy. In rats, GFAP expression increases after just 3 months of diabetes. In our laboratory this time point coincides with increased permeability to FITC-BSA and sodium fluorescein. In addition to effects on blood vessels, altered glial function would likely impact neuronal activity. Astrocytes and Muller cells have the exclusive property of glutamate metabolism in the retina and contain the requisite enzymes for conversion of glutamate to glutamine, notably glutamine synthetase and pyruvate carboxylase [7]. Reduction in available glutamate would reduce synaptic transmission whereas increased glutamate could contribute to excitotoxic neuronal cell death [32]. Indeed Lieth et al. have demonstrated impaired conversion of glutamate to

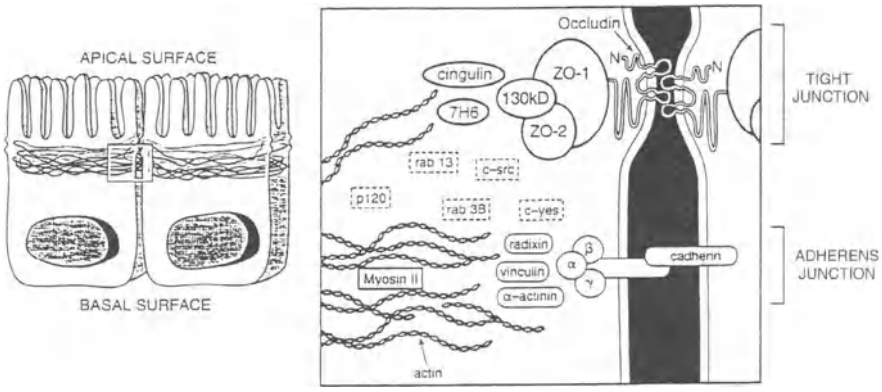

Fig. 2. Schematic representation tight junction proteins (from Anderson and van Itallie [33]).

glutamine in the retinas of rats with 3 months of experimental diabetes [submitted]. Thus, impaired retinal metabolism in diabetes may be reflected not only by increased expression of vasoactive agents, but also disturbance of neurotransmitter homeostasis. Reciprocal interactions between the three cellular elements of retinal tissue are clearly essential for proper function.

Tight junction proteins

Tight junctions have long been recognized as a morphologic entity in endothelial and epithelial cells, but the understanding of the molecular composition of these structures has emerged just within the last decade. Tight junctions are now recognized to represent a complex of at least 7 proteins in the peripheral cytoplasm and within the plasma membrane (Figure 2) [33]. ZO-1 was the first to be identified, characterized and cloned [34]. It is a 225 kDa phosphoprotein which resides just inside the plasma membrane in close physical proximity to other proteins of the junction, including ZO-2, p130, cingulin and the 7H6 antigen. Occludin, a 65 kDa protein, is thought to span the plasma membrane [35] and serve as the transmembrane protein which forms the cell-to-cell interaction of tight junctions. ZO-1 and occludin bind at their respective C-terminal ends [36], and the innermost junctional proteins probably interact with the peripheral cytoskeleton. In this way, the cytoskeleton and tight junction may act synchronously to regulate permeability.

The mechanism by which vasoactive agents regulate permeability has been the subject of much investigation and previous reports of observed changes in intracellular calcium F-actin content and distribution, vimentin reorganization and accumulation of inositol phosphate [37]. We have recently demonstrated that histamine specifically regulates ZO-1 tight junction expression by both histamine H_1 and H_2 receptors [38]. The EC_{50} for this effect is 3.3 nM in cultures of retinal endothelial cells. By contrast evidence has accumulated that

VEGF/VPF increases permeability by vesicular transport [39]. We have now observed (unpublished data) that VEGF reduces occludin and ZO-1 content and increases ZO-1 tyrosine phosphorylation, both in endothelial cells and retinas of normal rats. Thus, the vasoactive factors which are well recognized to increase permeability may do so by reducing tight junction protein expression. This mechanism would provide for a highly regulated and sensitive means of permeability control. It does not exclude the possibility of vesicular transport, but provides a possible molecular explanation which may allow for new routes of therapeutic control. The question that arises as to whether or not tight junction protein expression is altered in diabetes. It should be pointed out that neither ZO-1 nor occludin have been proven to be limiting in tight junction formation. However, the literature reveals a consistent correlation of a change in occludin content coincident with a change in permeability. Occludin content decreases when cells are exposed to a peptide to the extracellular domain of occludin and decrease transepithelial electrical resistance [40]. Thus, occludin, and probably ZO-1 content correlate with barrier permeability. Interestingly, 3 months of experimental diabetes causes a significant decrease in occludin content [submitted]. As pointed out above, this is the same point in time of experimental diabetes at which vasoactive factors are increased, glial reactivity is occurs, glutamate metabolism is altered and permeability to FITC-BSA is increased.

It should also be pointed out that the neural retina also releases factors that may oppose the vasodilatory response. Chakrabarti and Sima [41] have recently described increased immunoreactivity for endothelin-1 and endothelin-3 in the eyes of rats with diabetes. These proteins are also expressed by neuronal and glial cells. This finding suggests that the retina attempts to compensate for the vasodilation by elaborating vasoconstrictor substances.

Why does the retina respond to diabetes with the production of vasoactive agents and increasing vascular permeability and blood flow? The retina may be trying to compensate for some other defect by increasing the delivery of nutrient substrates to the retina. The clinical manifestations that are recognized as diabetic retinopathy could then, in fact, be viewed as the consequence of a compensatory mechanism gone awry. Various aspects of glucose toxicity ranging from protein kinase C activation, polyol pathway activation or non-enzymatic glycation have been proposed as explanations to account for diabetic retinopathy. However, it must be kept in mind that insulin deficiency is the primary metabolic defect in diabetes. Neurons are, in fact, sensitive to insulin and insulin is an important survival factor for retinal ganglion cells in culture [42]. Also, insulin increases glutamine synthetase in glial cells [43]. Taken together, these observations suggest the possibility that the insulin signalling deficiency or insulin unresponsiveness of diabetes *per se* may contribute to altered metabolism of the retinal neurons and/or glia, and the increased permeability and blood flow may be a pathogenic mechanism by which the vasculature responds. Because the neurosensory retina is normally transparent, it is

not possible to assess its function by routine clinical observation. Nevertheless, patients with diabetes, but without any observable retinopathy, clearly have reductions in the oscillatory potentials on the electroretinogram, reduced contrast sensitivity and altered blue-green color perception [44]. These findings strongly suggest that alterations in the neural retinal metabolism indeed occur prior to the onset of detectable vascular lesions. Together, these observations strongly suggest that diabetic retinopathy may be a consequence of a sequence of compensatory mechanisms which begins with the disturbed cellular metabolism of diabetes, followed by the production of vasoactive factors by neurons and/or glia, and the impact of these vasoactive factors on tight junction proteins. These data should then provide understanding of the mechanism by which new therapeutic avenues for the prevention and treatment of diabetic retinopathy are developed.

Microangiopathy as classically defined begins after prolonged diabetes, when the cells of the retina can no longer compensate for their altered metabolism. Could the concept of tissue regulation of vascular permeability and flow as demonstrated in the retina also occur in the kidney? Markle et al. demonstrated increased renal histamine production in the diabetic rat [45]. Thus, renal production of histamine could also contribute to glomerular permeability. In fact, it was demonstrated nearly 20 years ago that histamine and histamine receptor antagonists regulate microcirculation in the rat [46]. More recently VEGF protein and receptor expression has been demonstrated in renal glomeruli, mesangial cells and medulla [47, 48]. Although no evidence has yet been published that renal VEGF expression increases in diabetes, this is an intriguing possibility that could provide further evidence for similarities between the reaction of the retina and kidney to diabetes.

Clinical considerations

The currently proven therapies for diabetic retinopathy include intensive metabolic control [49] and photocoagulation [50, 51]. Intensive metabolic control is achieved by the administration of insulin and the increased availability of insulin may allow retinal neuronal cells to function more normally, and reduce the production of vasoactive compounds which act on tight junctions. While the most dramatic affect of intensive insulin therapy is in patients who have no retinopathy at the initiation of their intensive control, photocoagulation is used for patients who have advanced vascular lesions. Whether photocoagulation decreases the metabolic demand [52] and/or increases oxygen delivery [53] the effect of photocoagulation is nevertheless on the neurosensory retina. Aiello et al. have shown that photocoagulation reduces production of the VEGF/VPF [19]. In the same manner, photocoagulation may also have similar effects on the elaboration of vasoactive compounds and reduce the impact on tight junction proteins. Thus both intensive insulin therapy and photocoagulation may, in fact, be acting on retinal neurons and glial cells. This suggests

that therapy aimed at correcting abnormalities in the interactions between neurons, glial cells and vascular cells could prevent visual impairment from diabetes. To this end a randomized clinical trial of the histamine H_1 receptor antagonist, astemizole (Hismanal, Janssen) is in progress. The goal of this study is to determine if blocking the effect of histamine (presumably on vascular tight junctions) can reduce macular edema. If so, it may give impetus to control of vasoregulation in the kidney. Ultimately, however, correction of the underlying diabetic state itself, including deficient insulin action, will be needed to prevent the secondary affects on retinal and renal metabolism that consequently lead to diabetic retinopathy and nephropathy.

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12. Angiogenesis in diabetic retinopathy: a history of accomplishment, discovery and promise

LLOYD PAUL AIELLO

Editors' Comment:

Current theory holds that in diabetic retinopathy, retinal hypoxia stimulates production or increased activity by the retina of angiogenic growth factors. Angiogenesis resulting in vitreous hemorrhage and retinal traction are components of diabetic retinopathy that lead to blindness in one-half of those untreated within five years. Identification of specific angiogenesis promoting substances will permit antiangiogenic treatment strategies. Current antiangiogenic therapies for proliferative diabetic retinopathy require destruction of viable retinal tissue by laser photocoagulation or localized cryotherapy. Vascular endothelial growth factor/vasopermeability factor (VEGF) produced by many ocular cell types including retinal pigment epithelial cells is the candidate compound that best fits the model of hypoxemia-induced angiogenesis. Elevated concentrations of VEGF have been found in both vitreous and aqueous fluid samples of patients with active proliferative diabetic retinopathy but low in control patients and in diabetic subjects without retinopathy. A fascinating and promising approach, based on altering the molecular biology of retinal cells, to blunting proliferative diabetic retinopathy is described. An extraordinary 17 different angiogenesis inhibitors manufactured by over 120 biotechnology and pharmaceutical companies are in clinical trial. The race to block retinal angiogenesis by pharmacologic intervention will benefit diabetic individuals no matter who wins.

Introduction

Diabetes mellitus represents a major worldwide medical problem affecting more than 16 million Americans, only approximately half of whom are aware that they have the disease [1]. The numerous systemic complications associated with prolonged diabetes produce considerable morbidity, primarily as a result of the three classic microvascular complications (retinopathy, nephropathy and neuropathy) as well as increased cardiovascular disease. Indeed, individuals with diabetes mellitus experience renal, ocular and neurologic complications at a rate 17×, 25× and 50× higher than that of the general population, respectively [1]. The estimated societal costs associated with diabetes mellitus exceed $20 billion dollars annually [2].

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One of the most feared complications of diabetes mellitus is diabetic retinopathy. Although diabetic retinopathy is characterized by numerous histologic, biochemical and hemodynamic changes in the retinal microvasculature, the majority of severe and irreversible visual loss occurs as a result of increased intraocular vascular permeability and the proliferation of new blood vessels within the eye. This uncontrolled angiogenesis commonly results in vitreous hemorrhage, due to the fragility of the newly proliferating vessels, and retinal traction and/or detachment due to progressive fibrosis of the vascular fronds. Without treatment, the risk of legal blindness within 5 years exceeds 50%.

Fortunately, with advances in the use of laser photocoagulation achieved during the past 2–3 decades, this risk can be reduced to only 4% at 5 years if therapy is delivered in a timely and appropriate manner [3]. Nevertheless, due to suboptimal identification of patients at risk and instances where the disease progresses despite appropriate therapy, diabetic retinopathy remains the leading cause of new onset visual loss among working age Americans [4, 5]. In addition, there are significant side effects associated with laser photocoagulation since treatment destroys areas of viable retina in hopes of preserving better vision than would be obtained without treatment. Thus, it is apparent that therapies which inhibit the angiogenesis associated with diabetic retinopathy without inducing additional retinal damage would be medically desirable. Based on the detailed clinical findings associated with diabetic retinopathy and similar disorders, it has been suggested for nearly one half century that the underlying etiology for intraocular angiogenesis may involve the elaboration of growth factors by the retina. Although the actual molecules responsible and their mechanisms of action have remained incompletely understood for many years, a number of recent developments have significantly improved our understanding of the major angiogenic factors involved. These findings have suggested novel approaches to the inhibition of angiogenesis in diabetic retinopathy which promise to circumvent the destructive nature inherent with current laser photocoagulation therapies. Several anti-angiogenic approaches have already been used successfully to reduce intraocular neovascularization in animals and some compounds are in or nearing clinical trials. This manuscript briefly reviews the current accomplishments, new discoveries and theoretical novel therapies for angiogenesis associated with diabetic retinopathy.

Diabetic retinopathy

One of the most devastating microvascular complications of diabetes mellitus is diabetic retinopathy. The early stages of diabetic retinopathy are characterized by histologic changes (pericyte loss and basement membrane thickening), hemodynamic alterations (changes in retinal blood flow and areas of capillary nonperfusion), vascular abnormalities (micraneurysms, intraretinal microvascular abnormalities and venous beading) and reduced vascular integrity (increased permeability and retinal hemorrhages). Some degree of retino-

pathy occurs in nearly all patients with diabetes of 20 or more years duration [6]. The latter stages of diabetic retinopathy are characterized by the complications which induce visual impairment: primarily diabetic macular edema and proliferative diabetic retinopathy [7]. In proliferative diabetic retinopathy newly developing vessels commonly arise from the retina but may also be found on the iris and the trabecular meshwork. These new vessels are fragile and prone to breakage, resulting in vitreous hemorrhage, and often undergo fibrosis and contraction inducing distortion and detachment of the retina. Severe visual loss can result. If vessels proliferate on the iris or trabecular meshwork, the normal outflow of the aqueous fluid may be impaired and neovascular glaucoma and permanent optic nerve damage may result. All vessels undergoing angiogenesis are highly permeable and my leak serum components into the retina with subsequent edema and lipid deposition. Diabetic macular edema, however, may arise even in the absence of active angiogenesis. The most sight threatening stages of proliferative diabetic retinopathy (PDR) and macular edema are called high risk PDR and clinically significant macular edema (CSME), respectively. There are two distinct forms of diabetes mellitus: Type 1, juvenile onset or insulin dependent diabetes mellitus (IDDM); and Type 2, adult onset or non insulin dependent diabetes mellitus (NIDDM). There is a higher risk of severe ocular complications in Type 1 diabetes [8]. Approximately 25% of Type 1 patients have retinopathy after 5 years, increasing to 60% and 80% after 10 and 15 years, respectively. However, since there are more adult-onset cases than juvenile-onset cases, Type 2 disease accounts for a higher proportion of patients with visual loss.

Despite significant improvements over the past quarter of a century in our understanding and treatment of the ocular complications of diabetes mellitus, patients with diabetes have 25 times the risk of visual loss compared with the general population [1] and diabetic retinopathy remains the leading cause of new onset blindness in Americans age 20–74. In the United States alone, there are an estimated 700 000 persons with proliferative diabetic retinopathy (PDR), 130 000 with high risk PDR, 500 000 with macular edema and 325 000 with clinically significant macular edema (CSME) [9–13]. An estimated 63 000 cases of PDR, 29 000 high risk PDR, 80 000 macular edema, 56 000 CSME and 5000 new cases of legal blindness occur each year as a result of diabetic retinopathy [9, 10]. This accounts for 12% of all new cases of blindness [4, 5], annual losses of over 400 000 person-years-sight and federal government expenditures of over $620 million per year [11, 12]. Thus, the management and treatment of diabetic retinopathy remains one of medicine's most significant challenges.

Fortunately, there are extensive data available on most aspects of diabetic retinopathy from numerous epidemiological studies and clinical trials which provide a solid basis for current evaluation and management guidelines and which have detailed the effectiveness of current therapeutic approaches. Four national multi-center clinical trials have provided especially important information with regard to current therapy: the Diabetic Retinopathy Study (DRS)

[14–16], the Early Treatment Diabetic Retinopathy Study (ETDRS) [17, 18], the Diabetic Retinopathy Vitrectomy Study (DRVS) [19, 20] and the Diabetes Complications and Control Trial (DCCT) [21–23]. Individuals interested in the specific methods, outcomes or recommendations with regard to the current treatment of diabetic retinopathy should consult this source material.

Current antiangiogenic therapies

Current antiangiogenic therapies for proliferative diabetic retinopathy entail destruction of portions of the retina using either laser photocoagulation or, infrequently, localized cryotherapy. Both approaches destroy areas of viable retina, but they often induce regression of the neovascularization and thus preserve better vision than that obtained in the absence of such treatment. Without laser photocoagulation, eyes with high risk PDR have a 28% risk of severe visual loss within 2 years and a 50% risk at 5 years [15]. Severe visual loss is defined as best corrected acuity of 5/200 or worse on two consecutive visits 4 months apart and is significantly worse that the minimal level of visual loss defined as legal blindness (20/200). If laser photocoagulation therapy is delivered in a timely and appropriate manner, the risk of severe visual loss can be reduced to only 4% after 5 years [3]. Thus, with ideal delivery of medical and ophthalmologic care, greater than 90% of visual loss resulting from diabetic retinopathy can be prevented [3]. In addition, intensive insulin therapy resulting in improved glycemic control has now been conclusively demonstrated to delay the onset of any retinopathy and to slow the progression of retinopathy once it occurs [21–23]. Laser photocoagulation also has the benefits of being an outpatient procedure and usually associated with little pain.

Although laser photocoagulation can be remarkably effective [24, 25] there are significant associated side effects (ie. decreased peripheral and night vision), complications (i.e. lenticular or foveal burns and vitreous hemorrhage) and some patients progress to visual loss despite timely and appropriate treatment. In addition, laser photocoagulation is less effective at preventing visual loss from diabetic macular edema as compared with proliferative diabetic retinopathy, despite implications that both conditions may be induced by similar growth factors. Thus, substantial efforts have been directed at developing non-destructive therapies with reduced side-effects.

The growth factor theory of angiogenesis

Intraocular neovascularization is characteristic of only certain clinical conditions, most notably a diverse group of disorders known as ischemic retinopathies. Ischemic retinopathies, such as diabetic retinopathy, are characterized by areas of poor perfusion to the retina and subsequent development of intraocular angiogenesis [26, 27]. It has been appreciated for many years that retinal

neovascularization commonly arises at the border of perfused and nonperfused zones and is always associated with increased vascular permeability. The worse the retinal perfusion, the greater the risk of neovascularization in branch retinal vein occlusions [28] and lens removal during cataract surgery in patients with diabetic retinopathy is associated with an increased risk of iris neovascularization [29].

These observations supported the hypothesis first proposed by Dr I. Michaelson in 1948 that a factor or factors released by the retina was responsible for inducing intraocular angiogenesis [30]. The model was later expanded by Ashton and others to suggest that hypoxia of the retina probably stimulated the production or increased the activity of the angiogenic growth factors [31]. This model placed several restrictions on the attributes that such mediating factors should posses.

If a single molecule were responsible for all the clinical findings, it would most likely be synthesized in retina, induced by hypoxia, secreted and diffusable within the eye, and capable of binding endothelial cell-specific receptors that would subsequently stimulate blood vessel growth. Clinically, one should observe elevated intraocular concentrations of this factor during active proliferative stages of diabetic retinopathy, but low levels where no active proliferation exists. In addition, intraocular levels would be expected to decrease following laser photocoagulation which results in regression of angiogenesis.

Identification of potential mediators of angiogenesis

One of the most important ramifications of the growth factor model of angiogenesis described above is that if such molecules are required for the proliferation of vessels, then inhibition of the growth factor itself should suppress angiogenesis and prevent the subsequent complications which produce visual loss. This potential for a novel and theoretically non destructive treatment has prompted a wide variety of studies aimed at determining which factors may be mediating the angiogenesis of diabetic retinopathy. At present, the molecules which have received most attention include basic fibroblast growth factor (bFGF), growth hormone, insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor/vasopermeability factor (VEGF). Although each of these compounds are capable of stimulating endothelial cell growth, only VEGF has characteristics which thus far fully correlate with those predicted by the model. However, it is unlikely that a process as complex as angiogenesis is regulated by a single substance and each of the molecules discussed below are likely to contribute in part to the clinical neovascular response.

Basic fibroblast growth factor is a protein capable of stimulating endothelial cell proliferation, migration and vasculogenesis [32, 33]. However, bFGF is tightly associated with the extracellular matrix [34, 35] and is not secreted from cells since it lacks a consensus secretion signal peptide [36]. It is unlikely

that a nonsecreted protein can efficiently stimulate angiogenesis at sites distant from its area of production as is frequently observed clinically in diabetic retinopathy. Although a variety of alternative pathways for bFGF release have been postulated [37, 38] and bFGF exists in the retina [39], no causal relationship with proliferative diabetic retinopathy has been identified. Basic FGF can be absent from ocular fluids of individuals with proliferative diabetic retinopathy or present when no proliferation exists, raising further questions as to whether bFGF is the principal factor mediating angiogenesis in diabetic retinopathy [40].

A role for growth hormone in the mediation of proliferative diabetic retinopathy was suggested in 1953 after proliferative diabetic retinopathy was observed to regress following infarction of the pituitary during pregnancy [41, 42]. Subsequently, hypophysectomy was advocated as a treatment of diabetic retinopathy with some success [42–44]. However, the severe, life-threatening brittle diabetes which developed after hypophysectomy prompted rapid abandonment of this therapy once laser photocoagulation was introduced. The effects of growth hormone are mediated by the insulin-like growth factors (IGF-1 and IGF-2) [45]. IGF-1 can stimulate migration and proliferation of retinal cells in culture, and induce neovascularization of the rat cornea [46]. Although intraocular neovascularization has not been demonstrated in response to IGF-1, retinal vascular tortuosity, hemorrhage, hyperemia and increased fluorescein leakage occurs in animals. However, these findings are only present at IGF-1 concentrations exceeding 10 000 times those measured during clinical neovascularization [47].

Clinically, one study demonstrated a mean increase in serum IGF-1 levels in patients with proliferative diabetic retinopathy [48] and another population based study has noted a correlation of increased frequency of proliferative diabetic retinopathy with elevated intravitreal IGF-1 levels [49].

However, serum IGF-1 levels and retinopathy status has been evaluated in a comprehensive study of over 1000 well characterized patients at both baseline and after 6 years follow-up [50]. Serum IGF-1 was not associated with the 6 year incidence or progression of diabetic retinopathy even when the diabetic patients were subdivided into those with older or younger onset or use of insulin. Finally, in clinical disorders such as acromegaly, where growth hormone and IGF-1 levels are dramatically elevated, retinal proliferation is rarely observed. These findings suggest that IGF-1 is not likely to be a primary mediator of angiogenesis in diabetic retinopathy.

A recent study in transgenic mice expressing a growth hormone antagonist demonstrated a modestly reduced (34%) capacity of the animals to produce retinal neovascularization under hypoxic retinal conditions [51]. IGF-1 addition alone or transgenic mice expressing a growth hormone agonist did not increase retinal neovascularization. Thus, growth hormone and IGF-1 may play a permissive role in neovascularization, rather than serving as the primary angiogenic stimulus.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), also known as vasopermeability factor(VPF), is a molecule which has received considerable attention over the past few years since its attributes closely match those predicted for a major mediator of angiogenesis in diabetic retinopathy. Vascular endothelial growth factor is a highly conserved glycoprotein [52] that has both vasopermeability [53] and angiogenic activity [54, 55]. VEGF is secreted from the cell and certain isoforms are freely diffusible within the eye. VEGF is 50 000 times more potent at increasing dermal microvascular permeability than is histamine [56]. Interestingly, VEGF and bFGF act synergistically, producing a far greater angiogenic stimulus together than that observed with either factor alone [57].

VEGF primarily stimulates growth of endothelial cells [58, 59] and it can induce angiogenesis in the cornea and chorioallantioc membrane [55]. However, growth stimulation of retinal pigment epithelial cells also occurs *in vitro* [60]. Both retinal endothelial cells and retinal pericytes possess VEGF receptors [61–63]. There are two types of high affinity VEGF receptors called fetal liver kinase 1 (Flk-1, also known as KDR) and fms-like tyrosine kinase (Flt). Retinal endothelial cells possess more high-affinity VEGF receptors per cell than other cell types reported to date [64].

VEGF is produced by numerous ocular cell types including retinal pigment epithelial cells, pericytes, endothelial cells, glial cells, Muller cells and ganglion cells [63, 65–67]. Expression of VEGF is greatly enhanced by hypoxia, as first demonstrated in viable tumor cells surrounding areas of necrotic tissue [68]. In ocular cells, hypoxia can increase VEGF mRNA levels up to 30 fold at oxygen concentrations consistent with retinal ischemia [63]. VEGF secreted from hypoxic retinal cells is capable of stimulating retinal endothelial cell growth in culture.

In a neonatal mouse model of ischemic proliferative retinopathy which closely approximates the proliferative stages of diabetic retinopathy both clinically and histologically [69], VEGF mRNA levels increase over 3-fold within twelve hours of relative retinal hypoxia and remain elevated for 17 days [70]. Subsequently, mRNA levels slowly decline as clinically apparent neovascularization regresses. VEGF mRNA and protein are most prevalent in the inner nuclear layer of the retina, the ganglion cell layer, cells with Muller-like morphology and in regions adjacent to retinal neovascularization. Similar findings are observed in cat and rat models of ischemic retinopathy [71, 72]. VEGF mRNA and protein concentrations also correlate with iris neovascularization in a monkey model of retinal vein occlusion [73].

Since VEGF is both an endothelial mitogen and a vascular permeability factor, it might also account for the increased vasopermeabilty characteristics of diabetic macular edema. Indeed, diabetic rats retinas [74] and human ocular melanoma specimens [75] exibit spatial correlation between VEGF and retinal vessel hyperpermeability. Recently, it has been directly demonstrated that

VEGF rapidly increases retinal vascular permeability over three fold in the rat through activation of the β-isoform of protein kinase C [76].

In a clinical evaluation of ocular fluid specimens obtained from patients undergoing intraocular surgery, 31 patients were without neovascular disorders while 143 samples were obtained from patients with diabetes. Of the samples from patients with diabetes, 8 had no retinopathy, 24 had nonproliferative retinopathy, 41 had quiescent proliferative retinopathy and 70 had proliferative diabetic retinopathy with active intraocular neovacularization [77]. VEGF concentrations were markedly elevated in both vitreous and aqueous of patients with active proliferative disease; however, VEGF levels were low in control patients, patients without retinopathy, or in patients with diabetes in whom the intraocular neovascularization was no longer active. In all six patients where intraocular fluid samples were obtained both before and after successful laser photocoagulation treatment of active proliferative diabetic retinopathy, the intraocular concentrations of VEGF were reduced by an average of 75%. Similar findings were observed in another study where VEGF concentrations in 8 eyes of patients with proliferative diabetic retinopathy were statistically higher than that observed in 12 patients without associated neovascularization [78]. In addition, of 8 different growth factors studied, only VEGF has been consistently observed in all samples from patients with diabetes [79]. Histopathologic studies have demonstrated that vascular endothelial growth factor is present within the retina of human eyes and is elevated even at early stages of diabetic retinopathy [80, 81]. VEGF also increases retinal blood flow as has been clinically observed in patients with diabetes mellitus [82].

Growth factor inhibitors

If VEGF is a critical mediator of angiogenesis and permeability in diabetic retinopathy, then inhibition of its activity should suppress new vessel growth and/or vascular leakage. Several types of molecules which inhibit the action of VEGF have been designed or are in the process of development. Efforts are directed towards preventing VEGF production (adenosine receptor antagonists, antisense oligodeoxynucleotides), inactivation of VEGF activity itself (neutralizing antiVEGF antibodies, VEGF receptor chimeric proteins), inhibition of VEGF receptor expression or activation (small molecule inhibitors, antisense oligodeoxynucleotides, ribozymes) or inhibition of intracellular signal transduction pathways (protein kinase C inhibitors).

The essential role of VEGF in mediating intraocular angiogenesis has been established in 2 different animal models of ischemia-induced intraocular neovascularization and in a rat model of vascular permeability using 4 different inhibitory agents. VEGF receptor chimeric proteins [83] and neutralizing antiVEGF antibodies [84] both bind to VEGF and prevent it from stimulating its receptor on endothelial cells. The chimeric receptor proteins are capable of preventing both VEGF and hypoxia induced retinal endothelial cell growth

in vitro. They suppress ischemia-induced retinal neovascularization in the mouse in a dose-dependent manner when injected into the vitreous prior to the development of retinal neovascularization. The two different chimeric receptor proteins tested reduced histologically evident neovascularization in 25 of 25 (100%) and 21 of 22 (95%) animals studied, respectively. Up to 77% of the neovascularization was inhibited in these animals with a mean suppression of approximately 50%. Similar findings were observed using VEGF neutralizing antibodies in the primate model of iris neovascularization due to retinal vein occlusion where intravitreal injections given every other day for 14 days resulted in inhibition of iris neovascularization in the 9 animals studied [84].

Antisense phosphorothioate oligodeoxynucleotides have been utilized to prevent production of VEGF in the same murine model of retinal neovascularization discussed above [85]. Two different antisense molecules reduced new blood vessel growth 25% and 31% in 19 of 25 (76%) and 6 of 9 (67%) animals studied as compared with control molecules. The antisense therapy resulted in a 40–66% reduction of VEGF protein in the retina.

In these studies, inhibition of angiogenesis has been substantial, but incomplete. This suggests that factors other than VEGF may also be involved in the neovascularization process. It is probable that other growth factors, such as those discussed earlier, play a role in mediating a biological process as complex as ocular angiogenesis. However, it is not certain that maximal effectiveness of the inhibitors was obtained in any of these studies. Nevertheless, VEGF appears to account for at least half of the neovascular response in ischemic retinopathies.

Other inhibitors have demonstrated various levels of effectiveness in reducing VEGF mediated cell growth in culture. Recent studies addressing the mechanisms of hypoxic induction of VEGF and intracellular signal transduction following VEGF stimulation have suggested numerous targets for intervention in these processes which should subsequently suppress angiogenesis. Elevated adenosine concentrations which occur under hypoxic conditions can increase VEGF expression in both retinal endothelial cells and pericytes through activation of adenosine A2 receptors and subsequent stimulation of adenylate cyclase and protein kinase A [86]. Adenosine A2 receptor antagonists prevent hypoxia from stimulating VEGF expression in these cultured cells.

In retinal and aortic endothelial cells, VEGF binding to its receptor increases tyrosine phosphorylation of PLCγ and PI-3 kinase [87–89], resulting in increased diacylglycerol levels and rapid activation of PKC α, βII and δ isoforms [76, 89]. Interestingly, both VEGF's growth stimulation of endothelial cells and its effect on increasing retinal vascular permeability appear to be specifically mediated by the β-isoform of PKC 76, 89]. The recent development of PKC β-isoform specific inhibitors which can achieve therapeutic serum concentrations following oral administration [90] raise the theoretical possibility that orally administered systemic medications might be developed to suppress VEGF-mediated angiogenesis in diabetic retinopathy. Indeed, when the PKC β-isoform specific inhibitor is given orally to rats for only one week,

the ability of VEGF to increase retinal vascular permeability is almost completely suppressed [76].

Inhibitors are also being studied which target aspects of the angiogenic response which occur subsequent to growth factor activation. Integrin antagonists prevent cellular interactions with the extracellular matrix and thus interfere with a cell's ability to proliferate [91]. Indeed, subcutaneous injections of intergin antagonists suppress retinal neovascularization in the mouse [92]. Inhibitors of enzymes such as metalloendoproteases which are required for cells to degrade extracellular matrix prior to proliferation are also being evaluated. Finally, there are inhibitors of angiogenesis with currently unknown mechanisms of action such as Thalidomide under study [93].

Current status and future expectations

At the current time, 17 angiogenesis inhibitors are in clinical trial and over 120 biotechnology and pharmaceutical companies are developing antiangiogenic agents [94]. Most of these are being initially evaluated for their effectiveness in suppression of cancer although Thalidomide and the PKC β-isoform selective inhibitor are being used in human ocular studies for age-related macular degeneration and diabetic retinopathy, respectively. If an antiangiogenic compound is found effective in suppressing tumor growth as first proposed by Folkman in 1971 [95], it is also likely to be effective in preventing proliferative diabetic retinopathy. Indeed, VEGF inhibitors suppress tumor growth in mice [96].

The findings discussed above suggest that compounds which inhibit the action of VEGF and other growth factors may be therapeutically useful for the suppression of retinal neovascularization and macular edema in patients with diabetes. Theoretically such therapies would lack the retinal destruction and associated side effects of current laser photocagulation therapies and could be used to augment such therapy when treatment is ineffective. However, several important issues remain that will probably only be resolved once results from clinical trials become available. Drug delivery methods that are clinically useful must be developed. Many of the agents discussed require intraocular injection for effective concentrations to reach the retina. Repeated intraocular injections that would be required for chronic dosing in a condition such as diabetic retinopathy are not a viable therapeutic option due to the potentially devastating complications of retinal detachment and endophthalmitis. VEGF might also serve a crucial role in the maintenance of the normal retina since numerous cells produce low but detectable levels of VEGF even in the absence of hypoxia. In addition, angiogenesis is known to be important in normal reparative processes such as would healing and vascular collateralization. Thus, antiangiogenic therapies may need to be tightly monitored and regulated so as to permit desirable angiogenic responses when needed.

Conclusions

Historically, diabetic retinopathy has been one of the most visually devastating of all ophthalmic diseases. Even today it remains the leading cause of blindness among working age Americans. However, the current visual prognosis for patients with diabetes is far better than at any time in the past. Careful glycemic control can significantly delay the onset of retinopathy and reduce the progression of the disease while laser photocoagulation can reduce legal blindness from proliferative diabetic retinopathy by over 90% and reduce moderate visual loss from diabetic macular edema by approximately 50%. As mechanisms for improved glycemic control such as the insulin pump and pancreas transplants are refined, and as appropriate ophthalmologic care reaches a greater proportion of the diabetic population, outcomes should benefit even further.

Despite these accomplishments, there is considerable progress to be made before visual loss from diabetic retinopathy is eliminated. A wide array of recent discoveries have substantially improved our understanding of the mechanisms underlying angiogenesis in diabetic retinopathy. These findings have resulted in the development of novel therapeutic approaches offering the promise of orally administered therapies without the retinal destruction inherent with current treatment methods. Some of these new compounds have already proven successful in animal studies and clinical trials are beginning. Whether any of these approaches will ultimately prove clinically useful will not be determined until clinical trial results are available. However, as the 21st century approaches, the 19th century admonition given by Dr Elliot P. Joslin to his diabetic patients more than 20 years before the discovery of insulin remains poignantly true today: "Live, so that you may profit from some new discovery". If effective, safe, and clinically feasible anti-angiogenic regimens can be developed, millions of patients with diabetes may further benefit from this sage advise.

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13. Surgical management of proliferative diabetic retinopathy

STEVE T. CHARLES

Editors' Comment:

Laser photocoagulation is highly effective in halting progression and often induces regression of proliferative diabetic retinopathy. Appropriately timed vitrectomy, when indicated, adds a further surgical intervention in the struggle to preserve sight in diabetes. Charles emphasizes the distinction between a need for immediate surgery in traction retinal detachment involving the macula, neovascular glaucoma, and anterior vitreous cortex fibro-vascular proliferation and a temporizing strategy of unilateral vitreous hemorrhage when the contralateral eye retains functional vision. Recognizing that patients with multi-system diseases may require vitrectomy for 'emotional and social reasons', individualized evaluation for surgery is appropriate. Deciding when to do which surgical procedure for the diabetic eye demands a mixture of experience and patience. For the non-retinal specialist, two key points may be emphasized: (i) A unilateral vitreous hemorrhage in the absence of other enumerated risk factors is not in itself reason for vitrectomy and may be followed by repetitive ultrasound scans. (ii) After a vitreous hemorrhage, delay in referral to a surgeon skilled in vitreous surgery may miss the opportunity to restore sight.

Introduction

Better medical management and laser photocoagulation has significantly reduced the need for vitrectomy for the complications of proliferative diabetic retinopathy (PDR). Vitrectomy when indicated has an excellent prognosis when appropriate patient selection and techniques are utilized.

Case selection

It is useful to divide blindness from diabetic retinopathy into two subgroups: those cases requiring immediate surgery and those in which surgery is elective. Traction retinal detachment involving the macula (MTRD), neovascular glaucoma (NVG) and anterior vitreous cortex fibro-vascular proliferation (AVCVP, RLNV) are permanently blinding if left untreated. In contrast, the visual potential does not change in the treatment of vitreous hemorrhage if there is substantial delay before surgery is performed.

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Vitreous hemorrhage

Early experimental work incorrectly concluded that vitreous hemorrhage caused neovascularization via organization of the blood clot. Vitreous hemorrhage is a result of neovascularization rather than the cause. Although longstanding vitreous hemorrhage can deposit iron on many intraocular structures, there is usually no retinal damage from typical vitreous hemorrhage. Retinal detachment and ischemia, as well as optic nerve function determine the ultimate visual prognosis when longstanding vitreous hemorrhages are removed, not the hemorrhage *per se.*

If the other eye has functional vision, a unilateral hemorrhage can be followed indefinitely with ultrasound. B-scan ultrasonography repeated at each visit should be a part of frequent followup. Ultrasonic evidence of posterior pole detachment should indicate immediate vitrectomy. The usual question of duration of a vitreous hemorrhage plays a less important role in the surgical decision making process than other factors. If it does not appear that near-term clearing will occur, bilateral hemorrhage should indicate immediate surgery on the eye with the best visual prognosis. Vitreous hemorrhage in an only eye, just as in the better eye of bilateral cases, should be operated on to improve function. Those patients with shortened lifespan and multi-system disease need immediate visual rehabilitation for emotional and social reasons. Sub-posterior vitreous detachment (PVD) and preretinal hemorrhages clear far more rapidly than does hemorrhage in the vitreous cortex. For this reason, patients with bilateral or only-eye sub-PVD or preretinal hemorrhage can be followed for as long as the patient's emotional and social needs allow. If one eye has macular ischemia and the better eye develops a vitreous hemorrhage, vitrectomy may be indicated to improve the patient's overall visual function.

Traction retinal detachment

Traction retinal detachment (TRD) can be diagnosed by ophthalmoscopic or ultrasonic means. If the macula is detached, vitreous surgery should be performed within two weeks in the majority of cases. If there is extensive active neovascularization, it is better to perform panretinal photocoagulation (PRP) before vitrectomy. Because of extensive exudation and cellular proliferation, panretinal cryopexy should not be utilized. If vitrectomy indications are present, endo-PRP can be combined with vitrectomy. If vitrectomy is postponed until PRP-induced or spontaneous involution of neovascularization occurs, the incidence of postoperative neovascular glaucoma (NVG) and anterior vitreous cortex fibro-vascular proliferation is dramatically reduced.

Because of the relatively high rate of biologic complications and medical risk factors, vitrectomy is not indicated in extramacular TRD. This is true even if progression toward the macula or a similar condition in the other eye seems to 'threaten' the macula. It is best to operate on actual rather than predicted

visual loss. The rate of progression of extramacular TRD to include the macula is about 15% per year. After several years, progression to MTRD stabilizes at a cumulative rate of about 30%, and there are many cases with 5 to10 year-old extra-macular TRDs surrounding the macula with good vision and no surgery.

Cataract surgery can result in anterior movement of the vitreous with progression of extramacular TRD to macular involvement. Vitreous surgery should only be performed if the macula becomes elevated.

Contraindications

The absence of light perception indicates glaucomatous optic atrophy, ischemic optic neuropathy, or extensive retinal vascular occlusive disease and contraindicates vitreous surgery. Corneal changes; corneal, lid, or conjunctival infection; or the inability to withstand local anesthesia are obvious contraindications.

Iris neovascularization is a relative contraindication, because vitrectomy with retinal reattachment and endo-PRP can cause involution of the iris vessels. More frequently, however, vitrectomy in an aphakic eye with iris neovascularization, particularly it if is advanced, will have rapid and severe progression of postoperative NVG unless extensive PRP can be performed intraoperatively.

Cases of several years' duration typically exhibit extensive white vessels and retinal atrophy. If the temporal arcades are not perfusing the macula, there is no need for vitreous surgery because visual improvement will not occur. If the retina is extremely thin and atrophic but PRP has not previously been performed, this is an indication that minimal visual improvement will occur with reattachment. There are, however, cases of 3 to 4 years' duration that have improved to ambulatory vision levels after vitrectomy. As a rule, these late cases have a lesser incidence of NVG and anterior vitreous cortex fibro-vascular proliferation.

Surgical sequence and techniques

Vitreous surgery for PDR requires a planned sequence of surgical steps but with multiple branches depending on different intra-operative scenarios. As in all vitreous surgery, a full complement of accessory equipment must be immediately available in sterile conditions.

Anesthesia

The frequency of cardiovascular and renal disease in the diabetic requires careful preoperative medical evaluation. The anesthesiologist should play a role in the medical workup. Local anesthesia with monitoring by an anesthesiologist should be used in virtually all cases. An intravenous line, EKG, pulse oximetry, blood pressure monitoring and oxygen mask must be utilized in all

cases. The anesthesiologist or anesthetist should make liberal use of intraoperative blood sugar testing.

Incisions

One to two mm wide limbus-based flaps are preferable to a fornix-based flap or 4–5 mm flaps. The 4-mm infusion cannula is placed 3-mm posterior to the limbus if the eye is to be aphakic, or 4 mm back if the intent is to save the lens. The incision for the endoilluminator is made nasally just above the center of the medial rectus, and the active instrument incision is made temporally just above the center of the lateral rectus. The MVR blade is used for all sclerotomies. The sew-on infusion cannula should be inspected with the operating microscope before initiating infusion.

Management of the lens

Lens removal correlates with an increased incidence of postoperative NVG but prevention of anterior vitreous cortex fibro-vascular proliferation. The anterior vitreous cortex (AVC) and lens act as barriers to the anterior diffusion of vasoproliferative factor and their presence reduces NVG. Therefore, a specific attempt should be made to leave the AVC if the lens is retained during vitrectomy. Endocapsular lensectomy should be used in diabetics to preserve the anterior lens capsule and enable sulcus implantation of a posterior chamber lens after the vitrectomy is complete. Endocapsular lensectomy was developed by the author to facilitate anterior capsular retention and eliminate iris damage. This method starts with anterior vitrectomy, then posterior capsular rhexis with the vitreous cutter, then hydro-dissection and delineation, then sculpting of the nucleus and then cortex, and finally aspiration of the cortex.

Contact lenses should be utilized judiciously because of decreased corneal sensitivity. Spectacle correction is surprisingly well tolerated in these patients. Implantation of posterior chamber lenses (PCLs) after endocapsular lensectomy or phako-emulsifcation surgery tends to keep the AVC and posterior capsule intact and decrease NVG. Endocapsular lensectomy (ECL) with an aspiration-type 20-gauge ultrasonic fragmenter (Alcon) is preferable to phakoemulsification combined with vitrectomy. A judgment should be made preoperatively as to whether cataract will interfere with surgical visualization and endocapsular lensectomy performed. Endo-PRP should be performed in all cases with active neovascularization of the disk (NVD) or elsewhere (NVE), especially if the eye is aphakic or lens removal is required.

Vitrectomy

The continuity of the posterior vitreous cortex (PVC) is a critical concept in the planning of vitreous removal. The PVC will either be completely adherent

to the retina, partially detached, or completely detached from the retina. The 'core' vitrectomy misconception stems from the tendency of rotating full-function probes with syringeoperated suction to pull vitreous into the central portion of the eye. Complete sectioning or truncation of the PVC posterior vitreous cortex rather than 'band cutting' or 'core vitrectomy' must be understood before surgical success can be obtained. These concepts apply whether the vitreous is opaque, semiopaque, or clear.

Procedure if posterior vitreous detachment present

If the posterior vitreous cortex is detached from the retina, a central opening in the vitreous should be created and linear suction with a straight 20-gauge cannula performed if any sub-vitreous erythroclastic or hemolytic material is present. This step is also known as vacuum cleaning or extrusion. When a clear effluent is obtained from this fenestration, the opening should be enlarged until only a small 'skirt' at the confluence of the anterior and posterior vitreous cortex remains. Particular care must be taken to trim the superior 'skirt', because it can hang down postoperatively and obscure the seated patient's view. Linear suction with a straight 20-gauge cannula should be performed to remove all preretinal blood products and permit better visualization, less postoperative erythroclastic glaucoma and enable photocoagulation without damaging the retina. If a complete PVD is present, there is no perpendicular force on the retina and only tangential force from an epiretinal membrane (ERM) can cause TRD. Vascular attachment points of the ERM should be treated with the endophotocoagulator and the elevated vessels with bipolar diathermy only if they bleed intra-operatively or appear active.

Procedure if partial posterior vitreous detachment

If a partial PVD has occurred, the vitreous is therefore adherent to the retina at one or more epicenters. Typically, the optic nerve and vessels serve as attachment points because of glial proliferation. As the vitreous contracts, these attachment points become the apex (apices) of the now conical PVC. This is the most common vitreous configuration encountered in PDR. The goal in these patients is to section or truncate the posterior vitreous cortex to completely eliminate any anteroposterior traction. Vitreous bands do not exist as such but are actually more opaque portions of the PVC continuum. The posterior vitreous cortex truncation is initiated in an area indicated by preoperative ultrasound or indirect ophthalmoscopy to the attached retina. In the absence of this information, the first opening should be made nasally in the midperiphery to avoid the macula and to be easily treatable if a retinal break occurs. After an opening is made, vacuum cleaning through the opening must be continued until a clear effluent is obtained. It is at this time that the novice vitreous surgeon could mistake voluminous old blood trapped behind the

vitreous for active bleeding. When the retina is visualized through the opening, a safe circumferential truncation of the posterior vitreous cortex can proceed from this point and extend for 360°. It is not necessary to make multiple openings in the posterior vitreous cortex or to dissect the layers of the posterior vitreous cortex prior to this step. The 'skirt' must be trimmed as described above, and the portion connected to the retina trimmed down to near the retinal surface. Any areas of PVC connected to two or more retinal points should be sectioned with the vitrectomy instrument, if there is sufficient clearance for the tip, or with the 20-gauge, horizontal scissors. If these bridging areas of PVC are vascularized, they should be precoagulated with bipolar diathermy.

Procedure in absence of a posterior vitreous detachment

It is unusual in diabetes for the entire PVC to be adherent to the retina. In the unlikely event that the PVC is only slightly adherent, membrane peeling can be performed. Caution must be exercised in attempting to peel the PVC in a single sheet because retinal breaks can occur in sites remote from the peeling. Areas of photocoagulation can bind the PVC to the retina and even to the choroid and sclera. If strongly adherent zones are present, the PVC should be allowed to remain in these locations, and the scissors or vitrectomy instruments used to sever all tangential traction. Posterior vitreous cortex truncation must be completed in every case, but can be performed with scissors in the case of shallow PVDs or following inside-out delamination if a TRD is present. This latter method, developed by the author, was later termed 'en-bloc' but described by others using a less safe outside-in direction of dissection.

Traction retinal detachment

Traction retinal detachments result from posterior vitreous cortex (antero-posterior) as well as surface (tangential) traction. Although less apparent in cases with clear media, adherence of the posterior vitreous cortex to the retina is virtually always present when TRD exists. Clear media and excellent endoillumination are essential to visualization of the clear posterior vitreous cortex. The posterior vitreous cortex apex (apices) may be single or multiple and the adherence zones may be small or large. The least complex TRD's involve single-point posterior vitreous cortex attachment with a small zone of adherence on the temporal arcades elevating the macula. Posterior vitreous cortex truncation alone is sufficient to reattach the retina in this situation. If multiple apices with small adherent zones are present, again, posterior vitreous cortex truncation alone is sufficient to cause retinal reattachment.

Epiretinal membrane delamination

Broader zones of adherence (ERMs) are more difficult and require inside-out scissors delamination of the ERM to release tangential traction. Delamination

was developed by the author to allow more complete removal of epi-retinal membranes with less trauma to the retina. ERMs are attached to the retinal surface at individual points where glial and vascular proliferation has penetrated the internal limiting lamina (ILL). Although ERM's may appear to be integral to the retina, ERMs are actually adherent via discrete glial attachment points. Horizontal, 20-gauge scissors (Griesehaber 612.01) are utilized for delamination. It is better to avoid any traction on the ERM with forceps; as it can be gently reflected back with the endoilluminator. The membranes are usually delaminated from the retinal surface in a single sheet in an inside-out direction. Inside-out delamination is better than outside-in because the central retina is stronger, the retina redundant centrally, and the view better. On occasion, limited segmentation can assist in access and visualization. Delamination should usually begin centrally and proceed in an inside-out. The ultimate goal in scissors delamination is to remove all ERM. At times the membrane is extremely vascular, or the retina very atrophic, making this goal unachievable. Scissors segmentation of ERM is utilized when the membrane is markedly adherent to extremely atrophic retina. A minimal goal is to convert all angulated retinal contours to gently rounded contours. Scissors segmentation has been largely supplanted by scissors delamination, which has advantages over segmentation in that virtually all ERM can be removed, eliminating the cut edges of ERM as a source of fibrin bridges and glial reproliferation. Delamination prevents persistent small areas of retinal elevation causing later rhegmatogenous detachment and increased VEGF production.

Membrane peeling

On occasion membranes are minimally adherent to the retinal surface, but more frequently they are tightly adherent with the extensive glial attachment points. If peeling occurs spontaneously during scissors delamination-segmentation, it can be utilized to advantage. More frequently, peeling causes bleeding, retinal breaks, and defects in the ILL that may lead to recurrent glial proliferation. Inappropriate peeling of the ILL can be recognized by the many tiny white fibers stretching from the membrane to the peeled surface. For these reasons, scissors delamination is preferred to membrane peeling in almost all situations.

Retinectomy

When extensive retinal foreshortening, especially from glial recurrences, prevents a delamination approach, retinectomy should be used. If internal drainage of SRF followed by internal fluid-air exchange demonstrates non-conformation of the retina to the RPE, as signified by subretinal air and scissors delamination has already been completed to its maximum safe level, retinectomy is indicated. Circumferential retinectomies are usually preferred to radial. Large retinecto-

mies are managed by the use of silicone. Retinectomy can also be utilized for undissectable glial reproliferation in a starfold configuration.

Endocoagulation

Endocoagulation causes tissue shrinkage and retinal necrosis. Prophylactic coagulation of vessels that are not to be transected is unsafe and unnecessary. As blood can function as a matrix for postoperative glial proliferation, bleeding should be minimized. The endophotocoagulator should be used for glial attachment points, which are more difficult to treat with bipolar diathermy

Internal fluid-gas exchange

Intraocular air (gas) is only utilized if a retinal break is suspected or seen. The only role of air (gas) is to restore the transretinal pressure gradient via surface tension. Air (gas) surface tension management has no role in the treatment of traction retinal detachment without retinal breaks. If a retinal break is seen, internal drainage of sub-retinal fluid with fluid air exchange should be used, followed by focal confluent, single row photocoagulation.

Drainage of subretinal fluid

If a retinal break is present, internal drainage of subretinal fluid (SRF) followed by internal fluid-air exchange and then completion of internal drainage of SRF should be used. If no retinal break is present, it is typically unnecessary to drain SRF unless the detachment is extremely high and there is concern about the ability of the retina to conform to the retinal pigment epithelium (RPE). If no retinal break is present and a decision is made to drain SRF, needle drainage of subretinal fluid is useful.

Silicone surface tension management

Silicone should be utilized when large or multiple retinal breaks or retinectomies are present to eliminate the need for retinopexy and reduce reproliferation. Internal fluid-air exchange with the air pump and internal drainage of subretinal fluid with linear should precede air-silicone exchange.

Silicone oil acts as a barrier to the anterior diffusion of VEGF and virtually eliminates anterior segment neovascularization if no inferior iridectomy is present. Because silicone re-compartmentalizes the eye, fibrovascular proliferation can occur at the retina-silicone interface. For this reason extensive PRP is our best hope for these cases. Silicone may prevent oxygen diffusion from well perfused to hypoperfused retinal areas, thus causing visual loss.

Retinopexy

All breaks require retinopexy in spite of evidence that an occasional untreated break will not result in detachment. Endocryopexy is unsafe and should never be utilized. The laser endo-photocoagulator is used to treat all breaks unless they are very extensive, indicating long term silicone surface tension management.

Scleral buckling

Any retinal breaks that cannot reach the RPE with internal fluid-air exchange and internal drainage of SRF can be supported by a scleral buckle, but retinectomy is usually preferred. Circumferential silicone explants are used for peripheral and midperipheral breaks with unresectable traction. A prophylactic encircling band is not indicated

Endopanretinal photocoagulation

Patients with active retinal neovascularization are high-risk cases for postoperative NVG and RLNV. These cases should have all nondetached, nontreated areas of retina treated with endo-PRP. Focal treatment is reserved for bleeding vessels and is never used on the disk or larger vessels. Endo-PRP decreases NVG and anterior vitreous cortex fibro-vascular proliferation in high-risk cases but is not required in cases with involutional neovascularization. TRD cases are probably at higher risk than are hemorrhage cases, but unfortunately endophotocoagulation cannot be utilized on elevated retina. PRP should not be performed on a formerly detached retina because a thin layer of subretinal fluid always remains and over-treatment is inevitable. Over-treatment results in fibrin syndrome.

Results

Greater than 80% of diabetic TRD patients managed in the above manner will sustain visual improvement with vision greater than 5/200. Ninety-seven per cent of the retinas of the patients are attached at the two-week postoperative visit, but even after reoperation, 5% of the patients are blind from glial recurrence and secondary retinal detachment. The incidence of glial recurrence decreases with the utilization of delamination. Glial recurrence never occurs in truncation-only cases.

Some of the aphakic patients with attached retinas ultimately become blind from NVG in spite of careful management. Neovascular glaucoma correlates with the presence of active retinal neovascularization. Anterior vitreous cortex fibrovascular proliferation causes permanent blindness in some of the phakic cases.

Some of the patients with attached retinas do not have improved vision because of photoreceptor damage and retinal ischemia. Some of the successfully operated cases ultimately become blind from ischemic optic neuropathy. Some become blind from open angle glaucoma. Case selection has a large impact on success rate, but the goal is to help everyone possible, not to improve the success rate by elimination of difficult cases. Patients with good results at 6 months have excellent long-term success.

Complications

Hemorrhage

Immediate postoperative intraocular hemorrhage can occur from ERM vascular attachment points, non-treated new vessels, or sclerotomies. Approximately 50% of phakic cases develop post-operative vitreous hemorrhages. In aphakic cases, this hemorrhage will clear in 1 to 2 weeks, but the phakic cases can take 2 months or longer. If ultrasound indicates that the retina is attached, and there is vision in the other eye, no reoperation is necessary. If ultrasound shows the retina to be detached, immediate reoperation is indicated. If the patient is bilaterally blind, emotional and social needs dictate the need for reoperation.

A full vitrectomy setup with two conjunctival incisions and all three sclerotomies is better than so-called washouts. In this way, ERM and bleeding vessels can be approached and endo-PRP is possible. Two-needle washout can be used if medical conditions do not permit surgery under monitored local anesthesia. If any neovascularization is present, endo-PRP should be combined with the procedure.

Post-operative cataract

If a visually significant cataract occurs in the best or only-vision eye, it should be removed using phako-emulsification and PCL implantation. If the cataract occurs in the poorer vision eye, the patient can be observed with ultrasound

Erythroclastic (Hemolytic) glaucoma

Erythroclastic (hemolytic) glaucoma is best prevented by linear suction with the blunt 20-gauge cannula, trimming of the vitreous skirt, and coagulation of all bleeding vessels. If the pressure exceeds 25 mm Hg, topical carbonic anhydrase inhibitors and beta-blockers will usually control the pressure. On rare occasions, re-operation may be needed to control the pressure.

Neovascular complications

Just as retinal neovascularization is the most significant complication of the unoperated PDR eye, NVG and RLNV are the most severe problems in the

post-vitrectomy eye. An understanding of the pathogenesis is necessary to reduce and manage neovascular complications.

Anterior segment and anterior vitreous cortex neovascularization are due to VEGF released from hypoxic but non-infarcted retina. PRP is successful in reducing VEGF by causing destruction of hypoxic retinal areas, release of an inhibitor substance, and increased choroidal oxygenation to the retina. Trabecular meshwork neovascularization without peripheral anterior synechia or apparent iris neovascularization can cause severe glaucoma. It is no longer thought anterior segment NV is secondary to a circulatory disturbance, or that iris neovascularization somehow migrates to the trabecular meshwork. Although vitrectomy can induce changes in the oxygen distribution in the globe, this observation does not explain the transmissibility of ocular neovascularization from human vitreous specimens to bio-assay systems.

The barrier concept

Vasoproliferative factor encounters sequential barriers in its anterior diffusion en route to ocular egress through the trabecular meshwork. In non-operated eyes, NVE and NVD occur along the back surface of the PVC. If vitrectomy has removed the PVC, neovascularization occurs along the back surface of the AVC. Anterior vitreous cortex fibro-vascular proliferation as first reported by the author was previously incorrectly thought to be due to 'fibrovascular ingrowth' from the sclerotomies.

In aphakic eyes or when released in high concentrations, VEGF encounters the trabecular meshwork barrier, causing neovascular glaucoma. Iris neovascularization serves to indicate the presence of VEGF in the anterior segment and releases fibrin and blood. Trabecular meshwork neovascularization, however, has a direct role in neovascular glaucoma. If a successful filtering procedure is performed in a diabetic, aphakic, vitrectomized eye, anterior segment neovascularization will frequently disappear. This is analogous to the disappearance of NVE and NVD after removal of the PVC by vitrectomy. In these filtered cases, neovascularization occurs on the inside of the bleb, which can be thought of as an additional barrier.

If any neovascularization is seen in the postoperative course, immediate laser PRP should be performed. It is not advisable to wait for pressure elevation, which may obscure the view and lead to irreversible NVG. While PRP may not affect the intraocular pressure, it decreases fibrin release and hemorrhage from the iris vessels that relate to the phthisis process. Although on occasion iris neovascularization will disappear spontaneously or stabilize, it is better to treat all cases of iris with PRP.

If the pressure exceeds 25 mm Hg, topical timolol or Betaoptic may be effective and can be used in combination with propine, iopidine, and topical carbonic anhydrase inhibitors. If topical treatment cannot keep the pressure in the mid-20s, glaucoma surgery may be required. Presumably because of poor

perfusion, diabetics have poor tolerance for elevated pressure. Filtering procedures are effective in some of these patients but have a tendency to cause hypotony with resultant repeated intraocular bleeding. Cyclocryopexy should be performed, preferably on bare sclera, for six clock hours, 4 to 5 mm posterior to the limbus to avoid the trabecular meshwork. The treatments are held at 80°C for 1 minute. Although this has been quite effective in controlling the pressure using a single treatment, many of these patients go on to further fibrin release, cyclitic membrane formation, fibrovascular proliferation, and phthisis bulbi. Endo-cyclophotocoagulation is better than cyclocryopexy, trans-scleral laser and ultrasonic cyclo-destructive procedures because of reduced inflammation and better visual prognosis.

Anterior vitreous cortex fibro-vascular proliferation

If anterior vitreous cortex fibro-vascular proliferation develops, a glial proliferative factor will cause cellular migration and proliferation on the AVC. The membrane causes a characteristic ring-like equatorial TRD followed by total retinal detachment. This configuration can be noted on ultrasound and must be operated immediately. The retrolental or cyclitic membrane can be detected by looking obliquely at the slit lamp to allow early treatment. Treatment requires endocapsular lensectomy, removal of the cyclitic membrane with bipolar diathermy to the resected edges, and internal fluid-air exchange. Long term silicone surface tension management is usually required in these cases. Extensive PRP is necessary to reduce neovascularization.

Rhegmatogenous retinal detachment

Peripheral rhegmatogenous retinal detachment is relatively infrequent in these cases. If it occurs, it is usually related to retinal breaks missed at the time of the original surgery. Post-vitrectomy retinal detachments usually cannot be managed with scleral buckling alone. A greater success rate is usually obtained by using a vitrectomy revision approach with a search for residual traction or glial recurrence, internal drainage of SRF internal fluid-air exchange, focal endo-photocoagulation and air-gas exchange.

Glial recurrence

Epiretinal surgery, especially with peeling can result in a recurrence of glial proliferation. Contrary to previous teaching, no vitreous substrate ('scaffold') is required for a glial recurrence. Glial tissue can proliferate directly on the retinal surface, especially if it is devoid of the ILL. Fibrin from ERM epicenters can constitute a bridge-like substrate along which glial tissue can reproliferate.

Glial recurrences are managed with scissors delamination and internal drainage of SRF, internal fluid air exchange and focal endo-photocoagulation if

there is a rhegmatogenous component. Frequently, retinectomy is required. These membranes are tightly adherent to the retina and cannot be treated with membrane peeling.

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14. Heart disease in patients with the diabetic renal–retinal syndrome: clinical considerations

LUTHER T. CLARK

Editors' Comment:

Every physician treating diabetic patients knows that the greatest risk of death lies in coronary artery and/or other heart disease. According to the United States Renal Data System, heart disease accounts for the most deaths in ESRD patients treated with peritoneal or hemodialysis or given a kidney transplant. Minimizing morbidity and mortality from heart disease in diabetic patients requires the same strategy that is applicable to the population at large, mainly normalizing blood pressure and lipid levels. In preparation for a kidney transplant, the question of invasive (angiography) versus noninvasive cardiac testing is of major concern. Clark does not advise angiography because fewer than one-half of subjects will have significant lesions that may benefit from revascularization. Dobutamine echocardiography is the preferred procedure especially in diabetic subjects unable to exercise maximally because of coexisting obesity, peripheral vascular disease, degenerative joint disorders, or peripheral neuropathy. Correction of coronary artery insufficiency may be affected either by angioplasty or bypass surgery. Studies to date suggest that in diabetes, bypass surgery imparts a survival advantage over angioplasty though the authors caution that the addition of coronary artery stenting to angioplasty has been incompletely assessed. We agree that vigilant cardiac monitoring must be continued in stable diabetic ESRD patients who express no symptoms of coronary disease. Only by detection and correction of silent coronary disease will deaths from sudden heart disease be lessened.

Introduction

Cardiovascular disease is the major cause of morbidity and mortality in patients with both type 1 and type 2 diabetes mellitus, accounting for about 80% of all deaths and more than 75% of all hospitalizations of diabetic patients [1–4]. In patients with end-stage renal disease (ESRD), coronary heart disease (CHD) is common in both diabetic and non-diabetic nephropathy [2]. According to USRDS 1997, approximately 45% of deaths during the first year of renal replacement therapy are due to cardiac disease [5] (Table 1). Progression of coronary disease occurs frequently with all modalities of renal replacement

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Table 1. Causes of death in diabetic and non-diabetic ESRD patients 1993–1995 (USRDS 1997)

Cause of death	Deaths per 1000 patient years (%)		
	Diabetic	Non-diabetic	Total ESRD
Cardiac Disease	103.7 (44.9)	58.6 (40.1)	71.5 (41.7)
Cerebrovascular Disease	14.2 (6.2)	7.3 (5.0)	9.3 (5.4)
Infection	31.3 (13.6)	18.0 (12.3)	21.9 (12.8)
Aids	0.3 (0.1)	2.2 (1.5)	1.6 (0.9)
Malignancy	4.1 (1.8)	7.0 (4.8)	6.1 (3.5)
Hemorrhage	2.9 (1.2)	3.1 (2.1)	3.0 (1.8)
Hyperkalemia	4.7 (2.0)	2.7 (1.8)	3.2 (1.9)
Others/unknown/missing	69.4 (30)	47.2 (32.3)	54.6 (31.9)
Total	230.6 (100)	146.1 (100)	171.2 (100)

Table 2. Vascular disease in diabetes mellitus

Macrovascular disease
- coronary artery disease
- peripheral vascular disease
- cerebrovascular disease

Microvascular disease
- retinopathy
- nephropathy
- neuropathy
- peripheral microvascular disease

therapy, but prognosis is better following renal transplantation than with chronic dialysis [6].

Both microvascular (nephropathy, retinopathy, neuropathy, peripheral small vessel disease) and macrovascular (coronary artery disease, cerebrovascular disease, peripheral vascular disease) disease are associated with diabetes. The incidence and severity of microvascular disease is directly related to the duration and severity of hyperglycemia in type 1 diabetes. However, macrovascular complications are multifactorial in origin with many contributing factors, some of which are interlinked (Table 2). These include hypertension, dyslipidemia, obesity, insulin resistance, and hemorrheological abnormalities [7, 8]. The degree and duration of hyperglycemia may also contribute directly to macrovascular complications, but this has not been proven. Complications from macrovascular disease in diabetics appear early in life, affect women as often as men, and are more often fatal than in non-diabetics.

Coronary heart disease

Coronary heart disease is the major cause of morbidity and mortality among patients with diabetes mellitus. Compared to non-diabetics, diabetics are more likely to have multivessel disease, to have episodes of silent ischemia, and have greater morbidity and mortality following myocardial infarction.

Silent myocardial ischemia

Diabetic patients have a higher frequency of silent ischemia or ischemia with atypical symptoms than their non-diabetic counterparts. In the Framingham Heart Study, 32–42% of diabetic patients with myocardial infarction had atypical symptoms (dyspnea, fatigue, confusion, nausea, vomiting) compared to 6–15% of those patients without diabetes [9, 10]. Atypical symptoms during myocardial ischemia is an important clinical problem because patients may not recognize the need for medical care and there is an increased risk of myocardial infarction, malignant arrhythmias, and sudden cardiac death [11]. The increased frequency of silent ischemia in diabetics may be due to the cardiac autonomic neuropathy that may be seen in these patients. Optimal glycemic control slows the development of cardiac autonomic neuropathy and may lessen propensity for silent ischemic episodes.

Myocardial infarction

Diabetic patients are at increased risk for myocardial infarction. They present with first myocardial infarction at a younger age than non-diabetics, more often present without chest pain, and have higher complication rates, including arrhythmias, congestive heart failure, and recurrent infarction. Patients with myocardial infarction may present with unexplained heart failure, malignant arrhythmias, or even with acutely uncontrolled blood sugars and diabetic ketoacidosis. New onset dyspnea and congestive heart failure should always arouse suspicion of myocardial ischemia.

The in-hospital mortality for diabetic patients following myocardial infarction 1.5–2 times higher than for non-diabetics [1]. Women have particularly poor outcomes with mortality rates that are increased almost twofold compared to diabetic men. The excess in-hospital mortality and increased frequency of clinical heart failure occur despite similar residual left ventricular ejection fractions as in non-diabetics.

Early thrombolytic therapy during acute myocardial infarction results in improved left ventricular function and reduced mortality. The benefits are similar in diabetics and non-diabetics [12]. However, diabetics have an overall poorer outcome because of more severe preexisting disease, a higher rate of reinfarction, and a greater frequency of left ventricular dysfunction and congestive heart failure.

Congestive heart failure

Diabetes increases the likelihood of development of heart failure from all causes. Congestive heart failure is twice as prevalent in diabetic men and five times as common in diabetic women as in their non-diabetic counterparts [13]. This is largely a consequence of the higher rates of coronary heart disease and hypertension. However, even after adjustments for coronary disease, age, blood pressure, weight, rheumatic heart disease and serum cholesterol, diabetics are still at increased risk.

Hypertension and ventricular dysfunction

Hypertension is common in patients with diabetes and may result in left ventricular hypertrophy and heart failure. Patients with diabetic nephropathy are at particular risk since 75–85% of those with overt diabetic nephropathy will have hypertension [14, 15]. These patients may have diastolic dysfunction, systolic dysfunction, or both. In patients with normal left ventricular wall motion and ejection fraction, particularly in the presence of left ventricular hypertrophy, the predominant problem is usually diastolic dysfunction. Vigorous treatment of hypertension for optimal blood pressure control (less than 135/85 mmHg) and regression of left ventricular hypertrophy is the key to improvement of symptoms and risk reduction. Even in the absence of coexisting hypertension, diabetes may be associated with increased left ventricular mass and diastolic dysfunction [16].

Diabetic cardiomyopathy

Diabetic cardiomyopathy is a distinct pathologic entity which results in myocardial hypertrophy, increased interstitial connective tissue and microvascular pathology with systolic and diastolic ventricular dysfunction. The cardiomyopathy appears to be multifactorial in origin and may occur in the absence of coronary, valvular, or other known cardiac disease. Clinically, these patients usually have a dilated cardiomyopathy. In the Framingham Heart Study, approximately 15% of the individuals with diabetes and congestive heart failure were believed to have diabetic cardiomyopathy [17]. Hypertension and left ventricular hypertrophy are frequent associated findings and portend a poor prognosis.

Treatment and prevention of heart failure

Primary prevention of heart failure involves vigorous control of hypertension and other risk factors for CHD. Secondary prevention following myocardial infarction involves control of myocardial ischemia and remodeling. In selecting pharmacologic therapy for patients with predominantly systolic dysfunction, a

traditional stepped care approach to therapy is appropriate, including angiotensin converting enzyme (ACE) inhibition and diuretic, digoxin, vasodilators (nitrates, hydralazine), and sometimes beta blockade.

In patients whose heart failure is due predominantly to diastolic dysfunction, drug therapy can be problematic. Unlike with systolic dysfunction, clinical studies to direct patient care are inadequate. In addition to vigorous treatment of hypertension and other CHD risk factors, weight loss in obese patients is particularly important since obesity is a strong stimulus to ventricular hypertrophy. Angiotensin converting enzyme inhibitors, calcium channel blockers, selective alpha-1 blockers and beta-blockers may be beneficial. Diuretics must be used cautiously to control pulmonary and systemic congestion and to avoid intravascular volume depletion. Digoxin should be avoided. Arterial vasodilators may at times be necessary, but can be problematic, and should be used cautiously to avoid hypotension. Control of myocardial ischemia is essential since muscle relaxation and ventricular function are worsened during ischemia.

CHD risk assessment and pretransplant evaluation

Accurate diagnosis of coronary artery disease and risk assessment in patients with diabetes and ESRD are important for prevention and risk reduction counseling, for interpretation of patient symptoms, and for patient evaluation prior to renal transplantation. In candidates for renal transplantation, detection and treatment of coronary disease are especially important since CHD is the leading cause of death following kidney transplantation [18]. However, accurate risk assessment can be difficult and may present special challenges. Classic ischemic symptoms are often not present and the results of noninvasive studies may be inconclusive.

The prevalence of significant coronary artery disease in diabetic renal transplant candidates has been quite consistent in angiographic series ranging from 25–40% of patients who have one or more fixed coronary artery stenoses of at least 50% [8, 19–21]. In the series where patients with significant disease did not receive pre-transplant coronary revascularization, the 2 year mortality rate for diabetic ESRD patients was at least 50%. Outcome was improved in patients who had revascularization prior to surgery [21]. In the series by Koch and colleagues [8], routine coronary angiography was performed on 105 consecutive diabetic patients during the first 6 months of dialysis. Coronary artery disease was present in 47% (49) of patients. Thirty six percent (38) of patients had one or more significant stenoses and 10% (11) had lesions that were less than 50%.

Because of the high prevalence of coronary artery disease and the limitations of clinical symptoms and noninvasive studies, some investigators have advocated routine coronary angiography prior to renal transplantation [8]. We do not find routine coronary angiography an appealing strategy since less than half of patients will have significant lesions that require revascularization.

Noninvasive testing

The reported sensitivity, specificity, and predictive accuracy of noninvasive testing for detection of coronary disease in patients with diabetes and ESRD have been highly variable [22]. In most diabetic patients with significant coronary artery disease, there will be electrocardiographic (ECG) evidence of ischemia upon adequate exercise cardiac stress testing. Stress testing with thallium scintigraphy is more sensitive and specific than routine treadmill exercise tests, especially in patients with baseline ECG abnormalities. However, because of microvascular abnormalities, false positive results may occur in diabetic patients (particularly those with ESRD and left ventricular hypertrophy) and simultaneous evaluation of left ventricular function (i.e. with echocardiography) improves predictive accuracy. On the other hand, diabetic patients with coronary artery disease may have painless abnormal thallium scintigrams consistent with ischemia.

Normal stress thallium tests in patients who achieve target heart rate goals are usually highly reliable. If target heart rate is not achieved, a negative study should be considered inconclusive and non-diagnostic. In patients with left ventricular hypertrophy, and in particular, when hibernating myocardium is suspected, dobutamine echocardiography may offer advantages. A frequently encountered problem is that patients are unable to exercise sufficiently to achieve an adequate exercise stimulus to the heart rate because of coexisting morbidities such as obesity, diabetic foot ulcers, degenerative joint disease, claudication, amputations, or severe peripheral neuropathy. In these patients, pharmacologic stress testing with dipyridamole, dobutamine, or adenosine may provide a better evaluation of the coronary circulation. Dobutamine echocardiography is our preferred modality when assessment for ischemia and dynamic ventricular function are desired.

Coronary angiography

Coronary angiography is the gold standard for the diagnosis of coronary artery disease. It is indicated prior to transplantation in patients with evidence or symptomatic or asymptomatic myocardial ischemia or abnormal noninvasive studies. In very high risk patients, coronary angiography may be performed without prior noninvasive testing. Patients with severe coronary artery disease should get coronary revascularization prior to transplant surgery. Patients who have severe diffuse coronary disease who are not candidates for revascularization and those with severe left ventricular dysfunction will generally not be candidates for transplantation.

Coronary revascularization

Both coronary angioplasty and bypass graft surgery are effective revascularization techniques in patients with uremic diabetes and immediate results are

similar to those in patients without diabetes or uremia. However, these patients are at increased risk for poor long-term outcome.

Coronary artery bypass surgery

The greatest benefits from coronary artery bypass surgery in terms of survival and improved quality of life have been seen in patients with left main coronary stenosis and in those with triple vessel disease and left ventricular dysfunction. Most recent studies have shown similar perioperative mortality between diabetic and non diabetic patients. However, diabetes is associated with significantly greater perioperative morbidity because of wound infections, longer hospital stays, and reduced long-term survival compared to non-diabetics [23–25]. The poorer long-term prognosis in diabetics is due to more progressive disease in both the non-bypassed and bypassed native coronary arteries and a greater prevalence of associated risk factors [8]. The long-term benefits of bypass using internal mammary artery grafts is similar in patients with and without diabetes. However, many surgeons avoid the use of bilateral internal mammary artery grafts in diabetic patients because of increased risk of sternal wound dehiscence.

Percutaneous transluminal coronary angioplasty

Percutaneous transluminal coronary angioplasty is an effective strategy for many patients requiring revascularization because of lower morbidity than the surgical alternatives, shorter hospital stays, potential for performance as an outpatient, and reduced costs. Furthermore, recent technological advances and improvements in expertise make PTCA an option in some patients with multivessel disease and complex lesions who previously would have had only a surgical option. Initial success and complication rates are similar in diabetic and non-diabetic patients. However, restenosis rates are higher in diabetics following PTCA [26–27] and following coronary artery stenting [28]. Diabetics also have poorer long-term survival rates, a higher incidence of myocardial infarction, and are more likely to require repeat angioplasty or bypass surgery [23, 26].

The results of the Bypass Angioplasty Revascularization Investigation (BARI) trial has led to a recent reevaluation of the role of PTCA in diabetic patients with multivessel coronary artery disease [29, 30]. In the BARI investigation, a large randomized trial comparing CABG to PTCA in patients with multivessel disease, diabetic patients treated by angioplasty had a 15% lower 5-year survival than those who had bypass graft surgery. The survival advantage was observed only in the subgroup of diabetic patients who received at least one internal mammary artery graft. In this subgroup of patients the mortality was 2.9% compared to 18.2% when only saphenous vein grafts were used. The latter rate was very similar to that of patients receiving PTCA (20.6%). Further

analyses by the BARI investigators found that there was an increased frequency of development of new lesions in arteries that were instrumented. The investigators concluded that the combination of diabetes and an artery that was instrumented during PTCA are additive, increases the risk of development of new lesions in that artery [31], and that this may explain the poorer patient outcomes.

Data from several other revascularization trials provide conflicting results. In the diabetic sub-group of the Emory Angioplasty Versus Surgery Trial (EAST), there was no significant difference in 5-year survival after PTCA compared with CABG [32]. However, in the Coronary Angioplasty versus Bypass Revascularization Investigation (CABRI) there was improved survival at 2 years in the diabetic patients undergoing bypass surgery [33]. In a large prospective cohort of patients with multivessel disease from the Duke registry, the presence of diabetes was associated with a similarly worse long-term outcome following PTCA and CABG in patients with multivessel coronary artery disease [25].

Angioplasty versus bypass surgery

Most randomized trials comparing angioplasty with bypass surgery have reported similar outcomes. This may not be the case in patients with diabetes although it is premature to conclude at this time that bypass surgery is preferable to angioplasty for all patients with diabetes and multivessel disease. However, surgery should probably be recommended for patients with diffuse disease in whom more complete revascularization can be achieved with bypass surgery and at least one internal mammary artery graft is used. Although coronary artery stenting is now commonly utilized, the clinical trials to date have compared only balloon angioplasty with bypass surgery. The impact of coronary stenting as an option in these patients must await the results of further investigation.

CHD prevention and risk reduction

Cardiovascular disease in patients with diabetes and ESRD is highly prevalent. However, because of the high frequency of modifiable risk factors, opportunities for prevention and risk reduction are great. The cornerstones of an effective prevention strategy are glycemic control, aggressive risk factor modification, and ongoing patient surveillance and monitoring to facilitate early disease detection and prompt intervention (Table 3).

Glycemic control

The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive diabetes management in patients with insulin-dependent diabetes

Table 3. Prevention of macrovascular disease in patients with type 2 diabetes

Glycemic Control
Aggressive risk factor modification
• dyslipidemia
• hypertension
• smoking
• physical inactivity
• obesity
Ongoing surveillance and monitoring for typical and atypical syndromes

mellitus (IDDM) improves glycemic control and reduces risk of development of microvascular disease [34]. There was also a reduction in some coronary risk factors suggesting a potential benefit on macrovascular complication [34–35]. However, there are currently no clinical trial results that show an overall outcome benefit of intensive therapy on the development of macrovascular disease. The United Kingdom Prospective Diabetes Study (UKPDS) was designed to compare the efficacy of several different treatment regimens (diet, sulfonylurea drug, metformin, and insulin) on glycemic control and complications [35]. The results of this study should be available within the next year. Currently available data would appear to support glycemic control. However, until there is conclusive evidence of a benefit, this approach must be balanced against the greater difficulty of the regimen for patients, the possibility of more hypoglycemia, and the additional cost.

Dyslipidemia

Diabetic patients frequently have lipid abnormalities. In patients with type 1 diabetes with good glycemic control, lipid and lipoprotein concentrations are similar to those in non-diabetic populations. However, patients with type 2 diabetes often have the lipid triad of elevated triglyceride levels, low HDL-cholesterol and a small, dense form of LDL-cholesterol. Data from several large clinical trials have shown that patients with diabetes benefit at least as much from lipid-lowering therapy as do non-diabetics [36–38]. Vigorous efforts should be made to lower LDL levels and achieve the NCEP treatment goals for patients with and without established CHD. Triglyceride levels should also be lowered with improved glycemic control, dietary measures, and pharmacologic therapy if necessary.

Hypertension

Hypertension should be vigorously treated in patients with diabetes mellitus to achieve the recommended goal of less than 130/85 mmHg [39]. Lifestyle

modifications, especially weight loss should be the cornerstones of therapy. When pharmacologic therapy is required, ACE inhibitors, alpha-blockers, calcium antagonists, and low dose diuretics are preferred because of fewer adverse effects on glucose homeostasis, lipid profile, and renal function.

Smoking

Cigarette smoking is a powerful risk factor for coronary and other atherosclerotic diseases. Smoking acts synergistically with diabetes as an amplifier of risk for coronary and peripheral atherosclerosis. Smoking cessation should be pursued with vigor since smoking cessation may lower CHD risk by as much as 50% over a period of a year.

Obesity and physical activity

Weight reduction in obese patients and regular physical activity in all patients should be encouraged. Weight reduction and increased physical activity, if maintained, have beneficial effects on glycemic control, dyslipidemia, hypertension, platelet-coagulation abnormalities and insulin resistance.

Conclusions

Cardiovascular disease, and in particular coronary heart disease, is the major cause of morbidity and mortality in patients with diabetes and end-stage renal disease. Many of the factors contributing to the excess risk in these patients are modifiable and thus, opportunities for prevention and risk reduction are great. In patients with established CHD, optimal management and therapy require a coordinated multidiciplinary effort of physicians, nurses, nutritionists, and other providers. Patients may derive special benefit from cardiac rehabilitation where the emphasis is on patient education and vigorous modification of CHD risk factors. These patients should also be targeted for ongoing surveillance and monitoring for clinical and subclinical cardiovascular disease which may present atypically but is nevertheless potentially lethal. One of the medical tragedies we must strive to prevent is that the patient with diabetic nephropathy who has received a successful kidney transplant and return to a normal lifestyle then dies of unsuspected and untreated cardiac disease.

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15. NO and diabetic complications

ROBERT F. FURCHGOTT

Editors' Comment:

Furchgott's epochal discovery in 1980 of endothelium-dependent relaxation of arteries by acetylcholine led to identification of a labile material released by endothelial cells first termed endothelium-derived relaxing factor and later shown to be nitric oxide (NO). NO is now recognized as a key mediator in multiple physiologic processes that are perturbed in diabetes. In induced diabetes in the rat, for example, the hypotensive response to acetylcholine infusion is sharply attenuated, perhaps due to the action of advanced glycosylated endproducts. Another illustration of impaired NO effect in diabetes is the finding that penile corpora cavernosa obtained from impotent diabetic men show total absence of relaxation on exposure to acetylcholine. While an exact role for NO in the pathogenesis of diabetic complications has yet to be defined, there is no doubt that it will. Therapeutic initiatives to restore normal NO equilibrium are rational objectives once the pathophysiology of diabetes is elucidated.

Introduction

The first full paper on endothelium-dependent relaxation of isolated arteries by acetylcholine (ACh) was published in 1980 [1]. In that paper it was shown that ACh and other muscarinic agonists stimulate the release of some non-prostanoid, highly labile material from the endothelial cells, which on diffusing to the adjacent vascular smooth muscle cells, causes them to relax. This material was later referred to as the endothelium-derived relaxing factor or EDRF. Within a few years, a large number of agents in addition to muscarinic agonists were found to produce endothelium-dependent relaxation of arteries (e.g., ATP, ADP, substance P, bradykinin, histamine, serotonin, arginine, vasopressin, thrombin, and the calcium ionophore A23187). (For an early review, see Ref. 2.) However, it should be stressed that endothelium-dependent relaxation with any given agent is often limited with respect to the species of animal used, and also sometimes limited with respect to specific arteries within a given species. It should also be noted that increases in shear stress on endothelial cells due to increases in flow were also found to stimulate increases in release of EDRF [3, 4].

By 1983, it had been shown that EDRF stimulates soluble guanylyl cyclase in vascular smooth muscle and that the resulting increase in cyclic GMP (cGMP) somehow produces the relaxation [5–7]. However, the chemical

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nature of EDRF remained unknown until 1986, when both Ignarro and the present author independently at the same symposium presented findings that led them to propose that EDRF is NO [8, 9]. Among these findings were the very rapid inactivation of both EDRF and NO by oxyhemoglobin and by superoxide anions. The identification of EDRF as NO was substantiated in perfusion-bioassay procedures, using both endothelial cells *in situ* (on arterial segments) and cultured endothelial cells on microcarrier beads [10–12]. By 1988, Moncada and colleagues had shown that the substrate for NO synthesis in endothelial cells is L-arginine, that the nitrogen in the NO derives from a guanidino–nitrogen of the L-arginine, that the enzyme is an oxygenase, that NADPH is a co-substrate and that citrulline is a co-product with NO [13]. They also showed that N^G-monomethyl-L-arginine (L-NMMA) is a competitive inhibitor of the nitric oxide synthase (NOS). L-NMMA and other inhibitors of NOS have been very useful tools in evaluating the physiological as well as pathophysiological significance of NO (for reviews, see Refs. 14, 15). With respect to physiological significance, it should also be noted that endothelium-derived NO (EDNO) is an inhibitor of platelet aggregation and adhesion, of leukocyte adhesion, and of the neointimal proliferation of vascular smooth muscle cells [14, 15].

Shortly after NO was shown to be synthesized in endothelial cells, it was also shown to be synthesized by neurones in the central nervous system [16, 17], where its potential roles in memory formation and other functions have been the subjects of much study [18]. In the peripheral nervous system, NO was demonstrated to be the neurotransmitter released by the so-called NANC (nonadrenergic, noncholinergic) nerves which mediate some forms of neurogenic vasodilation and relaxation of smooth muscles in the respiratory, gastrointestinal and genitourinary systems [19–20]. In addition, NO was identified as the active cytotoxic and cytostatic principle synthesized from L-arginine in activated rodent macrophages [21–23], and this led to the finding that NO is generated in large quantities during host defense and immunological reactions as a result of induction of an NO synthesizing enzyme in a variety of cell types (including vascular smooth muscle cells) [24, 25].

Of the three isoforms of nitric oxide synthase (NOS) now recognized, two are constitutive, namely the eNOS first found in endothelial cells and the nNOS first found in neuronal tissue, while the third is the inducible iNOS (for reviews, see Refs. 27–29). The activity of both eNOS and nNOS is dependent on Ca^{2+}/calmodulin, while that of iNOS is not.

Evidence for either impaired production or effectiveness of endothelial NO in blood vessels has been reported in a number of cardiovascular disorders both in man and in experimental animals, including hypertension and atherosclerosis (for reviews, see Refs. 15, 29). The present paper will be a short review of some of the literature dealing with deficiencies in endothelium-derived NO production or action in diabetes mellitus, and also the role of NO in the vasodilator action of insulin. In addition, it will discuss briefly recent reports

on the possible role of NO released in excess in both islet β-cell destruction and glomerular damage during the onset of diabetes.

Impaired endothelium-dependent vasodilation in diabetes

There are a number of reports showing that aortic ring segments and isolated resistance vessels from streptozotocin-induced diabetic rats, alloxan-induced diabetic rabbits, and genetic diabetic rats exhibit impaired relaxation in response to ACh and some other endothelium-dependent relaxing agents (for reviews, see Refs. 29–31). However, it has been found in some preparations that the impaired relaxation is not simply due to a decrease in the release of NO by the endothelial cells of the vessels of the diabetic animals as compared to the non-diabetic. For example, in aortic rings from alloxan-induced diabetic rabbits, it appears that the decreased relaxation to ACh may be the result of release of endothelium-derived contractile factors that counteract the relaxation caused by the NO released by ACh. According to Cohen and Tesfamariam [31, 32], these counteracting contractile factors in the diabetic arteries consist of ACh-released vasoconstrictor prostanoids combined with an excess of oxygen free radicals which can scavenge NO. Those investigators found that either cyclooxygenase inhibitors or superoxide dismutase (SOD) added to their organ chambers could largely restore endothelium-dependent relaxation of the diabetic vessels to the level found in normal vessels. Also, SOD was found to overcome some impairment of ACh-induced endothelium-dependent relaxation exhibited by aortic rings from streptozotocin-induced diabetic rats [33]. In contrast, in studies on isolated mesenteric resistance vessels from spontaneously diabetic BioBred (BB) rats, impaired endothelium-dependent relaxation in response to both ACh and bradykinin was not at all ameliorated by added SOD [34]. However, since the NOS inhibitor N^G-nitro-L-arginine (L-NOARG) only partly inhibited the relaxation produced by these agents in both the diabetic and control vessels, the relaxation may be due to a combination of EDNO and some endothelium-derived hyperpolarizing factor (EDHF), and therefore an unambiguous case cannot be made for a deficient production of NO being the sole cause of the impaired relaxation in the diabetic vessels.

In a study of the effect of streptozotocin-induced diabetes in rats on the response to i.v. injections of graded doses of ACh, Bucala et al. [35] found a marked attenuation of the acute falls in mean arterial pressure in response to this agent in the diabetic animals as compared to controls. The attenuation of response was fully developed after about 2 months of diabetes. A similar attenuation of the acute hypotensive response to i.v. glyceryl trinitrate was found in the diabetic rats. Since ACh-released EDRF is considered to be NO and since glyceryl trinitrate is thought to be a 'prodrug' for NO, the authors proposed that in the diabetic animals the attenuation of responses to ACh was the result of a rapid quenching of the released endothelium-derived NO by the advanced glycosylation end-products (AGEs) that accumulate in the diabetic state. (For a review of AGEs in diabetes, see [36]). Experimental evidence for

rapid quenching of NO by AGEs *in vitro* was presented [35]. However, the acute hypotensive responses to bolus i.v. injections of ACh are probably not solely due to release of endothelial NO, so that quenching of NO by AGEs may be only one factor contributing to the decreased responses to ACh in the diabetic rats. A stronger argument for the significance of NO quenching by AGEs in the *in vivo* experiments could be made if AGEs added to non-diabetic arteries *in vitro* could be shown to inhibit relaxation by ACh and other endothelium-dependent vasodilators.

The first evidence for impairment of endothelium-dependent relaxation in human diabetes came from experiments on isolated penile corpora cavernosa [37]. The relaxation by ACh of the smooth muscle of the microvessels in such preparations obtained from nondiabetic men was almost completely absent in preparations from impotent diabetic men. Subsequently, studies on the effects of various agents on blood flow and vascular resistance in the forearm or leg clearly showed impairment of endothelium-dependent vasodilation in diabetes. Creager and coworkers found that patients with insulin-dependent diabetes mellitus (IDDM) exhibited considerably less increase in blood flow (vasodilation) in the forearm in response to infusions of the muscarinic agonist methacholine into the brachial artery than did healthy controls, whereas the increases in forearm blood flow on infusions of nitroprusside or verapamil (endothelium-independent vasodilators) were similar in the two groups [38]. Baron, Steinberg and colleagues in their studies on effects of intra-femoral artery infusions of methacholine on leg blood flow found that the vasodilator effect of this endothelium-dependent agent was very much attenuated in subjects with non-insulin-dependent diabetes mellitus (NIDDM) as compared to lean control subjects [39]. They also found that obese insulin-resistant subjects exhibited much less increase in leg blood flow in response to methacholine than did the lean control subjects. Moreover, under conditions of controlled euglycemic hyperinsulinemia, which augmented the leg blood flow response to methacholine in lean control subjects about 50%, there was no augmentation of the response in the NIDDM and obese insulin-resistant subjects. In connection with the augmentation of methacholine-induced vasodilation observed in the control subjects under euglycemic hyperinsulinemia, it should be remembered that hyperinsulinemia itself increases blood flow to skeletal muscles in normal subjects by increasing NO synthesis/release (see next section).

Role of NO in vasodilation induced by insulin

It is now well established that circulating insulin can produce vasodilation and an increase blood flow in skeletal muscle vascular beds, and that this vasodilation is considerably blunted in NIDDM and insulin-resistant states of obesity (for reviews see 40, 41). In studies using continuous intravenous insulin infusions, the increase in blood flow to the muscles of the forearm or leg does not occur immediately but gradually attains a steady state about two hours after the start of the infusion. Baron and coworkers have shown that the increase

in blood flow to the leg produced by hyperisulinemia under a euglycemic 'clamp' in normal subjects is completely blocked by an infusion of the NOS inhibitor L-NMMA into the femoral artery (42). Similarly, Scherrer and coworkers have demonstrated that the same NOS inhibitor infused at intervals into the brachial artery during the course of a two-hour intravenous infusion of insulin completely blocked the increase in blood flow and decrease in vascular resistance that normally develop during the course of such an infusion [43]. Thus, the increase in blood flow to skeletal muscles produced by circulating insulin appears to be due to an increased synthesis and/or release of NO that develops during the course of the infusion.

Considering the time-course of the increase in muscle blood flow during the course of insulin infusion, it would appear that insulin does not directly stimulate an increase in synthesis and/or release of NO (as in the case of muscarinic agonists and bradykinin) but is somehow potentiating the synthesis and/or release that would be present in the absence of insulin. How this is brought about is not clear. Baron and coworkers postulate that insulin somehow acts on the endothelial cells of the skeletal muscle resistance vessels to enhance their release of NO in response to chemical and physical stimuli [42]. Scherrer considers the possibility that insulin acts on the neural pathways of NANC nerves (now called nitrergic nerves) to enhance the release of NO in the vascular beds of skeletal muscles [43]. However, this seems unlikely since there is presently no clear evidence for nitrergic nerves activating vasodilation in skeletal muscles the way they activate vasodilation in brain [44].

There have been some studies on the effects of insulin on the contractile behavior of blood vessels *in vitro*. In some but not all studies of which the author is aware, exposure of the vessels (either segments of rat aorta or mesenteric resistance vessels) to insulin produced modest reductions in the contractile responses to norepinephrine [45, 46]. In one study, evidence for an insulin-induced increase in endothelial NO was obtained by showing that a reduction in contractile response to norepinephrine in the presence of insulin no longer occurred if a NOS inhibitor was present [46]. In studies on isolated arteries from experimental animals, there have been no reports of a relaxing effect of insulin when added to a precontracted preparation. Interestingly, there was one early report that insulin acutely produced an endothelium-dependent relaxation of precontracted rings of human coronary, pulmonary and radial arteries [47]. Unfortunately, there has been no report of which the present author is aware confirming these findings. In the present author's laboratory experiments attempting to show either a direct relaxing effect of insulin or a potentiating effect on ACh-induced relaxation of precontracted rings of rabbit aorta have been uniformly negative.

A role for NO in the onset of diabetes

Two recent reviews by Nerup and colleagues [48] and Corbett, McDaniel and colleagues [49] present a strong case for NO released by iNOS in pancreatic

islets having a major role in the destruction of β-cells associated with the onset of autoimmune diabetes. The reader is referred to those reviews for details. Both groups of investigators present experimental evidence that cytokines, particularly interleukin-1 (IL-1), induce iNOS in pancreatic islets *in vitro*, leading to generation of NO in concentrations that are damaging to the β-cells. Neither group attributes the damage and inhibition of insulin secretion to the NO alone. One group provides evidence that the cytokines also lead to induction of enzymes generating excess deleterious oxygen free radicals in the β-cells [48]. The second group has shown that IL-1 induces the expression of the COX-2 isoform of cyclooxygenase along with its induction of iNOS in the β-cells, so that there is an overproduction of proinflammatory prostaglandins and thromboxanes as well as NO [49]. Furthermore, they found that the NO produced by iNOS directly stimulated both the constitutive and inducible isoforms of COX, thus further augmenting the overproduction of potentially cytotoxic agents in the β-cells.

The possibility has also been considered that excess NO production in the kidney may be responsible, at least in part, for the high glomerular blood pressure and hyperfiltration in early diabetes that may be a contributing factor in the subsequent development of glomerular damage. Raij and Baylis in an excellent review on the glomerular actions of nitric oxide [50], cite a few reports that provide the experimental evidence suggesting that possibility. The evidence, which has been obtained with streptozotocin-induced diabetic rats [51, 52], is provocative but not yet definitive. In view of the evidence for impaired endothelium-dependent vasodilation in vessels from animals and humans with well established diabetes, Raij and Baylis [50] propose that the L-arginine pathway is involved in a bimodal fashion in diabetes, characterized by an early NO excess followed by a functional deficiency when the disease advances.

In another recent review on the regulation of glomerular filtration by mesangial cells [53], Stockard and Sansom point out that since NO enhances mesangial cell relaxation, high levels of NO acting on these cells could counteract the usual contraction of these cells in response to increased transmural pressure and thus allow for the glomerular hyperfiltration observed in early diabetes. They speculate that the high ambient glucose of early diabetes might somehow induce increases in local NO levels in the mesangial cells, but present no experimental evidence to support this.

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16. AGE and the kidney: an update

HELEN VLASSARA

Editors' Comment:

Debate over the deleterious effect of hyperglycemia in the pathogenesis of diabetic complications has ended. Large prospective controlled studies of the relationship between glycemic control and progression of diabetic retinopathy, nephropathy, and neuropathy all point in one direction: hyperglycemia is injurious and is directly linked to tissue damage. Exactly how a high ambient glucose concentration causes protein damage is a story that has been progressively told over the past 20 years starting with Cerami's observation that the level of glycosylated hemoglobin correlated with the degree of glucose regulation. As reviewed by Vlassara, a prime contributor to establishing the central role of advanced glycosylated endproducts (AGEs), these toxic molecules may be incriminated in pathologic changes of aging, atherosclerosis, Alzheimer's disease as well as diabetes. Nephrologists suspect that AGEs may be linked to the crippling amyloidosis with arthritis that afflicts long-term hemodialysis patients. In this report, a newly recognized threat of AGEs absorbed from cooked foods is assessed. There is hope of muting the damage caused by AGEs in diabetic individuals by at least three strategies: (1) limiting synthesis of AGEs by optimizing glucose regulation; (2) pharmacologic intervention to block the glycoxidation pathway using inhibitors like aminoguanidine; (3) breaking existing AGE-cross-links by administering novel chemical agents now in investigation. Clinical trials to determine whether aminoguanidine will favorably alter the course of nephropathy in type 1 and type 2 diabetes, as well as the high mortality of diabetic patients treated by maintenance hemodialysis, are nearing completion. It is probable that this fresh direction in the therapy – if successful – may reduce the often futile striving to sustain euglycemia as the only means of preserving eyes and kidneys in diabetes.

Background

Diabetic nephropathy (DN) is the most common cause of renal failure in the United States [1–3]. It is estimated that DN afflicts 30–40% of patients with IDDM of greater than 20 years duration. A proportion that is only expected to rise as overall survival increases with improved management of insulinopenia in this population [4]. Although of unknown nature, both environmental and specific gene defects have been suggested in the pathogenesis of this complication.

It has now been confirmed that sustained hyperglycemia is a prerequisite for DN [5]. Several well-known mechanisms already have been identified by

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which hyperglycemia can induce reversible or irreversible cellular and extracellular damage. As recently detailed [6–9], such mechanisms include interrelated glucose-driven pathways: (1) the polyol pathway and the associated alterations in the redox state of pyridine nucleotides [10, 11]; (2) the *de novo* synthesis of diacylglycerol, which can lead to the activation of several isoforms of protein kinase C [6, 7]; (3) decreased cellular uptake of myoinositol, leading to reduced Na/K-ATPase activity [8]; and (4) advanced glycation [9].

The latter, the subject of this discussion, refers largely to late rearrangements of glucose-mediated modification of proteins and lipids, also called Advanced Glycation Endproducts (AGE), which slowly accumulate in renal and extrarenal tissues with aging and at a more rapid rate in diabetes [12]. In addition, tissue and serum AGE levels are markedly elevated in diabetics with renal insufficiency [13]. Numerous studies have indicated that reactive AGEs can directly alter physical and structural properties of the glomerular matrix, for instance, by inducing collagen crosslinking [14], basement membrane thickening, and covalent trapping of plasma proteins [15]. In addition, AGEs are known to elicit a wide range of cell-mediated responses, thereby 'indirectly' mediating phenotypic changes leading to vascular dysfunction [16, 17], and matrix expansion [18, 19].

AGE and renal cells – *In vitro* studies

These studies, mentioned above, have suggested that AGEs may contribute to the pathogenesis of DN by interacting with renal and other cells through cell-specific AGE receptors [20–28]. In subsequent studies the expression of surface-associated AGE-receptors by mesangial cells (MC) and their specific involvement in matrix upregulation was confirmed; in the presence of anti-AGE-receptor antibodies, the AGE-mediated increase in collagen IV mRNA by MC was blocked [18, 24, 25]. Additional matrix components that were enhanced by AGEs in cultured MCs included laminin A, B1, B2 and heparan sulfate proteoglycan [18]. Moreover, the AGE-receptor-induced increase of collagen α1(IV) appeared to be mediated via Platelet-derived Growth Factor (PDGF), based on studies using anti-PDGF blocking antibody. This suggested that PDGF could be one factor that contributes to AGE-receptor-enhanced matrix synthesis by these cells *in vivo* under conditions of augmented AGE deposition within the glomerulus, e.g. diabetes, although other growth factors could also be involved, e.g. TGFβ1 [19].

AGEs upregulate gene expression of ECM and growth factors – animal studies

A subsequent study was undertaken to determine the contribution of AGEs to intact glomerular structure *in vivo* by examining selected molecular events involved in mesangial cell responsiveness, such as the induction of specific genes for ECM or growth factors. The study focused on microdissected glomer-

uli following the injection of AGE-mouse serum albumin (MSA) into healthy mice [19]. We noted that α1(IV) collagen mRNA increased by 1.7 fold in AGE-MSA treated mice (AGE-MSA vs MSA or CL, $p < 0.01$), but remained normal in AGE + aminoguanidine-treated mice. Similarly, laminin B1 mRNA was increased by 2.2 fold in the AGE-MSA mice ($p < 0.01$), while this was prevented with the simultaneous administration of aminoguanidine (AGE-AG vs AGE-MSA, $p < 0.01$). Although in this study increases in PDGF-B mRNA were not detected, the mRNA of another growth-promoting molecule, TGF-β1 mRNA, was found increased by 1.5 fold in AGE-MSA mice ($p < 0.05$); again in AGE-AG mice, TGFβ mRNA level was within normal range [19]. In connection with AGE-MSA treatment, the glomerular volume/body weight ration was increased by 39% in AGE-MSA mice ($p < 0.001$), while this increase was largely abrogated in AGE + AG mice (AGE-MSA vs AGE + AG $p < 0.005$). The data supported the premise that AGEs can influence glomerular homeostatic mechanisms *in vivo* by regulating specific gene products (e.g. collagen α1(IV), but *not* collagen I, independently of other systemic metabolic alterations of diabetes, e.g. hyperglycemia.

Long-term AGE treatment induces glomerulosclerosis and albuminuria; more animal studies

We further inquired whether AGEs could trigger changes in glomerular ECM protein secretion *in vivo* in the absence of diabetes. To explore this, low doses of AGE-modified rat albumin (25 μg/kg, i.v.), sufficient to elevate serum levels of AGE to the range found in human diabetes, were administered daily to healthy rats alone or in combination with the AGE-inhibitor aminoguanidine. After 4 months of treatment, the AGE content of renal tissues rose to 50% above control ($p > 0.025$). Light and electron microscopic analysis of kidney samples from AGE-treated rats revealed glomerular hypertrophy associated with deposition of PAS-positive material, mesangial and basement membrane thickening and mesangial ECM increase, consistent with significant glomerulosclerosis, compared to untreated ($p < 0.002$) or rat albumin-treated controls ($p < 0.002$) [29]. These changes were associated with significant protein ($p < 0.005$) and albumin ($p < 0.002$) loss in the urine of AGE-treated rats. Co-treatment with aminoguanidine markedly limited both structural and functional defects [29]. These *in vivo* findings further strengthened and expanded the evidence that AGEs independently can influence glomerular structure and function, in a manner which is consistent with the pattern of glomerulosclerosis in diabetic nephropathy.

AGE turnover, renal clearance and relationship to kidney function – human studies

Based on earlier studies, it was speculated that tissue macrophages, via their AGE-receptor system, represent a principal degradation mechanism for AGE-

modified tissues and cells. Tissue degradation leads to the release of small soluble AGE-peptide fragments which, combined with extracellular proteolysis of glycated matrix components, gives rise to a circulating low molecular weight (LMW) fraction likely to contain variable amounts of reactive glycation moieties depending on the underlying tissue levels and the activity of AGE removal systems. These degradation products of tissue AGE-modified constituents are believed to be released in the circulation to be cleared by the kidneys [13]. However, if not readily cleared, they can readily react with other target molecules e.g. lipoproteins, vessel wall components, to perpetuate injury. In this context, marked elevations in AGE-ApoB were found in plasma samples from diabetic patients with end-stage renal disease ($p < 0.001$), compared to normal and diabetics with normal renal function [30]. The extent of AGE-modification of plasma ApoB in non-diabetic patients with ESRD was also ten-fold higher than those with normal renal function. Given the short plasma half-life of LDL, these levels of AGE-ApoB could be attributed to ambient glucose alone. Rather, the data are consistent with the proportionally increased reactive pre-AGE and AGE-peptides found in the serum of both diabetic and non-diabetic uremic patients [13]. Thus, uncleared serum reactive AGEs are likely to prove an important potentiating factor of tissue damage for those diabetics with nephropathy.

In normal individuals LMW-AGE clearance is estimated to be 0.72 ml/min [31], and otherwise AGE levels correlate with renal function [13]. While diabetic individuals with normal glomerular filtration rate (GFR) can clear AGE-peptides at the same rate, progressive loss of kidney function correlates with increasing circulating LMW-AGE levels, up to eight-fold in diabetic patients with end-stage renal disease (ESRD) requiring dialysis [13]. Of note, most current modes of hemodialysis or peritoneal dialysis are apparently inefficient in removing AGE-peptides when compared to creatinine, supporting renal transplantation as the only truly effective long-term treatment [30].

Diabetic patients with ESRD are known to be particularly susceptible to cardiovascular complications [32] due to accelerated atherosclerosis. Thus, the pronounced increase in serum AGEs observed in diabetic anephric patients, while indicative of inefficient clearance by current dialysis modalities, also raised the possibility that uncleared re-circulating AGEs can participate in undesired interactions with the vascular tissues and plasma lipoproteins, e.g. LDL-ApoB [33], potentially accelerating ongoing pathology. The latter was supported in a recent study on ESRD patients followed for a 2 month period of hemodialysis, using high flux (AN69) membranes. A significant decrease of serum AGE levels was associated with a marked decrease (30%) in total serum ApoB (Figure 1) [33].

AGE and diet

A potentially critical finding has recently been the observation that significant amounts of reactive AGEs present in cooked foods are orally absorbed and

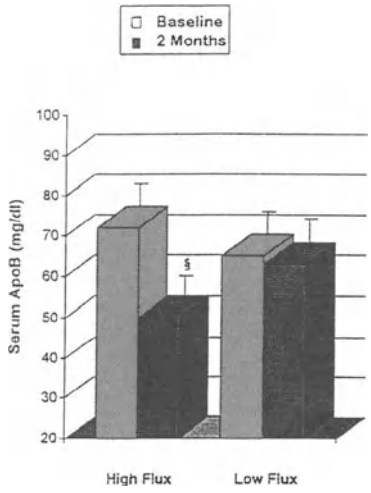

Fig. 1. Serum ApoB levels in diabetic patients with end-stage renal disease (ESRD), treated with high flux (AN69) and low flux (F8) hemodialysis, at baseline and at 2 months. Data are expressed as mean $\pm$ SD of total serum ApoB mg/dl, determined by the Incstar SPQ Test System. §AN69, baseline versus 2 months, $p = 0.02$.

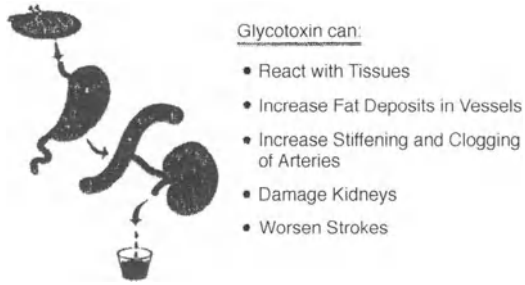

Fig. 2. Schematic representation of the *in vivo* generation of AGE.

only partially (<30% of the amount absorbed) excreted in the urine [34]. Diabetic patients, especially those with renal disease, exhibit impaired clearance of such diet-derived AGEs, also called glycotoxins, in a manner which is proportional to the underlying renal insufficiency, but can be as low as 3% of amount absorbed in severe nephropathy [34]. Importantly, food-derived circulating AGEs were shown to include reactive derivatives with protein-protein crosslinking activity, inhibitable by aminoguanidine [34], which can potentially attach to multiple target tissues, as shown schematically in Figure 2.

Since a significant portion of food-derived glycotoxins remain in the body for long periods of time, it follows that they may pose additional risk for renal

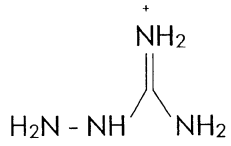

Fig. 3. The chemical structure of the AGE-inhibitor aminoguanidine.

and vascular damage, as demonstrated by the infusion of exogenously produced AGEs in normal rats and mice [19, 35]. Thus, in addition to current therapies, future management or prevention of diabetic nephropathy may include, in addition to AGE-inhibitors such as aminoguanidine, restricted dietary intake of AGE-rich foods.

Anti-AGE strategies

Pharmacologic intervention of the glycoxidation pathway has been in the last phases of clinical active evaluation. An important inhibitor of this process has been the nucleophilic compound aminoguanidine (Figure 3), shown to be a potent and specific inhibitor of glucose-mediated crosslinking and tissue damage *in vitro* and *in vivo* [36]. The terminal amino group of aminoguanidine, by virtue of its low pKa, reacts specifically with glucose-derived reactive intermediates and prevents protein-protein or protein-lipid AGE crosslinks from forming. This mechanism of action of aminoguanidine has now been confirmed in a number of studies, in which aminoguanidine was shown to prevent diabetes-related vascular complications in experimental animals [37–43]. From these extensive studies, it is apparent that aminoguanidine can be used to prevent advanced glycosylation and the AGE-mediated tissue in animal models of diabetes and aging. Of direct relevance to atherosclerosis, in a clinical study aminoguanidine caused a 28% decrease in LDL-cholesterol, a 20% decrease in total cholesterol and a 20% decrease in triglycerides [44]. Aminoguanidine and related advanced glycosylation inhibitors may eventually find widespread use in diabetics or in individuals at risk of age-related vascular sequelae. Development of aminoguanidine is now at phase II/III efficacy trials. A subsequently discovered agent, effective at 'breaking' AGE-crosslinks is under intense investigation as it offers the promise of reversing a number of AGE-mediated adverse effects [45].

It has been recently suggested that individuals with high levels of AGE-LDL in serum exhibit enhanced AGE deposition in their vessel walls and may therefore be at increased risk of atheroma formation [46]. High levels of circulating AGEs in non-diabetic and otherwise healthy adults may also be a reflection of dysfunctional AGE-receptor-mediated clearance mechanism since receptor expression is likely to be highly variable. While these questions are under study, early testing for AGE-related metabolic markers, such as AGE-

ApoB [37, 47] or AGE renal clearance [13, 31] may prove prudent as early warnings to those persons that are especially at risk to complications, enabling timely adjustments in dietary and life style prior to pharmacologic/invasive interventions.

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17. Xenotransplantation of encapsulated porcine islets

ANTHONY M. SUN

Editors' Comment:

Interspecies tissue and organ transplants (xenografts) have been imagined but not attained for thousands of years. Type 1 diabetic individuals depended for survival on insulin of xenogeneic origin (pigs and cows) from Banting and Best's first report in 1922 until the introduction of recombinant human insulin in the 1980s. A logical though until now impossible extension of the use of xenogeneic cell products is direct implantation of living xenogeneic cells. Researchers attempting this objective have had to deal with two key problems: (i) How many islets (or beta cells) are needed and where should they be implanted? (ii) What protective measures must be applied to protect islet viability? Sun, recounts his efforts to overcome both immunorejection and autoimmune rejection by immunoisolation of pancreatic islets. By first encasing rat islets in a semipermeable alginate–polysine–alginate microcapsule and then injecting encapsulated islets into induced diabetic mice, islet viability of 70–90% was attained for 3 months. Subsequently, a reproducible technique for porcine islet isolation was used to treat spontaneously diabetic cynomolgus monkeys with seven of nine monkeys rendered insulin independent for as long as 804 days. Sporadic successes using diverse techniques to implant islets in type 1 diabetic humans have been reported for two decades, each time engendering enthusiasm that a solution had been found. There is enough promise in the concept of microencapsulating xenogeneic islets to prompt an active clinical trial in China.

Introduction

Although the administration of insulin by injection is clearly a life saving intervention for patients devoid of β cells, this approach falls short of the remarkable titration of insulin delivery and consequent control of glucose levels achieved by normal, healthy individuals. In the absence of the physiological control of the plasma glucose concentrations, the daily injection of insulin has not been able to prevent the common complications of the disease, namely nephropathy, retinopathy, neuropathy as well as vascular complications. This has been confirmed in the recent Diabetes Control and Complications Trial (DCCT) which has demonstrated that intensive treatment of patients with insulin-dependent diabetes mellitus (IDDM) with tight glycemic control close to the control range effectively delays the onset, and slows the progression, of

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the various diabetic complications [1]. Therefore, it becomes mandatory to develop methods, applicable early in the course of the disease, and in any type 1 diabetic patient, for obtaining perfect metabolic control without increasing the risk of severe hypoglycemia. Consequently, the transplantation of islet tissue, either as whole pancreas or as isolated islets has been pursued because these techniques can provide near-normal blood glucose control and thus have the potential to prevent diabetic complications

In view of the many problems associated with the whole pancreas transplantation, grafts of isolated pancreatic islets represent the most promising approach to the restoration of the endocrine function of the pancreas in patients with IDDM. However, the problem of immune rejection remains. This has meant that the transplantation of insulin-producing tissue has largely been confined to diabetic recipients who already have a kidney transplant or who are receiving a kidney simultaneously with an islet transplant because of the risks of long term immunosuppression which are considered to outweigh the potential benefits of transplantation in a newly diagnosed diabetic patient.

To tackle the problem of immune rejection, a number of studies have investigated techniques to decrease the immunogenicity of transplanted islets and thus reduce for immunosuppression. These approaches known as immunoalteration are based on the hypothesis that islet immunogenicity is due to passenger leukocytes [2] or dendritic cells [3] and not the endocrine cells. Manipulations designed to destroy the dendritic cells and decrease islet immunogenicity include: culturing the islets for prolonged periods at room temperature [4] or in 95% O_2 [5], exposing the islets to ultraviolet radiation [6], cryopreservation [7] of islets and pretreating the islets with antibody to Ia antigen plus complement [8]. Despite considerable progress in the use of immunoalteration over the past years, this approach continues to have serious limitations. There is still no effective method of consistently protecting islet transplants from rejection by means of immunoalteration, with or without immunosuppression. No successful transplantation of immunoaltered islets into large animals has been reported, and indications are that such transplants would require immunosuppressive therapy in combination with donor islet pretreatment. While depleting donor tissue of passenger leukocytes before transplantation may temporarily prevent rejection, it does not eliminate the possible vulnerability of the transplanted tissue to a recurrence of the original autoimmune attack.

In view of the difficulties with overcoming the problem of immunorejection and autoimmune rejection the concept of immunoisolation has been advanced. This is achieved by enclosing the pancreatic islets by semipermeable and biocompatible membrane (bioartificial pancreas). The enclosed islets would act in the host as they had in the donor provided the surrounding membrane was impermeable to higher molecular weight antibodies but permeable to oxygen, glucose, other substances and/or the internally generated hormones. Thus, the encapsulated cells would respond to external substrate concentrations (e.g.,

blood glucose), and the required hormone (e.g., insulin) would be secreted into the systemic circulation. The clinical benefit of this approach is that diabetic patients would be provided with normal pancreatic islets which not only would be protected from immunorejection but would secrete, in addition to insulin, other hormones such as glucagon, somatostatin, pancreatic polypeptides and possibly other islet proteins – in response to physiological demand. This approach has the potential not only to allow allogenic transplantation without immunosuppression, but also to allow the use of xenografts and semipermeable membranes has great clinical potential for a wide range of diseases requiring enzyme or endocrine replacement therapy.

The alginate–polylysine–alginate (APA) microcapsule

In our approach to immunoisolation, we developed semipermeable, alginate–polylysine–alginate (APA) biocompatible capsules to enclose individual islets (microencapsulation).

The original microencapsulation procedure was developed by Lim and Sun in 1980 [9] and since that time the technology has undergone a great number of critical modifications and improvements. In its present form the membrane is composed of polylysine sandwiched between two alginate layers [10].

Scanning electron microscopy studies have shown that the APA microcapsules have a relatively smooth surface. The smoothness of the outer surface inhibits surficial cell attachment, enabling the microcapsules to remain semipermeable and effective inside the body for extended periods. The high water content (90% w/v) and negative charge of the outer alginate membrane also inhibit surficial growth. The high degree of biocompatibility of the APA biocapsules was demonstrated in numerous *in vivo* transplantation experiments in which microencapsulated pancreatic islets remained functional for as long as 26 months [11, 12]. Significantly, following intraperitoneal implantation of the microcapsules it is fairly easy to retrieve an overwhelming majority of the capsules by a simple lavage of the peritoneal cavity by a warm saline solution. Typically, in our experiments, following intraperitoneal implantation of empty APA microcapsules into mice or rats, 95–98% of the capsules were recovered 3 months later. When microcapsules containing rat pancreatic islets were used, 70–90% of them could be retrieved 3 months post-transplantation.

Capsule construction has improved considerably over the past years. It has been demonstrated that the capsule size is an important factor in the kinetics of insulin release by encapsulated pancreatic islets and in the easy access to nutrients and oxygen. Glucose tolerance in diabetic mice improved significantly after the mice received rat islets enclosed in capsules measuring 0.3 mm in diameter. By contrast, mice treated with islets enclosed in 0.8 mm capsules consistently experienced impaired glucose tolerance. The smaller capsules allow for:

1. Increased cell viability, as encapsulated cells have easier access to oxygen and nutrients.
2. Faster cell response to glucose fluctuations, since the dead space in the capsules is reduced.
3. Significant reduction in the volume of capsules needed for transplants.
4. Less susceptibility to cell overgrowth on capsular surfaces, as the smaller capsules have greater mobility.

The development of the electrostatic droplet generator [8] resulted in considerably smaller capsules (0.25–0.35 mm in diameter) with improved sphericity, surface smoothness and, most importantly, the strength of the capsules. When transplanted, the smaller capsules cause much less contact irritation, which in turn leads to a considerably smaller probability of cell overgrowth on capsular surfaces. *In vitro*, pancreatic islets encapsulated in the new capsules showed a response to glucose challenge which was comparable to that of free unencapsulated islets. The development of the droplet generator represents perhaps the single most important improvement in the microencapsulation procedure since its conception.

The chemical composition of the alginate has been considered by some investigators as crucial in attaining a high degree of biocompatibility of their microcapsules. The fibrosis of implanted microcapsules experienced by several groups was attributed to the mannuronic acid residues acting as cytokine inducers [9–12] while another study claimed that the final coating with a high mannuronic acid content alginate would actually reduce the amount of fibrosis around the microcapsules [13]. In our experience, the purity of the alginate – pyrogen and mitogen free – is much more important for the biocompatibility of the capsules than the alginate composition. However, the presence of both the mannuronic and guluronic acids is very important for the construction of the capsule. The proper polyelectrolyte complex formation between the anionically charged alginate (COO^-) and the cationically charged polylysine (NH_2^+) is of a crucial importance for the strength of the resulting membrane and elimination of either the mannuronic or guluronic acid would result in considerably weaker capsules.

The importance of the purity of the alginate was later confirmed by other investigators. Zimmerman et al. [14] have produced a method of preparing mitogen-free alginates. This work has incorporated the development of a mixed lymphocyte response assay to test the purity of the alginates and, as already suggested, has demonstrated that it is the purity and not the chemical composition of the alginate that affects the biocompatibility of the capsules. The nature of the contaminants was not determined in this study, but the authors commented that they could be oligomers of mannuronic and guluronic acids. Detailed descriptions of purification protocols such as that described by Zimmerman et al. [14] are essential if comparisons of encapsulated transplant experiments are to be made, and if the encouraging results of some groups are to be repeated by others who are not sharing the same degree of success.

In summary, the construction of perfect capsules in terms of their size, sphericity, strength, surface smoothness, biocompatibility and purity of the component substances is of the uppermost importance for the proper physiological function and survival of the encapsulated graft.

It should be noted here that considerable effort was made in a number of laboratories around the world to develop alternative macroencapsulation techniques, whereby a number of pancreatic islets are immunoisolated within diffusion chambers and placed intraperitoneally, subcutaneously or in other sites. Numerous devices of this type have been evaluated during the last several years. These include disc-shaped diffusion chambers, Millipore cellulosic membranes, hollow fibers diffusion chambers and wider bore tubular membrane chambers [15]. Membrane materials used to fabricate these devices include polyvinylchloride, polyamides (nylon), polypropylene, polycarbonate, cellular nitrate and cellulose triacetate. The common feature of this approach is that a great number of islets are placed within the chamber of the device. Consequently, the problem of cell death or dysfunction as a result of oxygen and nutrient supply limitations, or accumulation of wastes or other agents is likely going to be severe. Chambers retrieved several weeks after implantation often contain a central necrotic core. Another problem is the frequent breakage of the diffusion chambers which can cause not only loss of islet function but also intraperitoneal inflammatory responses. The problems posed by the large volume of implanted devices as well as a possible fibrosis of the chamber surfaces can further aggravate the situation. Typically, the use of these devices can result only in a short term amelioration of diabetic hyperglycemia.

The proponents of the various different macroencapsulation approaches freqently criticize the microencapsulation concept as for the overall volume of the graft. In fact, this is not an issue, taking into consideration the fact that the capsule volume is *directly proportional to the cube of the capsule radius.* Thus, if one million microencapsulated islets should be necessary to normalize hyperglycemia in a type 1 diabetic patient, the overall volume of the intraperitoneally implanted capsules of 300 μm in diameter would only amount to 14.1 ml, dropping to just 8.1 ml if the capsule diameter decreases to 250 μm. At the same time the transplanted microcapsules will cause very little contact irritation to the surrounding tissues as compared to the implantation of the various different types of diffusion chambers used by the proponents of the macroencapsulation concept.

Transplantation of microencapsulated pancreatic islets

In our past studies in small animal models we have clearly demonstrated that both allografts and xenografts of microencapsulated pancreatic islets were protected from immunorejection and that in both streptozotocin-induced and spontaneously diabetic recipients diabetes was reversed for extended periods of time [11, 12, 21–26]. At the same time these studies have unequivocally

advanced the use of xenografts as the most promising approach to the treatment of diabetes. Consequently, a prerequisite for future clinical transplantation of pancreatic islets into diabetic patients is the development of a reliable method of isolating donor islets from large animals. Since porcine pancreata are readily available and the molecular structure of porcine insulin resembles closely that of human insulin, the isolation of porcine islets becomes very attractive in the effort to develop a plentiful source of pancreatic islets.

The method of porcine islet isolation developed in this laboratory [27] is reproducible in terms of both the quantity and quality of the isolated islets. It involves a gentle manual processing of the pancreas, thus avoiding mechanical stress associated with the use the various different mechanical devices.

This development of a plentiful supply of pancreatic islets led to the initiation of a preclinical study in which microencapsulated porcine islets were xenotransplanted into spontaneously diabetic cynomologus monkeys [28]. In this study, porcine pancreatic islets were encapsulated in alginate–polylysine–alginate microcapsules and transplanted intraperitoneally into nine spontaneously diabetic cynomologus monkeys. Following one, two or three transplants of $3–7 \times 10^4$ islets per recipient, seven of the monkeys became insulin independent for periods ranging from 120 to 804 days with fasting blood glucose levels in the normoglycemic range. Glucose clearance rates in the transplant recipients were significantly higher than before the graft administration and the insulin secretion during glucose tolerance tests was significantly higher compared to pretransplant test. Porcine C-peptide was detected in all transplants recipients throughout their period of normoglycemia while none was found before the graft administration. Hemoglobin A_{1C} levels dropped significantly within 2 months after transplantation. While ketones were detected in the urine of all recipients before the graft administration, all experimental animals became ketone free two weeks after the transplantation. Capsules recovered from two recipients 3 months after the restoration of normoglycemia were found physically intact with enclosed islets clearly visible. The capsules were free of cellular overgrowth. Examination of internal organs of two of the animals involved in the transplantation studies for the duration of 2 years revealed no untoward effect of the extended presence of the microencapsules.

An examination of the glomerular membrane thickness revealed that while the GMT of exogenous insulin-controlled monkeys was abnormally widened after 6 months of diabetes (502 ± 79 nM), a much smaller thickening was observed in transplant recipients following the same period of time (369 ± 57 nM), and a normal value was found in the non-diabetic controls (228 ± 38 nM).

In this study we have for the first time demonstrated that spontaneous diabetes can be reversed for significant periods of time in a large animal model by immunoprotected islet xenografts without recourse to exogenous insulin therapy or to immunosuppression. The long survival of the microencapsulated graft can be attributed to two major factors: The strength of the capsular

membrane and the purity of the porcine islet tissue. The greater strength is of the capsules is directly related to their smaller size (0.25–0.35 mm in diameter). The improved capsular strength results virtually in no breaks of the grafted capsules as demonstrated in our previous study [29], thus significantly improving the graft survival. In addition, the smoother surface of the new capsules is conducive to a more effective coating with both the poly-L-lysine and alginate, thus improving the biocompatibility of the capsules.

The long-term survival of the encapsulated xenograft previously demonstrated in rodents was reconfirmed in this preclinical study in primates. We have shown in a large animal model the ability of the encapsulated graft to achieve physiological glucose-insulin kinetics. Of equal importance is our finding that the until now elusive porcine islets can be isolated while retaining their physiological competence and that the immunoisolated porcine xenografts can effectively reverse diabetes in long-term experiments in primates.

The use of large animals for transplantation of pancreatic islets encapsulated in alginate–polylysine–alginate microcapsules was also reported by Soon-Shiong et al. [30, 31]. In their first study microencapsulated canine islets were allotransplanted into spontaneously diabetic dogs. Insulin independence was achieved for an average of 105 days. Immunosuppression with cyclosporin A was employed for the entire duration of the experiments. In subsequent studies [31] the authors further extended the allograft survival, however immunosuppression with cyclosporin A was still used for the first thirty posttransplantation days. Calafiore [32] achieved insulin independence in one out of three dogs with alloxan-induced diabetes by transplants of alginate–polylysine–alginate–microencapsulated human islets, and transiently in one human patient. However, the microcapsules in that study were deposited in artificial prostheses directly anastomosed to blood vessels.

More recently, Soon-Shiong et al. [33] has reported insulin independence achieved in a type 1 diabetic patient following two transplants, 6 months apart, of human islets encapsulated in alginate–polylysine–alginate microcapsules. The exogenous insulin had been gradually discontinued over a 9 month period until insulin-independence was established. Again, immunosuppression with cyclosporine A was used. The authors have not yet shown whether similar results can be obtained without immunosuppression.

As reported by Clayton in his review article [34], the alginate–polylysine–alginate capsules have been the most widely reported of all encapsulation techniques. Several other methods have been described for islet microencapsulation. Braun et al. [35–37] used cellulose sulfate and polydimethyldiallylammoniumchloride and demonstrated good *in vitro* insulin release after five weeks in culture. Gin et al. [38] have reported using a membrane of polyacrylamide and agarose. However, after three weeks *in vivo* the capsules had become cloudy, presumably due to cell overgrowth, and the islets did not respond to changes in glucose concentrations. Sefton [39] and Sugamori [40] reported the use of a polyacrylate membrane. However, their results demonstrated that

this material was neither sufficiently permeable nor biocompatible for use in transplantation.

Tatarkiewicz [41] published the results of diffusion studies using islets in alginate droplets which had been coated with protamine sulphate and heparin to form the semipermeable layer. Although the results demonstrated good diffusion of insulin and glucose across the membrane and impermeability to immunoglobulins, there were no data relating to the biocompatibility or *in vivo* function of these capsules. Iwata [42–45] used agarose for islet encapsulation and reported excellent *in vitro* insulin release, even after 100 days in culture, and transplants into non-obese (NOD) diabetic mice have survived for over 100 days, with little evidence of graft fibrosis at 102 and 192 days post transplant.

Lanza et al. [46, 47] have reported the use of bovine or porcine islets encased in uncoated spherical hydrogel microspheres composed of calcium alginate. One to four islets were contained in each microsphere, or 'bioreactor', the diameter of which varied in different experiments from 0.8 to 4.0 mm. The 'bioreactors' were xenografted into diabetic mice and rats. The grafting resulted in the reversal of diabetic hyperglycemia for a number of weeks. Implantation of the microsheres was also reported [44] to have completely supplanted exogenous insulin therapy in spontaneously diabetic dogs for 60 to > 120 days. The microspheres used in their studies were produced from alginates with a high guluronic acid contents. The microreactors were permeable to molecules with a molecular weight of up to > 600 kD (including IgG and the various proteins of the complement system). Thus, the ability of uncoated microspheres in these studies is quite surprising, and would appear to suggest – according to the authors – a significant role for cell-mediated immunity in the process of xenograft rejection in the employed models.

The chemically synthesized copolymer HEMA-MMA has been evaluated as a material for capsule construction by our group in 1987 and by Sefton in 1995. Two major problems were identified: (i) The membrane thickness (100 μm). Although the membrane is very strong and does not break easily, its thickness greatly obstructs the passage of nutrients and metabolites and the diffusion of hormones; (ii) In general, capsules prepared from synthetic polymers suffer from the poor biocompatibility. Currently, their use has been abandoned.

The most essential criteria required for the generation of functional APA capsules can be summarized as follows: (i) purified, sterile, pyrogen-free sodium alginate; (ii) the molecular weight of poly-L-lysine determines the molecular cut-off point of the capsule; therefore for any given purpose of the capsules, the appropriate molecular weight of the poly-L-lysine has to be carefully selected; (iii) the need for an intact and smooth membrane; here, the formation of alginate beads represent a crucial starting point; the use of the droplet generator results in a significant improvement of the smoothness of the capsule membrane as well as the sphericity of the capsules; (iv) the reaction time with poly-L-lysine affects the membrane thickness, and in some cases also the

porosity of the membrane; (v) the second sodium alginate coating determines the biocompatibility of the capsule; if it is not performed properly, the exposed poly-L-lysine tends to quickly attract macrophages; (vi) the reaction time with sodium citrate must be strictly controlled if strong capsules are desired; a prolonged reaction time can weaken the capsule.

In summary, there are many factors contributing to the quality of the capsule. In order to make sure that the capsules can be used in long-term transplantation studies, the finished membrane needs to undergo extensive physical and chemical analyses, such as testing for the capsule size and sphericity, the membrane thickness and smoothness, the percentage of water content, the strength and flexibility of the membrane, the porosity of the membrane, the possible presence of holes or slits, the completeness of the outside coating with sodium alginate, as well as the determination of the zeta potential of the membrane.

Recently Wong et al. [48] has modified the APA membrane by using a combination of sodium alginate, cellulose sulfate, polyethylene-co-guanidine, $CaCl_2$, and NaCl. The modification resulted in an increase in the membrane thickness as well as its durability. Again, the greater thickness negatively affects the diffusion dynamics of the membrane. Consequently, the greater thickness does not really result in an improved capsule.

The development of successful techniques for transplantation of pancreatic islets has led to a search for techniques that would allow long-term storage of islet tissue, which would have a number of advantages for clinical application. These would include easy transport between centers, the possibility of transplanting islets from multiple donors, as well as the flexibility to separate the donor and recipient both in distance and in time between harvest and transplant. This would buffer the current imperfections in isolation results and decrease reliance on local donor availability. Finally, large scale production, quality control, and the surveillance for biological contaminants will require a storage capacity. Cryopreservation remains the most practical method for long-term storage, but despite many studies, an ideal method for freezing islets has not emerged, although a wide range of techniques have been described.

Cryopreservation, although documented in its ability to preserve islets [49–51] often results in lost function and the retrieval of diffuse, broken, and clumped islets contaminated by tiny fragments. As the production of smooth, fully biocompatible and strong encapsulated grafts requires solid, intact, and discrete islets, an ability to freeze the islet after encapsulation is an absolute and pressing requirement. In addition, as the graft preparation is highly specialized, it is envisioned that the processing will be fully completed at a centralized point, after which the transplant-ready grafts would be frozen and shipped to the user site for thawing and transplantation with minimal on-site requirements. Finally, it is postulated that the capsule may actually serve to protect the fragile islet from storage-related damage, thus improving the poor yield cur-

rently being obtained. This is especially important in considering the extreme fragility of porcine islets.

To study the feasibility and prospective benefits that may be gained by storing microencapsulated islets of Langerhans, rat islets, well established donor islets with very reproducible fresh and cryopreserved endocrine indices, were studied [52]. Cryopreserved encapsulated rat islets were assessed *in vitro* for insulin secretory capacity and in transplantation studies for function and biocompatibility.

The results showed that the insulin response of cryopreserved encapsulated rat islets was comparable with fresh islets. Transplantation of 800–900 banked rat islets resulted in the normalization of the metabolic blood glucose perturbation, body weight, and general health characteristics of all transplanted diabetic mice for the study duration of 90 days. Whereas free islets are easily fragmented and lost during the freezing process, the capsule protects the islets from freezing damage, increasing the retrieval rate from $79.5 \pm 9.8\%$ to $97.2 \pm 1.3\%$.

In subsequent studies we have endeavored to develop a technique for cryopreservation of microencapsulated porcine islets while applying the findings of the previous study on rat islets. The porcine islets were isolated as previously described [28, 29], microencapsulated in alginate–polylysine–alginate membranes and cryopreserved using the BioCool III-80 cooling apparatus as described in the previous publication [51].

Although some degree of islet fragmentation occurs during the isolation procedure, the freezing procedure did not result in any further fragmentation of the microencapsulated islets. By this procedure we were able to recover 96–98% of the prethaw number of encapsulated islets. This is a considerable improvement over the 66% recovery value reported by Marchetti et al. [53] for the prethaw porcine islet equivalent number.

In preliminary *in vitro* results, seven out of ten cryopreserved lots of porcine islets responded to the high glucose static challenge. *In vivo*, intraperitoneal transplantation of 2000 cryopreserved microencapsulated porcine islets into diabetic mice resulted in the reversal of hyperglycemia in six out of ten recipients for the entire duration of the ninety day study. Although a considerable degree of protection of the delicate porcine endocrine pancreatic tissue during the cryopreservation process and the subsequent long-term storage has been demonstrated in this study, it appears that the cryopreservation procedure will need to be further developed to improve the retention of viability of the thawed islets.

Conclusion

The shortage of human islets for transplantation purposes advanced the idea of the use of immunoisolated islet xenografts as a future approach to islet grafting into human diabetics. The pig has generally been considered as a prime canidate, mostly because of its wide availability and the similarity of the

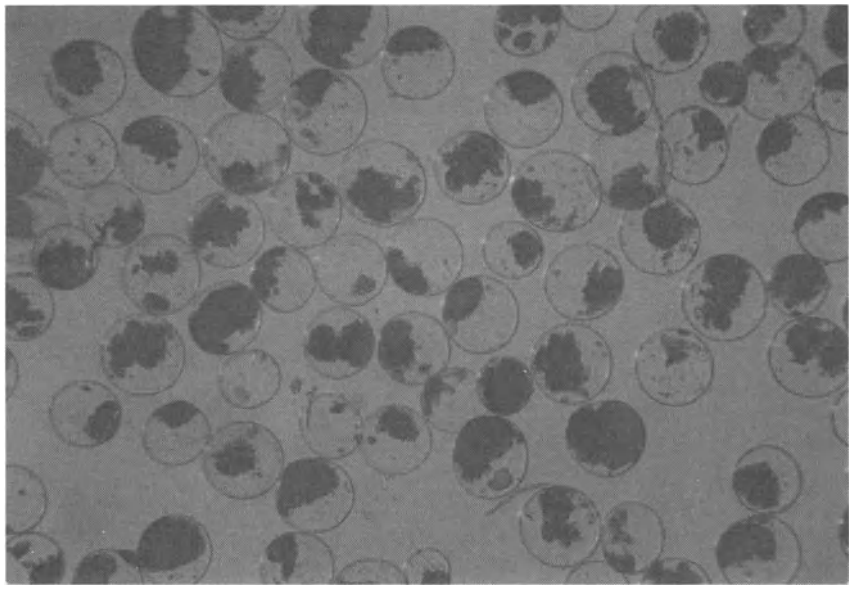

Fig. 1.

molecular structure of its insulin to human insulin. Despite these clear advantages, the use of porcine islets has been hampered by several factors. The technical difficulties associated with their isolation plus the relatively high cost of their procurement suggest that more original thinking has to be done to overcome these problems. To this effect, establishment of a national research center dedicated to selecting a suitable genetical line of donor animals, as well as to the development of proper procedures for isolation porcine islets and of the necessary quality control guidelines appear to be a pressing requirement. In addition, the necessary work aimed at assuring compliance with the regulatory authorities' guidelines for the future clinical transplantation will also have to be carried out. The foresight and the willingness to make available the necessary financial resources to attain the goal appears to be essential. At the same time it seems imperative to search for alternative types of insulin-producing cells which could form the basis for the next generation of transplants. These might conceivably involve, for example, the construction of artificial beta cells by means of genetical engineering.

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18. Generation of non-immunogenic islet cells using genetic engineering

MICHAEL BROWNLEE

Editors' Comment:

Can non-immunogenic insulin-producing pancreatic cells be created by genetic engineering? Brownlee relates the rationale for and substantive progress to date in this 'Star Wars' level research. By inserting an immortalized cell line with no potential for malignant mutagenesis, murine β-cells from transgenic mice expressing a rat insulin promoter-driven immortalizing gene plus an internal suicide gene were synthesized. Brownlee envisions combination of several genetic engineering strategies to produce a non-immunogenic β-cell line that will be clinically effective. Protection against autoimmune destruction would be included by transducing these cells with an anti-apoptotic gene. Alternatively, the insulin-producing cells would be encapsulated as a means of avoiding rejection. How long will it be before genetic engineering makes obsolete all previous treatment regimes for type 1 diabetes? As in so many fields of medicine, traditional physicians observe the rapidly arriving future in a state of wonderment. Clearly, 21st Century diabetes care is likely to be unrecognizable in the protocols now becoming obsolete.

Introduction

A major long-term goal of diabetes research is to re-establish normal glucose homeostasis in insulin-deficient patients by transplantation of insulin-producing pancreatic cells without the need for toxic immunosuppression [1–3]. A second major long-term goal of diabetes research is to overcome the inability to produce a sufficient number of islet cells of whatever origin for widespread clinical application.

A number of different approaches to circumventing human islet cell allograft rejection are currently under development. Broadly conceived, these approaches focus either on specific modification of the recipient's immune system, or on modification of the islet tissue to be transplanted.

Approaches of the first type include creation of full or mixed chimerism [4–6], thymic islet implantation [7, 8], and the use of co-stimulator antagonists such as CTLA4Ig [9, 10]. Each of these has shown promise in animal models, but none are yet ready for translation to humans. Creation of chimerism currently requires potentially harmful bone marrow irradiation [4–6], thymic involution in adults may attenuate the tolerizing effects of islet implantation,

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and it is likely that several different co-stimulator molecules exist in humans in addition to B7 [10].

Approaches of the second type include immunoisolation of graft tissue inside various permselective polymers [11–15] and genetic engineering of cells to circumvent allorejection [16–18]. Immunoisolation strategies have had increasingly encouraging results in recent years, but the large volume of polymer-enclosed material required due to oxygen-flux limiations on packing density and the difficulties associated with polymer retrieval and replacement limit clinical applicability at present [19].

Genetic engineering of cells to circumvent allograft rejection of islets has also had encouraging results in recent years [16, 18]. One approach is based on the observation that when CD8 positive T-cells become activated, they express CD95, a molecule which delivers an apoptotic signal when bound by its ligand, CD95L. It is thought that this is a mechanism by which activated lymphocytes are eliminated at the end of an immune response [20]. When syngeneic myoblasts were genetically engineered to express CD95L, composite grafting of allogeneic islets protected the islets from rejection for over 80 days [18]. General activation of CD95 results in fulminant liver destruction and rapid death in mice [21–23], however, and the possibility of cumulative tissue damage from protease-released CD95L molecules remains a concern.

Elimination of MHC Class I molecules on islet cells prevents allograft rejection

A second genetic engineering approach to circumventing allograft rejection of islets has been taken by our group. This approach is based on the observation that islets from β2-microglobulin knockout mice transplanted into allogeneic recipients survive nearly indefinitely [24], suggesting (a) that down-regulation of class I MHC is sufficient to abrogate allorejection, and (b) that cells lacking class I MHC are not destroyed by NK cells. Since genetic engineering of human islet cells will have to use a dominant negative strategy to eliminate class I MHC expression, the first iteration of such an approach utilized an adenoviral protein, gp19K, which retains class I molecules in the endoplasmic reticulum of infected cells [25].

Transgenic mice were made which expressed this gene under the control of the rat insulin promoter, and transplantation experiments were performed using gp19K-expressing B6D2 donors (genotype $H2^{b/d}$) into BALB/c (genotype $H2^{d}$) recipients. These islets remained viable for at least 91 days. Corresponding non-gp19k expressing islets were rejected between 14–21 days. However, transplantation of gp19K-expressing B6D2 donors (genotype $H2^{b/d}$) into B6 (genotype $H2^{b}$) were not successful, suggesting that the known differences in binding affinities of gp19K for various MHC class I alleles were important determinants of outcome [16, 26, 27]. This same strategy has also prevented the development of autoimmune diabetes in the transgenic lymphocytic choriomeningitis virus model of Type I diabetes [28], although much evidence suggests that auto-

immune destruction of islets in other models occurs through the indirect pathway via $CD4^+$ effector cell secretion of cytokines and reactive oxygen species.

In summary, these experiments in which the adenovirus protein gp19K was expressed in murine pancreatic beta cells have demonstrated that introduction of genes which downregulate cell surface expression of MHC class I molecules can prevent allorejection [16]. However, significant differences in binding affinities of gp19K for various mouse and human MHC class I alleles [26, 27] limit this effect to specific inbred murine strains. Thus, this particular protein will not be useful for the universal downregulation of class I expression required for human islet allotransplantation, and other dominant negative strategies utilizing other class I/peptide-inhibiting gene products must be used.

Creation of apoptosis-resistant islet cells may prevent loss inside immunobarrier devices due to recurrent autoimmunity

Although separation of islet cells from immune system elements such as IgG and lymphocytes appears to be another promising strategy for overcoming allorejection, but cell-loss of allografts and xenografts from hypoxia-induced apoptosis remains a concern. More worrisome for treatment of Type I diabetes is the failure of immunobarrier techniques to protect islet grafts from recurrent autoimmune destruction in animal models [29]. Recent data suggest that autoimmune destruction of such islets is due to cytokine-and reactive oxygen-induced β-cell destruction by apoptosis [30]. Because the proto–oncogene bcl-2 has been shown to prevent apoptosis in many cell types [31–34], genetic engineering strategies using this gene product to block apoptosis have the potential to prevent autoimmune-mediated allo- and xenograft destruction. To test this hypothesis, our group utilized a replication-defective HSV-1 amplicon vector to transfer the bcl-2 gene into both murine and human β-cells. Cytokine-induced apoptosis was blocked in cells expressing bcl-2, while cells expressing a marker gene showed nucleosomal fragmentation characteristic of apoptosis [35].

Regulatable islet cell growth may overcome the problem of limited tissue supply

A second major long-term goal of diabetes research is to overcome the inability to produce a sufficient number of islet cells of whatever origin for widespread clinical application. It is estimated that the current availability of human islets would limit transplantation to only several hundred patients a year [36]. Production of pure islets from animal tissue is extremely labor intensive at the present time. In addition, the potential problem of unknown human disease being caused by endogenous animal retroviruses is a serious unsolved issue. To provide sufficient numbers of human cells for eventual widespread clinical use, β-cell lines may offer a significant advantage. However, permanently

immortalized β-cell lines have an altered glucose-stimulated insulin secretion [37, 38] and in the case of SV40 large T antigen transformed cells, ultimately become neoplastic [39, 40].

To circumvent these problems, two approaches have been taken, using rodent cell models. One approach involves stably transfecting transformed β-cells (RIN cells) with the genes for the high Km β-cell glucose transporter (GLUT2) and glucokinase [41]. The cells remain transformed, however, and require further genetic engineering to restore normal glucose-coupled insulin secretion. A second approach has been to express an immortalizing gene in β-cells using an inducible promoter. Our group at Einstein has used the tetracycline-inhibitable transactivating system as a first iteration [42]. In this system, a fusion protein containing the DNA-binding domain of the tetracycline repressor protein and the activating domain of the herpes simplex vp16 protein is constitutively expressed from the insulin promoter. In the same β-cells of double transgenics, the T-antigen gene is driven by a minimal promoter combined with tetracycline operator sequences. In the absence of tetracycline, the constitutively expressed fusion protein binds to the operator sequences and transactivates expression of T antigen. Addition of tetracycline prevents binding of the fusion protein and thus shuts off T antigen production. Other, perhaps tighter inducible expression systems could presumably be used instead of the TET repressor system [43, 44].

Problems with this approach include unavoidable basal expression of the transforming gene, and the potential for mutagenic reactivation *in vivo*. An alternative approach that we are currently pursuing may overcome both of these problems. To provide a renewable source of β-cells having normal insulin secretory characteristics and no potential for mutagenesis, we have constructed a conditionally immortalized murine β-cell line using transgenic mice with a loxP-flanked (L) bicistronic transgene (LRTITL) expressing rat insulin promoter-driven (R) SV40 T Antigen (T) as the immortalizing gene and an internal ribosome entry site (I)-controlled suicide gene, HSV-thymidine kinase (T), to destroy non-inactivated cells after excisional inactivation by a Cre recombinase-expressing herpes simplex virus-derived viral vector (Ju, Q., and Brownlee, M., unpublished data).

SV40 T antigen has been used successfully to generate β-cell lines in transgenic mice [45, 46], including the tetracycline-regulated system described above. Likewise, the Cre-loxP recombination system of bacteriophage P1 [47, 48] has been shown to be capable of mediating loxP site-specific recombination in transgenic mice [49, 50]. Combining these strategies will provide a β-cell line for growth and expansion in the laboratory, and subsequent excisional inactivation of the immortalizing gene which will preclude both basal expression of the oncogene and potential reactivation *in vivo*. Since the Cre recombinase is not 100% efficient, even when a nuclear localization signal is included [49], we are co-expressing HSV thymidine kinase in in the construct, so that non-recombined cells can be selectively deleted by treatment with

gancyclovir [51–53]. Both genes are expressed from a single promoter, the rat insulin promoter [45], using an internal ribosome entry site from encephalomyocarditis virus [54–57], in order to avoid the possibility of selective inactivation of a second promoter driving TK. The recombinase itself is introduced when desired by means of a replication-deficient amplicon-based HSV virus-derived vector [58, 59]. This approach has several potential advantages over Cre expression from an inducible transgenic construct. First, it avoids the possibility of premature recombination due to basal expression of the enzyme. Second, it achieves a higher level of Cre expression because of the use of a strong viral promoter. Third, it avoids the possibility of continuous Cre expression, since expression from these episomal vectors stops after several weeks [60–62]. The ability to completely eliminate foreign gene products prior to transplantation may be particularly important for transplantable cells in light of recent reports that transgene expression can lead to cytotoxic lymphocyte generation [63–65].

Schematic model

A scheme can be envisioned in which the various genetic engineering strategies discussed above are combined to produce a non-immunogenic β-cell line which can be expanded for use in clinical treatment of diabetes. First, a human (-cell line expressing the loxP-flanked SV40 large T antigen–IRES–thymidine kinase construct would be made using a viral vector that integrates into non-dividing cells (Ju, Q., and Brownlee, M., submitted). These cells would then be transduced with an anti-apoptotic gene such as bcl-2 or superoxide dismutase, in order to protect from autoimmune destruction. These cells would be further transduced with MHC-I/peptide inhibiting genes such as gp19K, in order to protect against allorejection. Alternatively, the cells could be excisionally activated and then encapsulated. Prior to transplantation, immortalization is reversed by addition of HSV-1 virions expressing Cre recombinase. From experiments with the tetracycline system discussed above, it is known that these transformed β-cells reassume their normal glucose-stimulated insulin secretory pattern. Finally, non-recombined and therefore still immortalized cells will be eliminated by the addition of gancyclovir, since they and they alone will still express thymidine kinase. The excisionally inactivated cells can then be cryopreserved until needed for clinical application.

Acknowledgements

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19. Insulin-independence for > 10 years in 32 pancreas transplant recipients from an historical era

J. S. NAJARIAN, A. C. GRUESSNER, M. B. DRANGSTEVEIT, R. W. G. GRUESSNER, F. C. DOET & D. E. R. SUTHERLAND

Editors' Comment:

Until microencapsulation of islets or β-cells or genetically engineered insulin-producing cells are in hand, the best option for preventing microvascular complications in type 1 diabetes is a allogeneic pancreas transplant. True, the surgery is difficult mandating long hospital stays. Also, when compared with a kidney transplant alone, both morbidity and mortality are greater in recipients of a combined pancreas and kidney transplant. Najarian reviewed his extraordinary experience with 867 pancreas transplants performed since July 1966. In this report, the 20 pancreas recipients transplanted between 1978 and 1986 who were alive with a functioning graft 10 to 17 years posttransplant were analyzed. By means of a mail questionnaire, responses on life quality were obtained from 14 survivors of whom all 14 preferred their sometimes tenuous life with a pancreas graft to that of medical diabetes management. Indeed, a remarkable 13 of 14 respondents thought that they would now be dead had they not received a pancreas transplant. Our limited, and thus far, short term observation of pancreas recipients confirms such broad patient acceptance. Physicians must deliver what is available today while eyeing a better future. Pancreas transplants though difficult, expensive, and stressful are nevertheless the preferred therapeutic option for all those with type 1 diabetes who are younger than age 45.

Introduction

Between July, 1966 and August 1997, 867 pancreas transplants were performed at our institution. Of these, 200 were done during a pioneering and learning era (July 1978 – August 1987) using azathioprine & prednisone or cyclosporine, azathioprine and prednisone for immunosuppression [1]. Of these, 32 functioned for > 10 years. During this period, the emphasis was on solitary pancreas transplants (alone, PTA, $n = 107$; after a kidney, PAK $n = 75$), and only 18 were simultaneous with a kidney (SPK, all bladder drained (BD), of which 7 are still functioning).

Of the solitary transplants, 15 were open duct (OD, 1 functioning > 10 years), 3 were ligated, 41 were duct injected (DI, 1 functioning > 10 years), 85 were enteric drained (ED, 15 functioning > 10 years), and 38 were BD

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(7 functioning > 10 years). Of the PTA, 41 were living donor (LD, 9 functioning > 10 years) and 66 CAD (6 functioning > 10 years); of the PAK, 19 were LD (5 functioning > 10 years) and 56 CAD (5 functioning > 10 years). The overall technical failure rate was 27%. For PTA, 1 and 10 year graft survival (GS) rates were 35% and 14%; for PAK 32% (53% for LD) and 13% (26% for LD). (The 1978–86 results of solitary pancreas transplants contrast to those in the most recent era, 1994–97, in which 1 year GS for CAD PAK ($n = 65$) was 77% and for CAD PTA ($n = 27$) was 61%). The value of being able to monitor for rejection indirectly via a kidney was seen in the 1978–86 subset of 19 PAK recipients of LD pancreas from the same donor of the previous kidney; of 11 that were TS, 1 year and 10 year GS were 91% and 46%.

Indefinite pancreas graft function seems possible, the longest currently for LD being 17 years for a PAK (DI) and 16 for a PTA (ED) and for CAD being 17 years for a PAK (OD) and 14 years for a PTA (DI).

The 20 patients transplanted from 1978 to 1986 (the 7 for 1987 were not surveyed) who were alive with a functioning graft at 10 to 17 years post-transplant were sent a questionnaire on their current status; 14 answered. Of these, 8 were working full time (57%), 2 part-time (14%), and 4 were not working, 2 by choice (14%) and 2 because of disease (14%). Quality of life health status (Karnosfsky's) showed 6 were normal with no complaints (42%), 6 engaged in normal activity with some disease symptoms (42%), and 2 required occasional assistance (14%). Eleven were very satisfied (79%) and 3 were fairly satisfied (21%) with life, while 7 were very satisfied (50%) and 7 were fairly satisfied (50%) with health. On ability to manage life, 9 were very (64%) and 5 were fairly (36%) independent.

In response to the question, "If you had not had the pancreas transplant, how would you picture yourself today?", 13/14 responded "Dead". In response to the question, "Do you feel you would be better off dealing with diabetes or dealing with the side effects of immunosuppressions?", all 14 (100%) responded, "Dealing with immunosuppression is better."

Conclusion

Pancreas graft function for > 10 years occurs with all duct management techniques, regardless of donor source or recipient category. Pancreas graft chronic rejection rates between 1 and 10 years are similar to that of other organs. The 1 year GS rates are now much higher than in the pioneering era, and we predict that thousands of patients will achieve > 10 years of insulin independence within the next decade.

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20. Giving up: discontinuation of dialysis

CARL M. KJELLSTRAND

Editor's Comment:

From the establishment of Scribner's "Who Shall Live Committee," to the present struggle to include all who might benefit from ESRD therapy, Carl Kjellstrand has served as a conscience for nephrologists. Illustrative of the breadth of Kjellstrand's understanding of the socio-medico-psycho-economic interactions in renal failure therapy are his insights into the pathogenesis of withdrawal from dialytic therapy. Why, when there is competition to gain life-sustaining treatment will some who have been "fortunate" in gaining admission to a program opt to discontinue their treatment? While no single answer is self-evident, behavior of diabetic patients who so often are maimed and "beaten" by co-morbid diabetic complications offers important clues as to the rationale underlying the election of death.

Introduction

> *"It is not a question of dying early or later, but of dying well or ill, and dying well means the escape from the danger of living ill."* (Seneca, 4 BC)

Every technical breakthrough brings with it unforeseen ethical problems. Dialysis is no exception. The two main ethical problems with the procedure have been unplanned or ill-planned rationing, mainly along irrational and discriminatory gender and age lines, and discontinuation of dialysis [1]. The second ethical problem, discontinuation of dialysis with the knowledge that death will inevitably occur shortly, will be the subject of this chapter.

Until the mid 1980s, stopping treatment as a cause of death of diaylsis patients was either unknown, not well known or not acknowledged, as such a cause of death was never reported from the large dialysis registries. Some patients were stated to have died of failure to thrive, uremia or social causes, code words for discontinuation, but also other causes such as accidents or suicide. Since the first description of this cause of death [2] there has been an increase in the recognition of it, and the number of patients who stop dialysis and are reported is rapidly increasing.

How common is discontinuation?

From being not recognized at all, discontinuation of dialysis has now become the second leading cause of death reported by the dialysis registries in USA,

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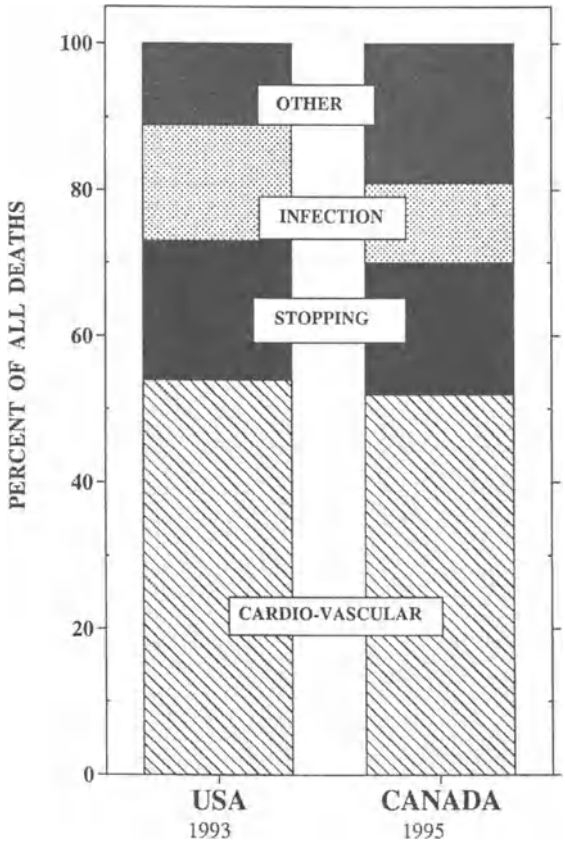

Fig. 1. Cause of death, as reported by the most recent dialysis registries in USA and Canada. Most patients die of cardiovascular disease, but stopping dialysis is the second leading cause, more common than deaths from infections.

Canada and Australia [3–7]. Figure 1 summarizes the data from USA and Canada. Discontinuation is responsible for between 15 and 25% of all deaths, secondary only to cardiovascular death which is usually responsible for approximately 50% of all deaths. Data from the US dialysis registry indicate that in USA between 1 and 2% of all dialysis patients discontinue treatment and die every year [3].

Who stops dialysis? 1: demography

Age

In USA, Australia, Canada and Europe the percentage of death caused by discontinuation of dialysis is related to age [3–6]. While few young patients

stop, and only 3–7% of patients below age 40 discontinue, it increases to 50% or 60% in the age group over 70 years and is the most common cause of death among the older dialysis patients. The same age-related pattern is described by the European reports. A different pattern is seen in Japan [7]. While the percentage of young patients in Japan that die due to discontinuation is the same as in the Western world, the percentage of such deaths declines, rather than increases, with age. Obviously cultural factors that reflect views of aging are at play.

Race

Racial factors and discontinuation of dialysis have been analyzed in the USA. In general discontinuation of dialysis is only half as common among black patients as among white [8].

Diagnosis

Diagnosis is of importance. Diabetic patients are at particular risk for this cause of death. Diabetic patients discontinue dialysis twice as often as non-diabetic patients [3].

Method of dialysis

The method of dialysis is of importance: stopping occurs twice as often in patients on peritoneal dialysis or on home hemodialysis as on in center hemodialysis patients [1–3, 9]. Struggling with dialysis difficulties at home, without the support and help with conflict solution by dialysis personnel several times a week, is an important problem that leads to discontinuation.

Dialysis method and diabetes

In the USA one of 30 diabetic patients on peritoneal dialysis discontinues every year, compared to 1 of 50 diabetic hemodialysis patients. The corresponding figures in patients with other diagnosis is 1 in 100 and 1 in 140 patients respectively [3]. Thus stopping dialysis is three times more common in diabetic patients.

Who stops dialysis? 2: independent risk factors

In one study at a large center, risk factors for discontinuation of dialysis included age, the presence of many co-morbid conditions, and home demodialysis [1]. In an analysis of the United States Registry, reasons for withdrawal from dialysis were most commonly due to 'failure to thrive' followed by medical

complications and access failures [3]. Approximately 1/2 of all reasons were due to failure to thrive.

In a study from the University of Alberta, 235 dialysis patients were prospectively followed, after a thorough somatic, biochemical and quality of life characterization, for up to 7 years. Ninety patients died, 13 did so by discontinuation [10]. In univariate analysis advanced age, divorced or widowed status, living in a nursing home, having no work and not participating in outdoor activities were risk factors for discontinuation. Among the quality of life scales, 'Perceived health' was lower in those patients who discontinued treatment. These patients also had pain much more often, more co-morbidity and were much less active on the Karnofsky activity scale. In a multivariate analysis co-morbid disease (doubling the relative risk of discontinuation), severe pain (increasing the relative risk four times), and divorced or widowed state (doubling the risk of discontinuation) were all independent factors that predicted stopping. However, no accurate statistical model could be created. A careful review of the patients' records revealed several acute problems, with one occurring on top of the other, often occurring in patients with many other diseases, to be the start of a chain of events that ultimately lead the lonesome patient without support at home and with underlying morale-crushing, severe pain to give up and stop treatment. Examples included patients admitted with an infection, patients treated with antibiotics that give rise to diarrhea with fluid loss and clotted accesses, or patients with one access problem after the other, with reported operations, infections, temporary catheters and antibiotic complications.

Religious views of discontinuation

Discontinuation of life support is of course not unique to dialysis. Particularly the Catholic Church, in its 'Declaration on Euthanasia' by the Sacred Congregation for the Doctrine of the Faith, has considered this problem. There is close to a 1000-year tradition, as some monks long ago questioned their bishop whether elderly brothers needed to be force-fed as they resisted feeding in their senile agitation. The answer, then as now, was no. The church allows the patient to carefully weigh what can be gained by the treatment versus the pain, indignity and surprisingly even economic sacrifice, and if in the judgement the scale tips in favor of saying no, this is not equal to suicide and thus not a sinful thing. Nurses and physicians who participate in this are also absolved. Clear statements along these lines also come from Protestants, Mormons and Methodists. The Jewish faith splits along liberal and conservative versus Orthodox Jews. The latter find the question non-existent. To an orthodox Jew it is so clearly a sinful thing to shorten life by refusing a method that prolongs it. There are no clear statements from either Coptic Christians, Muslims, Buddhists or Shintos [11].

There are also always dissident groups within each religion. Thus a daughter who much opposed her mother's decision to discontinue dialysis stated she thought this was a deadly sin and equal to suicide. Both mother and daughter were Catholics. When the Declaration of Euthanasia was quoted to the daughter and explained that this was based on teachings by Pius XII she angrily retorted 'The pope be dammed'.

Legal problems

There have been several court challenges to stopping dialysis, but the obverse from what is discussed here [11]. On several occasions physicians and/or hospitals have refused to stop dialysis when patients or family have requested this. Patients have been incarcerated and dialyzed against their wish while the families had to get a court order to discontinue the treatment. There are at least five such cases in the United States [11]. Only in one case, in Minnesota, were a hospital and a physician sued for stopping dialysis. An older woman became senile and confused on peritoneal dialysis and ripped out her catheter on several occasions. In discussions with her husband and religious leaders it was decided to discontinue her peritoneal dialysis and the patient died. Some months later the husband had a heart attack and died. Two children then sued the hospital and physician for abandonment of their mother. The judge, as has to be, placed value on the mother's life; in this case it amount to zero dollars and zero cents. The jury quickly acquitted the physician with a question why it had taken so long to make such a self-evident decision.

What is known about death from stopping dialysis?

In general death from advancing uremia after discontinuation appears relatively peaceful. The patients become increasingly lethargic and are usually found dead in bed one morning, probably from hyperkalemia. The mean time in hemodialysis from the last dialysis to death is approximately 8 days, while it is approximately 10 days in patients who discontinue peritoneal dialysis, probably because of the better preservation of renal function in the latter patients [1, 2, 8]. Some patients, though, will live up to 1 month and this should be made clear when discontinuation is contemplated. In Figure 2 is presented the survival days in 154 patients who discontinued hemodialysis.

There is an analysis of the quality of death of 11 patients who discontinued dialysis. In those patients that stopped because of acute complications or sudden changes in living situation, there was much anxiety, fright and worry; while those who had no acute problems but had contemplated their slow debilitation had a better quality of death. In this small study, there was a tendency for black patients to be more worried and frightened than white [12].

A large study with detailed interviews of patients after discontinuation, from several centers, and involving over 100 patients is presently in analysis and will

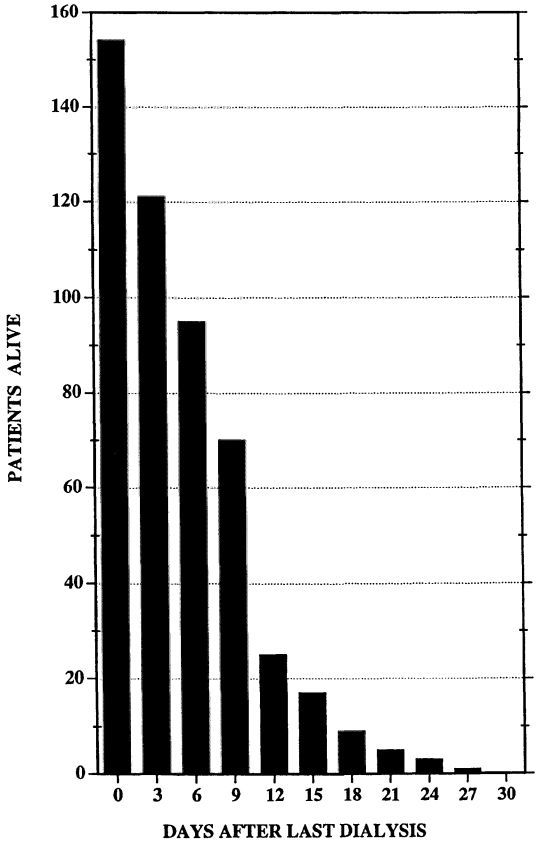

Fig. 2. Survival, after the last dialysis, of 154 patients who stopped dialysis. Half the patients died before 9 days and only three survived more than 3 weeks. The last patient died day 27.

probably be published in 1998. There are presently no preliminary data from this study (L. Cohen, personal communication 1997).

Prevention of stopping dialysis

Physicians have two obligations in dealing with discontinuation. The first is to try to prevent the discontinuation as a cause of death. In order to do so, one needs to consider the risk factors discussed above [1, 2, 8–10]. While one cannot do anything about the co-morbidity which the patient has when he or she arrives for dialysis, one should be able to deal with lonesomeness and pain. Moral and physical support of the lonesome patient is possible by all involved. Although the physician must shoulder a large part of this, because they care for so many patients, they will have most trouble with the time commitment that this is going to take. Dialysis nurses, on the other hand, do spend many

hours with each patient during the dialysis, and should be able to encourage and support the patients. Voluntary organizations such as the Kidney Foundation can obviously also be of great help by visiting patients at home and helping with the many small and vexing problems that the lonesome dialysis patient might find difficult to deal with.

It is also clear that pain management is very poorly done. In the prospective study, unknown to physicians who were making regular rounds of their patients, 6% of all patients complained of severe pain that was not adequately dealt with, and among the patients who stopped dialysis 33% complained of severe pain [10]. Attention to foot care and ischemic problems, which are particularly common in diabetic patients, and abdominal discomfort, perhaps from the multiple medications dialysis patients are given, and adequate pain management is obviously a must [13–16].

It is of course also necessary to make sure that the patients do not suffer from some acute treatable depression, somatic symptom or some socially remediable problem. In such cases the decision to stop should be overridden, the problem solved, and then the decision to discontinue reconsidered [17, 18].

Finally, it is necessary that physicians, who according to Feiffel [19] are more scared of death than others, come to grips on how to deal with this. The most common complaint, from 90 families of patients who had died by discontinuation, and who were contacted between 1/2 and several years thereafter, was that the physicians had not been candid in their discussions or were not available at all to discuss patients' problems to try to solve them [20, 21]; this included even discussing discontinuation of dialysis. Many families felt that resolution of this took too long a time and the discussion often had to be forced onto physicians by patients and their families. Other studies show an improved quality of life in desperately ill patients following candid discussions of choices of treatments or non-treatments in the dying patient [22]. Table 1 summarizes the considerations, philosophical and practical, that a physician needs to make to prepare for dealing with the patient who decides to stop dialysis in order to die [23].

Patients also have an obligation to let relatives and dialysis personnel know their wishes should they become incompetent. What do they want done when not in charge? Who do they want to make the decision for them? What latitude are they willing to give to such a decision maker? What factors are important in making the decision: pain, discomfort, dignity, quality of life and religious views? [24].

Smoothing the avenues of death

The last step everyone has to take alone. However until that moment having support is of great help. The patient must therefore not be abandoned by family or physician. They should be visited daily, they should specifically be asked about dyspnea and pain, and regular analgesic, not analgesic on demand,

Table 1. Philosophical, moral and practical considerations necessary for a physician dealing with patients who decide to stop dialysis to die.

I.	*Ethical framework* – Kantian deontology	
	Beneficence	Sanctity of life
	Non-maleficence	Do not cause suffering
	Autonomy	Respect dignity and difference
II.	*Intellectual/emotional analysis* – Existentialism	
	Weight burden vs. benefit: suffering vs. life gain	
	Non-maleficence and autonomy vs. beneficence	
III.	*Practical analysis I* – Classical utilitarianism – What are the alternatives?	
	Act	*Consequences*
	Do everything	Analyze and predict
	Change course	
	Do less	
	Stop everything	
IV.	*Practical analyses 2* – Common sense	
	The circumstances	Patient competent
		Temporary mental or physical stress
		If incompetent – who decides?
	Medical aspects	Disease understood
		Prognosis known
		Maximal care tried
		Do experts agree
	Who do you want to stop	Patient's best interest
		Family desperate
		Friends squeamish
		Team discouraged
		You depressed and tired
		Administrative power system
	Making the decision	Be prepared for the un-expected
		Discuss – listen – document
	Insoluble situations	Religious imperative
		Patient undecided or periodically lucid
		Family feuds
	What do you plan to do	Do not kill
		Do not practice medical scrupulosity
V.	*After stopping*	Visit patient DAILY, listen to chest, converse
		– Do no tests
		– Comfort medications only – fluid restriction – Uf.
		– Visit family

should be instituted to prevent rather than treat pain. Only comfort medications should be given, and there should be no dietary restrictions, except sodium and fluid restriction. The symbolic importance of listening to the chest, as well as the search for lung-fluid by physical diagnosis, is of true comfort to patients.

While death from uremia is a comfortable one, death from pulmonary edema is among the worst. One can ultrafiltrate the patient who develops pulmonary

edema, without doing any dialysis. All tests should be stopped and there should be no visiting restrictions. One worry physicians have is to be confronted by the dying patient with insoluble philosophical or religious questions such as the meaning of life, what happens after, is there a hell and so forth. Personal experience indicates that this is almost never the case. Discussions about pride in relatives, details about hobbies and important events in their life is what patients usually want to talk about. Although emotionally draining, the good deed of attention to the dying patient should also be a source of strength and pride for a true physician.

Some have suggested that families should be contacted shortly after or some time after death to solve questions, some physicians even attend the funeral. The value or not of this has not been evaluated.

Summary

Discontinuation of dialysis is secondary only to cardiovascular deaths in dialysis patients. It increases steeply with age and is three times as common in diabetic as in non-diabetic patients. Most religions have no prohibition but allows the weighing of gains and burdens. Patients who have become alone, have pain and have much co-morbidity are particularly at risk. Death usually occurs within one to two weeks and is in most instances comfortable. Much can be done to prevent this cause of death, and to smooth the avenues of death when unavoidable.

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Index

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