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STUDY OF NORMOALBUMINURIC DIABETIC NEPHROPATHY IN TYPE 1 DIABETICS

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CERTIFICATE

This is to certify that the dissertation titled "**Study of Normo albuminuric diabetic nephropathy in type 1 diabetics"** submitted by **Dr. P.K. Senthil Kumar** to the faculty of Nephrology, The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of D.M. Degree in Nephrology branch is a bonafide research work carried out by him under direct supervision and guidance for the years 2008 to 2011.

Professor and HOD
Department of Nephrology
Govt. Kilpauk Medical College
Chennai – 600 010.

Dean
Govt. Kilpauk Medical College
Chennai – 600 010.

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CONTENTS

INTRODUCTION

Diabetes is the leading cause of end stage renal disease throughout the world. Diabetic nephropathy occurs in 25-30% of type 1 diabetes. Increased albumin excretion rate has been considered the first clinical sign of diabetic nephropathy both in type1 and type2 diabetes(1). Though microalbuminuria is considered as the early marker of diabetic nephropathy, in some patients decrease in glomerular filtration rate and hypertension may precede(2). It has been proved that patients with normoalbuminuria and decreased gfr in type 2 and type 1 diabetic patients had significant glomerular changes in renal histopathology and also has been proved that these patients had rapid progression of diabetic renal disease(3). As albuminuria is not a predictor but a marker of diabetic nephropathy lot of researches had been undertaken to identify early predictor such as hyperfiltration, estimation of early gfr decline with both creatinine and cystatin c, plasma and urinary markers of inflammatory, oxidative pathways and fibrotic pathways as well as genetic variants that predispose patients to the onset and progression of diabetic nephropathy. Mogensen etal proposed the natural history of diabetic nephropathy in type1 diabetic patients. Accordingly stage1 is characterised by hyperfiltration and hypertrophy of glomeruli, stage 2 – basement membrane thickening and mesangial expansion, stage 3 – microalbuminuria (incipient nephropathy), stage 4- macroalbuminuria and decline in gfr (overt nephropathy), stage 5- end stage renal disease. This traditional view of the natural history of diabetic kidney disease is now challenged. It is clear that patients spontaneously regress from microalbuminuria and even overt nephropathy levels of proteinuria to normal levels of proteinuria and some patients never develop proteinuria at all prior to progressing on to decreasing gfr and end stage kidney

disease. So the true value of albuminuria is questioned. The existence of normoalbuminuric diabetic kidney disease was demonstrated in the united kingdom prospective diabetes study (UKPDS). A landmark study by perkins and krolewski showed that in 400 patients in joslin diabetes center with type1 diabetes were more likely to regress in their albuminuria than to go on and progress to nephropathy. They followed them for a baseline level of 2 years showing that they had persistent microalbuminuria for those 2 years, and then they followed them for an additional 6 years, and they averaged their albuminuria levels at 2 year intervals. At years 2,4, and 6 about 40% exhibited regression, all spontaneously whereas only 7-15% progressed on to nephropathy. Those who were younger , hemoglobin A1c less than 8%, systolic blood pressure of less than 115 and lower total cholesterol and triglycerides were more likely to regress.

Hovind etal at the steno diabetes center looked at patients with diabetes and nephrotic range of proteinuria and followed them for an average of 5-6 years and found that among 126 patients 22% remitted spontaneously. They also pointed out that younger patients, those with low mean arterial blood pressure and low serum cholesterol were those who had spontaneous regression

There are also reports saying that even typical nodular sclerosis can reverse following strict euglycemia as of following pancreatic transplant for type1 diabetes in a period of upto 10 years(5).

So, there is non proteinuric diabetic kidney disease and Proteinuric Diabetic kidney disease can also spontaneously regress(4). So both the positive and negative predictive values of proteinuria have been challenged.

This was also demonstrated in animal models many years back in cohen diabetic sensitive and diabetic resistant rats. In this study such animals were fed with diabetic diet and found that diabetic sensitive rat developed fall in gfr without proteinuria and they had typical diabetic histological changes.

So non proteinuric diabetic nephropathy should also be looked for instead of focusing only on proteinuria for detecting early diabetic nephropathy.

AIM OF THE STUDY

1. To confirm the existence of normoalbuminuric diabetic nephropathy in type1 diabetics

2.. To study the characteristics of these patients

REVIEW OF LITERATURE

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>300 mg/24 hours) (also referred to as macroalbuminuria or proteinuria), a steady decline in glomerular filtration rate (GFR), and elevated blood pressure (6). Two third of patients with diabetic nephropathy develop renal failure requiring either dialysis or renal transplantation. Diabetic nephropathy is the most common cause of chronic renal failure in india and accounts for more than one third of patients enrolled in long-term dialysis programs. Patients with nephropathy frequently develop other complications, in particular cardiovascular disease including hypertension and stroke, resulting in increased risk of early mortality . In patients with type 1 diabetes, 40 years after onset of the disease, the mortality rate is 90% for those patients with nephropathy but only 30% for those patients without renal disease (7). Thirty percent of diabetic patients die as a result of renal failure . Thus, renal complications of diabetes are important, and glomerulosclerosis and vascular disease are the most important causes of renal failure in the diabetic patient.

There are two main forms or types of diabetes, classified as type 1 and type 2 . . Type 1 diabetes encompasses the vast majority of cases that are primarily due to pancreatic β- cell destruction. The form named type 2 diabetes includes the most prevalent form of the disease, which results from insulin resistance with an insulin secretory defect. Type 1 diabetes accounts for 5 to 10% of people with diabetes and type 2 diabetes affects approximately 90% of those with the disease . The natural history, pathologic features, and pathogenesis of diabetic nephropathy are remarkably similar but not identical in patients with type 1 and type 2 diabetes.

Clinical Presentation

Frequency and Risk Factors

Incidence and Prevalence

Several studies from the 1980s showed a cumulative incidence of diabetic nephropathy of 25% to 40% after 25 years of type 1 diabetes with a declining trend in cumulative incidence compared with those patients diagnosed in the 1930s and in the 1950s. A study from Sweden published in 1994 reported a dramatic decline in the cumulative incidence of diabetic nephropathy in patients diagnosed before age 15 years (8). The investigators showed that the cumulative incidence of diabetic nephropathy after 25 years declined from 28% in those patients with an onset of type 1 diabetes between 1961 and 1965 compared with 8.9% in those patients with an onset between 1966 and 1970 and 5.8% in a cohort from 1971 to 1975. The patients with persistent albuminuria had higher mean glycosylated hemoglobin levels than did those without albuminuria. Furthermore, in all patients, the average glycosylated hemoglobin value decreased between 1980 and 1989. Most follow-up studies confirmed the declining trend of incidence of diabetic nephropathy in patients with type 1 diabetes (9,10). Some investigators have not found such a decline in incidence of diabetic nephropathy . Recent data reveal cumulative incidence of 13% to 16% of diabetic nephropathy in patients with type 1 diabetes 20 years after the onset of diabetes. The peak annual incidence of developing proteinuria in type 1 diabetes is approximately 3% between 10 and 20 years of diabetes duration (11,12). The prevalence of proteinuria in patients with type 1 diabetes is between 15% and 40% (11,13,14).

The prevalence and cumulative incidence of proteinuria (as a measure of diabetic nephropathy) in patients with type 2 diabetes is comparable but not identical with those of type 1 diabetes . The higher variability in the prevalence of proteinuria (2% to 20%) in patients with type 2 diabetes is, in part, due to ethnic differences in the prevalence of diabetic nephropathy. No evidence of declining cumulative incidence of diabetic nephropathy with increasing calendar year of diabetes onset (as demonstrated in some studies in patients with type 1 diabetes) has been demonstrated in a study of European White type 2 diabetic patients .

Duration of Disease

The incidence of diabetic glomerulosclerosis increase with the duration of diabetes. This correlation was shown most clearly by Andersent et al. in a large cohort study from Steno Memorial Hospital. These authors studied 1475 patients with type 1 diabetes for 25 or more years until death. These investigators found an increase in the annual incidence of renal disease through the first 16 years after diagnosis of diabetes with a decline in the number of patients developing renal lesions after that period. Only 4% of patients developed nephropathy after 35 years of diabetes. Rudberg et al in a study of adolescents with type 1 diabetes with a mean duration of disease of 10.9 years, found that duration of disease was an important factor in overall severity of glomerulopathy. Overt nephropathy caused by glomerulosclerosis first appears 10 to 15 years after the onset of type 1 diabetes and after 5 to 10 years in patients with type 2 diabetes (15). Although diabetic nephropathy is rare before 10 years of diabetes duration in patients with type 1 diabetes, approximately 3% of patients with newly diagnosed type 2 diabetes have evidence of diabetic nephropathy . Because the biologic onset and duration of type 2

diabetes is often uncertain and therefore, the biologic onset and clinical onset/diagnosis might be years apart, the course of diabetic nephropathy in this patient population is less well defined than in patients with type 1 diabetes. In addition, the more frequent co morbidities in patients with type 2 diabetes, such as hypertension and cardiovascular diseases, may alter the clinical presentation of nephropathy in patients with type 2 diabetes.

Gender

In earlier studies, more females have been reported to be affected than males (16). Another study demonstrated a clear predominance of males compared with females. More recent data show only a slight male predominance with a male-to-female incidence ratio of end-stage renal disease (ESRD) from diabetic nephropathy of 1.1:1. However, the finding that girls and women are diagnosed with diabetes at a rate of 1.5 times that of boys and men indicates that men are at greater risk of developing renal disease in diabetes than are women.

Racial and Ethnic Factors

Several distinct racial and ethnic groups have a greater incidence of type 2 diabetes than do other such groups. Nephropathy is also more common in the diabetic populations of various ethnic and racial groups. The prevalence of diabetic nephropathy is highest in Native Americans, followed by African Americans, Hispanics and Caucasians . The Pima Indians of the South Western United states have the highest prevalence of type 2 diabetes . Fully half of all Pimas develop type 2 diabetes by the time they have reached 35 years of age. A cumulative incidence rate of proteinuria as high as 50% has been reported in the Pima Indians 20 years after the

diagnosis of diabetes . African Americans also have a higher incidence both of diabetes and of the associated diabetic nephropathy. Increased prevalence of diabetic nephropathy is also noted in Hispanic Americans . These differences in prevalence of diabetic nephropathy among ethnic groups are present after adjustment for other risk factors such as hypertension, socioeconomic status, age, and prevalence of diabetes within the population . Asians and West Indians tend to developy type 2 diabetes at an earlier age, and in these patients diabetic nephropathy more often leads to renal failure and death .

Genetic Factors

Several lines of evidence support an important role for various genetic factors in the development of diabetic nephropathy. The fact that diabetic nephropathy develops only in a subset of patients with diabetes has long been interpreted as evidence pointing to genetic factors in the pathogenesis. Familial clustering of diabetic nephropathy in both type 1 and type 2 diabetes is another indication for probable genetic predisposition for the disease . Seaquist et al studied two sets of families with type 1 diabetes. In the first group, the probands had normal renal function, whereas in the second group, the probands had undergone renal transplantation necessitated by end-stage diabetic nephropathy. Two of 12 siblings in the first set of families developed diabetic nephropathy. However, 24 of 29 siblings of probands with previously documented nephropathy later developed nephropathy themselves. The only predictive factor of the later evolution of diabetic nephropathy was the presence of renal disease in the proband. No difference was noted between the two groups with regard to duration of diabetes, blood pressure, or glycosylated hemoglobin (a measure of glycemic control). Although a common environmental

factor is not excluded, the investigators believed that heredity plays a role in determining the susceptibility to diabetic nephropathy. A study from the Joslin Clinic (Boston, MA) examined the development of diabetic nephropathy in families with multiple siblings with type 1 diabetes . The cumulative incidence for overt proteinuria in siblings of patients with advanced diabetic nephropathy was 71.5% after 25 years compared with 25.4% in siblings of patients without persistent proteinuria. The authors of this study pointed out that this risk difference of 46.1% was sufficiently large to suggest that the genetic event is important and may signal a dominantly inherited gene. A genome scan in families with type 1 diabetes for linkage for diabetic nephropathy identified a linkage to a region that contains the angiotensin II type 1 receptor gene (17). In addition, several metabolic pathways and genes have also been proposed as candidates for genetic include the following :

1. Genes that mediate the synthesis and degradation of the glomerular capillary basement membrane and mesangial matrix components.

2. Components of blood pressure regulation and the renin-angiotensin system.

3. various cytokines, growth factors, signaling molecules and transcription factors.

4. advanced glycation processes.

5. genes that mediate glucose metabolism and transport.

A recent comprehensive study analyzing 115 candidate genes using a novel approach of transmission/disequilibrium test in patients with type 1 diabetes identified 20 genes with polymorphism (18). According to the investigators, at lease some of

these genes may have influence on the risk of diabetic nephropathy. However, inspite of a large number of studies that tested the association of candidate genes with diabetic nephropathy, the findings are mostly inconclusive and the genetic determinants of diabetic nephropathy are not yet fully understood.

Smoking

Cigarette smoking is a well-established independent risk factor for the development of the microvascular complications of diabetes. There are also date indicating that cigarette smoking contributes to the development of diabetic nephropathy. Cigarette smoking increase urinary albumin excretion (UAE) in patients with type 1 diabetes and also in type 2 diabetes . In addition, smoking cessation is associated with reduced UAE in patients with type 1 diabetes . However, as to whether smoking has a significant impact on renal functional deterioration in type 1 diabetic patients is some what controversial. Although several studies showed an association between cigarette smoking an progressive nephropathy in patients with type 1 diabetes, a recent large prospective observational cohort study failed to demonstrate an association between smoking status and the rate of decline in GFR with and without ACE inhibitor therapy.

Clinical Presentation

Clinical Stages

Mogensen et al (19) described five clinical stages in the evolution of diabetic glomerulosclerosis in patients with type 1 diabetes. stage I is characterized by the presence of both hyperfunction, as manifested by increased glomerular filtration rate (GFR), and hypertrophy, as recognized by enlargement of the kidneys affecting both

glomeruli and tubules. Stage II develops silently over many years in some patients and is marked by the evolution of glomerular lesions in the absence of clinical evidence of renal disease. In particular, the glomerular basement membrane (GBM) begins to thicken and the mesangial matrix expands (20). A correlation exists between elevation of glycosylated hemoglobin and increased GFR at this stage . Stage III marks incipient diabetic nephropathy. It is recognized by the occurrence of microalbuminuria, which is defined as ranging between 20 and 200 μg/min (30 to 300 mg/day) on repeated measurements (21). Renal function is preserved as determined by blood urea nitrogen (BUN) and serum creatinine. Typically, this stage is associated with intermittent proteinuria with slowly increasing urinary albumin excretion over all years . Once UAE attains levels of 0.075 to 0.1 g/day, the patient has sustained significant renal damage and clinical nephropathy is likely to occur . Increased blood pressure may be present at this stage, but it rarely occurs in patients with UAE less than 100 mg/day (22). Stage IV is characterized by the presence of overt diabetic nephropathy as clinically manifested by proteinuria >300mg/day and a declining GFR. The nephrotic syndrome has been reported in 6% of patients with overt nephropathy . Microscopic hematuria may be identified in 28% to 48% of patients with diabetic nephropathy (21). This stage is usually associated with rising systemic blood pressure and the presence of other diabetic complications. Development of overt nephropathy with albuminuria was once considered to be the "point of no return" with steady progression to ESRD. Aggressive antihypertensive treatment showed beneficial effects on the progression of diabetic nephropathy in both type 1 and type 2 diabetic patients . Stage V is the appearance of ESRD as a direct result of diabetic nephropathy.

Microalbuminuria and Risk for Diabetic Nephropathy

Based on values of albuminuria, the preclinical phases of diabetic nephropathy consist of normoalbuminuric and microalbuminuric stages. Approximately 70% of screened patients with type 1 and type 2 diabetes have normoalbuminuria. Microalbuminuria, defied as 30 to 299 mg/24 hours, a cutoff value adopted by the Ameircan Diabetes Association (23,24), is widely regarded as the best available marker for risk of later development of diabetic nephropathy in both type 1 and type 2 diabetic patients. Microalbuminuria is considered to be a promoter of renal functional deterioration, and a reduction of microalbuminuria or prevention of development of microalbuminuria is important in preserving renal function.

According to several studies, the prevalence of Microalbuminuria is less than 20% in patients with type 1 diabetes, and on average, slightly greater than 20% in patients with type 2 diabetes.

The rate of progression from microalbuminuria to proteinuria over 5 to 10 years in patients with type 1 diabetes is approximately 15% to 30% . The estimated incidence of progression from normoalbuminuria to microalbuminuria and subsequent proteinuria during a follow-up of 6 to 9 years in patients with type 2 diabetes is approximately 20% to 30% . A recent European study using data from more than 5000 patients with type 2 diabetes found that progression from normoalbuminuria to microalbuminuria occurred at a rate of 2.0% per year and from microalbuminuria to proteinuria at 2.8% per year .

Vibertia et al studied 87 patients with type 1 diabetes with follow-up of at least 14 years in 63 patients. These patients were divided in to two groups depending

on their UAE.Seven of the 8 patients with UAE rate (but still within the definition of microalbuminuria) developed proteinuria greater than 0.5g/day at follow-up, whereas only 2 to 55 patients in the low-UAE group had proteinuria at that time. The risk of developing clinical nephropathy was 24 times greater in the high-excretion group.Futhermore,38% had died at follow-up ,whereas only 9% were dead in the low-UAE group. These findings were confirmed by several follow-up studies . Warram et al determined UAE by albumin-to-creatinine ratio and found that 6% of patients with type1 diabetes for 1 to 3 years had microalbuminuria. At 10 years ,this prevalence had increased to 20% and to 52% at 20 years. After 30 years, the prevalence of overt nephropathy was 27%.The finding suggests that some of the 52% with microalbuminuria at 20 years did not progress to overt renal disease, whereas those with microalbuminuria in the first 10 years almost always progressed to overt nephropathy. These authors also determined that the median transition time from microalbuminuria to overt nephropathy is 9 years.However,not all patients with microalbuminuria early in the course of type 1 diabetes progress to overt nephropathy, as indicated in a study by Shield et al .

Also, in a significant proportion of patients with type 1 or type 2 diabetes, microalbuminuria can revert to normoalbuminuria. In a large European study, a regression rate of 50.6% has been demonstrated in type 1 diabetics with microalbuminuria after 7 years of follow-up(25). In the Steno-2 study,46 (31%) of 151 patients with type 2 diabetes and microalbuminuria reverted to normoalbuminuria during 7.8 years of follow-up(26). Antihypertensive therapy and improved glycemic control were independent predictors for remission(26).

End Stage Renal Disease(ESRD) in Diabetics

End-stage renal disease usually occurs 3 to 20 years after the onset of persistent proteinuria . Once overt proteinuria occurs, progression to ESRD seems to be inevitable despite good metabolic control. Patients who have type 1 diabetes and proteinuria develop ESRD more often than those who have type 2 diabetes and proteinuria .The cumulative incidence of ESRD is 50% in patients with type 1 diabetes 10 years after the onset of proteinuria (8) compared with 3% to 11% in proteinuric type 2 diabetes patients . However, because of the higher prevalence of type2 diabetes, 80% or more patients with ESRD secondary to diabetes have type 2 diabetes. The incidence of ESRD is also higher in women, reflecting the demographics of diabetes and diabetic nephropathy in the general population. Although there are considerable differences between various countries in the prevalence of ESRD among diabetics, a trend of increasing prevalence of ESRD in the population has been noted in all countries over the last 10 years.

Natural History and Progression of Diabetic Nephropathy

The natural history of diabetic nephropathy in patient with type 1 diabetes is well characterized showing various but steady decline in GFR ranging from 1 to 24mL/minute−1/year−1 (mean 12 mL/minutes−1/ year−1) and a concomitant rise in alubuminuria and in arterial blood pressure.

Although the great majority of patients with diabetic nephropathy have type 2 diabetes, there are far fewer studies and date available on the natural progression of diabetic nephropathy in patients with type 2 diabetes . In 1996,Nelson et al published the first study on the natural course of kidney function in patients with type 2 diabetes

in pima Indian followed for 4 years. The GFR was elevated at the onset of type 2 diabetes, remained elevated while normal albumin excretion or microalbuminuria persisted, and declined progressively after the development of macroalbuminuria, The average decline in GFR was 11mL/minute/year. Higher renal plasma flow, alubuminuria and body-mass index predicted a more rapid decline in GFR,Whereas blood pressure and HbA1C did not. IN a study from Steno Diabetes Center from Denmark,12 normotensive or borderline hypertensive male patients with type 2 diabetes were followed for 55 months . No treatment for hypertension was implemented, and the low average rate of decline in GFR (4.5mL/Minutes/Year)was similar to that seen in normotensive patients with type 1 diabetes. No significant correlation between putative progression promoters , such a blood pressure values, alubuminuria, or hyperglycemia, and the rate of decline of GFR were identified. This study indicated that the progression of diabetic nephropathy in normotensive untreated patients with type 2 diabetes is slow.

Plugh et al compared the course of ESRD in patients with type 1 diabetes and those with type 2 diabetes. These investigators found that hyperglycemia was more prominent in the patients with type 1 diabetes and renal disease, whereas hypertension was more frequently seen in the patients with type 2 diabetes and renal disease. In agreement with other studies, they found that the course to ESRD was shorter in patients with type 2 diabetes. They also noted that patients with type 1 diabetes more frequently suffered from other microvascular complications such as retinopathy or neuropathy, whereas patients with type 2 diabetes more often have myocardial infarcts or congestive heart failure.

Progression Factors and Renoprotection

Because only 30% to 40% of diabetics develop diabetic nephropathy, and the occurrence of such lesions decreases life expectancy, it is important to determine predictors of nephropathy as well as means to delay or prevent the progression of this disease. Several factors including onset of type 1 diabetes later in life, parental type 1 diabetes, edema, and an abnormal electrocardiogram independently predict progression of renal disease in diabetes. However, the most important factors include hypertension and the degree of metabolic control.

Blood Pressure Control

The high prevalence of hypertension in patients with type 1 diabetes (40%) and type 2 diabetes (70%), even before the onset of microalbuminuria, is well established (27). The impact of hypertension on the progression of diabetic nephropathy is also well documented .Ravid et al(28) followed 195 patients with type 2 diabetes with normal blood pressure at the outset of the study for 14 years and examined the relationship between their blood pressure and the progression of renal disease. The investigators found that the patients who became hypertensive showed and increased propensity for developing proteinuria(60.0% compared with 20.8% in the normotensive group) despite similar degree of metabolic control. Furthermore, among the patients who developed proteinuria, the hypertensive patients showed a greater decline in the GFR .Parving et al examined the effect of long-term antihypertensive therapy on progression of diabetic renal disease compared with the natural history of the disease. These investigators studied 45 patients with type 1 diabetes and nephropathy for a median follow-up of 16 years. The cumulative death

rate was 45% 16 years after the onset of diabetic nephropathy compared with 94% recorded by Krolewski et al. The latter study was an examination of the natural history of the disease without antihypertensive therapy.

There is strong association between blood pressure. Increase in alubuminuria and the rate of decline in glomerular filtration rate (GFR) in both type 1 and type 2 diabetic patients . Early studies showed that long-term antihypertensive treatment in type 1 diabetic patients with nephropathy reduces alubuminuria and the rate of decline of GFR This finding has been confirmed by several follow-up studies. Treatment of hypertension also significantly reduces the risk of development of microalbuminuria. In patients with type 2 diabetes, a reduction of systolic blood pressure form 154 to 144 mm Hg reduced the risk of the development of microalbuminuria by 29%. Effective control of hypertension has also been shown to slow the rate of progression to renal failure even in patients with overt nephropathy. Sawicki et al compared patients with long-term diabetes who received either routine antihypertensive therapy or intensive treatment for hypertension. The patients were followed for a mean of 68 months. Four percent of the patients in the intensive-treatment group died compared with 28% in the routine-treatment group. Renal replacement therapy was required in only 9% of the patients in the intensive-therapy group compared with 23% in the routine-treatment group, and progression of renal disease occurred in 27% of the intensively treated group compared with 59% of the other group.

Antihypertensive treatment not only slows down the rate of decline of GFR but in comes patients, may lead to regression of the disease(i.e.,delta GFR(upto1mL/Minute/year) equal to the natural aging process).In a study of 10 type 1 diabetic patients with nephropathy, Pravin et al showed slowing of the rate of

decline in GFR from 0.91mL/minute/month in a period of 29 months before aggressive antihypertensive treatment to a rate of 0.39 mL/minute/month during an interval of 39 months of aggressive antihypertensive therapy. This change was also accompanied by a decline in urinary albumin excretion (UAE).No difference was seen in degree of metabolic control during the two period of investigation.

Higher blood pressures, even in the range considered normal, are predictive of diabetic nephropathy risk. Patients with advanced diabetic nephropathy and type 1 diabetes had higher mean arterial blood pressure during adolescence . The risk of developing nephropathy is also enhanced in diabetic patients with a family history of hypertension. Krolewski et al studied 89 patients approximately 20 years after the onset of type 1 diabetes. Thirty- three of these patients had nephropathy at follow-up. The risk of nephropathy was tripled for those patients having a parent with hypertension. Furthermore, the patients with nephropathy had higher maximal velocity of lithium-sodium countertransport in red cells, a marker of risk for essential hypertension. The risk of developing nephropathy was increased still more in those patients with a history of poor metabolic control in their first 10 years of the disease. The authors of this study concluded that the risk of renal disease in diabetics is increased in patients with a genetic predisposition to hypertension, with a further increment in the risk resulting for poor metabolic control.

In addition to its beneficial effects on the rate of progression of diabetic nephropathy, aggressive blood pressure lowering therapy also significantly extends the median survival of diabetic patients with persistent proteinuria. Early studies showed an average survival of patients with persistent proteinuria owing to diabetic nephropathy of 5 to 7 years , the renal prognosis is similar in patients with proteinuria

who have type1 or type 2 diabetes . A significantly improved median survival time of 14 years or greater has been documented in patients treated with aggressive antihypertensive therapy . Data from the Framingham and Multiple Risk Factor Intervention Trial Diabetic Cohort showed that cardiovascular mortality was increased by a factor of two to four in diabetic patients, and there was clear association between systolic blood pressure and complications without any threshold value.

Glycemic Control

Studies beginning in 1982 indicated that microalbuminuria in diabetic patients is a good predictor of the later development of diabetic nephropathy and decline in renal function. Several groups showed that the extent of UAE correlates with the degree of Glycemic control in both type 1 diabetes and type 2 diabetes. This finding led investigators to examine the effect of strict metabolic control during the stage of incipient nephropathy on the later development of overt glomerulosclerosis. The importance of degree of metabolic control in the progression of diabetic renal disease is illustrated in the study of Krolewki et al from the Joslin Clinic . These investigators found that the degree of Glycemic control in the first 20 years of type 1 diabetes was a strong predictor of ESRD. The prevalence of ESRD was 36.3% in patients with the worst control compared with 14.4% with better control and only 9.2% in those with the best control. Stephenson et al found similar results in Europe in a study of 3250 patients with type 1 diabetes.

Bangstad et al performed a prospective study of patients with type 1 diabetes and microalbuminuria in two groups randomized to conventional treatment or to

continuous subcutaneous insulin infusion. Renal biopsies were taken at the beginning of the study and again between 26 and 34 months later. The investigators found that strict metabolic control was established in the infusion-treated group as determined by mean glycosylated hemoglobin levels. The GBM thickness increased in each group, but the increment of GBM change was larger in the conventional therapy group. Volume fraction of mesangial matrix was increased only in the conventional-therapy group. The investigators concluded that a close relationship was present between the level of blood glucose and the characteristic changes of early diabetic nephropathy.

Meta-analysis of several randomized studies comparing the effects of long-term intensive versus conventional blood glucose control on the risk of nephropathy progression in normoalbuminuric (80%) and microalbuminuric type 1 diabetic patients showed beneficial effects on the progression from normoalbuminuria to microalbuminuria by intensive treatment. Intensified Glycemic control in Diabetes Control and Complication study did not decrease the rate of progression to macroalbuminuria in patients with type 1 diabetes who were microalbuminuric at the beginning of the study. Strict Glycemic control in patients who were normoalbuminuric at the beginning of the study did, however reduce the occurrence of microalbuminuria by 39% and that of alubuminuria by 54% at the end of 6.5-year study. Also, improved Glycemic control reduced the renal functional deterioration in proteinuric with type 1 diabetes in another study.

Only a few studies analyzed the effects of glycemic control on the rate of progression of nephropathy in patients with type 2 diabetes. The progression rate of microalbuminuria to macroalbuminuria was reduced with intensive glycemic control in a Japanese study. Progressive beneficial effects of intensive metabolic control have

also been found by the U.K. Prospective Diabetes Study on the development of microalbuminuria and overt proteinuria. However, Several studies failed to demonstrate a significant correlation between Glycemic control and progression of GFR in albuminuric patients with type 2 diabetes.

It is now generally accepted that the degree of glycemic control is an extremely important factor in the development and evaluation of diabetic nephropathy. The impact of hyperglycemia on progression of diabetic nephropathy has been well documented ,especially during the later stages of the disease, once albuminuria has developed. The impact of hyperglycemia during the later stages of the disease once albuminuria has developed, is debated**.**

Blockage of the Renin-Angiotensin System

Blockage of the rennin-angiotensin system with angio-tensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) confers additional benefit on preserving renal function. The first large study of these agents was reported by Lewis et al, who examined the effect of captropril in a randomized controlled study of 409 patients with type 1 diabetes with a median follow up of 3 years. These investigators found that doubling of the serum creatinine occurred in 43 of the patients taking the placebo but in only 25 patients who received captropril. The risk of death, or the need for either dialysis or renal transplantation, was reduced by half in the captropril treatment group. These investigators concluded that captropril slowed the progression of diabetic nephropathy more effectively that simple control of blood pressure. Ravid et al examined 94 patients with type 2 diabetes, serum creatinine below 1.4mg/dL., and microalbuminuria between 30 and 300 mg/24 hours.

The patients were assigned to treatment with enalapril for 5 years or placebo. Each subject then had a choice of receiving enalapril or receiving no therapy for 7 years had stable microalbuminuria during the period. The untreated group showed an increase in microalbuminuria from 123 to 310 mg/24 hours in 5 years, with an additional increase to 393 mg/24 hours in the last 2 years of the study.The treated patients showed no change in the reciprocal of the serum creatinine . those who did not receive any therapy showed a decline of 13% in 5years and 16 % at 7 years.Treatment resulted in an absolute risk reduction of 42%. Discontinuation of therapy renewed progression of the renal disease. Six of the 33 patients (18%) who received treatment throughout the 7 year period progressed to macroalbuminuria, compared with 12 of 20 patients (60%) who were not treated with enalapril. Mean blood pressure was maintained at 108mm Hg or less in all patients.

The findings of three randomized, placebo-controlled studies in type 1 and type 2 normotensive and normoalbuminuric diabetic patients have suggested beneficial effects on the development of microalbuminuria . A meta analysis of 12 trials evaluating 698 nonhypertensive microalbuminuric patients with type 1 diabetes treated with ACE Inhibitors also showed decrease of the risk of progression to proteinuria by 60% and increase of the chances to revert to normoalbuminuria. It has been documented that in type 2 diabetic patients with hypertension and normoalbuminuria, the renoprotective effects of blood pressure reduction were similar in those treated with ACE inhibitors versus calcium antagonist , or beta blockade . Type 2 diabetic patients with microalbuminuria also benefit from treatment with ACE inhibitors In a double-blind randomized study, type 2 diabetic patients with microalbuminuria and normal blood pressure were receiving enalapril or placebo for 5

years. The renal function remained stable and only 12% of patient in the study group developed diabetic nephropathy. In contrast, the renal function in the placebo-treated group declined by 13% and 42% of patients in this group developed nephropathy. The short-term renoprotective effects of ACE inhibitors were similar to those of ARBs in reducing the albuminuria in patients with type 2 diabetes. In the MICRO-HOPE(Heart Outcomes Prevention Evaluation) study , the ACE inhibitor ramipril decreased the risk of overt nephropathy by 24% and the risk of cardiovascular death in patients with type 2 diabetes who were older than 55 years of age with one additional cardiovascular risk factor by 37%. The effect of these agents are complex but include alternations in glomerular hemodynamics resulting in decrease of hyperfiltration, inhibition of glomerular and tubular hypertrophy, and lowering of systemic blood pressure.

Effects of Treatment on Progression of Nephropathy

Additional evidence of the importance of various risk factors or predictors in the progression of nephropathy can be garnered from studies in which therapy is aimed at modifying these factors. Restricting dietary protein has been used as a mean to reduce glomerular hyperfiltration . Zellar et al (29) found that dietary protein and phosphorus restriction resulted in a slowing of progression of renal disease independent of blood pressure and glycemic control that was comparable in the two experimental groups.

Pathologic Findings

The possible association of specific glomerular lesions with diabetes mellitus was first recognized by Kimmelstiel and Wilson in 1936 (30). They described a series

of eight patients who, autopsy, had a striking formation of nodules. Seven of these patients had diabetes, whereas the remaining patient with moribund without available clinical history. Edema and heavy proteinuria were present, accompanied by high blood pressure in some patients. At the same time, Murakami in Japan described a similar histologic picture in a single patient. Additional studies since that time have described the histologic alterations that are characteristic of diabetic glomerulosclerosis and are accompanied by a stereotypical clinical course. The clinical significance of this disease has engendered a large body of work examining pathogenesis and maneuvers to alter the course of the disease.

Gross Appearance

The kidney of the diabetic patient may be increased, decreased, or normal in size. At early stages, it is invariably increased, particularly in patients with hyperfiltration. When diabetic glomerulosclerosis progresses with scarring and loss of nephrons, one sees a reduction in the size of kidney, but the end-stage kidney of the diabetic patient does not commonly show gross contraction. On cut surface, preservation of normal architecture is noted. With proper lighting, one may be able to detect hypertrophied glomeruli within the cortex. Frequently, the arteries at the corticomedullary junction are prominent because thickening of their walls resulting from arteriosclerosis prevents their walls resulting from arteriosclerosis prevents their retraction into the parenchyma. The main renal artery and it branches may show atherosclerosis.

Light Microscopic Findings

Glomeruli

The glomeruli show a constellation of findings. These include a diffuse form with mesangial sclerosis manifested as an increase in mesangial matrix and uniform thickening of capillary walls, a nodular lesion sometimes combined with microaneurysms, exudative or hyalinosis lesions, and the capsular drop. At early stages, glomerular volume is enlarged 70% as compared with glomeruli of nondiabetic subjects . Filtration surface area increased by 80% in a similar comparison.

Diffuse Lesion.

The diffuse lesion consists of widespread increase in eosinophilic, periodic acid-Schiff(PAS)- positive material within the mesangium as first described by Spuhler and Zollinger. Hypercellularity is uncommon , although it may occur. Uniform increase in the thickness of the capillary walls may be seen. The increase in glomerular basement membrane(GBM) thickness may be seen as early as 2 years after the onset of diabetes mellitus, and it is not usually accompanied by clinical evidence of renal dysfunction . The mesangial alterations progress in severity . Capillary walls continue to thicken at the same time, increasing with duration of disease . The GBM is thickened and mesangial volume and matrix volume fraction are increased by the time microalbuminuria appears clinically . The combination of mesangial expansion and thickened GBM Results in decreasing patency of capillary Lumina and decreased filtration surface area. As the glomeruli become obsolete, they do not shrink to the degree seen in other diseases affecting glomeruli resulting in solidified glomeruli.

However, one can find typical ischemic change in certain of the glomeruli because arteriosclerosis contributes to the evolution of diabetic renal disease. Bowman's capsule may be thickened in the more advance lesions. Advanced glycosylation end products(AGEs) are believed to play a role in the pathogenesis of the lesions in diabetic nephropathy. Such AGEs have been identified in the expanded mesangium of diabetic patients, but have not been seen in control specimens.

Nodular Lesion

The nodular lesion is the type of change first recognized by Kimmelstiel and Wilson(30). Typically, it is characterized by the accumulation of homogeneous eosinophilic material within the mesangium, often appearing as a rounded accentuation of the mesangial expansion observed in the diffuse form or diabetic glomerulosclerosis . Formation of Kimmelstiel- Wilson nodule is recognized when expansion of the mesangium attains a size at least one and one-half times that of the normal mesangial stalk. As such, it should measure more than 40μm in diameter, but it may be up to 100 μm. Such lesions are usually acellular although nuclei may be arranged at the periphery. Blood worth noted at least one nodule in 25% of kidneys studied in 200 consecutive autopsies of patients with diabetes mellitus. Several lobules may be affected within any given glomerular tuft, and in this case, the nodules often vary in size On occasion, glomeruli may have a single large nodule. These variants tend to have a laminated appearance, particularly when viewed with silver or reticulin stains they may have a separate pathogenesis. The number of glomeruli affected varies from case to case. The nodules are PAS-positive, the smaller lesions staining more intensely than the larger ones. They stain green with Masson's trichrome, blue with Mallory's stain, and black with silver stains. Falk et al reported

an increase in mesangial staining for fibronectin, laminin, and type IV and V collagen in early and moderately advanced mesangial lesions with an increase only of type V collagen in late nodules. Hennigar et al(31) found that 7.6% of diabetic patients had severe nodular lesions, and in their review of the literature discovered a range of 27% to 46% of diabetic patients with nodular lesions.

Two populations of nodular lesions exist. The smaller, more numerous nodules arise as continued mesangial expansion of the diffuse lesion . However, the larger, often solitary and laminated variants may originate in relation to microaneurysms which are defined in the context as cystic dilatations of the capillary measuring more than 35µm in diameter . Investigators have observed that the frequency of microaneurysms and large nodules in similar(9% to 25% of diabetic kidneys. Furthermore, microaneurysms may arise in association with mesangiolysis , and a progression of changes has been described wherein the GBM is loosened from its anchoring points as it reflects back over the mesangium. This change is accompanied by disintegration of mesangial matrix , the appearance of fibrillar material, resulting in the formation of several layers and a large module. Agents implicated in the development of microaneurysms include platelet factors, hemodynamic factors and possibly changes in elasticity of the GBM . Stout et al suggested a different pathogenetic mechanism for the Kimmelstiel Wilson nodule. In their hypothesis the first step is the formation of focal mesangiolysis, which progresses from an edematous to a proliferative stage characterized by a loose but organized fibrillar matrix . As this centralized matrix becomes more condensed, the lesion changes from focal nodular mesangial expansion to a simple Kimmelstiel Wilson nodule. Repeated injury then results in a similar progression of changes and

the eventual formation of the laminated lesion called the complicated nodule. Paueksakon et al combine the idea of repeated microvascular injury with the occurrence of mesangiolysis in association with Kimmelstiel Wilson nodules. They found increased plasminogen activator inhibitor-1(PAI-1) in such lesions, particularly those in which they found fragmented red blood cells, an indicator of microvascular injury.

Considerable overlap of the diffuse and nodular lesion occurs. However, it is useful to identify the occurrence of the nodular lesion because it signifies a more serious pathologic from a diabetic nephropathy and corresponds well to the presence of clinical signs and symptoms. Moreover, the presence of mesangial nodules should always arouse suspicion of diabetes.

Hyalinosis Lesion.

The hyalinosis lesion, which is another name for the so-called exudative lesion or fibrin cap , is often present in diabetic nephropathy. The initial change characteristic of this lesion is the accumulation of hynaline eosinophilic homogenous material between endothelial cells and the GBM of the capillary loops. As the lesion evolves, the material, which represents various plasma constituents, increases in amount and eventually occludes the capillary lumen. On occasion, lipid droplets and even lipid-laden macrophages may be present within the lesion . The epithelial cells overlying these lesions are frequently enlarged and may show vacuoles or protein droplets. Adhesions are often observed between the glomerular lobule containing such a lesion and nearby Bowman's capsule. The material within the capillary lumen stains intensely pink with PAS. Use of the methenamine silver stain with PAS and

hematoxylin and eosin counterstains differentiates these lesions from nodules. The combination of PAS and eosin intensifies the staining of the hyalinosis lesion, producing contrast with the silver-staining nodule. Furthermore, the silver stains the basement membrane and reveals the luminal location of the hyalinosis lesion.

The lesion is not specific for diabetic nephropathy, although it is seen in approximately 60% of diabetic kidneys. It is identical to the lesion characteristic of focal and segmental glomerular sclerosis with hyalinosis and may be seen nonspecifically in certain other glomerular diseases including various forms of glomerulonephritis and relax nephropathy. The pathogenesis of the lesion is not well understood, although its early development has been associated with endothelial injury and possible hemodynamic alterations. Because its prevalence in diabetic kidneys increases with the severity of the nephropathy and correlates with the degree of arteriosclerosis, other investigators have suggested that it is associated with ischemia.

The lesion called the capsular drop(32) is identified as a round, eosinophilic accumulation of material between the basement membrane and parietal epithelial cells of Bowman's capsule. It has the same staining qualities as the hyalinosis lesion. This unusual lesion is most frequently seen in diabetes, although it may be seen in other conditions.

Miscellaneous Glomerular Changes.

One of the early physiologic changes in diabetics is the occurrence of glomerular hyperfiltration accompanied by glomerular hypertrophy. Some of these patients then proceed to develop the characteristic histologic changes of diabetic

nephropathy described earlier, namely increased mesangial matrix, the nodular lesion, increased thickness of the GBM, and the hyalinosis lesion. As these lesions evolve, the solidified glomeruli fail to undergo contraction, and the result is a population of large obsolescent glomeruli may also emerge in response to the vascular disease that is a frequent companion to the glomerular disease in diabetes. Thus, these smaller glomeruli are identical to those seen in ischemic renal disease in other conditions . Furthermore, they are present in a distribution expected for vascular disease; that is they occur in stripes perpendicular to the capsular surface within the distribution of the affected vessel. Podocyte injury may also be seen. Reduction in the number of podocytes has been documented in both type1 and type 2 diabetes . This reduction in podocyte number is associated with increasing proteinuria . It has been further suggested that the loss of podocytes results in denudation of the GBM and may be an initiating factor in glomerulosclerosis.

Atubular glomeruli has also been seen in diabetic nephropathy(33).Atubular glomeruli are defined as those glomeruli that have open glomerular capilaries but have lost their connection to the proximal tubule and presumably do not produce filtrate. Accurate determination of these glomeruli requires serial sections. However small glomeruli surrounded by tissue with marked tubular loss are likely to be atubular .Najafian et al, using the appropriate morphometric techniques, found that 17% of glomeruli in diabetics were atubular and that an additional 51% were attached to atrophic tubules. They found that these glomeruli could account for much of the variation in glomerular filtration rate in diabetic patients.

Tubules

The tubules generally show changes that reflect the degree of glomerular alterations. Obsolescent glomeruli (of either variety) show atrophy of adjacent tubules with decreased size of epithelial cells and diminished luminal diameters. Similar changes are also seen in the tubules that belonged to now atubular glomeruli. Apoptosis has been detected in both proximal and distal tubules of the diabetic kidney and represents a possible mechanism for the loss of the tubular cells. The tubular basement membrane is often thicker than expected for the degree of atrophy. Occasionally, proximal tubular epithelial cells are finely vacuolated and contain lipid; this usually occurs in patients manifesting the nephrotic syndrome. The straight portion (S3) of the proximal tubules may show the glycogen-containing Armanni-Ebstein change, but this is seen only rarely. Tubular basement membranes are frequently thickened and may show splitting and lamination.

Blood Vessels

Both arteries and arterioles invariably show the typical changes of arteriosclerosis and arteriolosclerosis, respectively. In arteries, this type of injury is manifested by varying degrees of intimal thickening accompanied by reduplication of elastic lamina. Hyaline arteriolosclerosis is a frequent and early manifestation of diabetic renal disease and is more pronounced in diabetes that in other diseases of the kidney. It is characterized by often striking hyalin deposition in arterioles, and both afferent and efferent limbs may be affected. When this lesion is seen in young people , particularly without hypertension, the suspicion of diabetes must be raised. Bohle et al and Osterby et al noted increase in vascular disease associated with more severe

glomerular damage. The presence of intrarenal microaneurysms in arterioles has been documented using microangiography . Osterby et al assessed the ration of matrix to media in arterioles from diabetic patients with and without microalbuminuria . These investigators found that this ratio was increased in patients with microalbuminuria, a finding suggesting that arteriolar matrix accumulation may be similar to increased matrix elsewhere in diabetes and can occur early in the course of the disease. Furthermore, this change progresses with duration of disease . AGEs were identified in arteriolar hyalin and in intimal thickening in diabetic patients . Three – dimensional analysis of the vascular pole of the glomerulus demonstrated new vessel formation with anastomoses between glomerular capillaries near the hilus and peritubular capillaries .

Interstitum

Interstitial fibrosis is common in the diabetic kidney and may be accompanied by chronic inflammatory infiltrates compose chiefly of T lymphocytes and macrophages. The increase in interstitial volume is due largely to increase in cells at early stages of diabetic nephropathy associated with only mild glomerular changes. Increase in collagen and other matrix components occur later in the disease. Increased fibrosis of the interstitium is greatest in those cases with arterial and arteriolar narrowing; this has been verified by morphometric techniques. The presence of interstitial fibrosis, particularly when accompanied by inflammatory infiltrate, correlates with renal survival. The degree of interstitial fibrosis correlates inversely with the GFR . Taft et al and Bader et al showed a correlation between increase in interstitial fibrosis and final creatinine clearance in patients who had two biopsies. Lane et al demonstrated that mesangial expansion, arteriolar hyalinosis, global

glomerular sclerosis, and interstitial expansion are interrelated. However, the progression in each compartment is not stereotypical for all patients. Thus, in some patients, the severity of interstitial disease may be greater than that of glomerular lesions whereas in others, the reverse may be true.

Immunofluorescence Microscopy

Immunofluorescence techniques have been used in several studies. The typical finding is the occurrence of linear staining along the glomerular capillary walls with immunoglobulin G (IgG). The intensity of the staining varies among individual patients and does not correspond to the severity of the glomerular lesions. Linear staining of capillary walls has also been reported with IgM, the third component of complement(C3), fibrinogen, and albumin. Staining of the mesangium and Kimmelstiel- Wilson nodules is rarer and usually shows fainter staining than that reported for capillary walls.

Hyalinosis lesions usually stain brightly with IgM and C3, as they do in the identical lesions found in focal segmental glomerular sclerosis, phyelonephritis, and elsewhere.IgM and C3 are also present in hyaline arteriolosclerosis in diabetes as well as in vessels showing hypertensive changes. Hyalinosis lesions may also contain fibrinogen and lipoprotein of fibrinogen, complement ,β-lipoprotein, and small amount of IgG.

Vessels with hyaline arteriolosclerosis may show similar changes. Linear staining for IgG and albumin has also been reported along tubular basement membranes and Bowmen's capsule in patients with advanced diabetic glomerulosclerosis . The staining was thought to reflect structural changes in the

renal extracellular membranes, which permit entrapment of serum proteins,possibly as a result of changes in permeability. It is now thought that advanced glycosylation end products may bind to the basement membranes and change their properties.

Electron Microscopy

Glomerular Capillary Wall

In that capillary wall, morphologic changes are present in both the GBM, which is always thickened in most loops, and the epithelial cells, which show variable effacement of the foot process. Most authors agree that this increase in the thickness of the GBM is the earliest change . It should be mentioned that some loops may show thinning of the GBM. Huang , using guanidine treatment, showed that the increase in basement membrane thickness was due to increased amounts of material from the epithelial rather than from the endothelial cells. Changes in the foot processes are variable. At times, they remain discrete, whereas other cases have differing degrees of effacement. Osteby et al measured a mean width of the foot process of 352 nm in diabetics as compared with a mean width of 224 nm in nondiabetic control patients. Other investigators have confirmed this difference in foot process width Widening of foot processes appears with the onset of microalbuminuria and in the more recent literature shows a week correlation with the degree of albuminuria. Pagtalunan et al, in a study of Pima Indians, did not document widened foot processes until the stage of clinical nephropathy. Furthermore, these investigators noted as absolute loss in the number of visceral epithelial cells during clinical nephropathy concurrent with mesangial expansion. Thus, the remaining cells had to cover increased surface area. These investigators suggested that this podocyte loss may contribute to the

progression of diabetic nephropathy. This finding has also been confirmed by other investigators.

Thickened GBM is always seen in diabetic nephropathy. In fact, a recent study has described the isolated finding of thickened GBM as a possible manifestation of prediabetes or early diabetes in 23 patients who presented with proteinuria greater than 0.5g/day sometimes accompanied by hematuria but without clinical evidence of diabetes. Two years later seven patients remained normoglycemic, six had fasting blood glucose between 110 and 125mg/dL, three had impaired glucose tolerance, and seven had become diabetic . As duration of diabetes increase, greater variability in thickness of the GBM is observed. In part, this change is related to the thinner GBM in microaneurysms , but interindividual variation and interglomerular variation contribute to the large standard deviations. Vogler et al studied 15 patients with type 1 diabetes and found areas of attenuation of the GBM in 6 patients. The thinned areas were continuous with the outer aspect of the thickened GBM in sites where the membrane has apparent laminations. Mesangiolysis has been described in association with the formation of microaneurysms. Osterby et al observed similar basement membrane thinning in severely affected diabetic kidneys. In such cases, the GBM measured as little as 100nm and comprised 1% to 5% of the total capillary length in individual glomeruli. The pathogenesis of these alterations in GBM may be related to many factors, as discussed more fully beginning in the section on pathogenesis.

Changes in the biochemical composition of the GBM have been reported by various investigators. Spiro first recorded such alterations including an increase in hydroxylation of lysine and in the number of disaccharide units. Additional work form his laboratory has shown decreased heparan sulfate and laminin . Tamsma et al

confirmed the decrease of heparan sulfate and found a correlation between that decrease and the degree of proteinuria. Other authors, using immunohistochemical localization techniques, detected a change in change density resulting from alteration in location of the heparan sulfate rather that a absolute decrease in the amount Kim et al showed a change in the chains of collagen type IV in the GBM.

Mesangium

Mesangial widening and nodules are due to increased synthesis of mesangial matrix(198) and decreased degradation secondary to cross- linking of glycosylated collagens . Additional factors are considered in the section on pathogenesis. Dachs et al noted that the first change consisted of widening of the usually delicate starnds of mesangial matrix with increase of the numbers of mesangial cells. The number of mesangial cells and cellular processes may be slightly increased within the expanded mesangial matrix. These observations have been confirmed by morphometric analysis. Steffes et al showed increase in volume fraction of mesangial matrix per glomerulus and in mesangial cells per glomerulus in patients with type 1 diabetes compared with controls. The volume fraction of mesangium increases over the duration of the diabetes(34). In addition to occasional collagen fibrils, cell debris manifested as small calcific deposits, remnants of cell membranes, and scattered organelles are often present . Some nodules develop loosening of the matrix, namely, mesangiolysis, resulting in detachment to endothelial cells and loss of the anchoring points of the GBM to the mesangium. These alterations are thought to precede the exaggerated mesangial expansion of the single large nodules with laminated texture that are associated with microaneurysms.

The major component of the increased matrix is type IV collagen . Other components that are increased include type V and VI collagen, laminin, and fibronectin . Suzuki et al examined glomerular and interstitial expression of the mRNAs of metalloproteinase-3, tissue inhibitor of metalloproteinase-1, and type IV collagen in diabetic nephropathy. These investigators found that the expression of these entities was inversely correlated with the degree of mesangial expansion but directly correlated with the severity of interstitial disease. Expression of integrins is increased on all cell type in diabetic patients with moderate increase in mesangial matrix. As mesangial matrix increases in parallel in mesangial cells, whereas it remains the same on epithelial cells with decreased expression in endothelial cells. The pathogenesis of increased matrix production is discussed in the section on pathogenesis.

Hyalinosis

When the early hyalinosis or exudative lesion is observed with the electron microscope, it appears as a accumulation of homogeneous electron-dense material between the endothelial cell and the GBM. As more material collects, it fills the capillary lumen. On occasion, lipid droplets, cellular debris, or lipid- laden macrophages are present in association with these lesions. In obsolescent glomeruli, remains of the hyalinosis lesions formed earlier persist as denser areas within increased matrix and the GBM. Similar material may also be present within the mesangium. One must distinguish such hyalinosis accumulations from immune deposits, although this distinction may be difficult. This material is identical to that seen in the subintima of arterioles with hyaline arteriolosclerosis. Furthermore, the

capsular drop is made up of similar material lying between Bowman's capsule and the parietal epithelium. The pathogenesis of this lesion is not entirely clear.

Clinicopathologic Correlations

The renal morphologic changes in diabetic nephropathy affect all four renal compartments, i.e., glomeruli, tubules, interstitium, and vessels. The changes in each of these four compartments encompass a spectrum of morphologic alterations showing good overall correlation with the biologic duration of the disease. Detailed morphometric studies, especially in patients with type 1 diabetes, have documented that some of the structural-morphologic changes, including both glomerular and tubulointerstital changes, show close correlation with the clinical-laboratory parameters of renal dysfunction. Studies addressing structural-functional correlation in patients with type 2 diabetes have demonstrated, in general, less precise correlation between morphologic findings and renal functional parameters that those seen in patients with type 1 diabetes.

The characteristic glomerular changes in diabetes include glomerular enlargement, diffuse thickening of the glomerular capillary basement membranes, mesangial expansion primarily owing to matrix accumulation, and progressive glomerulosclerosis with development of globally sclerotic glomeruli. One of the earliest and easiest morphologic changes to detect in diabetic nephropathy is the diffuse thickening of the glomerular capillary basement membranes (35). Precise quantitative measurements demonstrated that in patients with type 1 diabetes, thickening of proximal tubular basement membranes is strongly related to the thickening of the glomerular capillary basement membranes . Although mesangial

matrix accumulation with mesangial expansion represents one of glomerulosclerosis, the increase in the mesangial fractional volume (i.e., the volume fraction of the glomerulus occupied by mesangium (Vv[Mes/glom]) can be documented only 4 to 5 years after the onset of diabetes in patients with type 1 diabetes(35). Once mesangial widening with matrix accumulation had developed in patients with type 1 diabetes, increases in the mesangial fractional volume (Vv [Mes/glom]) correlate precisely with the decrease in the peripheral glomerular capillary basement membrane filtration surface density . Also, evidence for close relation between mesangial fractional volume (Vv [Mes/glom]) and urinary albumin excretion rate in patients with type 1 diabetes has been provided in landmark studies involving elegant morphometric analyses by Maure et al and Caromori et al . Glomerular fractional volume (Vv [Mes/glom])is also a strong concomitant of hypertension. In patients with type 1 diabetes, thickness(width) of the glomerular capillary basement membranes directly correlates with the blood pressure and the urinary albumin excretion rate; however, the correlation is weaker that the one seen with glomerular fractional volume(Vv [Mes/glom]). The width of the glomerular capillary basement membranes is inversely correlated with the glomerular filtration rate (GFR) whereas total peripheral capillary filtration surface is highly correlated with GFR in patients with type 1 diabetes. In general, thickening of the glomerular capillary basement membranes and degree of mesangial expansion (i.e., glomerular fractional volume (Vv [Mes/glom]) increase in patients with type 1 diabetes from normoalbuminiuria to microalbuminuria to proteinuria; however, considerable overlap exists between these groups. In addition, some normoalbuminuric patients with type 1 diabetes can have advanced glomerular lesions and other clinical finding of advancing renal disease such as low glomerular filtration rate and hypertension. This is a indication that albuminuria, albeit a strong

predictor of progression, is still an imprecise indicator of diabetic nephropathy risk. Also, thought at one time to be part and parcel of diabetic nephropathy, glomerular capillary basement membrane thickening and mesangial expansion can progress at varying rates within and between patients with type 1 diabetes. In some patients, marked glomerular capillary basement membrane thickening can be seen without significant increases in the mesangial fraci\tional volume (Vv [Mes/glom]) whereas others may show the opposite pattern.

Tubulointerstital changes in diabetic nephropathy include interstitial expansion owing to increase in cellular content and/or fibrosis, and tubular atrophy. Mild interstitial expansion in patients with long-standing type 1 diabetes is largely due to an increase in interstitial cell component that antedates interstitial fibrosis . Increased fractional volume of interstitial fibrillary collagen appears at later stages of the disease,when the glomerular filtration rate is already reduced . This is different from the glomerular lesions in which mesangial matrix accumulation is the primary cause of mesangial widening even at the early stages of the disease. The extent/severity of interstitial fibrosis and global glomerulosclerosis have also been shown to correlate with the clinical manifestations of diabetic nephropathy in patients with type 1 diabetes (36,37). The extent of interstitial fibrosis has long been advocated as an independent predictor of chronic renal dysfunction as characterized by elevated serum creatinine not only in patients with diabetes including those with type 2 diabetes, but also in a number of various nondiabetic glomerular, tubulointerstital, and vascular diseases . However, during the early stages of diabetic nephropathy, the extent of interstitial fibrosis as a predictor of renal dysfunction as characterized by changes in the GFR and/or microalbuminuria is of limited value.

As already mentioned, the findings of studies addressing renal structural-functional relationship in patients with type 2 diabetes are less conclusive than those in patients with type 2 diabetes are less conclusive that those in patients with type 1 diabetes. In general patients with type 2 diabetes show greater morphologic heterogeneity of the renal lesions. This may, at least in part, be related to higher frequency of cardiovascular risk factors such as hypertension, hyperlipidemia, obesity, and accelerated arteriosclerosis in patients with type 2 diabetes. In a study of 34 unselected patients (age: 58± 7years) with type 2 diabetes and micro albuminuria, renal biopsies revealed renal morphologic heterogentity with less than one third of patients having "Atypical" changes with absent or only mild diabetic glomerular lesions included tubulointerstital fibrosis with or without arteriolar hyalinosis and with or without global glomerulosclerosis. Some patients did not show any significant morphologic changes. The presence of both typical and a typical patterns of renal pathologic findings was associated indication that hyperglycemia may contribute to different patterns of renal injury in this patient populations.. In a study by Osterby et al , renal biopsies of 20 patients with type 2 diabetes showed similar morphologic changes to those seen in patients with type 1 diabetes, i.e., thickening of the glomerular capillary basement membranes and increased mesangial volume ratios with mesangial matrix accumulation. However, the glomerular changes in this study were less advanced in patients with type 2 diabetes compared with historical controls of patients with type 1 diabetes with similar degree of urinary albumin excretion. In addition, patients with type 2 diabetes had higher GFRs than patients with type 1 diabetes with similar degree of urinary albumin excretion , possibly because of larger glomerular volumes in patients with type 2 diabetes, a progressive increase to the glomerular fractional volume(Vv[Mes/glom]) paralleled the clinical laboratory

presentation of diabetic renal disease from the normoalbuminiuria to microalbuminuria and overt nephropathy.

Numerical and structural abnormalities of the visceral epithelial cells (podocytes) have also been described in both type 1 and type 2 diabetic patients. A significantly decreased number of podocytes has been detected in both type 1 and type 2 diabetes. In patients with type 1 diabetes, widening of the foot processes of the visceral epithelial cells correlated with the urinary albumin excretion rate . A significant inverse correlation between the number and density of podocytes per glomerulus and proteinuria has also been reported in patients with type 2 diabetes. In pima Indians with type 2 diabetes the number or podocytes per glomerulus was the strongest predictor of progressive renal disease, with fewer cells predicting more rapid progression. Decreased glomerular expression of podocytes – associated nephrin, a protein known to have a significant role in maintaining normal permselectivity of the glomerular filtration barrier, has been described in patients with type 1 and type 2 diabetes. Decreased in nephrin expression correlated with the broadening of the foot process width. One study indicated that reduction in nephrin expression was due to down-regulation of expression rather than decreased number of podocytes in the glomeruli.

MATERIALS AND METHODS

Type 1 diabetic patients attending kilpauk medical college outpatient department were enrolled in the study . These patients were confirmed as type1 diabetic by documenting low c peptide level. Height , weight, body mass index and smoking habits were recorded . Time of diagnosis of diabetes was enquired and the presenting symptom at the time of diagnosis was recorded. The duration of diabetes was calculated. All these patients were questioned for hospitalisation for any illness and for comorbid conditions. Requirement for insulin and their compliance to insulin were recorded. These patients were enquired for other drug intake and indigenous medicine intake. Pulse rate, blood pressure and peripheral pulses were recorded. These patients were assessed for gastropathy, peripheral neuropathy and autonomic neuropathy. Electrocardiogram and for selected patients Echocardiography were also done. They were also examined for diabetic retinopathy by direct ophthalmoscopic evaluation and fluorescein angiography if necessary by ophthamologist. Fasting lipid profile was done in these patients. Serum electrolytes like potassium, sodium, magnesium and uric acid were measured. These patients were screened for viral markers like hepatitisB, hepatitis C and HIV. Ultrasonogram abdomen and KUB were done and kidney size was assessed.The urine was screened for proteinuria by sulfosalicilic acid method. Those who were negative for albuminuria were screened for microalbuminuria. Serum creatinine was measured on two consecutive days by jaffe's method and the mean serum creatinine was calculated. Gfr was calculated by cock grauft and gault formula and those patients with a low gfr (less than 90ml/min/1.73m2 were identified. Totally we had 37 patients with low gfr and normoalbuminuria(negative for microalbuminuria). These patients with low gfr by

cock grauft gault formula and negative for microalbuminuria (37patients) were screened for gfr by DTPA scan. After doing radionuclide study (DTPA scan) we had twenty patients with normoalbuminuria and negative for microalbuminuria and low gfr(less than 90ml/min) . These patients were subjected to renal biopsy after getting informed written consent. Renal biopsy was done as an outpatient proceedure and we had no complications. Two samples were taken and sent in formalin to renal pathologists for light microscopic studies. Electron microscopic examination was not done due to unavailability. Twenty patients with normoalbuminuria and normal gfr(>90ml/min by DTPA scan) who were age and sex matched with those with low gfr were registered as control. Duration of diabetes in the normal gfr and low gfr were compared. Serum magnesium level was compared between these two groups. Incidence of hypertension and retinopathy in both these groups were compared. The characteristic profile of patients with low gfr was analysed with respect to duration of diabetes, serum magnesium level, hypertension, retinopathy and renal biopsy features. Stastical method like chi square and student t test were applied.

RESULTS

Total number of type1 diabetic patients screened in this study were 95.

Of which we selected twenty patients with normoalbuminuria and low gfr by radionuclide scan. We compared these patients with age and sex matched twenty patients with normoalbuminuria and normal gfr (>90ml/min by DTPA scan).

Sex & age distribution:

Both these groups were sex and age matched.

Duration of diabetes:

The duration of diabetes in patients with low gfr ranges from five years to twenty nine years and among patients with normal gfr it was three years to nine years .The mean duration of diabetes in low gfr group was twelve years and among normal gfr it was five years. This was stastically significant.

Mean gfr:

The gfr in the low gfr group ranges from 25 to 89 and it was in the range of 92 to 105 in normal gfr group. The mean gfr in low gfr group was 68 and in the normal gfr group it was 97.

Serum magnesium:

The mean serum magnesium was 1.9 in the low gfr group and it was 2.4 in normal gfr group. This was stastically significant with a p value of less than .05

Hypertension & retinopathy:

Hypertension was seen in five patients (25%) in the low gfr group and it was seen only in one patient (5%) in the normal gfr group. Retinopathy was seen in eight patients (40%) in the low gfr group and only in one patient (5%) in the normal gfr group. This was found to be stastically significant with a p value of less than .05 .

Renal biopsy:

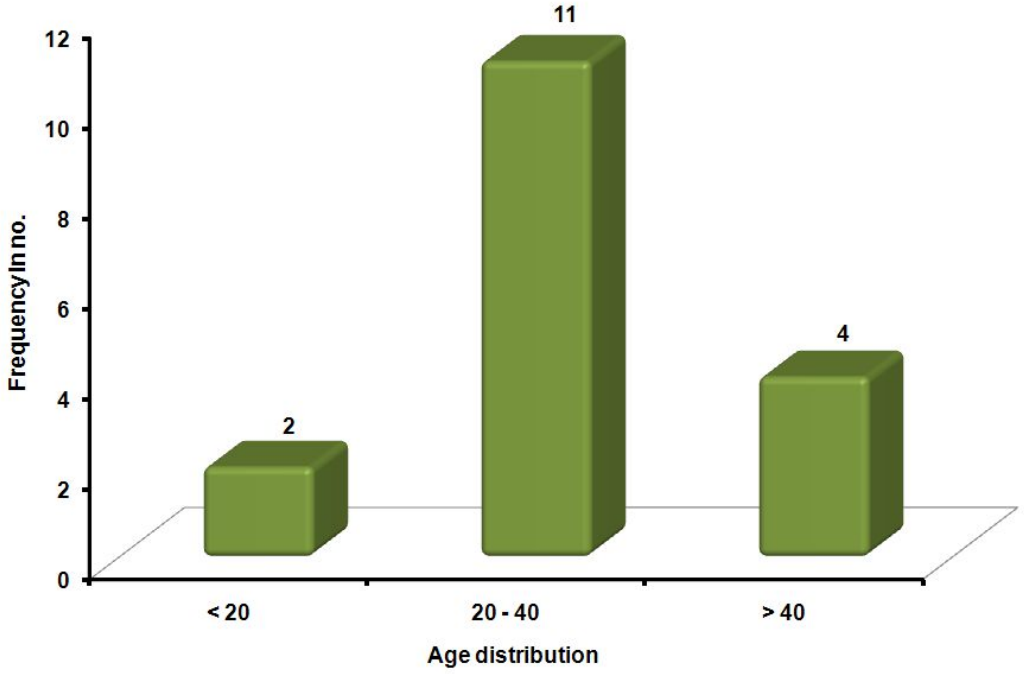
Renal biopsy was done in all the twenty patients in low gfr group of which fourteen patients showed characteristic diabetic changes of diffuse glomerulosclerosis and no other cause could be ascribed for low gfr and three patients showed nodular sclerosis along with diffuse changes. Three patients showed atypical pattern with predominant tubulointerstitial involvement with tubular atrophy, interstitial fibrosis and arteriolar hyalinosis.

Charesteristics of the patients with normoalbuminuric diabetic nephropathy:

We had seventeen patients with renal biopsy proven normoalbuminuric diabetic nephropathy.

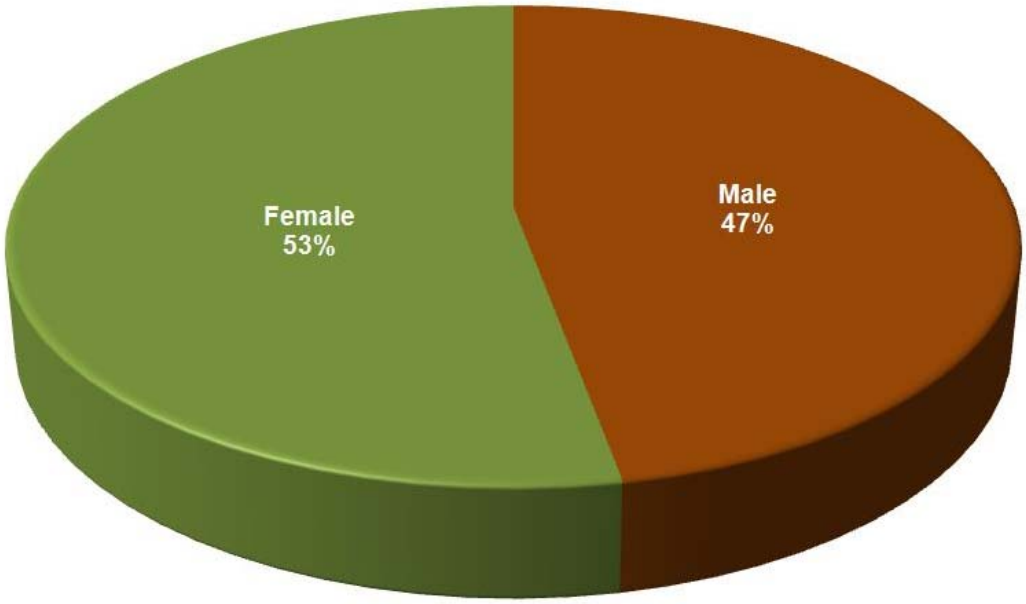
Age & sex distribution:

Age	No.	P - value
< 20	2	0.000
20 - 40	11	
> 40	4	



Of the seventeen patients eight (47%) were male and the age ranges from 19 to 47 years with a mean age of 33 years. Two patients were below 20years of age and eleven patients were between 20 to 40 years and four patients were more than 40 years.

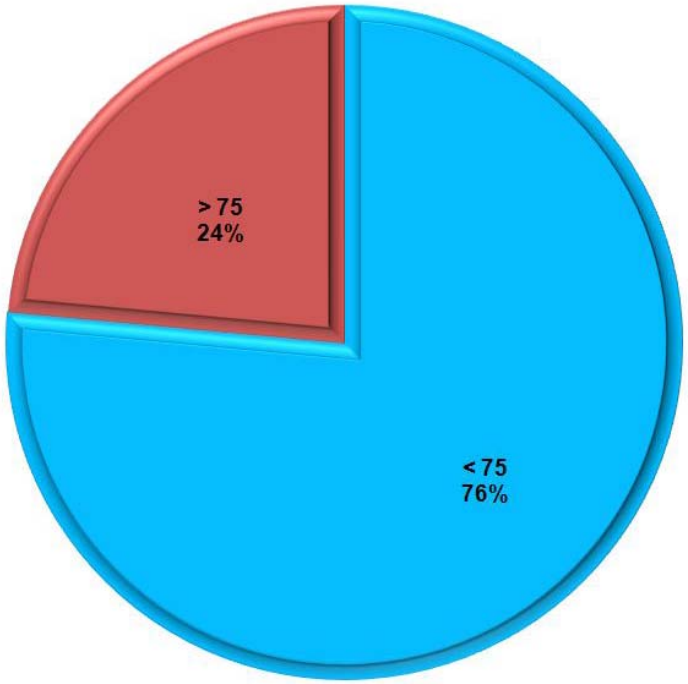
Gender	No.	P - value
Male	8	0.000
Female	9	



Gfr:

Thirteen patients (76%) had gfr of less than 75 and 4(24%) patients had gfr of more than 75. The mean gfr was found to be 67ml/min.

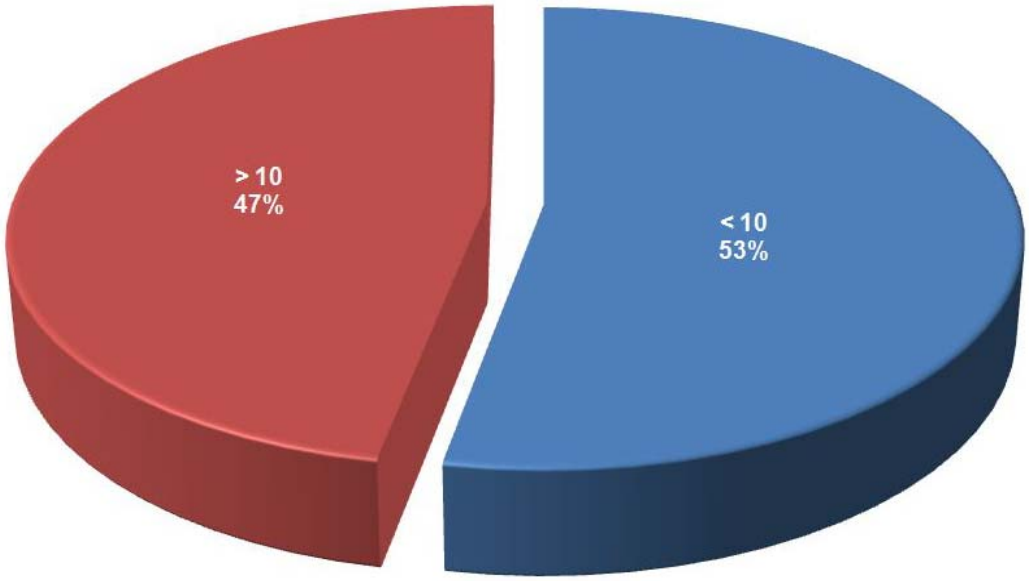
GFR	No.	P - value
< 75	13	0.000
> 75	4	



Duration of diabetes:

Nine patients had a diabetic duration of less than ten years and eight patients had a duration of more than ten years. The mean duration of diabetes in this study group was found to be 11years .

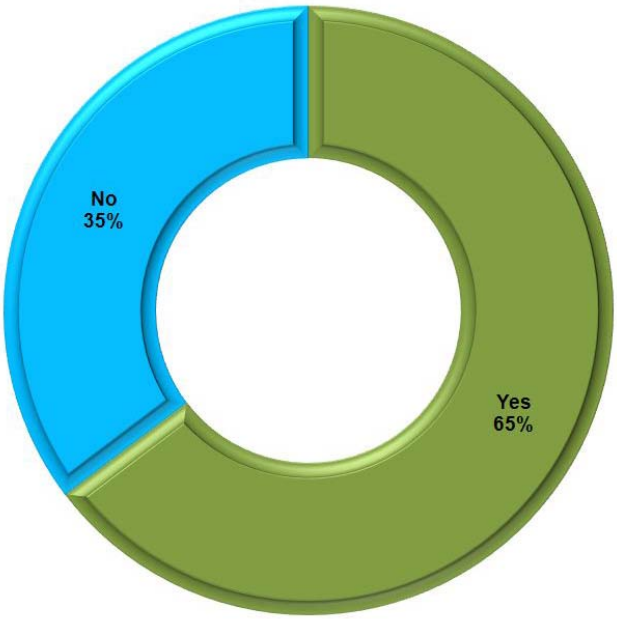
Duration	No.	P - value
< 10	9	0.000
> 10	8	



Serum magnesium:

Eleven patients (65%) had a serum magnesium of less than 2mg/l and only six patients had serum magnesium of more than 2 mg/dl.

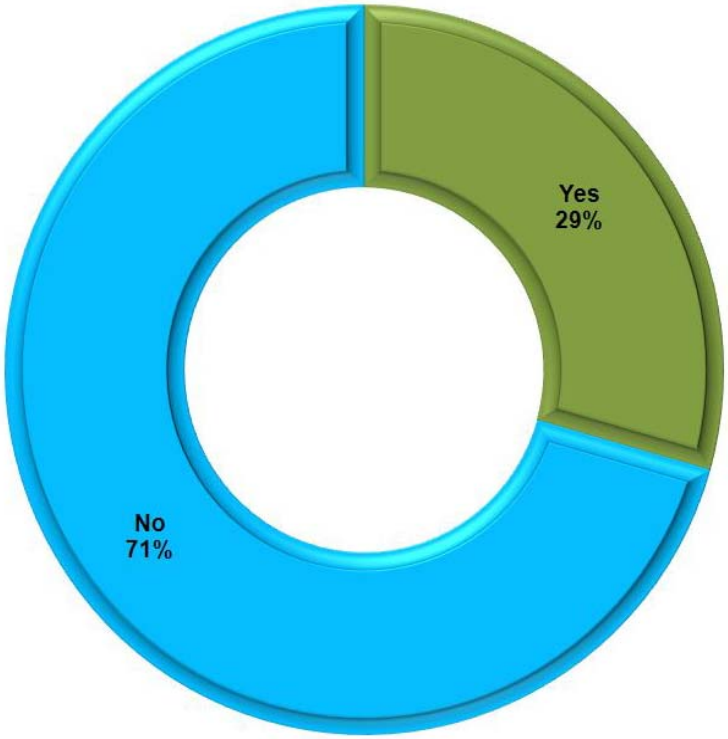
Magnesium	No.	P - value
< 2	11	0.000
> 2	6	



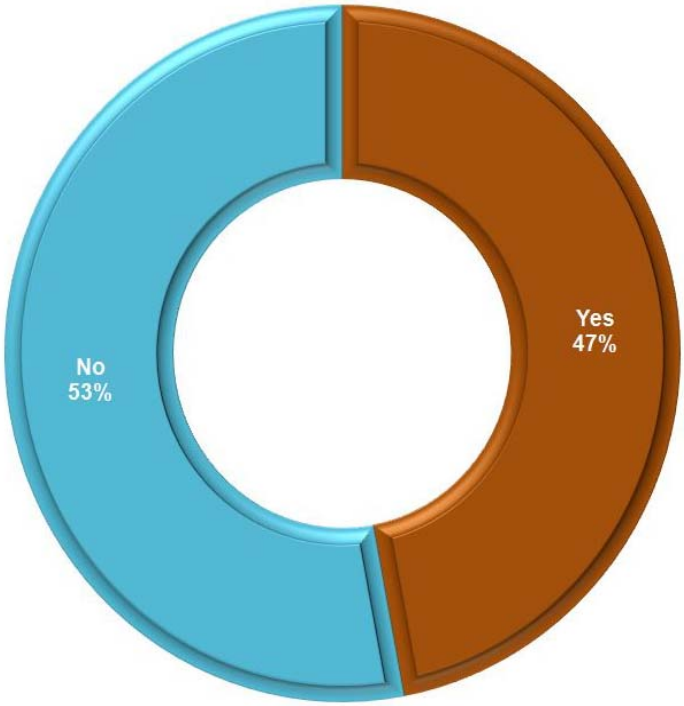
Hypertension & retinopathy:

Among the seventeen patients five patients (29%) had hypertension and eight patients (47%) had diabetic retinopathy changes.

Hypertension	No.	P - value
Yes	5	0.020
No	12	



Retinopathy	No.	P - value
Yes	8	0.002
No	9	



Comparing Duration of diabetes with hypertension & retinopathy:

Comparison of duration with Hypertension

Duration	Hypertension		P - Value
	Yes	No	
< 10	0	12	0.000
> 10	5	3	

Comparison of duration with Magnesium

Duration	Magnesium		P – Value
	< 2	> 2	
< 10	8	4	0.858
> 10	5	3	

Comparison of duration with Retinopathy

Duration	Retinopathy		P - Value
	Yes	No	
< 10	1	11	0.000
> 10	7	1	

Comparison of duration with Renal biopsy charecteristic of diabetic nephropathy

Duration	Renal biopsy		P – Value
	Yes	No	
< 10	9	3	0.139
> 10	8	0	

Comparison of GFR with Hypertension

GFR	Hypertension		P - Value
	Yes	No	
< 90ml	5	15	0.080
> 90ml	1	19	

Comparison of GFR with magnesium

Magnesium	N	Mean	Std. Deviation	Std. Error Mean	P - Value
< 2	20	1.9950	.24382	.05452	0.000
> 2	20	2.4750	.40246	.08999	

Comparison of GFR with Retinopathy

GFR	Retinopathy		P - Value
	Yes	No	
< 90ml	8	12	0.007
> 90ml	1	19	

Among eight patients with a duration of diabetes more than ten years five had hypertension and seven patients had diabetic retinopathy changes. This was stastically significant.

DISCUSSION

Long standing type1 diabetic patients with normal albumin excretion rate are still at risk of developing clinically significant nephropathy. It is therefore important to identify markers of increased nephropathy risk among these patients. One possibility is to perform kidney biopsy in such patients. However this is not very practical in most clinical settings. Thus we examined whether reduced gfr can be predictive of more advanced underlying glomerular lesions. It was first reported that reduced gfr in eight normoalbuminuric longstanding type1 diabetic women was associated with worse diabetic glomerular lesions. Shortly thereafter, a small group of normoalbuminuric long standing type1 and type2 diabetic largely female patients with reduced gfr was described. A similar prevalence of reduced gfr was reported among longstanding normoalbuminuric and normotensive type1 diabetic patients in brazil. However many other investigators did not encounter reduced gfr in normoalbuminuric type1 diabetic patients.

The existence of normoalbuminuric diabetic kidney disease was demonstrated in the united kingdom prospective diabetes study (UKPDS). In this cohort study they followed 4000 diabeteic patients with normoalbuminuria and normal creatinine clearance as estimated by cockcroft-gault formula. They followed them for a median of about 15 years. In this about 12% of patients with microalbuminuria manifested with a decreased creatinine clearance first. Of those patients with decreased creatinine clearance about 51% never had albuminuria at all. This existence of normoalbuminuric kidney disease has been replicated also in NHANES as well as in multiple cohort studies in australia , in japan and in italy.there are also multiple cohort studies in type 1 diabetes also showing the same. In this cohort there were 105

patients with type1 diabetes who had no albuminuria, and they underwent gfr measurement with iothalamate clearance, and they found that 23 of those 105 patients had a decreased gfr less than 90. There were also more prevalence of hypertension and retinopathy in the group with low gfr. On renal biopsy they found typical diabetic nephropathy changes on histology in low gfr group. The basement membrane width was increased and fractional mesangial matrix and mesangium per glomerulus was expanded in those with decreased gfr

In our study we analysed 95 type1 diabetic patients. 45 were female and 50 were male. Most of them were in the age group of 20-40yrs with a mean duration of less than 10yrs in 60 patients and more than 10yrs in 35 patients. In this study the mean gfr was low in female comparing to male and in those patients with long duration of diabetes (more than 10yrs). In this study we compared the 20 patients with low gfr and 20 patients with normal gfr. The sample size is small as we did study in type1 diabetic patients. There was no significant sex and age difference between these two groups as the patient selection was done by matching these parameters. The mean gfr was found to be 68ml/min in low gfr group and it was 97ml/min in the normal gfr group.

The duration of diabetes in the low gfr group is 12yrs and it is 5 years in normal gfr group. This data is highly significant and this emphasize the fact that the incidence of normoalbuminuric diabetic nephropathy increases as duration of diabetes increases. This is same as we see in albuminuric diabetic nephropathy.

The mean serum magnesium is 1.9 in patients with low gfr and it is 2.4 in normal gfr group. This is statistically significant. There are many studies showing that

lower magnesium level in diabetic patients are associated with increased incidence of comorbid illness (38,39,40). If this is proved right then these patients with low gfr are more prone for diabetic end organ complications.

Hypertension is seen in 5 patients with low gfr while it is seen in only one patient with normal gfr . Similarly retinopathy is seen in 8 patients with low gfr while it is seen in only one patient with normal gfr. These data are statistically significant. This confirms the fact that these patients with low gfr are placed in a high risk zone comparing to those with normal gfr. Generally there are heterogeneity in renal biopsy in diabetics. There are three categories identified . category 1- normal or near normal structure, just a mild amount of mesangial expansion. Category2- thickening of basement membrane, arteriolar hyalinosis and nodular mesangial expansion. Category3- atypical patterns of renal injury with more tubulointerstitial disease where the tubular basement membrane was thickened and that there was more tubular atrophy, a lot more interstitial fibrosis and much greater arteriolar hyalinosis(41).

In a study by nosadini, they looked at a large cohort of patients in italy. They followed them from the initial diagnosis of diabetes until they got to a gfr estimated with creatinine of about 60-75. Then , they made sure that that was actually their gfr measuring it with isotope labelled creatinine EDTA. They found that 30 of these patients had albuminuria and 27, again, had normal albuminuria. Then they went on to biopsy them. Interestingly , 93% of the patients who had albuminuria had the typical diabetic histopathology whereas only about 20% with normal albuminuria had this pathology. It was more often those with normal albuminuria who fell into category3. Very few of those with albuminuria exhibited this category. So they assumed that it is the difference in histology that accounts for this existence of normoalbuminuric

diabetic kidney disease and that infact this may be reflective of more the vascular outcomes in diabetes rather than the specific glomerular diabetic nephropathy that we always assume patients to have(42). In this study we did renal biopsy to those twenty patients with low gfr and we found that seventeen patients had typical diabetic nephropathy changes. Only three patients we had the histology showing predominant tubulointerstitial changes. So we have this data paradoxical to this Italian study.

When we analysed the 17 patients with typical diabetic nephropathy changes we could not find any statistically significant change with respcct to gender. Among them 13 patients had gfr less than 75ml/min. As we had 5 patients with hypertension among these seventeen patients, the incidence of hypertension among these normoalbuminuric diabetic nephropathy from our study is 35%. Similarly we had 8 patients with retinopathy in this group accounting for an incidence of 48%. So there is an increased incidence of hypertension and retinopathy in this normoalbuminuric diabetic nephropathy population.

CONCLUSION

1. There exists an entity – normoalbuminuric diabetic nephropathy in type1 diabetic patients

2. Incidence of normoalbuminuric diabetic nephropathy increases with duration of diabetes.

3. There is an increase incidence of hypertension(29%) and retinopathy(47%) in this group of patients.

4. Serum magnesium is low in these patients with normoalbuminuric diabetic nephropathy the significance of which is not clearly known yet there are studies to say that low magnesium is associated with poor outcome.

5. So along with screening for albuminuria periodic screening for GFR should also be done so that these normoalbuminuric diabetic nephropathy can be detected early. This patients should be treated as high risk and they should avoid radiocontrast and other nephrotoxic drugs if not mandatory.

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MASTER CHART - 2

gfr<90ml	s.no	male	female	age	gfr	duration of diabetes	hypertension	magnesium	retinopathy	renal biopsy
	1	yes		18	89	5	no	2.1	no	yes
	2	yes		47	73	2	no	2	no	yes
	3	yes		15	66	6	no	2.2	no	yes
	4	yes		41	79	18	yes	1.9	yes	yes
	5	yes		15	82	7	no	1.8	no	no
	6	yes		29	25	2	no	2	no	yes
	7	yes		35	64	11	yes	2.1	yes	yes
	8	yes		53	57	29	yes	1.7	yes	yes
	9	yes		28	88	1	no	2	no	yes
	10		yes	25	64	3	no	1.6	no	yes
	11		yes	24	46	10	no	2	yes	yes
	12		yes	22	64	4	no	2.1	no	no
	13		yes	35	81	12	yes	2.8	no	yes
	14		yes	39	71	13	no	2.1	yes	yes
	15		yes	25	52	2	no	2	no	no
	16		yes	27	62	5	no	1.8	no	yes
	17		yes	38	65	18	yes	1.9	yes	yes
	18		yes	39	65	23	no	1.8	yes	yes
	19		yes	28	67	11	no	1.9	yes	yes
	20		yes	42	60	9	no	2.1	no	yes

MASTER CHART - 2

gfr<90ml	s.no	male	female	age	gfr	duration of diabetes	hypertension	magnesium	retinopathy	renal biopsy
	1	yes		18	89	5	no	2.1	no	yes
	2	yes		47	73	2	no	2	no	yes
	3	yes		15	66	6	no	2.2	no	yes
	4	yes		41	79	18	yes	1.9	yes	yes
	5	yes		15	82	7	no	1.8	no	no
	6	yes		29	25	2	no	2	no	yes
	7	yes		35	64	11	yes	2.1	yes	yes
	8	yes		53	57	29	yes	1.7	yes	yes
	9	yes		28	88	1	no	2	no	yes
	10		yes	25	64	3	no	1.6	no	yes
	11		yes	24	46	10	no	2	yes	yes
	12		yes	22	64	4	no	2.1	no	no
	13		yes	35	81	12	yes	2.8	no	yes
	14		yes	39	71	13	no	2.1	yes	yes
	15		yes	25	52	2	no	2	no	no
	16		yes	27	62	5	no	1.8	no	yes
	17		yes	38	65	18	yes	1.9	yes	yes
	18		yes	39	65	23	no	1.8	yes	yes
	19		yes	28	67	11	no	1.9	yes	yes
	20		yes	42	60	9	no	2.1	no	yes

MASTER CHART. 1

gfr>90ml	s.no	male	female	age	gfr	duration of diabetes	hypertension	magnesium	retinopathy	renal biopsy
	1	yes		20	98	5	no	2.2	no	
	2	yes		45	92	3	no	2.4	no	
	3	yes		15	96	4	no	2.5	no	
	4	yes		41	99	7	no	2.7	no	
	5	yes		17	95	6	no	1.8	no	
	6	yes		29	102	9	no	1.4	no	
	7	yes		35	105	3	no	1.9	no	
	8	yes		55	97	2	no	2.7	no	
	9	yes		26	95	5	no	2.9	no	
	10		yes	22	99	8	no	2.5	no	
	11		yes	26	98	6	no	2.7	no	
	12		yes	24	93	9	no	2.2	no	
	13		yes	37	98	4	no	2.8	no	
	14		yes	24	100	3	yes	2.7	yes	
	15		yes	28	104	7	no	2.9	no	
	16		yes	27	97	3	no	2.8	no	
	17		yes	30	98	2	no	2.7	no	
	18		yes	39	96	3	no	2.8	no	
	19		yes	30	95	4	no	2.6	no	
	20		yes	41	92	6	no	2.3	no	