DIABETIC CHILDREN”

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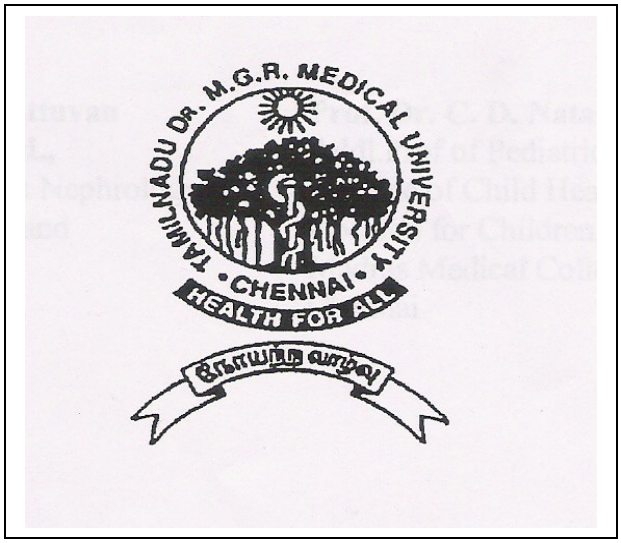
M.D. DEGREE EXAMINATION

BRANCH VII – PAEDIATRIC MEDICINE



INSTITUTE OF CHILD HEALTH

AND

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MADRAS MEDICAL COLLEGE

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CERTIFICATE

This is to Certify that this dissertation entitled "**EARLY DETECTION OF NEPHROPATHY IN DIABETIC CHILDREN"** is a bonafide work done by **Dr.V.RADHIKA,M.D**., Postgraduate student of Paediatric Medicine, Institute of Child Health and Hospital for Children, Egmore, Chennai – 8 attached to Madras Medical College, during the academic year 2004-2007.

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DECLARATION

I declare that this dissertation entitled ***"EARLY DETECTION OF NEPHROPATHY IN DIABETIC CHILDREN"*** has been conducted by me at the Institute of Child Health and Hospital for children, under the guidance and supervision of **Prof. Dr. PRABHA SENGUTTUVAN, M.D., DCH., DM (Nephro).** It is submitted in part of fulfillment of the award of the degree of M.D [Paediatrics] for the March 2007 examination to be held under The Tamil Nadu Dr. M.G.R. Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

Dr. V.RADHIKA.

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TABLE CONTENTS

INTRODUCTION

History

The development of protienuria in patients with diabetes mellitus has been described in the eighteenth century by Cotugno and it was Richard Bright who postulated in 1836 that albuminuria reflects renal disease. Although renal lesions in diabetic patients have been well known in the nineteenth century, they were usually regarded as non-specific consequences of hypertension. It was only in 1936 that Kimmelstiel and Wilson recognized nodular homogenous glomerular lesions (nodular diabetic glomerulosclerosis) as a diabetes specific complication [1, 2]. Later documented that the renal lesion was part of the more general clinical syndrome of microangiopathy.

Keen *et al* (1969) in the United Kingdom and Parving *et al* (1976) in Denmark were the first to note that urinary albumin excretion rates are elevated in some patients with types1 and 2 diabetes[3,4]. There is now consensus that a lowish but supranormal albumin excretion rate in the urine ('microalbuminuria') is a powerful predictor of renal (and cardiovascular) events [5].

Persistent albuminuria, that is, greater than 300 mg/24 h or 200 μg/min, is the clinical hallmark of the manifestation of diabetic nephropathy (DN). This clinical definition is valid in both types 1 and 2 diabetes.

Dimension of the Problem

In most countries, DN has become the leading cause of ESRD[6]. According to the United States Renal Data System[7] in 1999 DN was the primary diagnosis in 42.8 per cent (38,160 of 89,252) of incident patients[7] an increase by 238 per cent compared to 1990. In 2000, the proportion of diabetics amongst patients reaching ESRD varied considerably between different countries, for example, 14.6 per cent in the Netherlands, 22 per cent in Australia, 25 per cent in Sweden, and 36.1 per cent in Germany, but it was consistently on the increase in all countries.

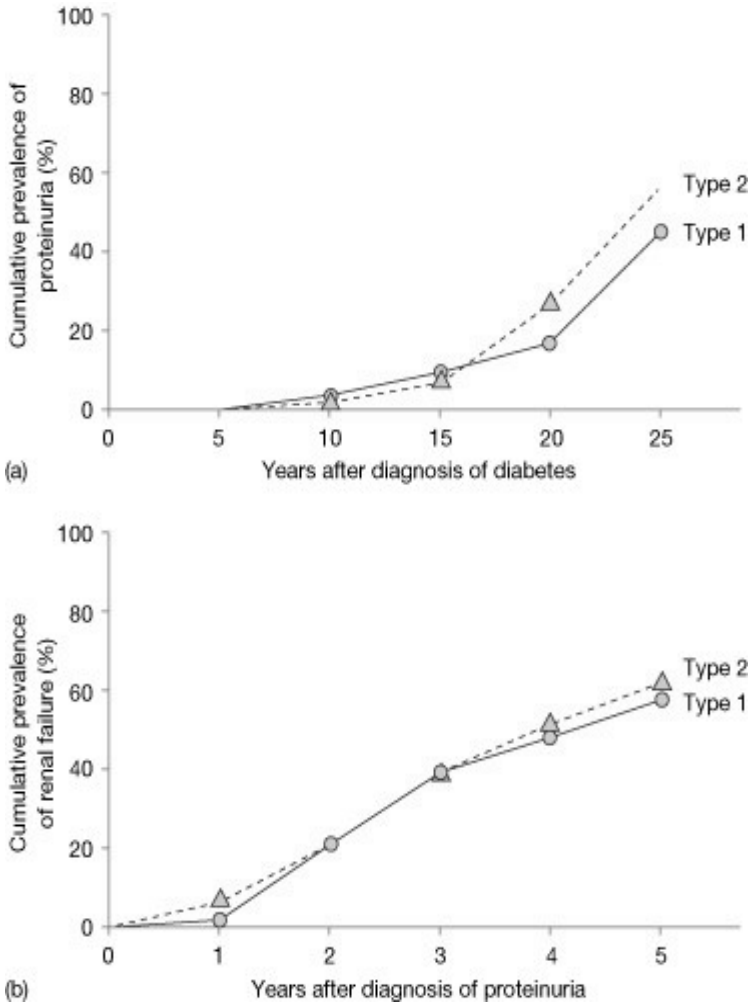
The prevention and management of diabetes and its renal complications is thus an immense global challenge. Registry figures tend to underestimate the renal burden posed by diabetes because it is under represented as illustrated by

our own observations[8]. In 1998–2000, diabetes mellitus was found as a comorbid condition in no less than 48.9 percent of patients admitted for renal replacement therapy in Heidelberg[8].

Classical features of Kimmelstiel Wilson's disease were observed in only in 60 per cent, that is, large kidneys, massive proteinuria (>1 g/24 h) with or without retinopathy. Atypical presentation consistent with ischaemic nephropathy was seen in 13 per cent and known primary renal disease (e.g. polycystic kidney disease, analgesic nephropathy, glomerulonephritis) with superimposed diabetes in 27 per cent of cases. For the diabetic patient with ESRD, survival is similar irrespective of whether he or she suffers from DN or primary non-diabetic renal disease[9].

Inadequate medical care for the diabetic with renal complications is illustrated by the finding that in 11 per cent of the patients the diagnosis of diabetes had not even been made at the time of admission to the renal unit. This may result from the fact that hyperglycaemia is often self-corrected when patients lose weight as a result of anorexia.

Fig. 1 Cumulative risks (a) to develop proteinuria and (b) to progress to renal failure in patients with types 1 and 2 diabetes[10].



PATHOMECHANISMS:

Pathology

Macroscopic Changes

Enlargement of the kidney is found in newly diagnosed patients with type 1 diabetes. Similarly, in experimental animals it is seen within 4 days of the onset of diabetes. It results from tubular hypertrophy and interstitial expansion[11]. It is thought to be related to hyperfiltration and stimulated active reabsorption [12].

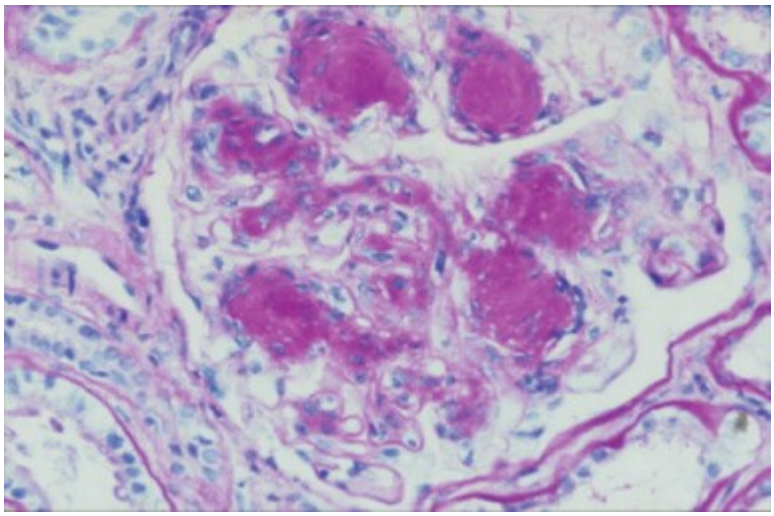
Light Microscopic Changes

The Glomerulus

Glomerular enlargement is an early feature of both human and experimental diabetes and is the result of increases in capillary length and diameter. Glomerular enlargement is also present in established nephropathy, mean glomerular volumes are 4–6 × 10^6 μm^3 in patients with types 1 and 2 diabetes compared to normal values of 1–2.3 × 10^6 μm^3 [13]. The reasons for enlargement are unclear—initially it may be because of altered glomerular haemodynamics and hyperfiltration, and later because of a compensatory response to capillary closure and global glomerulosclerosis.

The hallmark of diabetic glomerulopathy is diffuse mesangial expansion, associated with nodule formation in a minority of patients (Fig. 2). Most patients with long-standing diabetes show an increase in periodic acid–schiff (PAS)-positive matrix material, although these changes are much more prominent in those with clinical nephropathy. Initially, this material is central to the tuft but later it expands and effectively obliterates the capillaries eventually leading to global glomerulosclerosis[11].

Fig. 2 Nodular diabetic glomerulosclerosis. PAS reaction. Magnification 100×. Courtesy of Prof R. Waldherr, Heidelberg, Germany.



Nodules are more or less pathognomonic for diabetes and were the first glomerular pathological abnormality described by Kimmelstiel and Wilson. They comprise acellular, eosinophilic, and lamellated structures, usually located at the periphery of the tuft. Their pathogenesis is unclear, but they may represent obliterated capillary microaneurysms, or result from focal mesangiolysis at the junction of the glomerular basement membrane (GBM) with the mesangium. This would lead to endothelial cell detachment and subsequent ballooning into the capillary, quickly followed by matrix deposition[11]. These features are not universally found in patients with microalbuminuric type 2 diabetes patients [14]. A proportion of these patients have an appearance which is more suggestive of glomerular ischaemia or tubulointerstitial disease. Patients with type 2 diabetes with clinical nephropathy or retinopathy have glomerular changes similar to type 1 diabetes [15]. Capsular drops of PAS-positive matrix material lying between the matrix membrane and parietal epithelium of Bowman's capsule are commonly seen, but their significance is unclear.

In ESRD, glomeruli appear as sclerosed hyalinized structures and a percentage is actually resorbed. This global sclerosis is due to a combination of mesangial expansion and ischaemia secondary to afferent arteriolar hyalinosis [16].

Tubulointerstitium

Armanni–Ebstein lesions, that is, glycogen-rich granules in proximal tubular cells, result from glucose overload and are preventable by reversing glycosuria. Tubulointerstitial expansion contributes to whole kidney enlargement. It is also a feature of established nephropathy and is found even in a significant number of microalbuminuric type 2 diabetic patients. Its causes are almost certainly different in early diabetes and established nephropathy. Acute changes in the juxtaglomerular apparatus are seen within hours of development of diabetes in the rat. More subtle long-term abnormalities in this structure have also been described in patients with microalbuminuric type 1 diabetes and may reflect increased activity of the tubuloglomerular feedback mechanism and possibly an upregulation of the renin–angiotensin system (RAS) [17]. Afferent and efferent arteriolar hyalinosis is a characteristic feature of diabetic patients and is more prominent in those with microalbuminuria[18] or more advanced renal disease.

Immunofluorescence Microscopy

Non-specific linear staining for immunoglobulin (IgG) (mainly IgG4) and albumin is found in the GBM, tubular basement membrane (TBM), and Bowman capsule. No direct correlation is found with the severity of glomerulopathy or nephropathy. Its pathophysiological significance is unclear [11]

Electronmicroscopy

Glomerulus

Its structure is normal at the onset of type 1 diabetes, but GBM thickening up to three times the normal range of 270–359 nm is an almost universal feature in patients with duration of diabetes greater than 10 years. This thickening is more marked in patients with microalbuminuria and clinical nephropathy. There is an accumulation of type IV collagen with a net reduction in heparin sulfate proteoglycan. This combination disrupts both GBM structure and its electrostatic charge properties. It is thus thought to result in abnormal permselectivity and leakage of circulating proteins[11].

In normal man, the ratio of cellular to matrix components is about 1 : 1. The mean fractional volume of the mesangium is between 14– 20 per cent of the glomerular tuft[19]. In contrast, in patients with DN, the mesangium can comprise over 40 per cent of the total tuft volume. This accumulation disrupts the microfibrillar structure of the mesangium, altering its porosity to proteins and weakening attachments to the endothelium and GBM, thus perhaps predisposing to nodule formation[11].

Nodules comprise matrix of similar composition, but with a prominent accumulation of microfibrils. In advanced nephropathy, thin irregular segments of GBM lined by an abnormal looking endothelium may be found, representing either new capillary growth or microaneurysms. This may lead to microscopic haematuria that is occasionally seen in diabetes as it is in thin basement membrane disease.

Tubulointerstitium

The structure of the TBM is largely similar to the GBM, but it is almost twice as wide. In diabetes, the TBM increases to about two to three times its normal width and often appears split [20], possibly permitting penetration of macromolecules into the interstitial space and thus favouring the development of fibrosis. Early tubulointerstitial expansion results from an increase in cellular components[21] followed by collagen accumulation later.

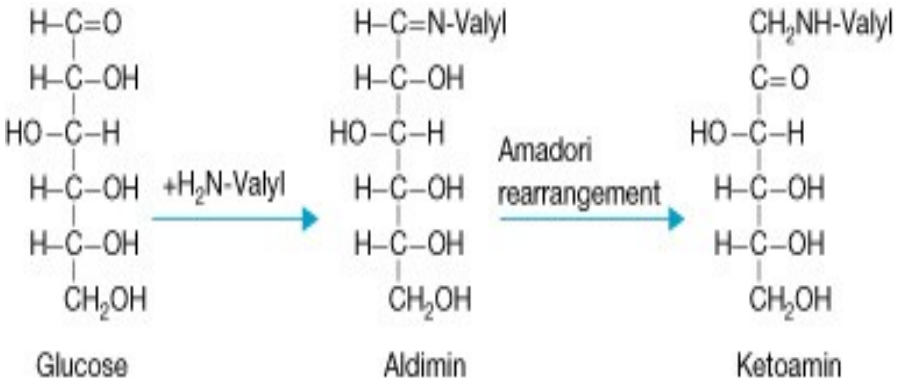
Pathomechanisms of Microvascular Damage:

The microvascular lesions of the kidney in diabetes mellitus had variably been ascribed to generation of advanced glycation endproducts (AGE), cumulation of sorbitol, activation of protein kinase C (PKC), and activation of the hexosamine pathway. A unifying concept has recently been proposed by Brownlee [22]. He provided evidence that in insulin-insensitive tissues hyperglycaemia increases the delivery of glucose-derived intermediates as the metabolic substrate for mitochondrial oxidation. Increased mitochondrial oxidation leads to the generation of reactive oxygen species (ROS). ROS are responsible for the following four metabolic abnormalities: (a) accumulation of methylglyoxal and other substrates leads to the generation of early Amadori products and late AGE respectively, (b) furthermore activation of PKC by ROS, particularly the β-isoenzyme, (c) activation of the polyol pathway causing accumulation of sorbitol, and finally (d) also activation of the hexosamine pathway.

Major attention has recently been paid to the pathogenic role of AGE. As shown in Fig. 3, glucose (but more importantly also other compounds such as methylglyoxal) interact with the α and γ amino groups of amino acids to form Schiff bases that rearrange spontaneously to yield Amadori products. These are non-enzymatically transformed into highly reactive early Amadori products, for example, methylglyoxal, dideoxyglucosone, deoxyglucosone, etc. In the course of weeks, heterocyclic advanced fluorescent AGEs are generated, which cross-link proteins and interact with several receptors, the most important of which is receptor for AGE (RAGE).

Fig. 3 Glucose (but more importantly also other compounds such as methylglyoxal) interact with the α

and γ amino groups of amino acids to form Schiff bases, which rearrange spontaneously to yield Amadori products.These are non-enzymatically transformed into highly reactive early Amadori products, for example, methylglyoxal, oxyglucosone,deoxyglucosone.



Activation of the RAGE triggers ROS formation and promotes translocation of the transcription factor NF-κB, a central switch for inflammatory processes into the nucleus. The relevance of AGE for DN is illustrated by the fact that the course of DN is accelerated in animals, which are transgenic for RAGE [23]. Important secondary mediators for the development of renal damage are transforming growth factor β (TGFβ) [24], locally generated angiotensin II [12], endothelin, and several other cytokines.

Renal Pathophysiology

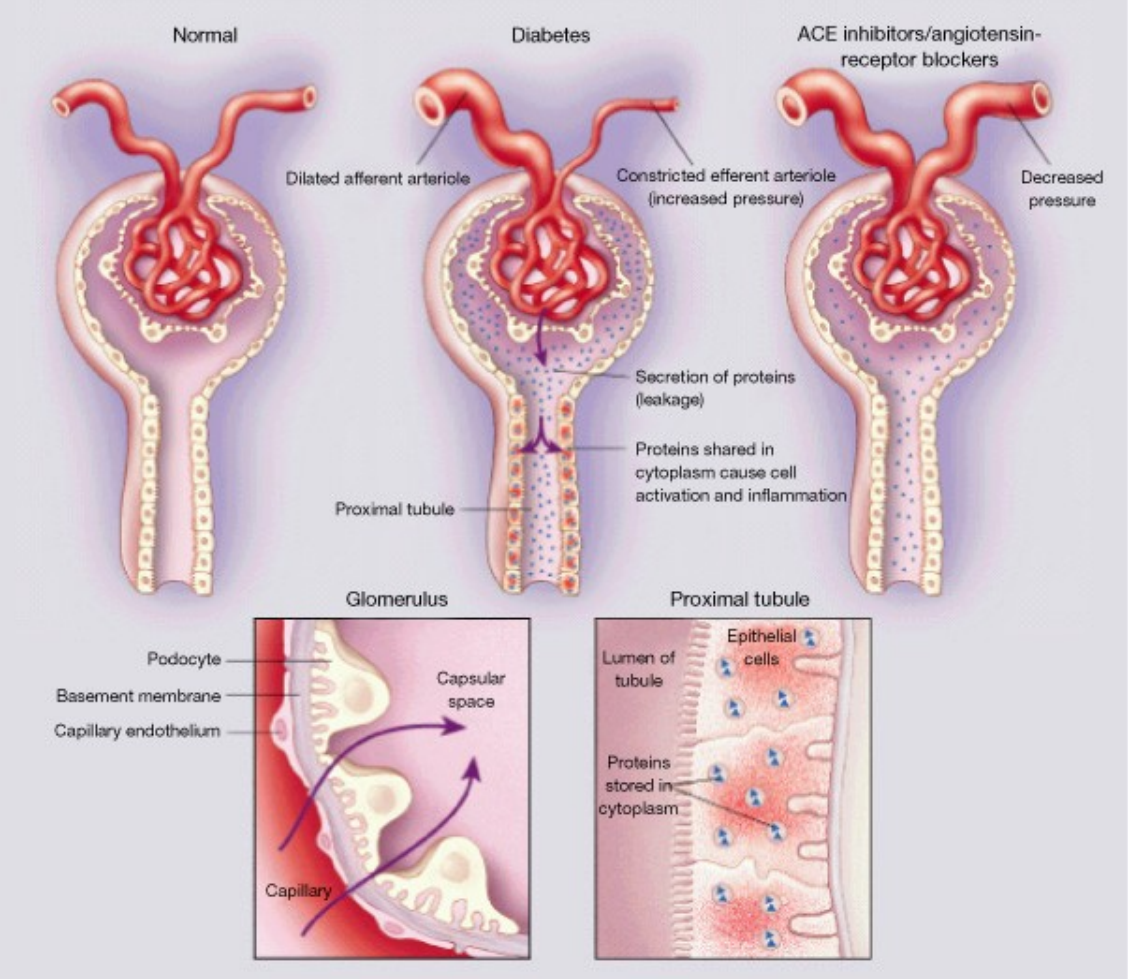
It has been known since decades that the glomerular filtration rate (GFR) is elevated in diabetes mellitus [25]. This has now been well documented in type 1 diabetes[26] and, although this has remained more controversial, equally in type 2 diabetes[27].

The mechanisms underlying hyperfiltration have been clarified in animal experiments [28]. In rats with diabetes, hyperfiltration as well as hyperperfusion and enhanced glomerular capillary hydraulic pressure have been well documented.

This constellation is due to preferential afferent renal vasodilatation with the resulting impairment of renal autoregulation. This was also documented in patients with type 1[29] and type 2 diabetes[30]. Dilatation of the preglomerular vessels will increase the vulnerability to hypertension (or conversely vulnerability to ischaemia from hypotension). A larger proportion of aortic blood pressure is transmitted into the glomerular capillary bed causing 'glomerular hypertension'. This fact also explains why even blood pressure (BP) values in the upper normal range are injurious to the vasodilated kidney of diabetic patients.

As shown in Fig. 4, glomerular hypertension resulting from afferent vasodilatation and efferent vasoconstriction, in conjunction with altered glomerular permeability causes proteinuria, activation of proximal tubular epithelial cells, renal fibrosis and ultimately nephron loss and renal failure. ACE inhibitors interfere with these processes as described in detail below.

Fig. 4 Schema of glomerular hypertension, injury from proteinuria, and the effect of ACE inhibitors.



Apart from elevated systemic and glomerular capillary pressure, proteinuria plays a major pathogenetic role. Remuzzi [31] had postulated that proteinuria is not only a marker of adverse renal prognosis, but actually a 'nephrotoxin'. This concept has been amply confirmed by studies showing that proximal tubular epithelial cells acquire an inflammatory phenotype and upregulate expression of angiotensinogen[12], endothelin[32], and cytokines, when they have been confronted with a protein overload in the tubular fluid. Albumin was shown to be less injurious than complement factors, iron-containing proteins and oxidized lipids[33].

Angiotensin II, endothelin, and cytokines are then secreted in an abluminal direction into the interstitium where they activate peritubular fibroblasts and lead to interstitial fibrosis. This sequence has also been shown *in vivo* by studies in the amphibian kidney[34].

In the past, it was felt to be a paradox that in diabetes the kidney responds so favourably to pharmacological blockade of the RAS, although the systemic RAS is suppressed. It has recently been documented, however, that hyperglycaemia upregulates the local synthesis of angiotensinogen[35] in proximal tubular epithelial cells, which possess all components of the RAS and are able to generate angiotensin II. In the tubular fluid and the interstitium, the concentration of angiotensin II is orders of magnitude greater than in the circulation [36].

Furthermore, the expression of the angiotensin II receptor subtype 1 is upregulated[37]. These facts may explain the finding that despite low plasma renin activity (PRA) in patients with diabetes, RAS blockade causes a pronounced increase in renal plasma flow[38] under hyperglycaemic, but not under euglycaemic conditions [39].

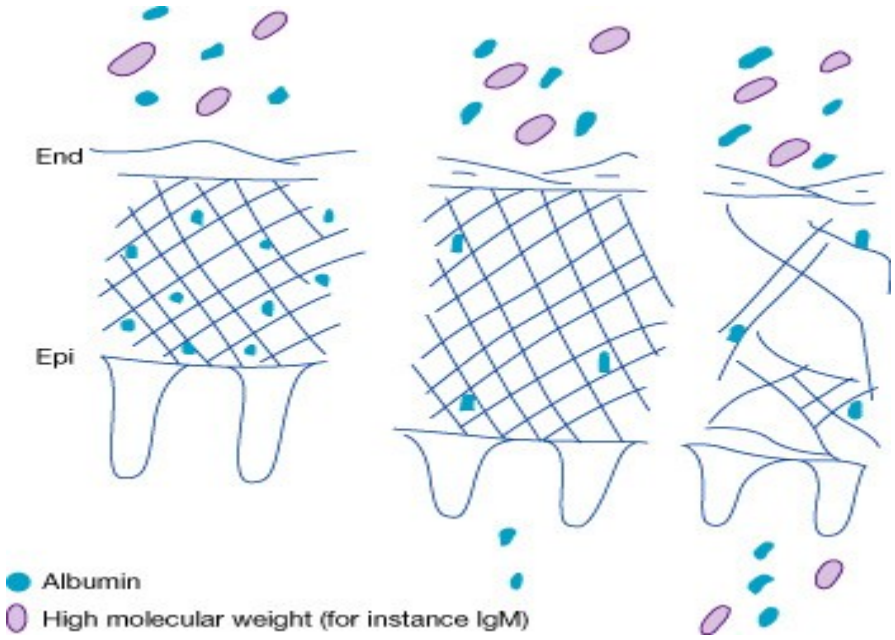
In diabetes, renal sodium (Na) handling is abnormal. This explains the frequent findings of Na retention and hypervolaemia. Proximal tubular reabsorption of Na is increased as a result of increased activity of the Na, glucose cotransporter [40]. As a result, distal Na delivery is diminished and GFR is increased via the tubuloglomerular feedback mechanism, leading to hyperfiltration [41]. In addition, insulin directly increases distal tubular Na reabsorption [42]. Furthermore, increased angiotensin II in tubular fluid was recently shown to also activate Na channels in the collecting duct [36]. Na retention is a prominent factor in the genesis of hypertension of the diabetic patient.

Why Do Diabetic Patients Develop Albuminuria And Proteinuria?

In the past, it had been thought that negatively charged molecules of the GBM are reduced, particularly sialic acid and heparan sulfate, normally repel negatively charged anionic albumin. As a result glomerular permselectivity would be reduced so that albumin molecules can escape into the glomerular filtrate. Reduced negative charge density of the GBM has not been consistently confirmed [43].

It was also thought that in later stages of DN, disruption of the texture of the basal membrane creates gaps and holes (Fig. 5) and allows high molecular weight serum proteins to escape into the filtrate.

Fig. 5 Schema of the genesis of selective albuminuria and non-selective proteinuria.



Recent insights into podocyte function and specifically identification of proteins of the slit membrane [44] have led to the concept that the podocyte is a prime player in the genesis of proteinuria. Indeed, in experimental diabetes the expression of one permeability controlling protein, nephrin, is abnormally low and is restored by administration of angiotensin II receptor blockers [45,46]. It is also of interest that in experimental diabetes, podocyte damage is the first sign of renal injury [47]. Diminished podocyte numbers have been documented in animals and patients with diabetes[48]. Podocytes are

postmitotic and can no longer proliferate. If glomeruli increase in size, each podocyte has to cover an ever larger domain, ultimately exceeding the capacity of the podocyte.

This discrepancy causes loss of podocytes by desquamation, apoptosis or necrosis, denudation of the basement membrane, synechia formation, and ultimately glomerulosclerosis [44]. When tubular epithelial cells are exposed to higher protein loads, they acquire an inflammatory phenotype and amplify renal damage as outlined above.

Stages of Diabetic Nephropathy:

There are few renal diseases where the cause is as predictable as in DN. This has led to a scheme proposed by Mogense`n which is valid in type1 diabetes but less consistently so in type 2. Diabetic nephropathy can be divided in to five stages[59, 60].

Stage-I

Occurs at the onset of the disease. It is characterized by a 30 % to 40 % increase in GFR above normal has been documented primarily is patients with new onset type 1 diabetes , this returns to normal within several weeks to a few months after instituting insulin therapy. This hyper filtration is associated with enlarged kidneys and increased intraglomerular pressure, which may causes transient increase in albumin excretion.

Stage-II

Characterized by normal albumin excretion < 20 mcg/ min or < 30 mcg/24 hrs regardless of the duration of the disease. Some patients maintain their hyperfiltration which may be a poor prognostic indicator for subsequent DN [61].

Stage-III (Incipient Nephropathy)

Characterized by microalbuminuria at rest .This is defined as elevated excretion rates of albumin between 20 –200 mcg /min (30-300 mg/24h). In general, patients with lowers amounts of microalbuminuria (20-70 mcg/min) have either elevated / normal GFR that are higher than those with

more microalbuminuria (70-200 mg/min). GFR begins to decline during the incipient stage of diabetic nephropathy.

At this stage, BP, are often higher (although in normal range) than in non diabetic subjects. It may increases to abnormally high levels during exercise. In general BP increase 3 to 4 mmHg per year.

Stage – IV (Overt Nephropathy)

Characterized by clinical protienuria that is a level of urinary protein that is detectable by simple tests. Thus overt nephropathy is clinical as urinary protein excretion exceeding 0.5 g/24 h. Albumin is not the only protein excreted at the stages, clinical protienuria corresponds to albumin excretion rates exceeding 200 mcg/min or 300 mg/24 hr. Early in the clinical course of overt diabetic nephropathy, the GFR may be in the normal ranges, but usually declines slowly but steadily.

Although the rate of fall in GFR may vary markedly among patients, the decrease in GFR is fairly constant within each patient. The average GFR decline is approximately 1 ml/min/month. During this stage of diabetic nephropathy, BP increases approximately 7mmHg per year; Thus hypertension eventually occurs in almost all patients. Aggressive treatment of elevated BP slows the rate of decline of the GFR. At this point, aggressive treatment of elevated glucose concentration has little effect. Once the process of nephron destruction is underway, it seems independent of the inciting cause, although lowering the BP is definitely helpful.

Stage – V –ESRD

It is similar to kidney failure resulting from any other cause. However disproportionate shares have diabetic nephropathy. Although only 3 % of the population is known to have diabetes, nearly 50 % of patients starting dialysis have RF caused by DM. Signs symptoms include progressive weakness, lethargy, fluid retention , anorexia, nausea, vomiting, diarrhea, hiccups, pruritis, difficulty in

controlling hypertension, anaemia & electrolyte disturbance.

However the patients with ESRD resulting from diabetes are more symptomatic at higher level of GFR than patients with nondiabetic causes of kidney failure, that is, GFR does not have to be as low for diabetic patients to start experiencing difficulties.

Table 1: The Stages Of Diabetic Nephropathy

Stage	Glomerular filtration	Albuminuria	Blood pressure	Time interval [a]
Renal hyperfunction	Elevated	Absent	Normal	At diagnosis
Clinical latency	High normal	Absent		
Microalbuminuria (Incipient Nephropathy)	Within the normal range	20–200 μg/min (30–300 mg/day)	Rising within or above the normal range	5–15
Macroalbuminuria/proteinuria (overt nephropathy)	Decreasing	200 μg/min (300 mg/day)	Increased	10–15
End Stage Renal failure	Diminished	Massive	Increased	15–30

Extrarenal Complications Associated With Diabetic Nephropathy

Table 2: Major microvascular and macrovascular complications in patients with diabetic nephropathy

Microvascular Complications
Retinopathy
Polyneuropathy Including Autonomic Neuropathy
Gastroparesis, Diarrhoea/Obstipation, Detrusor Paresis, Painless Myocardial Ischaemia, Erectile Impotence, And Supine Hypertension/ Orthostatic Hypotension
Macrovascular Complications
Coronary Heart Disease, Left Ventricular Hypertrophy, And Congestive Heart Failure
Cerebrovascular Complications (Stroke)
Peripheral Artery Occlusive Disease
Mixed Complications
Diabetic Foot (Neuropathic, Vascular)

Diabetic retinopathy is present in virtually all type 1 diabetic patients with nephropathy [49], but only 50–60 per cent of proteinuric patients with type 2 diabetes suffer from retinopathy [50], so that the absence of retinopathy does not preclude the diagnosis of Kimmelstiel Wilson glomerulosclerosis. Nevertheless absence of retinopathy is one argument, amongst others, favouring the indication for a renal biopsy (see below). The risk of blindness because of severe proliferative retinopathy or maculopathy is substantially greater in diabetic patients with nephropathy. Retinopathy tends also to progress more rapidly in patients with DN, so that more frequent ophthalmological examination is clearly indicated, that is, at yearly or half-yearly intervals.

Many patients with DN have ***polyneuropathy***. Sensory polyneuropathy is an important aspect of the diabetic foot problem . Motor and sensory neuropathy may cause areflexia, wasting, and sensory disturbances such as paraesthesia, anaesthesia, impaired perception of vibration, and pain, but the most vexing clinical problems are the result of autonomic polyneuropathy. It involves frequently the cardiocirculatory system [51] causing abnormally low pulse rate variability (RR interval) and unchanged heart rate in upright position, but more sophisticated techniques such as uptake of radioanalogues of norepinephrine by the heart show that subclinical abnormalities are practically always found. Further consequences of autonomic polyneuropathy are gastroparesis, that is, delayed emptying of gastric contents into the gut, and diarrhoea, or constipation (often alternating with each other).

These problems are caused by impaired intestinal innervation, often complicated by intestinal bacterial overgrowth because of stasis. Finally, urogenital abnormalities are frequent such as erectile impotence or detrusor paresis with delayed and incomplete emptying of the bladder.

The major macroangiopathic complications are stroke, coronary heart disease, and peripheral vascular disease. These complications are again up to five fold more frequent in diabetic patients with and without DN.

The onset of DN is a turning point in the life of a patient with diabetes. The presence of DN greatly increases the mortality of a patient with types 1 and 2 diabetes. This had been documented by [52], decades ago. They found that compared to the background population type 1 diabetic patients without proteinuria had only a slightly, that is, two- to threefold elevated, mortality. In contrast, mortality was increased by a factor of 20–200 in patients with proteinuria. The major increase in risk starts as soon as microalbuminuria has set in [53], but a higher risk is found even in upper quartile of normal

range albuminuria. The mechanistic link between microalbuminuria and cardiovascular death is not well understood. It is widely thought that the presence of microalbuminuria reflects generalized endothelial cell dysfunction and increased risk of atherogenesis. It is associated with many cardiovascular risk factors, such as elevated BP, dyslipoproteinaemia, increased platelet aggregation, increased C-reactive protein (CRP) concentration and others.

An added risk factor is presumably the association of microalbuminuria with autonomic polyneuropathy [51], which predicts deaths from myocardial infarction or arrhythmia (sudden death). Further cardiac abnormalities associated with microalbuminuria are cardiac hypertrophy [54] and impaired left ventricular diastolic function.

The prognosis is even grimmer in diabetic patients with proteinuria, that is, with clinically manifest DN [52]. In the past, the estimated 10-year cumulative death rate was up to 70 per cent in type 1[55] as well as in patients with type 2 diabetes [56]. It has substantially decreased in recent years with better control of hypertension, better care of coronary heart disease, and improved management of patients by renal replacement therapy. For instance in the Heidelberg series, mortality decreased substantially in the decades between 1966 and 1985 [57].

There is an ongoing discussion whether the high death rate in DN is explained by established cardiovascular risk factors or whether uraemia amplifies the cardiovascular risk because of the presence of additional non-classical risk factors.

In this context, it is of interest that experimentally even minor impairment of renal function, for example, uninephrectomy, causes drastic acceleration of atherogenesis [58].

Diagnostic Procedures

DN remains a clinical diagnosis based upon the detection of proteinuria, although many patients will also have hypertension and retinopathy. The diagnostic procedures to establish the diagnosis of DN and its complications comprise (a) determination of albuminuria or proteinuria, (b) blood pressure measurement, (c) estimation of GFR and in selected cases, and (d) renal biopsy.

Microalbuminuria:

Definition

Microalbuminuria is defined as excretion of 30 – 300 mg albumin per 24 hr (20 –200 mcg/min) in at least 2 out of 3 nonketotic sterile urine samples over a period of 6 months.

Methods:

Five methods are available for assessing for the presence of microalbuminuria.

1.) Measuring albumin in 24 hr urine.
2.) Measuring albumin in a specifically timed shorter urine collection period (Exampl:4 hrs).
3.) Measuring albumin in overnight urine sample (i.e. Between going to sleep and waking up in the morning).
4.) Measuring albumin to creatinine rate in a random urine sample.
5.) Measuring the albumin concentration.

Because urinary volume in so critical to the concentration of albumin measured, simply measuring the albumin concentration is not generally recommended as a screening test if one of the other approaches is available[62].

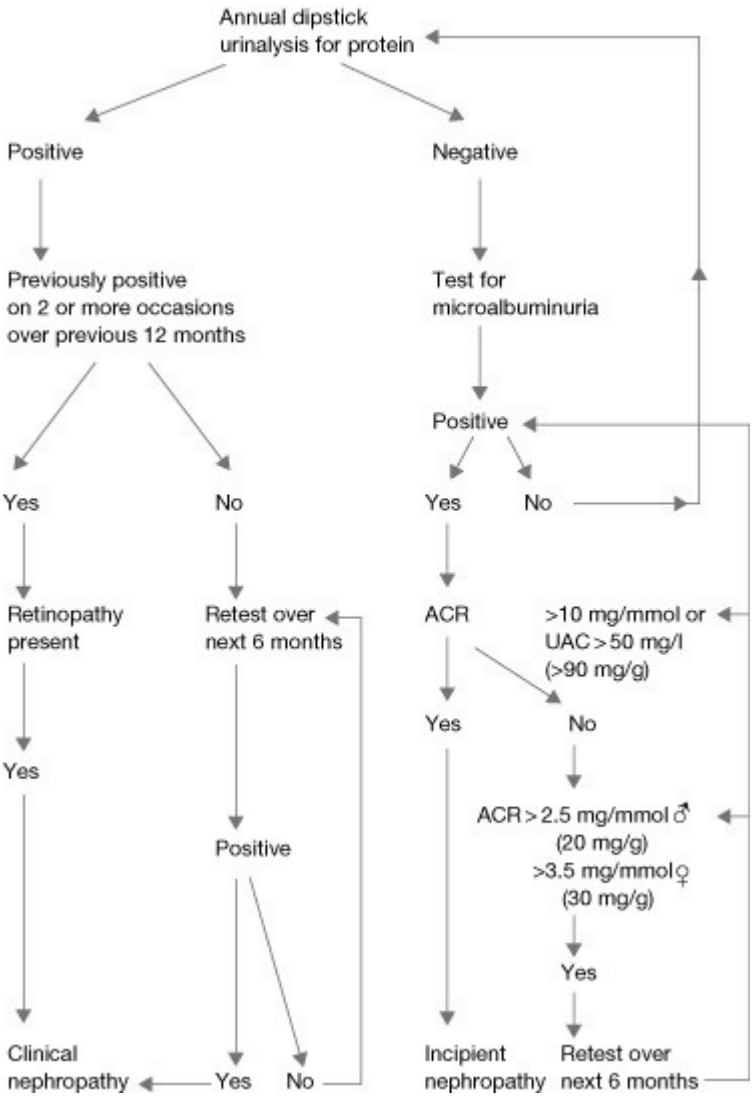
Timed urine collections & 24 hr urine collections are the least convenient for patients. An overnight urine collection is less inconvenient but albumin execretion rate overnight urine collection is less inconvenient but because of recumbency and little activity the albumin excretion rate overnight is approximately 25 % less than during the day (upright posture and exercise induced albumin excretion) this may not be a drawback,because an abnormal value in an overnight collection is more certain to be

a valid diagnosis.

The albumin / creatinine ratio is the simplest of four valid tests to assess microalbuminuria. ACR of 30 mcg /mg (3.5 m/m mol) or higher have a high degrees (95 % to 100 %) of both sensitively and specificity in predicting microalbuminuria when measured by 24 hr or timed urine collection[63, 64]. A ratio measured in a sample collected on rising in the morning correlate much more closely with microalbuminuria measured by other means.

The Council Diabetes Mellitus National Kidney Foundation [65] has recommended the simple ACR over other methods.The measurement of microalbuminuria is only reliable if confounding factors have been excluded such as fever, physical exercises urinary tract infection, non diabetic renal disease, hematuria from other causes, heart failure, and uncontrolled hypertension.

Flow Chart for Diagnosis of Incipient and Advanced Nephropathy:



Renal biopsy

Renal biopsy is certainly not indicated when a type 1 diabetic patient has retinopathy and when the time course is consistent with DN. Renal biopsy should be considered, however, when protienuria is present less than 10 years after the onset of type 1 diabetes. In type 2 diabetes, this argument is unreliable because the onset of type 2 diabetes is often not known. The presence of dysmorphic erythrocytes, erythrocyte casts, or cellular casts is not a feature of DN and should prompt investigations to exclude glomerulonephritis or vasculitis, if necessary by renal biopsy. Other indications are rapid deterioration of renal function or elevated serum creatinine without urine abnormalities. Needless to say that prior to renal biopsy renal ultrasonography is indicated which by itself may already yield a diagnosis.

Table 3: Potential Indications for Renal Biopsy in Patients with Diabetes

Biopsy not indicated when
Typical evolution of renal disease
Concomitant retinopathy
Biopsy should be considered when
Renal manifestations are seen atypically (<10 years) early in type 1 diabetes
Dysmorphic erythrocytes/casts are found (nephritic sediment)
Rapid deterioration of renal function of unknown cause is noted
Elevated serum creatinine without urine abnormalities
Heavy proteinuria (>5–8 g/day) persists despite lowering of blood pressure

MANAGEMENT OF MICROALBUMINURIA AND PROTEINURIA

Once urinary albumin excretion is raised, it may not be possible to stop progression of nephropathy completely but it is certainly possible to delay the process substantially.

Blood Glucose Control

Studies examining the effect of tight blood glucose control on progression of nephropathy have been too small or too short to demonstrate convincing benefit[66, 67]. However, it is obviously extremely important to maintain good blood glucose control for other reasons.

Reduction of Intraglomerular Pressure

As described above, raised intra glomerular pressure is the hallmark of diabetic nephropathy and a major factor in its progression. It rises primarily because of angiotensin II constrictor effects on the efferent glomerular arteriole. Thus first line therapy in the secondary prevention of diabetic nephropathy aims to reduce intraglomerular pressure using inhibitors of the renin-angiotensin system.

In young, type 1 diabetic patients with early nephropathy but ‘‘normal’’ blood pressure, numerous studies have demonstrated that prescription of an ACE inhibitor reduced progression to protienuria compared with placebo. Initial blood pressure was generally 130/80 mm Hg at entry to these studies and around 120/75 mm Hg on treatment. A meta-analysis has confirmed these beneficial effects, demonstrating an average 65% risk reduction in the development of protienuria and a threefold increase in the likelihood of regression to normal albumin excretion. [68]

Almost all of this effect was independent of changes in systemic blood pressure and thus has been attributed to specific, intraglomerular effects of ACE inhibition. In most of these studies, maximum doses of drug have been used.

In type 2 diabetes, several studies have been reported in microalbuminuric but ‘‘normotensive’’ individuals, show benefit with ACE inhibitors compared with placebo, in terms of reduction in the numbers progressing to protienuria and in one, stabilization of serum creatinine over five years[69] This benefit is also at least partly independent of blood pressure lowering. Several large studies have been performed in hypertensive patients. In type 1 diabetic patients with protienuria and rising serum creatinine, addition of ACE inhibition compared with placebo to blood pressure control using other classes of antihypertensive therapy, significantly reduced the numbers reaching a combined end point of death, need for dialysis, or doubling of the serum creatinine[70].

In microalbuminuric[71]or proteinuric[72, 73] type 2 diabetic patients, prescription of an angiotensin II receptor antagonist (ATIIRB) significantly reduced the rate of progression of nephropathy but had no effect on cardiovascular outcomes. In the diabetes subgroup of the Heart Outcomes Prevention Evaluation study, changes in albumin excretion were difficult to interpret because of methodological problems.[74] The apparent significant reduction in macro vascular end points in those patients

randomized to ramipril may have been due to a difference in 24 hour blood pressure not reflected in clinic blood pressure measurements. Thus in type 1 and type 2 diabetic patients with microalbuminuria or protienuria, prescription of an inhibitor of the renin-angiotensin system, titrated up to the maximum tolerated dose, is the first line in management, regardless of initial blood pressure. There is often concern that patients, particularly those with type 2 diabetes, may have atheromatous renovascular disease and that prescription of an ACE inhibitor or ATIIRB may precipitate acute renal failure.

There is no reliable screening test for Reno vascular disease and it is important not to deny patients the potential benefits of renin-angiotensin system inhibition. Thus renin-angiotensin system inhibitors should be tried in all patients, unless in the rare case where there is a definite contraindication. Patients should begin with the smallest dose, which should be titrated up gradually, with serum creatinine and potassium being checked 1–2 weeks after each change in dose. A small (20%) rise in serum creatinine is common, but this should plateau. If the creatinine rises steadily, the drug should be withdrawn.

Systemic Blood Pressure Control

Numerous studies have demonstrated the importance of reducing systemic blood pressure as well as intraglomerular pressure in delaying the rate of fall of glomerular filtration. It is well accepted that the rate of fall of glomerular filtration rate can be reduced from around 12 ml/min/year to 5 ml/min/year if arterial blood pressure is adequately controlled. With aggressive antihypertensive therapy, it is possible in at least some patients with persistent protienuria to reduce protein excretion into the microalbuminuric range for several years and to maintain the glomerular filtration rate[75] Likewise, in type 1 patients with nephrotic range protienuria, good blood pressure control can reduce protein excretion to ,600 mg/24 hours for at least one year and decrease the

rate of fall of the glomerular filtration rate[76] Particularly in type 2 diabetes, but also in type 1 patients with more advanced renal disease, systemic blood pressure will be high despite prescription of the maximum tolerated dose of ACE inhibitor or ATIIRB.

Addition of other agents to lower blood pressure further is imperative. In general, the number of agents needed increases as nephropathy advances and it is not uncommon for individuals with rising serum creatinine to require four or five different agents.

The choice of additional agents is individual, there being no good add-on trials. It is logical to use a diuretic (thiazide or loop) early: patients are often salt overloaded and many of the trials of ACE inhibitors/ATIIRBs included a diuretic in their regimen. Thereafter, the choice rests on the individual's circumstances.

NOVEL THERAPIES

A number of novel therapies have been demonstrated to reduce urine albumin excretion and prevent glomerulosclerosis in a variety of animal models of diabetes . To date, few of these have been tried in clinical practice, generally in small, short term studies. One therapy, already available, is aldosterone blockade. Activation of the renin-angiotensin system stimulates aldosterone secretion, which may subsequently be involved in renal damage. Aldosterone levels may ''rebound'' during treatment with inhibitors of the renin-angiotensin system.

There is also evidence that aldosterone, independently of the renin-angiotensin system, is an important pathogenic factor in progressive renal disease, promoting fibrosis and collagen formation[77] In

several experimental models, blockade of aldosterone reduces proteinuria.

In one small study of type 2 diabetic patients with early nephropathy already taking an ACE inhibitor, addition of spironolactone 25 mg/day resulted in a 40% decrease in urine albumin excretion and a significant reduction in left ventricular mass over 24 weeks[78] In a study reported in abstract of hypertensive, microalbuminuric type 2 diabetic patients, the selective aldosterone antagonist eplerenone reduced proteinuria at least as much as ACE inhibition[79] Dual blockade resulted in a further reduction in proteinuria.

Thus selective aldosterone blockade as monotherapy orin combination with inhibitors of the renin-angiotensin system is a potentially useful therapy for preventing progression of diabetic nephropathy.

REVIEW OF LITERATURE

Schultz et al.,[80] in a hospital based longitudinal study of 514 children at United Kingdom, who developed type 1 diabetes before the age of 16 yrs , found that 12.8% developed microalbuminuria and was persistent in 22 (4.8%) of the subjects. HbA_{1c} was worse in those who developed MA than in others (mean difference +/- SEM: 1.1% +/- 0.2, $P < 0.001$). In subjects who had been 5-11 years of age when their diabetes was diagnosed, the appearance of MA was delayed until puberty, whereas of those whose age was <5 years at diagnosis of diabetes, 5 of 11 (45%) developed MA before puberty.

Holl et al., [81] in a hospital based study comprising of 447 children and adolescents with type 1 diabetes at Germany, found that, after a duration of diabetes of 11 years, 5% of patients displayed persistent microalbuminuria (10% after 13 years). The duration of diabetes until persistent microalbuminuria was identical for patients with prepubertal or pubertal onset of diabetes. In addition to duration, female sex ($P < 0.03$) and insufficient long-term metabolic control ($P < 0.03$) contributed significantly and independently to urinary albumin excretion.

Based on repeated measurements in individual patients, the positive predictive value of one sample was 76%, the negative 99.5%. When related to the duration of diabetes, 12 patients with persistent microalbuminuria had duration of diabetes <5 years (range 0-3.1).

Stone et al., [82] in a hospital based longitudinal cohort of 972 children at Westmead Australia ,the incidence of persistent microalbuminuria was 4.6 (95% CI 3.3–6.1) per 1,000 patient-years. The median diabetes duration at the onset of persistent microalbuminuria was 9.3 years, and the earliest case was 1.6 years after diagnosis of diabetes. Predictors of persistent microalbuminuria from the first assessment using multiple logistic regression were high cholesterol [95% CI 1.2–4.0]) and borderline microalbuminuria (2.5 [1.2–5.2]). Predictors using Cox regression were HbA_{1c} (hazard ratio 1.4 [95% CI 1.1–1.7]), age at diagnosis (1.2 [1.1–1.3]), obesity (3.6 [0.8–15.5]), and insulin dose (2.7 [1.0–7.5]).

Cosmescu A et al., [83] in a hospital based study at Romania, Out of 110 children and teenagers suffering from type1 diabetes mellitus, persistent microalbuminuria (the incipient diabetic nephnropathy) was detected in 9% and intermittent microalbuminuria in 11.8%. In all the cases, a poor glycaemic control was associated, the average HbA_{1c} being higher than 10%.

Moore TH et al[84] in this centrally coordinated, cross sectional, multicentric study done in 22 pediatric diabetes outpatient clinics in UK & Ireland, found that out of 1007 patients aged between 10 to 20 years with IDDM , 9.7% had microalbuminuria. The mean duration of diabetes in this study group was 6 yrs. Significantly more girls than boys (3:1) and significantly more pubertal and post pubertal patients had abnormal albumin excretion. Microalbuminuria was not associated with raised blood pressure. The mean HbA_{1c} in patients with microalbuminuria was higher than in those with normoalbuminuria.

Jones C A et al.,[85] in a hospital based longitudinal study over 8 years in 233 children with Insulin dependant diabetes mellitus attending a single pediatric diabetic clinic at Liverpool UK, found that, 14.5% had persistent microalbuminuria. Longer duration of diabetes and raised median HbA_{1c} were found to be associated with persistent microalbuminuria. The onset of persistently raised ACR in 13 of 34 children was before puberty and in 23 of 34 children it was within first 4 years of diagnosis. The cross sectional prevalence of microalbuminuria was 12.9% at one year, 18.3% at 5 years and 33% at 10 yrs after diagnosis.

Francis J et al., [86] reported from Birmingham, a case of 12 yr old asian girl with a 4 year history of poorly controlled insulin dependant diabetes mellitus who developed overt diabetic nephropathy. Screening for microalbuminuria would have identified incipient diabetic nephropathy and highlighted the importance of good glycemic control. Although screening for microalbuminuria is recommended after five years from diagnosis it may be appropriate to undertake this annually in those with poor glycemic control.

Bruno G et al.,[87] found that out of 298 subjects with IDDM with 3 to 9 years duration, prevalence of microalbuminuria was 7% in subjects with duration 3-9 yrs, and 4% in subjects with

duration of 3 to 5 years. In almost 50% of the cohort, HbA_{1c} levels were over 9%, whereas only in 10.9% HbA_{1c} levels were lower than 6.6%. This shows a low prevalence of microalbuminuria in young subjects with short duration of IDDM, even if the glycemic control obtained was far from ideal.

Bojesting M et al., [88] in a 10 year follow up of 109 patients found that, at initial investigation, 81 patients had normal AER, 27 had microalbuminuria and 1 had microalbuminuria. Only 5 (19%) of the initially microalbuminuria patients developed, macroproteinuria during the 10 yrs follow up period, and in 15 (58%) patients, AER decreased to normal. Three (4%) of the normoalbuminuric patients developed microalbuminuria but none macroproteinuria. The initially microalbuminuric patients in whom AER normalized had improved their glycemic control over 10 yrs. Hence in the majority of patients with microalbuminuria in whom it is possible to obtain good glycemic control, microalbuminuria will disappear and the risk of nephropathy will be markedly reduced.

Powrei JK et al., [89] in a 10 yrs prospective study of a cohort of IDDM patients attending single out patience clinic at London found that, 8 of the 97 patients had developed microalbuminuria by the 10 yr follow up. Multiple linear regression analysis showed that urinary ACR at 10 yrs was influenced by initial albumin creatinine ratio, initial glycated hemoglobin concentration, & duration of diabetes. The development of microalbuminuria is determined primarily by poor long term glycemic control. There is a weaker relation with longer duration of disease and younger age at onset of diabetes, but blood pressure does not seem to be implicated.

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Rudberg S et al., [90] in a 15 yr follow up study from onset in 156 type I diabetic children at Sweden found that the cumulative incidence of microalbuminuria by duration was 24.2% at 15 yrs of diabetes. Eleven patents developed microalbuminuria after more than 5 years. Six patients had an onset before 5 years of diabetes. The relative importance of early versus later hyperglycemia, yearly blood

pressure, age, age at onset and duration of diabetes for increased albumin excretion rate after more than 5 years, was shown in a multiple regression analysis where the first 5 year mean HbA_{1c} was the only independent predictor.

Mathiesen ER et al [91] Studied a total of 102 diabetic children attending 2 diabetic clinics in the age group of 7 to 18 yrs with more than 2 years of diabetic duration and found that, 20% had persistent microalbuminuria. Microalbuminuria was only demonstrated in patients aged more than or equal to 15 years, prevalence of 37%. Arterial blood pressure was elevated in the microalbuminuria group compared to age matched normoalbuminuric diabetic group. HbA_{1c} was slightly higher in the microalbuminuric patients, $p<0.10$.

Barkai et al., [92] in a prospective study done in normoalbuminuric prepubertal (n=20), pubertal (n=28), & post pubertal (n=26) IDDM group matched for diabetic duration & long term metabolic control & were followed up for 3 years, found that AER increased significantly over a period of 3 years in the pubertal (p=0.001) & post pubertal (p=0.003) subjects but not in pre pubertal subjects. Six pubertal, 2 postpubertal and none of the pre pubertal subjects developed microalbuminuria. Multiple logistic regression analysis showed that puberty represents an independent risk for the development of microalbuminuria in diabetes.

Bravo RLE et al., [93] studied 160 Insulin Dependant Diabetic Children attending a single outpatient clinic and found that 8% had microalbuminuria and 3% had clinical protienuria. The abnormal excretion was more prevalent in females with the poorest metabolic control, the longest duration of diabetes, and the highest age (13-18 years).

Pedro Gonzalez et al., [94] studied 30 children with type I diabetes in the age group of 2 to 16

yrs and found that, microalbuminuria was found in 12 (40%) of patients, with a greater frequency in those in pubertal age and with greater time of evolution. Whereas sex and metabolic control did not influence.

STUDY JUSTIFICATION

Diabetic nephropathy is the single most common cause of end stage renal disease[95] .It accounts for 30 to 35 % of diagnoses leading to ESRD [96]. Approximately 35 to 45 % of Type 1 diabetics develop nephropathy [55].The devastating renal consequences of diabetes mellitus highlight its potential for significant morbidity and emphasize that better understanding of its pathogenesis is needed to heighten clinical suspicion of early kidney involvement.

Several longitudinal observations have provided insight into the natural history of diabetic nephropathy, facilitating identification of renal involvement in its earliest stages .A most significant

observation has been the recognition that microalbuminuria identifies the diabetic at risk for developing renal insufficiency. Its presence confers incipient nephropathy and heralds likely progression to end stage renal failure.

Viberti and co – workers first described microalbuminuria as a risk factor for renal disease , in that , patients with microalbuminuria were 20 times more likely to develop clinical protienuria than patient with normoalbuminuria [97] , an observation that was upheld by subsequent investigations [5,98, 99]. About 80% of Type 1 diabetics with microalbuminuria go on to develop clinical nephropathy. Recent works show that microalbuminuria is not only a marker of impending diabetic nephropathy, but indicates presence of abnormal glomerular metabolic function.

In previous studies microalbuminuria has been detected in 7- 20 % of young adults and children with IDDM [100,101,102,103]. American diabetes Association[104] and International Society for Pediatric and Adolescent Diabetology (ISPAD) [105], recommends screening for all patients with duration of diabetes > 5 years or with chronological age > 11 years. But many studies [85, 86] have also reported incidence of microalbuminuria and overt diabetic nephropathy in children < 5 yrs of diabetic duration.

Hence it is imperative that all diabetic children need to be screened for microalbuminuria to detect diabetic nephropathy in its earliest stage.

AIM

1. To determine the cross sectional prevalence of microalbuminuria in children attending our diabetic clinic.
2. To assess the factors likely to increase the risk of microalbuminuria.

SUBJECTS AND METHODS

Study Design : Descriptive Study.

Study Place : Institute Of Child Health and Hospital For Children - Chennai.

Study Period : March 2005 to July 2006.

Sample Size : 125

Study Population

Inclusion Criteria : All Children with Diabetes Mellitus Attending the Diabetic Clinic at ICH & HC.

Exclusion Criteria : Children with Known Renal Disease And Those on Reno Toxic Drugs Are Excluded.

Manoeuvre

125 children with diabetes mellitus attending the diabetic clinic ICH & HC were recruited consecutively, irrespective of duration of diabetes. Those with known renal isease and those on Reno toxic chemotherapy were excluded from the study. After obtaining informed consent from the parents, a preformed Proforma was filled.

Height was measured to nearest 0.1 cm using standardized Harpeden Stadiometer. Weight was

measured to nearest 500 gms using standardized bathroom scale. Blood pressure was measured in sitting posture in right upper limb using a standard sphygmomanometer after five minutes of rest.

Three morning urine samples 5ml from each patient was collected over a period of 1 year at 3 to 6 months interval. The presence of urinary tract infection or microhematuria was excluded by dipstick detection. Urine sample were not included in the study if either microhematuria or urinary tract infection were present. Girls were asked not to collect urine during menstruation and wait for three days after the last day of bleeding. The concentration of albumin in the urine was determined by radioimmunoassay and the concentration of creatinine by jaffe's method. The ACR was calculated for each patient for each collection.

Corresponding blood samples were taken to assess long term metabolic control by estimating HbA_{1c}.It was estimated by high performance liquid chromatography. Urea, creatinine and ultrasound abdomen was done to all patients at the time of recruitment, to rule out other existing renal diseases. Fundus examination was done to all patients once during the study period by the Ophthalmologist.

Statistical Analysis:

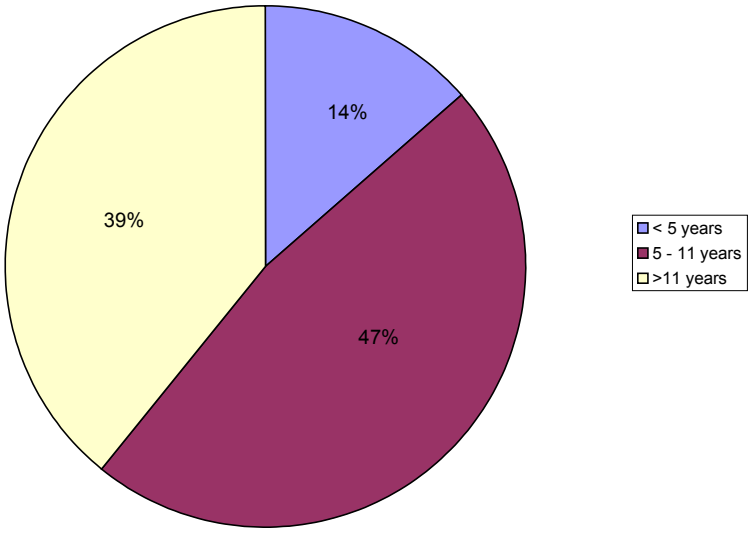
All results were tabulated and percentage was arrived by using windows MS Excel application and analysis was performed by using SPSS version 11.0-Software. Descriptive statistics like frequencies and percentages were obtained. Chi-square test was done to compare between various groups. P value less than 0.05%was considered significant.

DEMOGRAPHY

Table .1

AGE DISTRIBUTION OF STUDY POPULATION

CHART 1



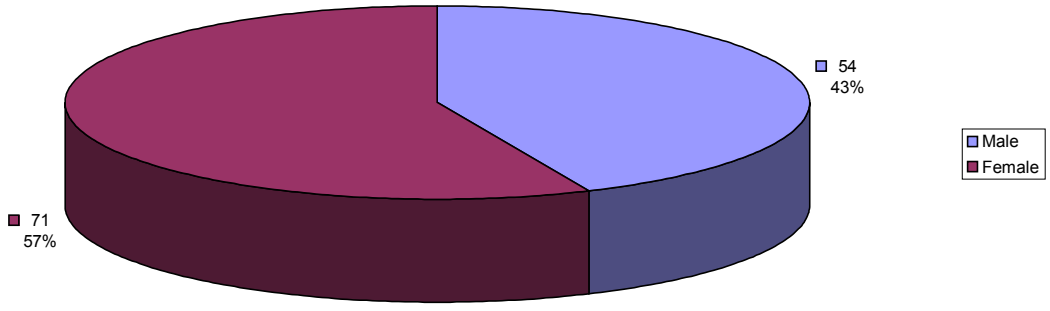
RESULT:

47.2% of children were between 5 to 11 years, and 39.2% above 11 years and 13.6% were below 5 years.76 children were prepubertal.

Table .2
GENDER DISTRIBUTION

Sex	N	%
Male	54	43.2
Female	71	56.8
Total	125	100

CHART.2



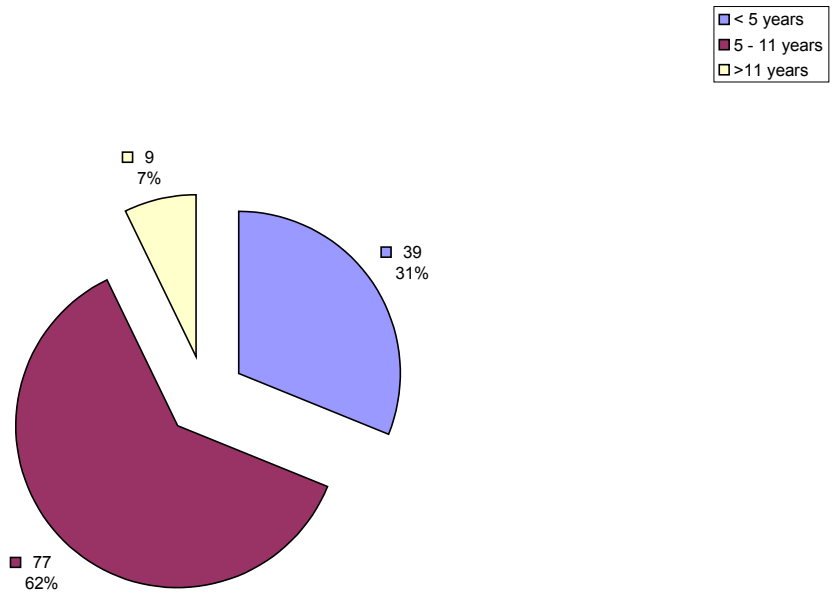
RESULTS

Slight female preponderance was noted in the study .Male female ratio Was 1:1.3.

Table 3
DISTRIBUTION OF CHILDREN AS PER AGE AT ONSET OF DIABETES

Age	n	%
< 5 years	39	31.2
5 - 11 years	77	61.6
>11 years	9	7.2
Total	125	100

CHART.3



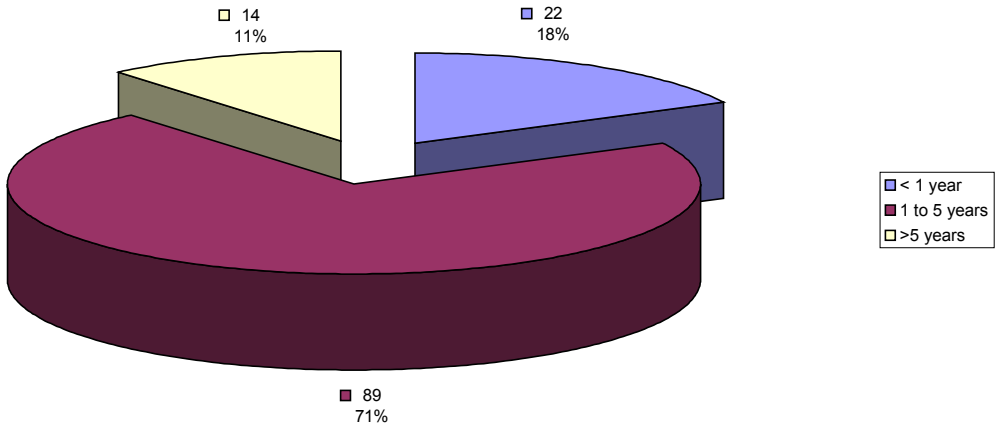
RESULTS:

61.6% were diagnosed to have diabetes between 5 to 11 years of age. 39(31.2%) were below five years at the time of diagnosis, followed by 9(7.2%) above 11years at the time of diagnosis.

Table 4
DISTRIBUTION OF DIABETIC AGE

Duration	n	%
< 1 year	22	17.6
1 to 5 years	89	71.2
>5 years	14	11.2
Total	125	100

CHART.4



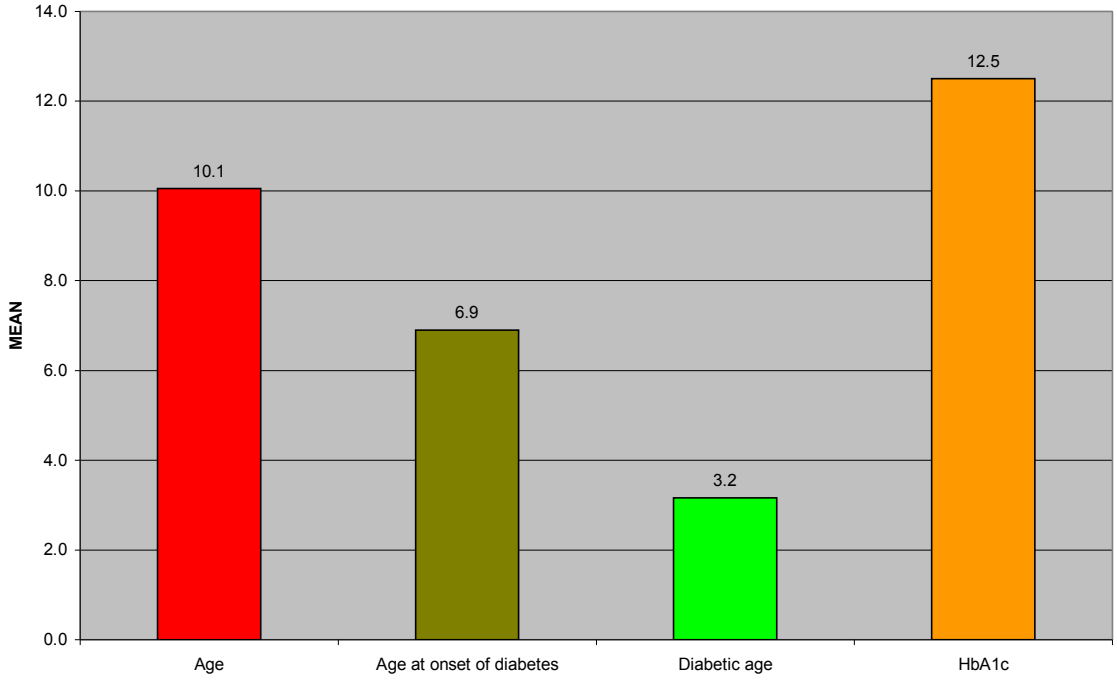
RESULTS:

71.2% children of the study population had diabetic duration of 1 to 5 years followed by 17.6% children with less than one year of duration. Children with more than five year of diabetic duration comprised 11.2 %.

Table 5
MEAN AND STANDARD DEVIATION OF STUDY PARAMETERS

Particulars	Mean	S.D.
Age	10.1	3.7
Age at onset of diabetes	6.9	3.3
Diabetic age	3.2	2.0
HbA_{1c}	12.5	3.0

CHART.5



RESULTS:

The Mean Age of Our Study Population Is 10.1 (SD 3.7).

The Mean Age at Onset Of Diabetes Is 6.9 (SD 3.3).

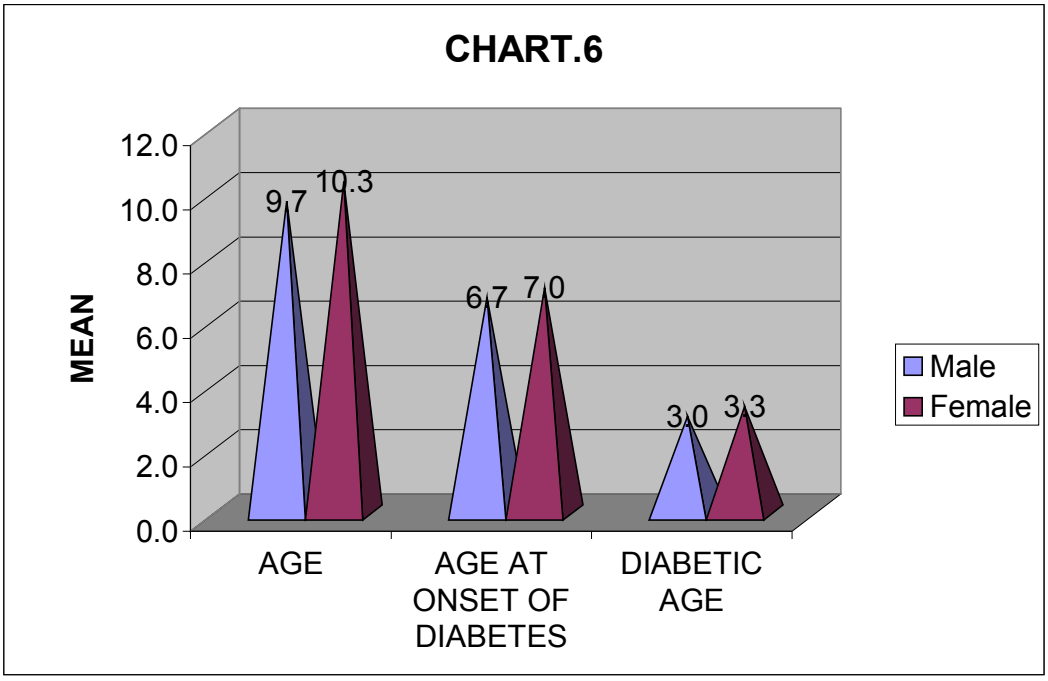
The Mean Diabetic Duration Is 3.2(SD 2.0).

The Mean HbA_{1C} Is 12.5(SD 3.0)

Table: 6

MEAN AND SD OF SEX DISTRIBUTION vs. AGE, AGE AT ONSET OF DIABETES AND DIABETIC AGE

	Male		Female	
	Mean	S.D.	Mean	S.D.
Age	9.7	3.4	10.3	3.9
Age at onset of diabetes	6.7	3.0	7.0	3.5
Diabetic age	3.0	2.0	3.3	1.9



RESULTS:

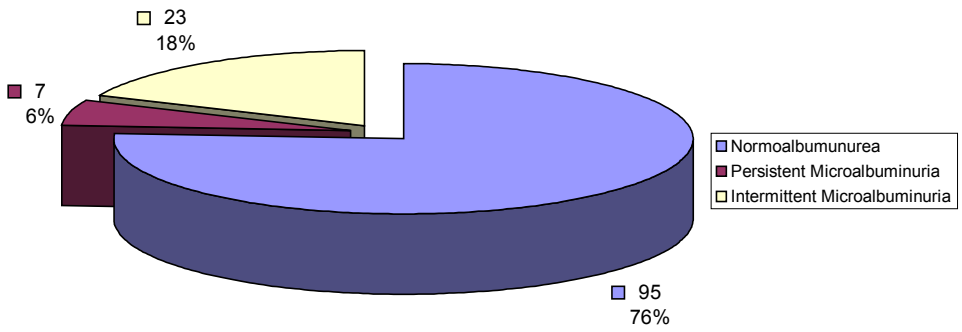
Mean of age, age at onset of diabetes and diabetic duration of the female children were found higher when compared to the male children.

Table 7
MICROALBUMINURIA IN THE STUDY POPULATION.

	n	%

Normoalbuminuria	95	76
Persistent Microalbuminuria	7	5.6
Intermittent Microalbuminuria	23	18.4
Total	125	100

CHART.7



RESULTS:

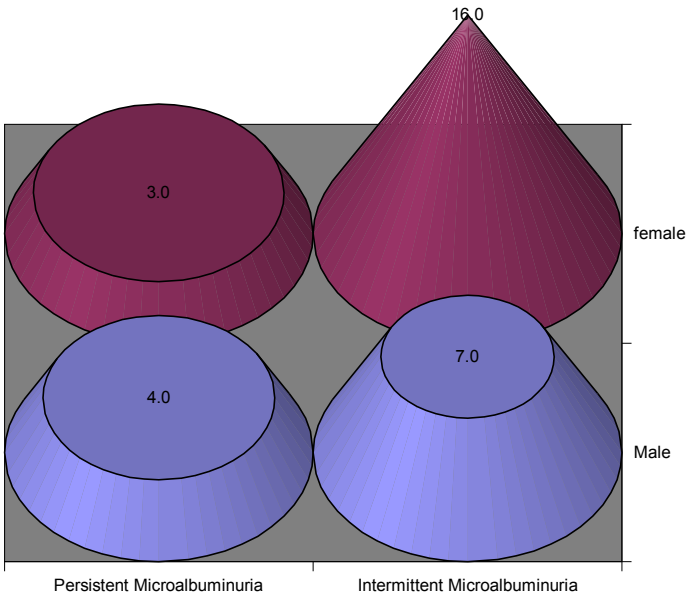
18.4% intermittently developed UAC in the microalbuminuric range (>30 mcg/mg) in only one of the 3 samples. 7(5.6%) children in the total cohort developed persistent microalbuminuria i.e., 2 out of 3 consecutive samples.

Table 8
GENDER DISTRIBUTION vs. MICROALBUMINURIA

	Male		Female		
	N	%	n	%	p value
Persistent Microalbuminuria	4.0	7.4	3.0	4.2	0.5
Intermittent Microalbuminuria	7.0	13.0	16.0	22.5	0.2

Microalbuminuria(Total)	11.0	20.4	19.0	26.7	0.5

CHART.8



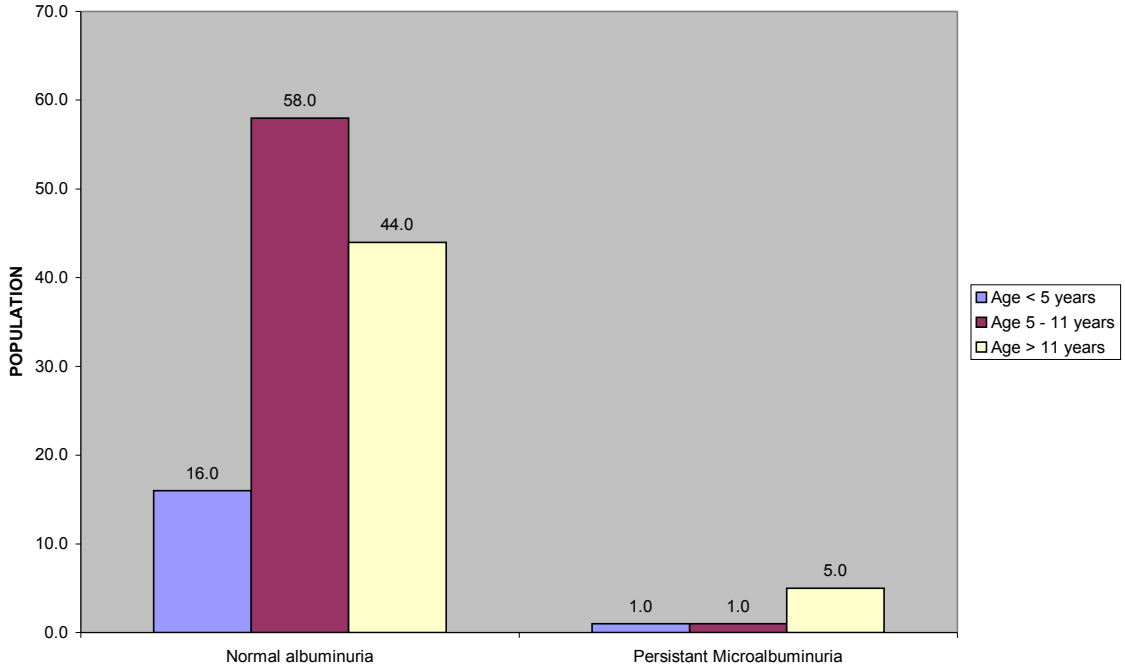
RESULTS:

There is no sex prepondence in children developing persistent microalbuminuria, with 3(4.2%) female and 4(7.4%) male children developing persistent microalbuminuria.

Table 9
PUBERTY vs. MICROALBUMINURIA

	N	%	Normoalbuminuria		Persistent microalbuminuria		P value
			N	%	n	%	
Age							
< 5 years	17.0	13.6	16.0	94.1	1.0	5.9	
5 – 11 years	59.0	47.2	58.0	98.3	1.0	1.7	
> 11 years	49.0	39.2	44.0	89.8	5.0	10.2	0.2

CHART.9



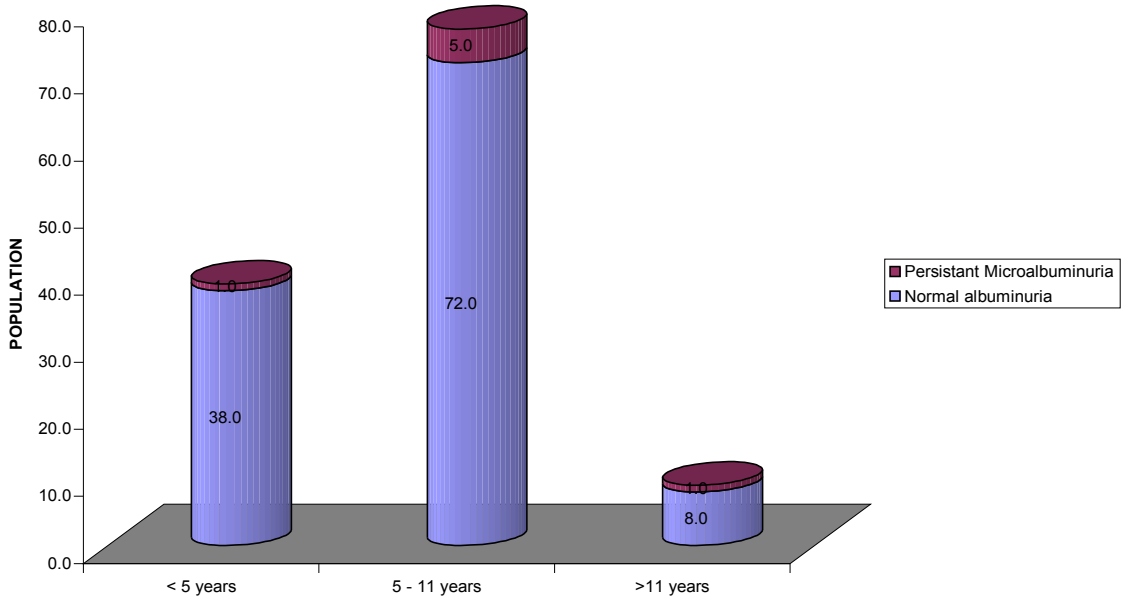
RESULTS:

5 out of 7 developed microalbuminuria after onset of puberty. Only 5 children (10.2%)above the age of 11years developed persistent microalbuminuria and this was not statistically significant. (p= 0.2)

Table 10
PUBERTAL ONSET OF DIABETES vs. MICROALBUMINURIA

	N	%	Microalbuminuria				P value
			Normal		Persistent		
			N	%	n	%	
Age at onset of diabetes							
< 5 years	39.0	31.2	38.0	97.4	1.0	2.6	
5 – 11 years	77.0	61.6	72.0	93.5	5.0	6.5	
>11 years	9.0	7.2	8.0	88.9	1.0	11.1	0.5

CHART.10

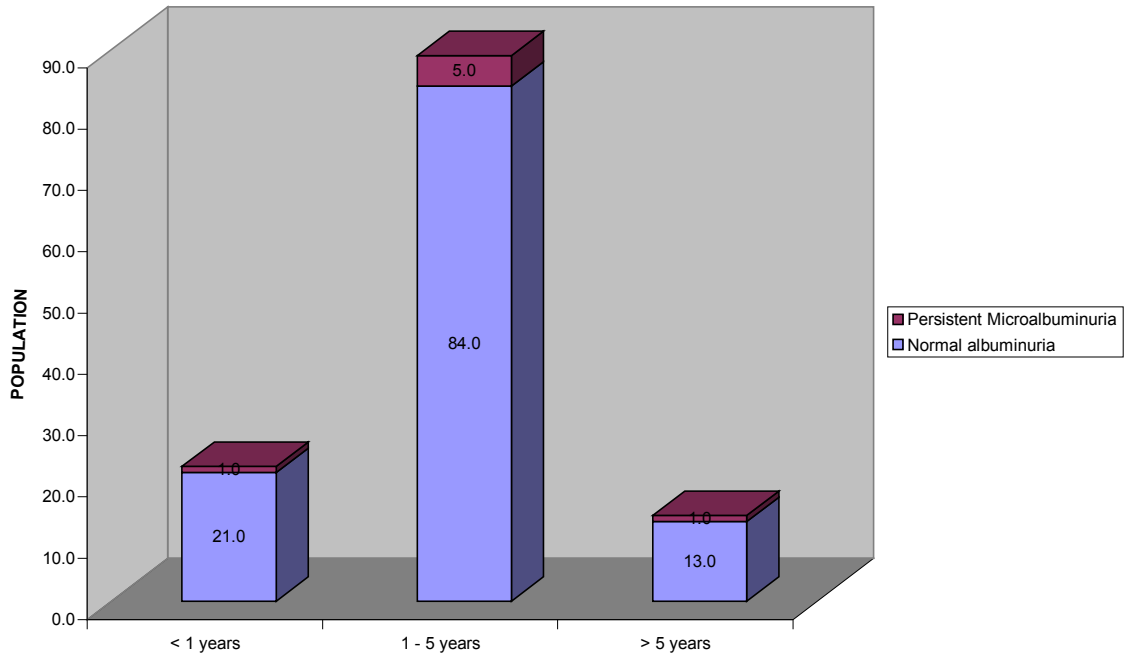


RESULTS:

In the above 11 years group, out of 9 children only 1 child 11.1% developed microalbuminuria. Most of the children developing persistent microalbuminuria were 5 to 11 years of age at onset of diabetes.

Table 11
DIABETIC DURATION vs. MICROALBUMINURIA

CHART.11



RESULTS:

In our study population out of 14 children with more than five years of duration only 1 child i.e., 7.1% developed microalbuminuria which is not statistically significant. Whereas 5(5.6%) out of 89 children with duration of 1 to 5 years developed microalbuminuria and 1(4.5%) child below one year age developed microalbuminuria.

Table 12
HbA_{1c}

	HbA_{1c}	
	Mean	**SD**
Normoalbuminuria	12.3	3.0
Persistent Microalbuminuria	13.3	2.4
Total study population	12.5	3.0

RESULTS:

The HbA_{1c} of our study population was 12.5(SD 3.0). the mean HbA_{1C} of children with persistent microalbuminuria was 13.3(SD 2.4) as against 12.3(SD 3.0) among normoalbuminuric

group. There was no Statistical significance in the mean HbA_{1c} of the two groups.

Table 13

Univariate Logistic Regression Of persistent microalbuminuria

Variables	O.R.	95% C.I.	p-value
Age at onset of diabetes			0.5
< 5 years	0.5	0.05 , 4.2	
5 - 11 years	1.0	Reference	
>11 years	2.5	0.2 , 26	
Duration of diabetes			0.9
< 1 year	1.0	Reference	
1 to 5 years	1.5	0.2 , 13.3	
>5 years	1.7	0.1 , 30.5	
HbA_{1c}	1.5	0.2 , 13.2	0.7

RESULTS:

Univariate logistic regression analysis of variables – age at onset of diabetes, duration of diabetes, and HbA_{1C} did not reveal any significance.

The sensitivity of one elevated urinary albumin excretion rate to detect persistent microalbuminuria was 64% and specificity was 90%. The positive predictive value of one urine albumin measurement was 39% and the negative predictive value was96% (95% CI 91%, 99%).

Using the accepted definition of severe hypertension in childhood systolic and /or diastolic blood pressure equal to or greater than the 95^{th} percentile for age and sex none of the children in our study population were hypertensive.

On Fundus examination none of our children showed evidence of diabetic retinopathy.

DISCUSSION

125 children recruited for study from our diabetic clinic were, in the age group of 2 to 16 years, the mean age was 10.2 +/- 3.0. The youngest child was 2.4years of age. Literature review reveals studies on microalbuminuria in IDDM children with a mean age of 13 +/- 4 years Bravo et al[93], 10-20 yrs in MIDAC study group[84]. How ever there is a similar study with age group of 2 to 16 years by Pedro et al [94].Most of the literature on microalbuminuria reveal the mean age of the population to be in the higher range, as adolescents are included in the pediatric population. 86.4% of our study group comprised of children above five years and only 13% of them were below 5 years i.e., pre-school children.

Gender distribution of our population revealed that female children predominated in this study. They consisted about 56.8%, whereas the total male children were 43.2%. The male female ratio was 1:1.3.On the contrary it was 1:3 in MIDAC study group[84].

Age at diagnosis of diabetes mellitus was taken as the age of onset of diabetes and this revealed 60% of our children were in the age group between 5 to 11 years and about 1/3rd were below 5 years. Only 7.2% were above 11 years of age at onset. This shows that most of our children had pre pubertal onset of diabetes mellitus (93%).

The diabetic age of our population revealed that majority of our children (89%) had a diabetic age of less than 5 years. Only 11% had diabetic duration over 5 years. The least duration being one month.

Mean age, age of onset of diabetes and diabetic age of the female children were found to be

higher when compared to male children, but this was not statistically significant.

Microalbuminuria:

Based on the albumin creatinine ratio of early morning urine samples, 18.4 % (23) of our study population intermittently developed microalbuminuria (ACR more than 30mcg/mg) in only one of the three consecutive samples. 5.6 %(7) of children in the total cohort developed persistent microalbuminuria i.e., ACR more than 30mcg/mg in two of the three consecutive samples. Of the seven children with persistent microalbuminuria 5 were in the age group of more than 11yrs and one child each in the age group of 5 to 11 years and below five year. The youngest being 4 years of age with diabetic age of 11 months.

The prevalence of microalbuminuria of 5.6% in our study is much lower than the studies done previously elsewhere in different population. 9.8% in MIDAC study group[84], 8% in the study by Bravo et al [93], 9% in the study by Cosmescu et al [83] and 12.9% by Jones et al [85]. The world wide prevalence is between 7 to 20 percent .The reasons attributable could be that the age group selected for the study in the first groups was much different from our study group. Most of our children were in the prepubertal age. Several Retrospective and cross sectional studies found a relationship between occurrence of microalbuminuria and the pubertal period. And suggested that puberty may play a critical role in the development of DN [100, 106,107,108].This findings suggest that the endocrine changes of puberty lead to an accelerated process of early kidney damage in diabetes. Studies of similar nature in pubertal children with type 1 DM would probably reveal higher prevalence than the present study which comprises predominantly of prepubertal children 61%.

Gender distribution, age at onset of diabetes and diabetic age did not reveal any statistical significance in children with persistent microalbuminuria which is in concordance with the study by

Pedro et al[94].

Various studies done by bravo et al, Holl et al [81], Cosmescu et al [83], Jones et al [85], MIDAC study group[84], ORPS[80], reveal that more girls than boys, more pubertal children and those with longer duration of diabetes(> 5 years) have increased risk of developing microalbuminuria.

The mean HbA_{1c} of our study population was 12.5 which indicate a poor glycemic control in our population. This could be attributable to lack of frequent home based blood glucose monitoring due to economic constraints in our study population. This again emphasizes that home based frequent BG monitoring is essential in diabetic children for long term glycemic control which is a must for avoiding long term complications in diabetes.

In this context, in our study comparing microalbuminuric group and normoalbuminuric group, there was no statistically significant difference in mean HbA_{1c}.

On the contrary studies by Bravo et al [93], Holl et al [81], Cosmescu et al [83], MIDAC study group [84], ORPS [80], show that poor metabolic control was significantly associated with development of microalbuminuria. The mean HbA_{1C} of the microalbuminuric children being 10% in Cosmescu et al [83]. In the study by Bruno et al[87] 50% of the cohort had HbA_{1c} levels over 9% and only 10 % had the levels below 6.6%. In ORPS[80], the mean HbA_{1c} of the total cohort was 9.9% +/- 1.7. The mean HbA_{1c} was significantly greater in children who developed microalbuminuria (mean difference+/- SEM : 1.1% +/- 0.2).

Among our study population 18 children were positive for microalbuminuria in the first sample.7 children among them had persistent microalbuminuria. 4 children tested negative in first

sample and positive in second sample.

The sensitivity of one elevated urinary albumin excretion rate to detect persistent microalbuminuria was 64% and specificity was 90%. The positive predictive value of one urine albumin measurement was 39% and the negative predictive value was96% (95% CI 91%, 99%).This was similar to the observation by Holl et al [81], with the sensitivity of 76% and specificity of 95.8%. Their PPV was 40.6% and NPV was 99.5%.

It has to be kept in mind that the predictive values of a positive screening result depend on the prevalence of the disease in the respective population. Persistent microalbuminuria is quiet rare in pediatric subjects upto early adulthood. In our study a single value in the microalbuminuric range conveys only a 39% chance to indicate persistent microalbuminuria in pediatric patients.

Medically, this demands that other potential causes of microalbuminuria have to be excluded (urinary tract infection and nondiabetic kidney diseases such as IgA nephritis, febrile proteinuria, etc.) and elevated albumin excretion has to be confirmed by at least one more measurement before a diagnosis of incipient diabetic nephropathy can be made and therapeutic consequences are justified. In addition, this high rate of false-positive results may lead to unnecessary psychological disturbance of patients and their families. However, the intra-individual variability of urinary albumin excretion should not be used to support a nihilistic approach to routine screening in pediatric patients.

CONCLUSION

- The prevalence of microalbuminuria in the pediatric population attending our diabetic clinic is 5.6%.
- Pubertal age and increasing duration of diabetes, appears to be associated with increased risk of microalbuminuria but is not statistically significant.
- Gender and age at onset of diabetes were not associated with increased risk of persistent microalbuminuria.
- In agreement with recommendations by the American Diabetes Association and the International Society for Pediatric and Adolescent Diabetology (ISPAD), the German Working Group on Pediatric Diabetology recommends screening for all patients with a duration of diabetes >5 years or with a chronological age >11 years. Following these guidelines, in our patient population 2 children with persistent proteinuria <11 years and 6 children with duration less than 5 years would have been missed.

Recommendations:

- Children with lesser diabetic age (<5years) also need to be investigated for microalbuminuria.
- Similar studies in larger groups are needed to identify the risk factors in our population so that necessary recommendations can be made to diagnose diabetic nephropathy much earlier in the risk group.

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ANNEXURE

PROFORMA

Diabetic Clinic No. :

Name :

Age :

Age at onset :

Age at Diagnosis :

Sex :

Diabetes Duration :

H/O Renal Disease :

Family History of DM :

On Examination :

Height in cms

Weight in Kg
Blood Pressure
Fundus Examination

Investigations :

- **Urine - Albumin**
  Ketone Bodies
  Puscells / Deposits

- **Blood - Urea**
  Serum - Creatinine

Test	Date /Sample I	Sample II	Sample III
Urine Microalbuminuria			
HbA_{1c}			

- **Ultra sound abdomen**

ABBREVIATIONS

- ACR — Albumin Creatinine Ratio
- AER — Albumin Excretion Rate
- AGE — Advanced Glycation End Products
- ACE — Angiotensin Converting Enzyme
- ATIIRB — Angiotensin II Receptor Antagonist
- CRP — C Reactive Protien
- DN — Diabetic Nephropathy
- ESRD — End Stage Renal Disease
- GBM — Glomerular Basement membrane
- GFR — Glomerular Filtration Rate
- IDDM — Insulin Dependent Diabetes Mellitus
- ISPAD — International Society for Pediatric and Adolescent Diabetology.
- MIDAC — Microalbuminuria In Diabetic and Adolescent children
- NPV — Negative Predictive Value
- ORPS — Oxford Regional Prospective Study
- PRA — Plasma Renin Activity
- PPV — Positive Predictive Value
- RAS — Renin Angiotensin System
- RAGE — Receptor for AGE
- ROS — Reactive Oxygen Species
- SD — Standard Deviation
- TBM — Tubular Basement Membrane
- UAC — Urinary Albumin Concentration