Study of microalbuminuria and retinopathy in prediabetes

Dissertation submitted in partial fulfillment of regulation for the award of M.D. Degree in General Medicine (Branch I)

The Tamilnadu Dr. M.G.R. Medical University

Chennai
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CERTIFICATE

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DECLARATION

I solemnly declare that the dissertation titled **" THE STUDY OF MICROALBUMINURIA AND RETINOPATHY IN PRE-DIABETES "** was done by me from **March 2007 to October 2009** under the guidance and supervision of **Prof.Dr.P.JAMBULINGAM**

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CONTENTS

INTRODUCTION

Diabetes is a disease which affects 150 million individuals[1] world wide. Diabetes mellitus (DM) has long been recognized as a major public health problem[1] with far reaching consequences, not only for its adverse health impact on individuals, but also for its economic burden on the health care system and the society at large . The International Diabetes Federation (IDF) in 2005 confirmed that diabetes is one of the most common non-communicable diseases globally[2] and constitutes the fourth or fifth leading cause of death in most developed countries as well as many developing and newly industrialized countries. In India, over 30 million people have been diagnosed with pre-diabetes.Pre-diabetes is a condition in which the glucose levels are higher than normal but enough for the diagnosis of diabetes mellitus .The microvascular complications starts in the pre-diabetic state itself so early diagnosis and treatment of this condition is important as this delay the progression of microvascular complications and onset of diabetes mellitus. As far as the pre-diabetic population is concerned, only China surpasses India, and by 2025,every fifth diabetic in the world will be an Indian.Pre-diabetes is a mounting problem in India. The prevalence of IGT is thought to be around 8.7 per cent in urban areas and 7.9 per cent in rural areas. It is thought that around 35 per cent of IGT sufferers go on to develop Type 2 diabetes. So, India is genuinely facing a healthcare crisis and the type of diabetes that its people suffer from differs considerably from what patients in the West suffer from. Pre-diabetes currently refers to people who have impaired fasting glucose (IFG) i. e. fasting glucose levels between 100- 125mg/dl or

impaired glucose tolerance (IGT), i. e. two hours post glucose load between 140-199mg/dl or both. Pre-diabetes raises short-term absolute risk of Type 2 diabetes five to six fold, and in some populations this maybe even higher[3]. People with diabetes are vulnerable to multiple and complex medical complications. These complications involve both cardiovascular disease (heart disease, stroke) and microvascular disease. Epidemiologic evidence suggests that these complications of diabetes begin early in the progression from normal glucose tolerance to frank diabetes[4]. Early identification and treatment of persons with pre-diabetic conditions has the potential reduce both the incidence of diabetes and related complications.. Early diabetic nephropathy in the pre-diabetic state can be detected by micro-albuminuriaand retinopathy by typical funduschanges.

Recognition of microalbuminuria Stemmed from Diabetes research decades ago.According to National Kidney Foundation Microalbuminuria is defined as a Urine Albumin Excretion Rate (UAER) of approximately 30-300mg/d in atleast two of three consecutive samples of nonketotic sterile urine. . Microalbuminuria [5]possibly reflects a state of increased renal endothelial permeability and is an easily measured marker of rather diffuse endothelial dysfunction, low grade inflammation and vascular disease burden. There are data supporting that this programme would have beneficial effects in detecting individuals at high risk for cardiovascular atherosclerotic disease. Regarding the determination of microalbuminuria, there had been considerable variation in the performance of diagnostic tests. 24 hour urine albumin excretion remains the gold

standard, but impractical for routine practice. Microalbuminuria can be diagnosed on the basis of three positive tests – Albumin creatinine ratio; urine albumin excretion rate or a combination of both

Almost 8% of individuals with pre-diabetes have detectable early diabetic retinopathy, according to research presented June 12 at the 65th Scientific Sessions of the American Diabetes Association (ADA) in San Diego.

Among those with very early diabetes (diagnosis within 6 to 12 months of the onset of diabetes), almost 13% have diabetic retinopathy, investigators found. ADA's chief scientific and medical officer, Richard Kahn, PhD, says the the complications of diabetes begin years before a typical diagnosis

Finally, the appearance of retinopathy in patients with pre-diabetes, and the suggestion that lesions are present at glucose levels below the current diagnostic thresholds for diabetes, could lead experts to reconsider those limits

.

Aims and objectives

1. To study the relative frequency of microalbumiuria and retinopathy in pre-diabetic individuals.
2. To study the correlation between blood sugar levels and microalbuminuria.
3. To study the correlation between blood sugar levels and retinopathy in pre-diabetics.
4. To study the correlation between microalbuminuria and retinopathy in pre-diabetics.

REVIEW OF LITERATURE

The term pre-diabetes include population whose blood sugar is higher than normal but not enough for the diagnosis of diabetes .

Defining Pre-diabetes:

. The American diabetic association defined pre-diabetes as condition in which fasting blood sugar {FBS} between 100-125mg/dl or two hour postprandial blood sugar {2hr PPBS} between 140-199mg/dl or both.

Symptoms of Pre-diabetes

Although most people with pre-diabetes have no symptoms at all, symptoms of diabetes may include unusual thirst, a frequent desire to urinate, blurred vision, or extreme fatigue.

Screening for Pre-diabetes[6]

1.over 45 years of age

2. have any risk factors for diabetes

3. overweight with a BMI (body mass index) over 25

4. belong to a high risk ethnic group

5.have a history of gestational diabetes or delivering a baby that weight more than 9 lbs

6.You have high triglycerides and low HDL cholesterol, central obesity and hypertension (the metabolic insulin resistance syndrome).

Pathophysiology of Pre-Diabetes

Substantial defects in insulin secretion have been noted inpatients with IFG and IGT.Elevated plasma glucose 2 h postchallenge [7]predicts defects in beta-cell function. Defective Insulin secretion have been demonstrated in people with normal fasting glucose and glycosylated hemoglobin concentrations with glucose values greater than 140 mg/dL or 7.8 mmol/L 2 hours after ingestion of 75 g of glucose orally. Thus, defects in insulin secretion[8] can be detected before the onset of overt hyperglycemia. Detailed study of insulin secretion in patients with IGT has demonstrated that consistent quantitative and qualitative defects are seen in this group. During oral glucose tolerance testing, there is a delay in the peak insulin response and first-phase insulin responses to an intravenous glucose bolus are consistently decreased in relation to ambient insulin sensitivity. Further, abnormalities in first-phase insulin secretion were observed in first-degree relatives of patients with type 2 diabetes who exhibited only mild intolerance to glucose, and an attenuated insulin response to oral glucose was observed in normoglycemic co-twins of patients with type 2 diabetes. This pattern of insulin secretion during the so-called prediabetic phase was also seen in subjects with IGT who later developed type 2 diabetes and in normoglycemic obese subjects with a recent history of gestational diabetes, another group at high risk for type 2 diabetes.Beta cell

abnormalities may therefore precede the development of overt type 2 diabetes by many years.

The temporal pattern of insulin secretory responses is altered in IGT and is similar to but not as pronounced as that seen in diabetic subjects . There is a loss of coordinated insulin secretory responses during oscillatory glucose infusion, indicating that the ability of the beta cell to sense and respond appropriately to parallel changes in the plasma glucose level is impaired. Abnormalities in rapid oscillations of insulin secretion have also been observed in first-degree relatives of patients with type 2 diabetes who have only mild glucose intolerance,further suggesting that abnormalities in the temporal pattern of beta cell function may be an early manifestation of beta cell dysfunction preceding the development of type 2 diabetes. Because an elevation in serum proinsulin is seen in subjects with diabetes, the contribution of proinsulin to the hyperinsulinemia of IGT has been questioned. The hyperinsulinemia[9] of IGT has not been accounted by an increase in proinsulin, although elevations in fasting and stimulated proinsulin or proinsulin/insulin ratios have been found by many, although not all, investigators. Correlation of elevated proinsulin levels in IGT as a predictor of future conversion to diabetes has also been observed.

Obesity and other insulin-resistant states are associated with a substantially greater risk for the development of type 2 diabetes. The ability of the pancreatic beta cell to compensate for insulin resistance determines whether blood glucose levels remain normal in insulin-resistant subjects or whether the subjects develop glucose intolerance

or diabetes. Kahn and co-workers studied the relationship between insulin sensitivity and beta cell function in 93 relatively young, apparently healthy human subjects of varying degrees of obesity. In human with normal glucose tolerance and varying degrees of obesity, beta cell function varies quantitatively with differences in insulin sensitivity.

The insulin resistance of obesity is characterized by hyperinsulinemia. Hyperinsulinemia in this setting reflects a combination of increased insulin production and decreased insulin clearance, but most evidence suggests that increased insulin secretion is the predominant factor. Both basal and 24-hour insulin secretory rates are three to four times higher in obese subjects and are strongly correlated with body mass index.

THE ROLE OF INCREASED HEPATIC GLUCOSE PRODUCTION IN IMPAIRED GLUCOSE TOLERANCE.

The disposal of glucose after meals depends on the ability of insulin to increase peripheral glucose uptake and simultaneously decrease endogenous glucose production. Although studies have suggested that the kidney can contribute up to 25% of endogenous glucose production,the defect in type 2 diabetes is primarily in defective regulation of glucose production from the liver . Two routes of glucose production by the liver are glycogenolysis of stored glycogen and gluconeogenesis from two- and three-carbon substrates derived primarily from skeletalmuscle. .The production of glucose by the liver is regulated primarily by the relative actions of glucagon and insulin to activate or suppress glucose production, respectively, although the nervous system

and glucose autoregulation of hepatic glucose production probably play less important roles. The ability of insulin to reduce HGO is an important mechanism for maintaining normal glucose tolerance. Under normal circumstances, insulin suppresses up to 85% of glucose production in normal individuals by directly inhibiting glycogenolysis, especially at lower insulin concentrations.Under circumstances in which glycogenolysis is enhanced by glucagon, the effects of insulin to suppress hepatic glucose production may be even greater. Glucagon increases glycogenolysis by activation of the classical protein kinase cascade involving cyclic AMP (cAMP)dependent protein kinase and phosphorylase[10] and also increases gluconeogenesis in part by increasing the transcription of phosphoenolpyruvate carboxykinase through the cAMP response element binding protein.

The indirect or peripheral effect of insulin in controlling glucose production by the liver is twofold. First, insulin has a profound effect on decreasing glucagon secretion by the alpha cell of the pancreas through systemic and paracrine effects. The decrease in glucagon secretion decreases the activation of glycogenolysis and gluconeogenesis. The second important peripheral action of insulin is decreasing FFA levels by suppressing lipolysis. FFAs[11] increase hepatic glucose production by stimulating gluconeogenesis.The suppression of glucagon secretion and decrease in FFA delivery[12] to the liver are additive in reducing liver glucose production.

Hepatic insulin resistance plays an important role in the hyperglycemia of type 2 diabetes,and the impairment in suppression of HGO appears to be quantitatively similar

to or even larger than the defect in the stimulation of peripheral glucose disposal. There is a direct relationship between increases in HGO and fasting hyperglycemia . Treatment of patients with metformin, which suppress hepatic glucose production, results in improvements in glucose tolerance.

Complications of Pre- Diabetes

Pre-Diabetes are characterized by hyperglycemia[13], a relative deficiency of insulin, and the development of diabetes-specific microvascular pathology in the retina, renal glomerulus, and peripheral nerve. Pre-diabetes is also associated with accelerated atherosclerotic macrovascular disease affecting arteries that supply the heart, brain, and lower extremities.Large, prospective clinical studies show a strong relationship between glycemia and diabetic microvascular complications . There is a continuous, though not linear, relationship between level of glycemia and the risk of development and progression of these complications Hyperglycemia and the dyslipidemia induced by insulin resistance both appear to play important roles in the pathogenesis of macrovascular complications.

SHARED PATHOPHYSIOLOGIC FEATURES OF MICROVASCULAR COMPLICATIONS

In the retina, glomerulus, and vasa nervorum, diabetes-specific microvascular disease is characterized by similar pathophysiologic features.

1.Requirement for Intracellular Hyperglycemia

Clinical data indicate that chronic hyperglycemia is the central initiating factor for all types of diabetic microvascular disease. Duration and magnitude of hyperglycemia are both strongly correlated with the extent and rate of progression of diabetic microvascular disease. Although all diabetic cells are exposed to elevated levels of plasma glucose, hyperglycemic damage is limited to those cell types (e.g., endothelial cells) that develop intracellular hyperglycemia. Endothelial cells develop intracellular hyperglycemia because, unlike many other cells, they cannot down-regulate glucose transport [16]when exposed to extracellular hyperglycemia. vascular smooth muscle cells, which are not damaged by hyperglycemia, show an inverse relationship between extracellular glucose concentration[17] and subsequent rate of glucose transport . In contrast, vascular endothelial cells show no significant change in subsequent rate of glucose transport after exposure to elevated glucose concentrations.

2.Abnormal Endothelial Cell Function

Early in the course of diabetes mellitus, before structural changes are evident, hyperglycemia causes abnormalities in blood flow and vascular permeability in the retina, glomerulus, and peripheral nerve vasa nervorum. The increase in blood flow and intracapillary pressure is thought to reflect hyperglycemia-induced decreased nitric oxide (NO) production[18] on the efferent side of capillary beds, and possibly an increased sensitivity to angiotensin II. As a consequence of increased intracapillary pressure and

endothelial cell dysfunction, retinal capillaries exhibit increased leakage of fluorescein and glomerular capillaries have an elevated albumin excretion rate (AER). Comparable changes occur in the vasa vasorum of peripheral nerve. Early in the course of diabetes, increased permeability is reversible; as time progresses, however, it becomes irreversible.

3.Increased Vessel Wall Protein Accumulation

The common pathophysiologic feature of diabetic microvascular disease is progressive narrowing and eventual occlusion of vascular lumina, which results in inadequate perfusion and function of the affected tissues. Early hyperglycemia-induced microvascular hypertension and increased vascular permeability contribute to irreversible microvessel occlusion by three processes:

The first is an abnormal leakage of periodic acidSchiff (PAS)-positive, carbohydrate-containing plasma proteins, which are deposited in the capillary wall and which may stimulate perivascular cells such as pericytes and mesangial cells to elaborate growth factors and extracellular matrix.

The second is extravasation of growth factors, such as transforming growth factor 1 (TGF-1), which directly stimulates overproduction of extracellular matrix components, and may induce apoptosis in certain complication-relevant cell types.

The third is hypertension-induced stimulation of pathologic gene expression by endothelial cells and supporting cells, which include glut-1 glucose transporters, growth factors, growth factor receptors, extracellular matrix components, and adhesion

molecules that can activate circulating leukocytes.The observation that unilateral reduction in the severity of diabetic microvascular disease occurs on the side with ophthalmic or renal artery stenosis is consistent with this concept.

Microvascular Cell Loss and Vessel Occlusion

The progressive narrowing and occlusion of diabetic microvascular lumina are also accompanied by microvascular cell loss. In the retina, diabetes mellitus induces programmed cell death of Müller cells and ganglion cells, pericytes, and endothelial cells.In the glomerulus, declining renal function is associated with widespread capillary occlusion and podocyte loss, but the mechanisms underlying glomerular cell loss are not yet known. In the vasa nervorum, endothelial cell and pericyte degeneration occur, and these microvascular complication.

4.Development of Microvascular Complications During Posthyperglycemic Euglycemia ("Hyperglycemic Memory)

Another common feature of diabetic microvascular disease has been termed hyperglycemic memory, or the persistence or progression of hyperglycemia-induced microvascular alterations during subsequent periods of normal glucose homeostasis. Hyperglycemia-induced increases in selected matrix gene transcription also persist for weeks after restoration of normoglycemia in vivo, and a less pronounced, but qualitatively similar, prolongation of hyperglycemia-induced increase in selected matrix gene transcription occurs in cultured endothelial cells.

Observations from clinical studies imply that hyperglycemia induces prolonged and

sometimes irreversible changes in long-lived intracellular molecules that persist and cause continued pathologic function in the absence of continued hyperglycemia.

5.Genetic Determinants of Susceptibility to Microvascular Complications

Clinicians have long observed that different patients with similar duration and degree of hyperglycemia differed markedly in their susceptibility to microvascular complications. Such observations suggested that genetic differences existed that affected the pathways by which hyperglycemia damaged microvascular cells.

A role for genetic determinant of susceptibility to diabetic nephropathy is most strongly supported by the demonstration of familial clustering of diabetic nephropathy.

MECHANISMS OF HYPERGLYCEMIA-INDUCED DAMAGE

Four major hypotheses about how hyperglycemia causes diabetic complications have generated a large amount of data as

well as several clinical trials based on specific inhibitors of these mechanisms. Until recently, there was no unifying hypothesis linking these four mechanisms together, nor was there an obvious connection between any of these mechanisms, each of which responds quickly to normalization of hyperglycemia, and the phenomenon of hyperglycemic memory . Increased Polyol Pathway Flux,Aldose Reductase Function.

Advanced Glycation End-Product Inhibitors[19]

The hydrazine compound aminoguanidine was the first AGE inhibitor discovered. In a large randomized, double-blind, placebo-controlled, multicenter trial , Aminoguanidine[20] reduced the progression of diabetic retinopathy (defined as an increase by three or more

steps in the Early Treatment Diabetic Retinopathy Study).

Future Drug Targets

The recent discovery that each of the four different pathogenic mechanisms discussed in this section reflect a single hyperglycemia-induced process suggests that interrupting the overproduction of superoxide by the mitochondrial electron transport chain would normalize polyol pathway flux, AGE formation, PKC activation, hexosamine pathway flux, and NF-B activation. Novel compounds that act as superoxide dismutase/catalase mimetics already exist,and these compounds have been shown to normalize hyperglycemia-induced mitochondrial superoxide overproduction. These and the other agents described in this section may have unique clinical efficacy in preventing the development and progression of diabetic complications.

RETINOPATHY, MACULAR EDEMA, AND OTHER OCULAR COMPLICATIONS.

Diabetic retinopathy is a well-characterized, sight-threatening, chronic microvascular complication that eventually afflicts virtually all patients with diabetes mellitus.Diabetic retinopathy is characterized by gradually progressive alterations in the retinal microvasculature, leading to areas of retinal nonperfusion, increased vasopermeability, and pathologic intraocular proliferation of retinal vessels.Epidemiological studies have shown that early retinopathy changes are seen in pre-diabetic patients.A study of retinopathy in pre-diabetes ,published in 65th ADA scientific sessions showed a prevalence of 8% compared to 13% in early diabetes. The complications associated with

the increased vasopermeability, termed macular edema, and uncontrolled neovascularization, termed proliferative diabetic retinopathy (PDR), can result in severe and permanent visual loss. The retinopathy in diabetic patients can be divided into Non-proliferative diabetic retinopathy(Mild,Mod,Severe),proliferative diabetic retinopathy and Cystoid macular oedema.

NEPHROPATHY IN DIABETES AND PRE-DIABETES

The development of proteinuria 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. 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 (Parving *et al* . 1982). 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). .

PATHOMECHANISMS FOR MICROVASCULAR DAMAGE The microvascular lesions [21]of the kidney in diabetes mellitus had variably been ascribed to generation of advanced glycation endproducts (AGE)[22], cumulation of sorbitol, activation of protein kinase C (PKC), and activation of the hexosamine pathway. A unifying concept has recently been proposed byBrown Lee. 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 Advanced Glycosylated End products (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. Glucose 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).Activation of the RAGE[23] triggers ROS formation and promotes translocation of the transcription factor NF-κB, a central switch for inflammatory processes into the nucleus.Important secondary mediators for the development of renal damage are transforming growth factor β (TGFβ) , locally generated angiotensin II, endothelin, and several other cytokines. Glomerular hypertension resulting from afferent vasodilatation and efferent vasoconstriction, in conjunction with altered glomerular permeability causes proteinuria[24] activation of proximal tubular epithelial cells, renal fibrosis and ultimately nephron loss and renal failure.

Apart from elevated systemic and glomerular capillary pressure, proteinuria plays a major pathogenetic role. Remuzzi *et al* . (2002) 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 , endothelin , 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 Angiotensin II, endothelin, and cytokines are then secreted i into the interstitium where they activate peritubular fibroblasts and lead to interstitial fibrosis..Mechanism of microalbuminuria 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 (**van den Born *et al* . 1995**). It was also thought that in later stages of DN, disruption of the texture of the basal membrane creates gaps and holes and allows high molecular weight serum proteins to escape into the filtrate.Recent insights into podocyte function and specifically identification of proteins of the slit membrane (**Mundel and Shankland 2002**) have led to the concept that the podocyte is a prime player in the genesis of proteinuria.. It is also of interest that in experimental diabetes, podocyte damage is the first sign of renal injury (**Gross *et al* . 2003**). Diminished podocyte numbers have been documented in animals and patients with diabetes (**Steffes *et al* . 2001**). Podocytes are postmitotic and can no longer proliferate.

Stages of diabetic nephropathy

There are few renal diseases where the course is as predictable as in DN. This has led to a scheme proposed by Mogensen which is valid in type 1 diabetes, but less consistently so in type 2 diabetes. An early stage of renal hyperfunction is followed by a stage of clinical latency which may last up to 20 years. It is subsequently followed in a very well-defined time course by the stages of microalbuminuria progressing to that of overt

nephropathy and renal failure.

The stages of diabetic nephropathy

Stages	Glomerular filtration	Albuminuria	Bloodpressure	Time interval
1.Renal hyperfunction	Elevated	Absent	Normal	At diagnosis
2.Clinical latency	High normal	Absent		
3.Microalbuminuria	Within the normal range	20–200 µg/min (30–300 mg/day)	Rising within or above the normal range	5–15yrs
4.Macroalbuminuria/proteinuria (overt nephropathy)	Decreasing	200 µg/min (300 mg/day)	Increased	10–15yrs
Renal failure	Diminished	Massive	Increased	15–30

The factors that increase the risk to develop diabetic nephropathy are;

1.Hyperglycemia.

2.Smoking.

3.Hypertension.

Obviously there is no DN without hyperglycaemia. The role of hyperglycaemia in the

development of DN is most convincingly demonstrated by transplantation studies. When normal kidneys are transplanted into diabetic recipients, they developed glomerulosclerosis in experimental or clinical observations (Mauer *et al* . 1976_). More excitingly isolated pancreatic transplantation in patients with type 1 diabetes and nephropathy led gradually after several years to the regression of diabetic glomerulosclerosis (Fioretto *et al* . 1998). There exists a wealth of information on the association between hyperglycaemia or HbA1c concentrations and development of microalbuminuria (Prirart 1978 ; Feldt-Rasmussen and Deckert 1993 ; Krolewski *et al* . 1996 ; Rudberg and Dahlquist 1996). The definite proof that the association is causal in nature has come from intervention trials, that is, the Diabetes Control and Complications Trial (DCCT) (The Diabetes Control and Complications Trial Research Group 1993) in type 1 diabetes and the Kumamoto trial (Shichiri *et al* . 1995) as well as the UKPDS study (UK Prospective Diabetes Study Group 1998_) in type 2 diabetes .

MICROALBUMINURIA

Concepts and definition:

The Invention of sensitive immunoassays in the beginning of 1960s allowed the measurement of previously undetectable amounts of albumin in urine. This is important because microalbuminuria is now documented and widely accepted as an important marker for early diabetic renal disease, and also of early vascular complications including early mortality. Recently clear treatment strategies based upon many

intervention trials were proposed.These are now well accepted by the medical community. Therefore screening for microalbuminuria and follow up of patients is now general practice in some countries.

Microalbuminuria is defined as UAER of approximately 30 – 300 mg/d (24 hour urine albumin excretion rate) in at least two of three consecutive samples of nonketonic sterile urine. Ideal is the first voided, mid stream morning sample (5ml sample).

ADA and NKF (American Diabetes Association and National Kidney Foudation) define microalbuminuria as an albumin creatinine ratio between 30 – 300 μg/mg in both men and women[7]. Sex specific ACR cutpoints: > 17 μg/mg in men, >25 μg/mg in women.

24 hour urine albumin excretion rate: 30 – 300 mg/d.

Overnight urine albumin excretion rates: 20 - 200μg/hr.

Albumin creatinine ratio : 2.5 – 25mg/mmol (for men) or

3.5– 25 mg/mmol (for women)

30 - 300μg/ mg in both men and women.

The 24 hour urine collection is considered to be the gold standard for assessing the low levels of UAER.

Techniques of measurement and monitoring:

Timed urine collections 24 hor or overnight remain the gold std.

Disadvantage: This is cumbersome to the patient, in repeated large scale screening, this may become a significant problem. For large scale screening use of

albumin creatinine ratio in early morning urine is a convenient and reliable screening method.

Potential confounders:

Potential confounders in the detection of microalbuminuria are :

Day – to –day variation of 40%. Several tests necessary.

False positives: Strenuous exercise, urinary tract infection, menstruation severe of dysregulation (newly diagnosed Type I diabetes),

Urine WBC, Hematuria, Ischemic heart disease, Renal disease.

Dipsticks exclusively detect albumin, sensitivity being for concentrations as low as 250 mg/ml. All the below mentioned methods can be used for quantitative estimation of proteins, all the available methods are imperfect. Urine albumin cretatinine ratio can be used in random urine samples. Measurements correlate well with those obtained by classical way. Urine albumin estimated by ELISA and creatinine by reaction – rate Kinetic principle RIA and Nephelometry.

Methods for detecting and measuring proteinuria

Method	Description	Detection limit	Coments
Turbidimetric	Addition of Trichloroacetic or sulfosalicylic acids after colloid properties and	50 – 100 mg/ml	Imprecise, different readings for albumin and

	produce turbidity to be read in densitometer (Benzathonium chloride also used)		globulin
Stick tests	Impregnated with indicator dye (bromocresol green) which changes colour in presence or protein.	100mg/ml	Reacts poorly with globulin. Usual clinical screening test.
Nephelometric	Specific anti albumin antibody used.		Measures albumin excretion, not total protein.

Factors that Microalbuminuira helps to predict:

Diabetic renal disease

Diabetic Retinopathy

Complications in pregnancy

Cardiovascular mortality and morbidity in

1. Type I diabetes
2. Type II Diabetes
3. Elderly Non diabetic people
4. Non diabetic people with hypertension

Association of Microalbuminuria with other cardiovascular risk factors:

There are several reports in non diabetic population of an association between microalbuminuria and obesity, increased levels of LDL, plasma fibrinogen, increased alcohol consumption, cigarette smoking, high fat diet & low HDL.

Microalbuminuria may reflect a prothrombotic state and impaired endothelial function as increased levels of von Willebrands factor, thrombodulin, Thrombin – antithrombin III complexes, and impaired fibrinolytic activity were described in subjects with increased UAER (Mennen et al 2001). These derangements may lead to augmented blood viscosity, more peripheral vascular resistance, there by to increase UAER and to diffuse atherosclerotic lesions. It is suggested that microalbuminuria constitutes the renal manifestation of generalized vascular endothelial dysfunction that probably underlies the link between increased UAER and high risk for cardiovascular disease. (Pendrinelli et al 1999).

Managing a case of Pre-Diabetes

A diagnosis of pre-diabetes identifies an individual at high risk

for developing type2 diabetes and increased risk of cardiovascular

deaths.The treatment of pre-diabetes[25] could alter the natural history of type 2 diabetes.

Lifestyle Interventions ;

Obesity is the most potent acquired risk factor for developing

Type 2 diabetes.Studies have shown physical inactivity[26],low fibre and high fat diet increase future risk of diabetes. The Da Qing study demonstrated that community based

diet and exercise reduced incident type 2 diabetes by 30-40% among Chinese subjects with IGT.The US Diabetes Prevention Progam(DPP) study

Founded that an intensive diet and exercise intervention reduced incident type 2 diabetes by 58%.

Pharmacological interventions;

The DPP study demonstrated that metformin decreased incident type2 diabetes by 31% relative to control.The STOP-NIDDM Trial demonstrated that Acarbose treatment to IGT patients reduced incident type2 diabetes by 25%.The XENDOS Trial examined the use of Orlistat in obese patients ,decreased incident diabetes by 37%.Recently the DREAM trial shown reduction of type 2 diabetes by 60% using Rosiglitazone.Analysis of large no of trial of ACE or

ARB could reduce risk by as much as 25%.

MATERIALS AND METHODS

The patients admitted in medical wards and those attending medical out patient unit in Medical College Hospital, Coimbatore with a diagnosis of Pre-diabetes were studied during a period starting from May 2005 to October 2006.

Number of study group : One

Sample size : 200

Study design : Prospective Analytical study

Statistical method:

Chi square test was used to study the relationship between microalbuminuria and other variables. P value was calculated for all the variables. For those variables with significant P value, odds ratio was calculated.

Ethical concern:

The study conforms to the guidelines of declaration of Helsinki 1964, revised in 1975. The study was subjected to the ethical revision board of the study center.

Study Design:

Patients over 30 years of age who was diagnosed to have Pre-diabetes were

included in the study, irrespective of the gender

Exclusion criteria:

1. Patients with age<30 years of age.
2. Patients with Hypertension(Blood Pressure >140/90mmHg)
3. Patients with Renal disease.
4. Patients with urinary tract infection by history or investigations.
5. Patients with raised serum creatinine [>1.5 mg/dl] or urine examination showing RBC, WBC, urine C & S yielding bacteria.

The diagnosis of Pre-diabetes was made by checking Fasting blood glucose,,ine FBS between 100-125 or OGTT between 140-199 are selected.FBS was taken after 8hrs over night fasting.OGTT done using 75 gram of glucose.

1. Both out-patients and in-patients are included.
2. A detailed case record was prepared for each patient on the basis of specially designed proof.
3. The study population was divided into two groups depending on Fasting blood sugar{FBS} or IFG and 2hour Oral Glucose tolerance test(OGT) or IGT.

1. IFG 100 – 109	1.IGT 140-159
2. IFG 110- 119	2.IGT 160-179
3. IFG 120–125	3.IGT 180-199

This was an arbitrary division done for the ease of comparison.

Fundus Examination: Done by direct ophthalmoscopy; pupillary dilatation achieved using 1% Tropicamide. According to Fundus findings, the patients were divided into 6 groups. (Refer to Review of Literature):

1. Fundus normal
2. Fundus not visualised
3. Mild Non-proliferative Diabetic Retinopathy
4. Moderate Non-proliferative Diabetic Retinopathy
5. Severe Non-proliferative Diabetic Retinopathy
6. Proliferative Diabetic Retinopathy

Investigations done included:

1. Fasting blood sugar,2Hour Oral Glucose Tolerance Test
2. Serum creatinine – Only those patients with normal value were taken up for the study.
3. Urine examination – Albumin, RBC, WBC, Bacteria, Urine culture, ketones. Only those patients with normal urine examinationresults were included. **Microalbuminuria**:

Was assessed by urine albumin: creatinine ratio (ACR) based on the recommendations of National Kidney Foundation and American Diabetic Association. The average of the ACR values from 3 urine samples were used. Urine albumin was estimated by turbidimetry. 1st voided midstream early morning sample of 5ml urine.Patients were asked to avoid exercise prior to collection, urine examination done in women in non menstrual phase.

Any value in between 30 – 300 µg/mg creatinine was taken as microalbuminuria.

Sex specific ACR cut off points: > 17µg/mg in men Sex specific ACR cut off points : > 25 µg/mg in women.

OBSERVATIONS

A total of 200 Pre-diabetic patients were studied.

Out patients - 132

In patients - 68

Table No:1 – Age Distribution

Age in years	No of patients	Percentage
30 – 39	26	13
40 – 49	36	18

50 – 59	59	29.5
60 – 69	68	34
70 – 79	11	5.5

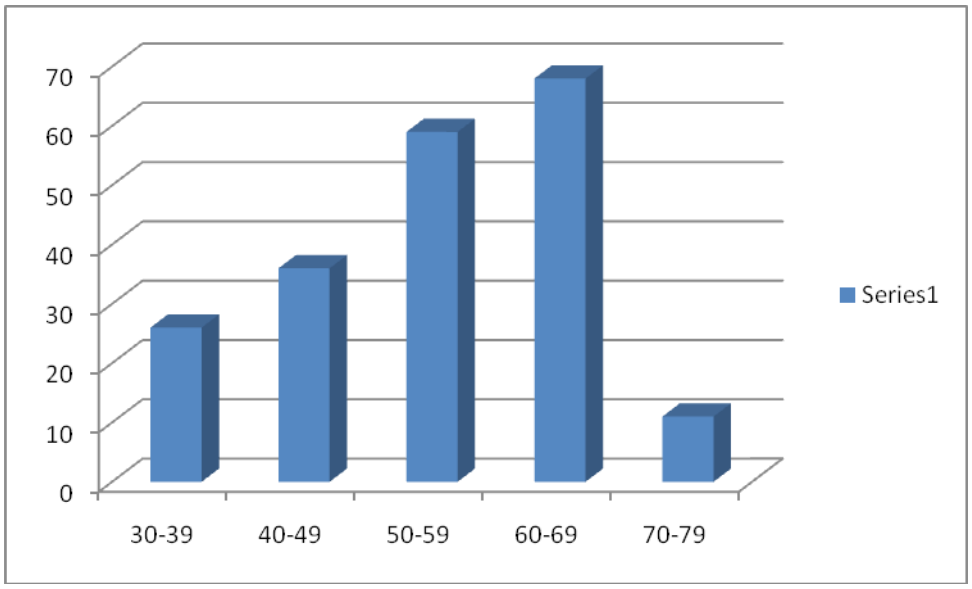


Table No:2 – Sex Distribution

Sex	No. of	Percentage

	patients	
Male	116	58
Female	84	42

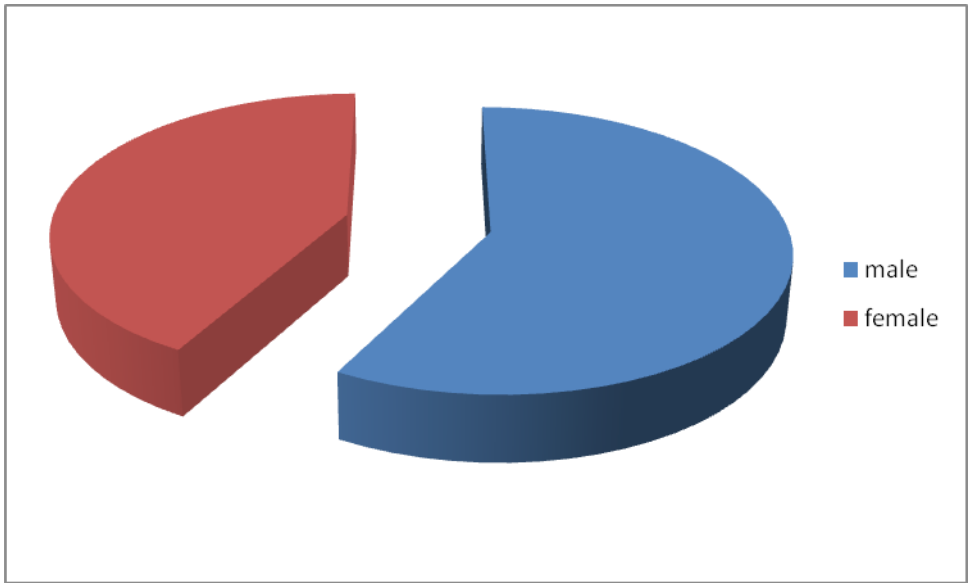
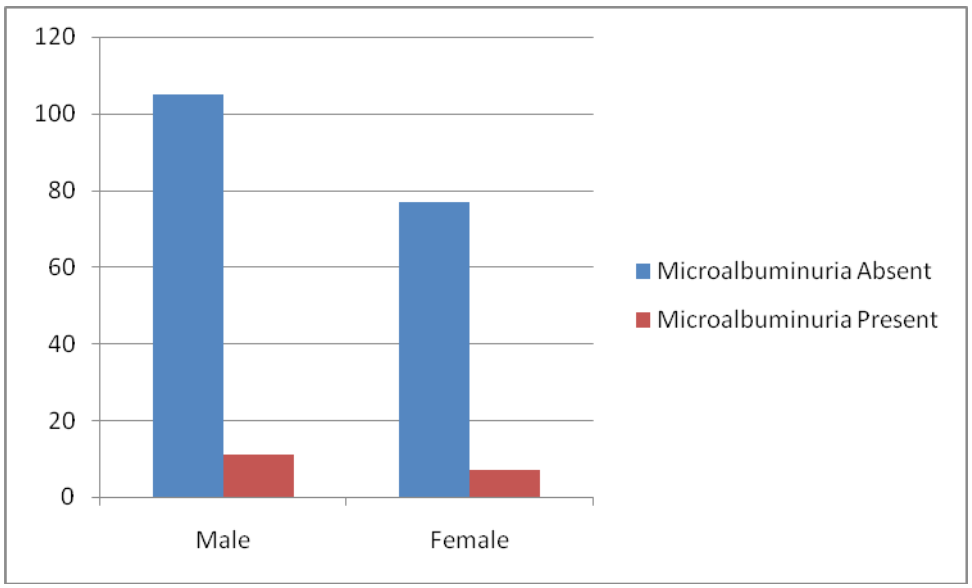


Table No:3 - Distribution of microalbuminuria among males & females

		Microalbuminuria				$\chi^2 = .05$
		Absent		Present		
		No.	%	No.	%	P = .
Sex	Male	102	89.5	12	10.5	81(Non
	Female	76	90.5	8	9.5	significant)

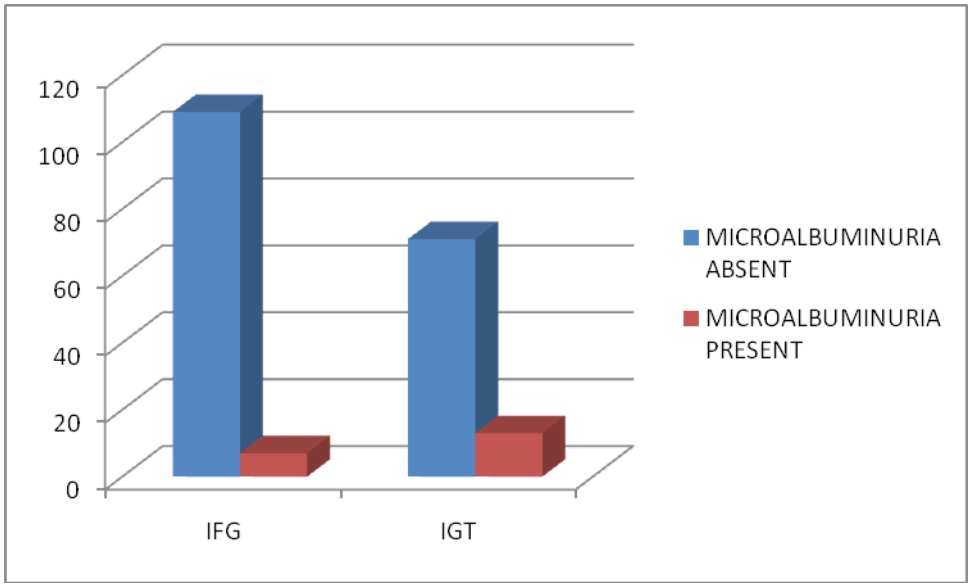


There was no statistically significant difference in the prevalence of microalbuminuria in male & female pre-diabetics(P=0.81).

	Absent	
	no	%
IFG	109	94%
IGT	75	85%

Table no:4-Distribution of Microalbuminuria in IFG and IGT.

	Microalbuminuria				
	Absent		Present		
	No.	%	No.	%	$\chi^2_{=4.83}$ P=.02
IFG	109	94%	7	6%	
IGT	71	84.5%	13	15.5%	

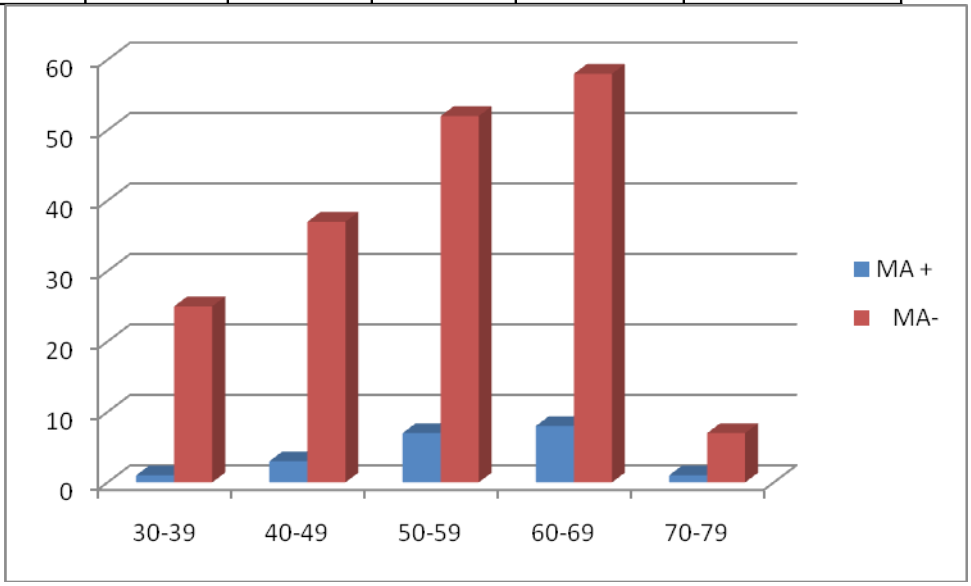


There is increased prevalence of microalbuminuria in IGT when

Compared to IFG, and it is statistically significant(P=0.02)

Table No:5–Distribution of microalbuminuria among different age groups

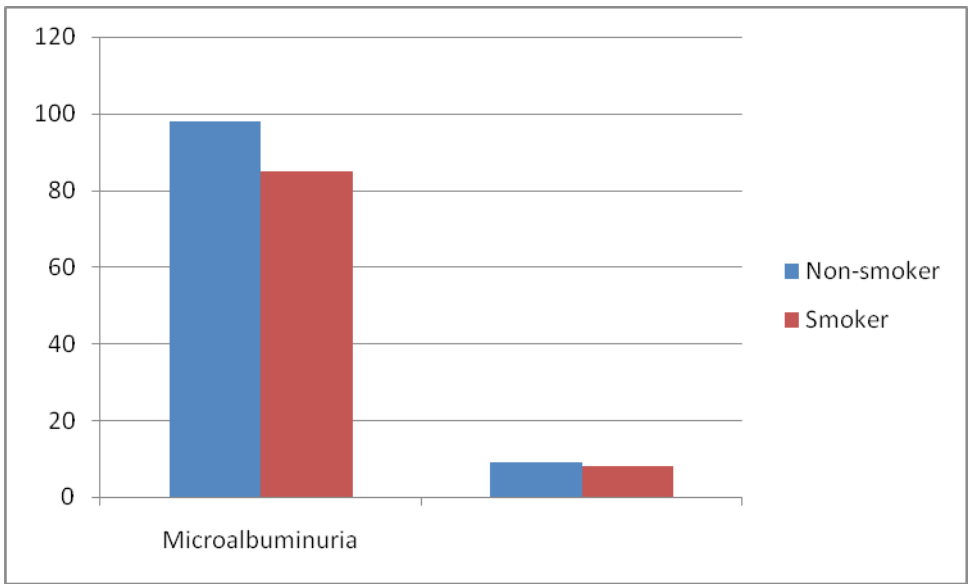
Age (years)	Microalbuminuria				
	Absent		Present		
	No	%	No	%	
30 – 39	25	92.3	1	7.7	χ^2=1.98
40 – 49	37	91.6	3	8.3	P =0.74
50 – 59	52	91.5	7	8.4	
60 – 69	58	91.1	8	8.8	
70 – 79	7	90.9	1	9	



It was observed that as the age advances,there is no statisticallysignificant increase in microalbuminuria.

Table No:6 – Distribution of microalbuminuria among smokers &non smokers

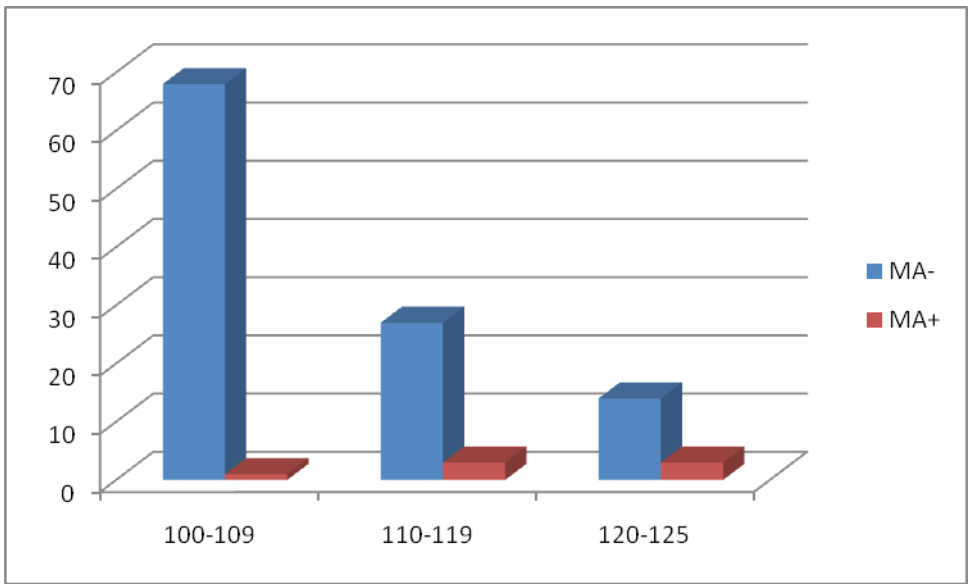
Smoking	Total No:	Microalbuminuria				χ^2 =0.02
		Absent		Present		
		No.	%	No.	%	
Nonsmoker	107	96	91.6	11	8.4	P=0.88
Smoker	93	84	91.4	9	8.6	



There was no statistically significant difference among the prevalence of microalbuminuria among smokers & non smokers.

Table No:7 – Microalbuminuria & the level of blood sugar in IFG

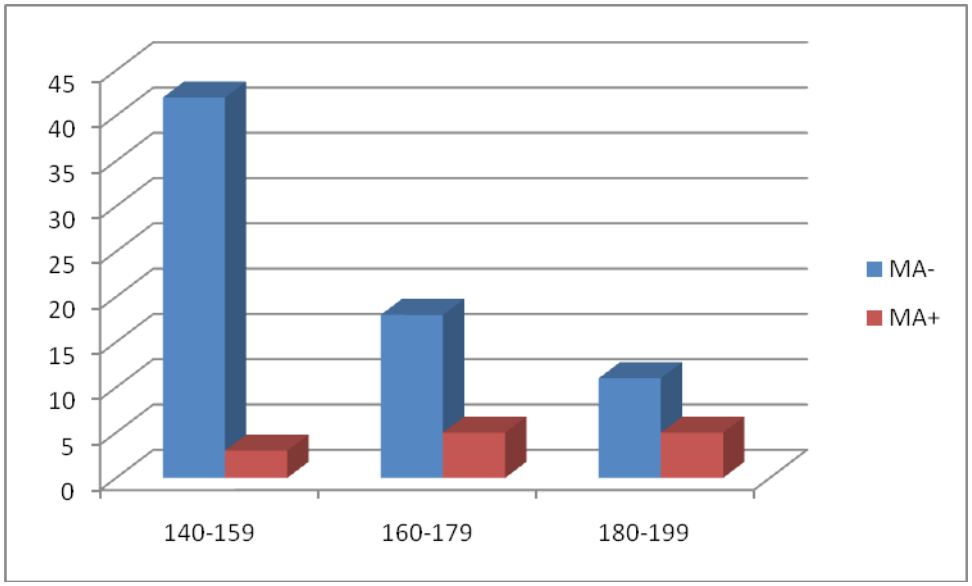
IFG	Microalbuminuria				χ^2 = 6.31
	Absent		Present		
	No	%	No	%	
100-109	68	98.6	1	1.4	
110-119	27	90	3	10	P<0.05
120-125	14	82.4	3	17.6	



The prevalence of microalbuminuria increases with increase in blood sugar in the pre-diabetic range and it is statisticaiiy significant(P value<0.05)

Tableno:8-Microalbuminuria and levels of blood sugar in IGT

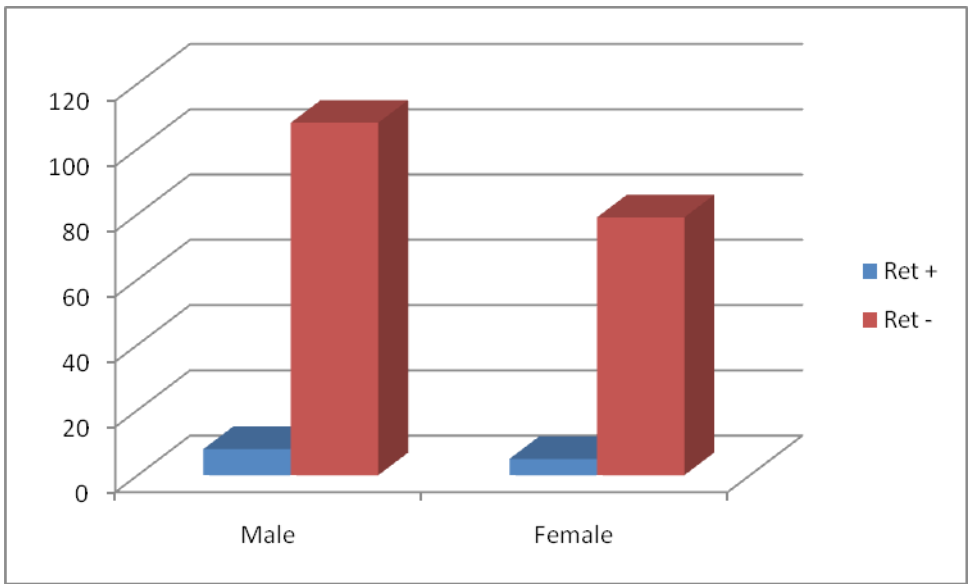
IGT	Microalbuminuria				χ^2 = 6.4
	Absent		Present		
	No	%	No	%	
140-159	42	93.3	3	6.7	P=.04
160-179	18	78.3	5	21.7	
180-199	11	68.8	5	31.2	



The prevalence of microalbuminuria increases as the blood sugar raises in the pre-diabetic range in IGT and it is statistically significant(P value=0.04).

Table no:8-Distribution of retinopathy in males and females

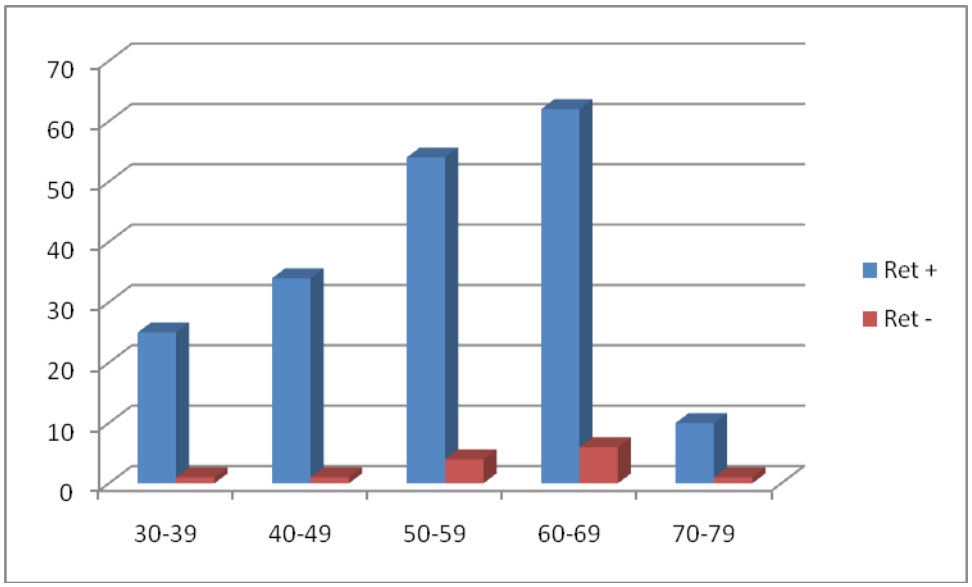
Sex	Retinopathy				
	Absent		Present		
	No.	%	No	%	χ^2 =0.07
male	108	92.2	8	7.8	P=.78
female	79	92.9	5	7.1	



It was observed that there was no statistically significant difference in prevalence of retinopathy in male and female pre-diabetic.

Table no:9-Distribution of retinopathy in different age groups.

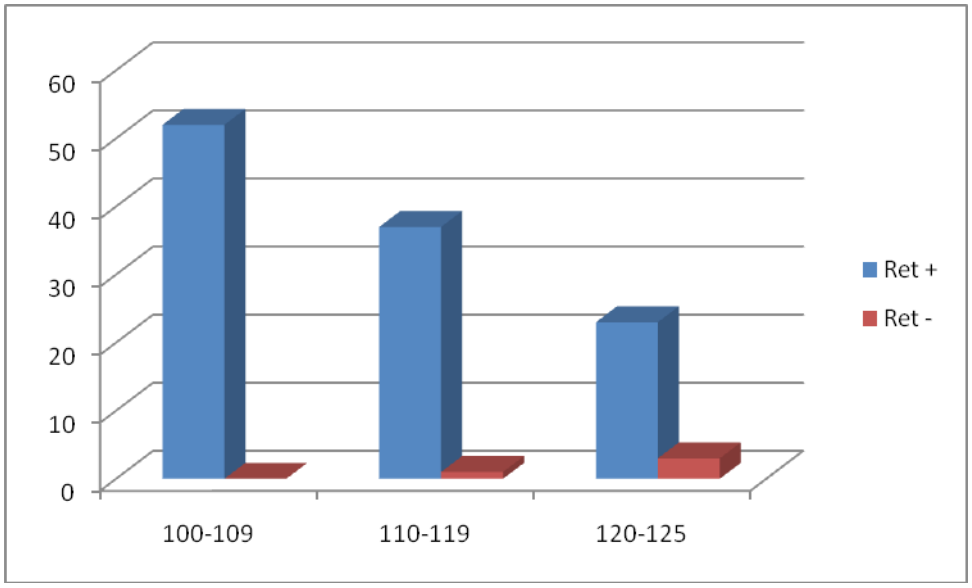
	Retinopathy				
Age	Absent		Present		
	No.	%	No	%	
30-39	25	96.2	1	3.8	
40-49	34	97.1	1	2.9	
50-59	54	93.1	4	6.9	χ^2
60-69	62	91.1	6	8.8	$=1.79$
70-79	10	90.9	1	9.1	P=0.77



As the age advances there is no statistically increase in prevalence of retinopathy.

Table no:10-Retinopathy and levels of blood sugar in IFG

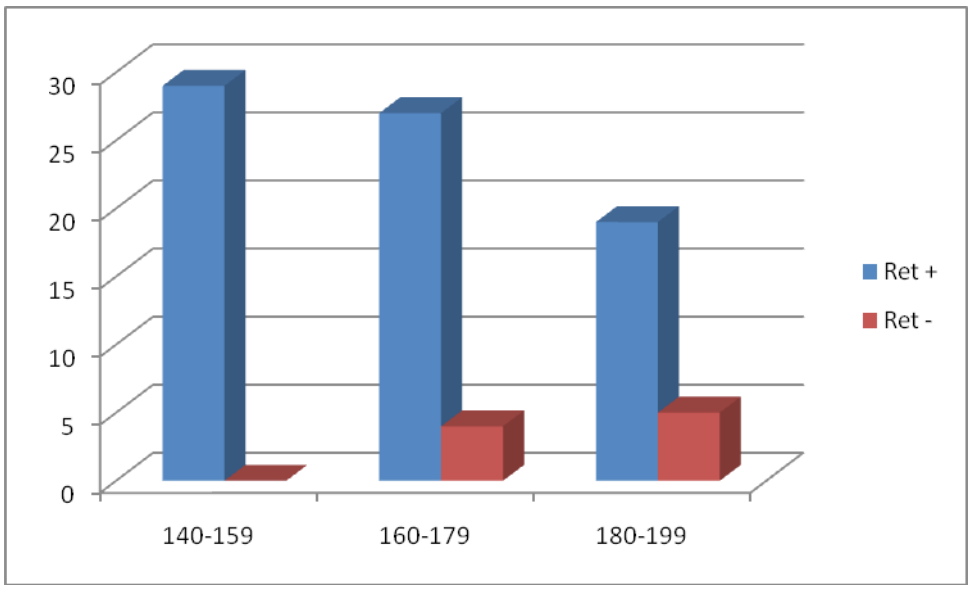
IFG	Retinopathy				
	Absent		Present		
	No	%	No	%	
100-109	52	100	0	0	$\chi^2 = 7.04$
`110-119	37	47.6	1	52.4	P = 0.029
120-125	23	82.2	3	17.8	



The prevalence of retinopathy increase as the blood sugar rises within the pre-diabetic range in IFG and it is statistically significant.

Table no:12- Retinopathy and levels of blood sugar in IGT

IGT	Microalbuminuria				
	Absent		Present		
	No	%	No	%	$\chi^2 = 12.4$ P = 0.001
140-159	29	100	0	0	
160-179	27	87.1	4	12.9	
180-199	19	79.1	5	20.8	



There is increase in prevalence of retinopathy as the blood sugar rises within pre-diabetic range in IGT and it is statistically significant.

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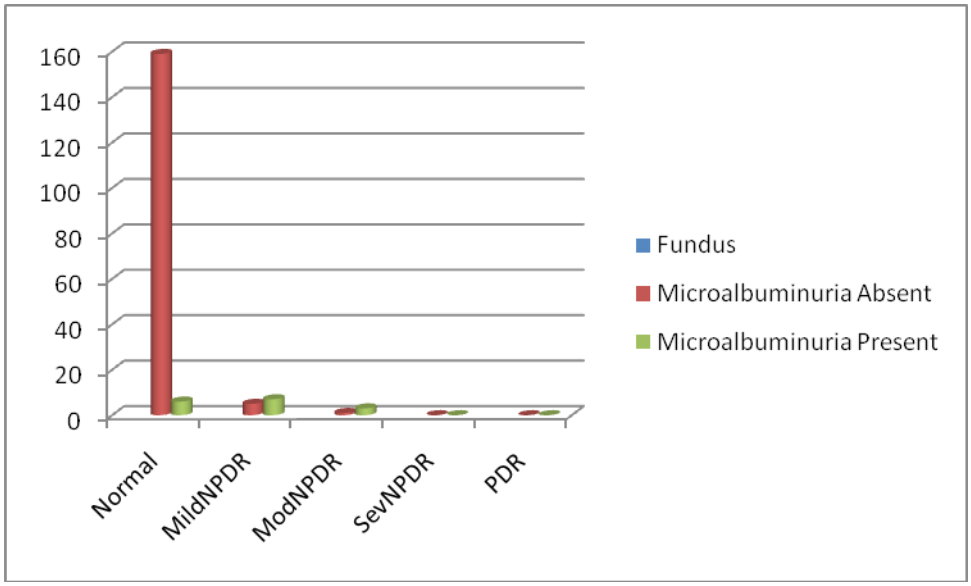
Table no:13-Microalbuminuria and retinopathy changes

Fundus	Total No: of cases	Microalbuminuria				
		Absent		Present		
		No	%	No.	%	
Normal	165	159	96.4	7	3.6	χ^2 =65.35
MildNPDR	11	4	41.7	7	58.3	P < 0.0001
Moderate.NPDR	2	0	33.3	2	66.7	
Severe.NPDR	0					
PDR	0					



A higher proportion of patients with early diabetic retinopathy had microalbuminuria compared to those with out retinopathy changes. the association between the two were statistically significant.[P<0.0001]

Abnormalities in Fundus Examination:

13 patients had the changes of Diabetic Retinopathy.

165 patients had normal fundus.

20 patients fundus not visualized(due to hazy media).

9 patients had associated microalbuminuria(69.2%)

4 patients had no microalbuminuria(30.8%)

DISCUSSION

The present study evaluated the prevalence of microalbuminuria and retinopathy

in pre-diabetic patients and correlation between microalbuminuria,retinopathy with age,smoking and aiso levels of fasting blood sugar and OGTT.

Prevalence : The prevalence of microalbuminuria in this study was 10% and that of retinopathy was 6.5%.The prevalence of microalbuminuria is more in IGT(15.5%) compared to IFG(6%).A similiar study by WangXL etal showed a prevalence of microalbuminuria as 11.1% in IGT and 5.8 % in IFG.When the blood sugar rises in the pre-diabetic range in IFG and IGT the prevalence of microalbuminuria also rises. The prevalence of retinopathy was also more in IGT(12%) compared to IFG(3-6%).The prevalence was higher in those with higher blood sugar both in IFG and IGT.The DPP Outcome Study(Diabetes Prevention Program) showed a prevalence of retinopathy as 7.6% in those with pre-diabetes using Fasting blood glucose.A higher proportion of patients with retinopathy had microalbuminuria and association prevalence was statistically significant.

Sex distribution of microalbuminuria and retinopathy: In the present study, for both microalbuminuria and retinopathy there was no significant difference of prevalence in men and women. In the HUNT study (Norway) a stronger association was observed between microalbuminuria and mortality in men than in women. In this study the interaction between sex and Albumin creatinine Ratio (ACR) was statistically significant (p= 0.003) and supported a sex difference, women had more prevalence. They attributed this difference to the higher incidence of asymptomatic UTI in women. They suggested the need for different ACR cut off values in men and women – because men

have greater muscle mass and higher creatinine excretion than women although albumin excretion levels are equal[13]. (HUNT – Nord Trondelag Health Study).

The prevalence of microalbuminuria and retinopathy was not statistically higher in the older age groups.The present study also looked into the relation between microalbuminuria and other risk factors for cardiovascular disease like smoking.No significant correlation was observed between smoking and microalbuminuria.. This observation is discordant with that seen in previous studies on this aspect. One possible explanation for this variability – may be an error in the classification of smokers and non smokers – this was done on the basis of history.

CONCLUSIONS

1.Pre-diabetes is a mounting health problem in the community early diagnosis and intervention delay the onset of diabetes and the microvascular complications.

2.The microvascular complications like retinopathy and microalbuminuria starts in the pre-diabetic stage itself.

3.Prevalence of microalbuminuria and retinopathy was more in IGT when compared to IFG.

4.There is significant correlation between microalbuminuria and retinopathy in pre-diabetic patients.

5.The IGT is more important cardiovascular risk factor than IFG .

6.Further studies including large population are needed to know the prevalence of retinopathy and microalbuminuria in pre-diabetic individuals.

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