STUDY OF ASSOCIATION OF SERUM URIC ACID WITH ALBUMINURIA IN TYPE II DIABETES MELLITUS

By

DR. PRITI S. LANGOTE

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Under the guidance of

Dr. M.S.MULIMANI **M.D.**

Professor and H.O.D,

Department of Medicine,

SHRI B.M. PATIL MEDICAL COLLEGE, VIJAYAPUR

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Date:

Place: Vijayapur **DR.PRITI S. LANGOTE**

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Place:

Date:

Dr. M. S. Mulimani M.D.
Professor and HOD,
Medicine Department,
Shri B. M. Patil Medical
College, Vijayapur.

B.L.D.E UNIVERSITY'S

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RESEARCH CENTRE, VIJAYAPUR.

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HOD of Medicine

Seal & Signature

Dr. M.S.MULIMANI
M.D.(Medicine)
BLDEU's Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur.

Date:

Place: Vijayapur

Principal

Seal & Signature

DR.M.S.BIRADAR
M.D.(Medicine)
BLDEU's Shri B.M. Patil Medical College, Hospital and Research centre, Vijayapur

Date:

Place: Vijayapur

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Place: Vijayapur

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DR.PRITIS LANGOTE

LIST OF ABBREVIATIONS USED

ACR	Albumin creatinine ratio
CBC	Complete blood count
CKD	Chronic kidney disease
DM	Diabetes mellitus
DN	Diabetic nephropathy
ESRD	End stage renal disease
FBS	Fasting blood sugar
GFR	Glomerular filtration rate
HbA1c	Glycosylated Haemoglobin
ICAM	Intercellular adhesion molecule
IDDM	Insulin Dependent Diabetes Mellitus
NIDDM	Non-Insulin Dependent Diabetes Mellitus
RAAS	Renin Angiotensin Aldosterone System
UA	Uric acid
UAER	Urine albumin excretion rate
SUA	Serum Uric Acid
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

ABSTRACT

BACKGROUND AND OBJECTIVES:

Diabetic nephropathy (DN) is the most common cause of chronic kidney disease worldwide.[1-2]In the wake of the current epidemic of diabetes mellitus (DM), the prevalence of DN and end stage renal disease (ESRD) is projected to rise.[3]

Different therapeutic strategies targeting DN have been explored such as tight glycemic control, [4] tight blood pressure control[5] and various inhibitors of the renin angiotensin aldosterone system (RAAS).[6-8] while these therapies appear to slow the progression of kidney disease due to diabetes, none of them are curative. Thus we require adjunctive therapeutic strategies; especially in patients with complications of treatment or lack of appropriate response.[9] hence there is a pressing interest to identify other potentially modifiable factors in the progression of DN.

Inflammation and endothelial dysfunction appear to play a central role in the onset and the progression of DN. Recent evidence has emerged in the last decade to suggest uric acid is an inflammatory factor and may play a role in endothelial dysfunction.

Studies suggest that treatment of diabetic nephropathy may be benefited by treatment with xanthine oxidase inhibitor.[10]

The aim of this study is to find an association between serum uric acid level and albuminuria in type 2 diabetes mellitus (T2DM).

METHODS:

This study was carried out in B.L.D.E.U's Shri B.M. Patil Medical College Hospital and Research Centre, Vijayapur, Karnataka; during the period from December 2013 to July 2015. A total of 56 patients who were known case of Type 2

diabetes mellitus were included in the study. The blood of the selected subjects were analysed for Serum uric acid and Urine albumin creatinine ratio.

RESULTS:

Serum uric acid levels for, and

1. Normoalbuminuric patients=4.09±1.36 mg/dl
2. Microalbuminuric patients=5.21±1.60 mg/dl
3. Macroalbuminuric patients=7.38±0.87mg/dl

Serum uric acid level correlated positively with urinary albumin creatinine ratio ($r = 0.559$, $p = <0.001$).

CONCLUSION:

Serum uric acid had a significant positive correlation with albuminuria in type 2 diabetes mellitus.

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INTRODUCTION

Uric acid is end product of purine metabolism; it is filtered in glomeruli and excreted in urine. Hyperuricemia is defined by serum uric acid concentration greater than 7 mg/dl in man and 6 mg/dl in women.[11] Hyperuricemia due to genetic abnormality in the purine metabolism is a well-known cause of gout. Hyperuricemia is present in normal population by about 14% in males and 1% in females as compared to its 32% prevalence in type 2 diabetic patients.[12] The frequency of hyperuricemia is more in the obese type 2 diabetic patients than those without obesity.[13] Similarly other literature has mentioned its association with metabolic syndrome or insulin resistance.[14]

Apart from this, hyperuricemia is an associated factor with multiple other disorders. Uric acid is a common risk factor for, vascular diseases, [15] hypertension, [16] type 2 diabetes mellitus and diabetic nephropathy.[17] Hyperuricemia has an association with type 2 diabetes mellitus and hypertension independently of each other. It is more commonly associated with albuminuria than those without albuminuria in type 2 diabetic patients.[17]

Though the cause and effect relationship of hyperuricemia and diabetic nephropathy is debatable, however some literature mentions the detrimental effects of high uric acid level on the kidney functions.[18] The main detrimental effect of high uric acid level as a part of the obesity and metabolic syndrome is through its injurious effects on the endothelium and inducing chronic inflammation.[19] Some studies have shown slowing the progression of renal disease by treating the hyperuricemia with allopurinol.[16]

Diabetic nephropathy is leading cause of end stage renal disease worldwide contributes to patient morbidity, mortality and increases nation's health care costs.[20]

Diabetic and non-diabetic patients with albuminuria have increased cardiovascular mortality.[21, 22]

Accumulating data reveal that inflammation, endothelial dysfunction and procoagulant imbalance are associated with nephropathy, retinopathy, and cardiovascular disease in Diabetes Mellitus (DM).[23-25] The association of serum UA levels and dyslipidemia with inflammation and endothelial dysfunction are also shown.[26, 27] However, the putative association between serum UA levels and albuminuria is not clear.

The pathogenesis of diabetic nephropathy is incompletely understood but may include glycosylation of circulating and intrarenal proteins and abnormal intrarenal hemodynamics. Hyperglycemia may cause an increase in mesangial cell glucose concentration and glycation of matrix proteins that lead to increased matrix production and mesangial cell apoptosis.[28] Cytokines activation, inflammation, and vascular growth factors may be responsible in the matrix accumulation in diabetic nephropathy.[29] Glomerular hypertension and hyperfiltration are also responsible for development and progression of in diabetic nephropathy, hence, blockade of the renin-angiotensin system is beneficial in the treatment of disease.

Detection of microalbuminuria is the screening method of choice for identifying of early stage of diabetic nephropathy.[30] Correlation of serum uric acid (UA) with diabetic nephropathy was shown in several studies. For example level of serum UA was shown significantly higher in type 1 diabetic patients with persistent macroalbuminuria, compared to patients with normoalbuminuria.[31] Elevated UA is associated with endothelial dysfunction, insulin resistance, development of hypertension, and cardiovascular disease.[32] Elevated serum uric acid may be also associated with progression of non-diabetic renal disease.[33]

Currently renin-angiotensin system blockade is the gold standard in diabetic nephropathy treatment that leads to slowing the renal impairment but not arrest or reverse of the disease. Thus we require adjunctive therapeutic strategies, especially in patients with complications of treatment or lack of appropriate response.

Recently, some prospective randomized controlled trials suggested that lowering of uric acid with allopurinol could decrease the severity of proteinuria and probably slow the progression of renal failure in diabetic patients[34] and also in the patients with hyperuricemia and non-diabetic chronic kidney disease.[16] Mechanism of beneficial effect of xanthine oxidase inhibitor may be related to preventing uric acid-induced renal inflammation.[10] Indeed, allopurinol decrease serum uric acid level and reduce oxidative stress, it is not exactly obvious the main beneficial mechanism of allopurinol in the diabetic nephropathy.

In conclusion, it seems that further studies are needed to clarify the effect of uric acid in initiation and progression of diabetic nephropathy and effect of uric acid lowering drugs on preventing or slowing of disease progression.

AIMS AND OBJECTIVES OF THE STUDY

To find out association of serum uric acid level with albuminuria in type 2 diabetic patients.

REVIEW OF LITERATURE:

HISTORICAL REVIEW:

Diabetes was for many years regarded as the disease of the kidneys. This was the opinion of Aretaeus, Capadcian in second century AD. The view was still held by Erasmus Darwin in 1801. The presence of proteinuria in diabetes mellitus had long been known. Contunniues (1770), Rollo (1798), Darwin (1801), Rayer (1840), Van Noorden (1912), all had described the association of dropsy with diabetes. Vacuolization of tubular epithelium was observed by Armani (1875) and Ebstein (1881) and was shown to be due to glycogen infiltration by Ehlrich in 1888. Kimmelstiel and Wilson were the first to attribute specific glomerular lesions entitled inter capillary lesions in the glomeruli of kidney. These peripheral hyaline masses are known as Kimmelstiel Wilson lesions. These histological features were associated with clinical features of diabetes, hypertension, nephrotic syndrome and renal failure. Fahr demonstrated soon after the diffuse lesions in 1942

Several major studies among diabetics have been undertaken (Tuft et al 1956, Farqahar et al 1959, Gellman et al 1959, Hatch et al 1961, O Sullivan et al) to demonstrate proteinuria especially when diffuse changes are considered.[35]

In 1963 Keen and Chlouvervakis developed sensitive and specific Radioimmunoassay for detecting human albumin in low concentration i.e. microalbuminuria, which indicate earliest stage of diabetic renal disease.[36]

The first suggestion, that pro-inflammatory cytokines could participate in the development of DN, was made in 1991 by Hasegawa et al.[37,38] They reported that glomerular basement membranes from diabetic rats induced significantly greater

amounts of TNF-α and IL-1 in cultured peritoneal macrophages than when these cells were incubated with basement membranes from nondiabetic rats.[37]

Elevated SUA itself may increase the risk for development of renal disease in both the general population and patients with diabetes. A study from Japan in 2001 showed that hyperuricemia emerged as the only significant risk factor of renal failure besides age.[39]

Bo et al in 2001, examined 2,113 patients with type 2 diabetes in Italy and found that hyperuricemia was associated with insulin resistance and with early onset or deterioration of diabetic nephropathy.[40]

In 2005, a study was done among 343 adults with type 2 diabetes in Taiwan, China, [41] in which hyperuricemia was correlated with albumin excretion rate. The respective uric acid levels for normoalbuminuria, microalbuminuria and macroalbuminuria were 5.2± 1.6 mg/dl, 5.6 ± 1.9 mg/dl and 6.7± 2.1 mg/dl ($P < 0.001$).The standardized regression coefficient for Albumin Creatinine ratio (ACR) and the odds ratio for abnormal albuminuria for every 1 mg/dl increment of uric acid after adjusting for calculated creatinine clearance and other confounders were 0.138 ($P < 0.05$) and 1.183 (1.025–1.364), respectively.

In 2009, an animal study was done in type 2 diabetic db/db mice to study effect of lowering uric acid on renal disease.[10] Diabetic (*db/db*) mice were treated with allopurinol or no treatment for 8 weeks. They concluded that *db/db* develop hyperuricemia, which may have a role in mediating tubulointerstitial injury associated with diabetes. Lowering uric acid improved renal function, proteinuria, and tubulointerstitial damage, and the mechanism is likely due to blocking uric acid-induced intrarenal inflammation.

A double-blinded randomized controlled trial was carried by Momeni A et al,[34] in 2010, on 40 patients with T2DM and DN. Allopurinol (100 mg/d) was compared with placebo. Administration of antihypertensive and renoprotective drugs continued for both the groups. And it was observed that serum levels of uric acid (P = .02) and 24-hour urine protein (P = .049) were significantly lower in the patients on allopurinol group, after 4 months of receiving allopurinol, compared with the control group.

Bonakdaran S et al[17] carried a study in 2011, in 1275 type 2 diabetic patients. Serum uric acid levels for normoalbuminuric, microalbuminuric and macroalbuminuric patients were 4.49 ± 1.22 mg/dl, 4.84 ± 1.52 mg/dl, and 6.15 ± 1.68 mg/dl, respectively. The association between serum uric acid concentration and degree of urinary albumin excretion was significant even after adjustment for estimated GFR.

Xiao-ling C et al, [42] in 2011, carried a study in China. Multivariate Logistic regression analysis was performed to evaluate the predictors of albuminuria. Result showed that diabetic duration, Systolic Blood Pressure, Creatinine and Serum uric acid (SUA) are the independent predictors of albuminuria. And all are positively correlated with it. (For SUA, p=0.001)

A Korean study in 504 type 2 diabetic patients by Kim ES, et al[43] showed that Uric acid level remains a significant predictor for abnormal albuminuria after adjusting for Metabolic syndrome presence as well as the use of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) (OR 1.414, 95% CI 1.071-1.868).

A cross-sectional analytical study conducted in 2013, by Behradmanesh S, Horestani MK, Baradaran A, et al[44] in 60 patients with T2DM without a history of gout, showed a significant positive association of serum uric acid with the level of proteinuria was seen ($r = 0.47$, $P < 0.001$), after adjustment of weight.

A study was carried in Pakistan in 2013[45] in 163 diagnosed type 2 diabetic patients. In this study, Hyperuricemia was more prevalent in patients with diabetic nephropathy 50% (n=16) than those without nephropathy 19% (n=25).

A retrospective study was carried out by Akbas et al[46] in Turkey in 2013-2014, involving 645 diabetic patients. Positive correlation was determined between serum UA and albuminuria (r = 0.108, $P = 0.006$) and it was found by logistic regression analysis that albuminuria is associated independently with serum UA levels.

EPIDEMIOLOGY OF DM AND DN

GLOBAL CONSIDERATION

The prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 382 million in 2013.Based on current trends, the International Diabetes Federation projects that 592 million individuals will have diabetes by the year 2035.In 2013, the prevalence of diabetes in individuals from age 20-79 ranged from 23-37% in different countries. In India, it was 65.1million. Centers for Disease Control and Prevention (CDC) estimated that the incidence and prevalence of diabetes doubled from 1990-2008, but appears to have plateaued from 2008-2012.

The global burden due to diabetes is mostly contributed by type 2 diabetes which constitutes 80-95% of total diabetic population.

PREVALENCE IN INDIA

Prevalence of diabetes in India in 1970's was 2.3% in urban area and 1.5% in rural areas, as shown by multicentric study by the Indian Council of Medical Research (ICMR).In 2000,the prevalence has risen to 12% to 19% in urban areas and to 4% to9% in rural areas. A study from rural Andhra Pradesh reported a prevalence of 13.2%.

National studies or population based studies on diabetic complications are sparse in India. A few population based studies indicate the prevalence of retinopathy to be 18%to 27% and overt nephropathy to be about 2.2% with a large percentage (27%) having microalbuminuria. Peripheral vascular disease is prevalent in 6.3%, peripheral neuropathy in 26% and coronary artery disease (CAD) is detected in 21%.

DIABETIC NEPHROPATHY

Definition and Diagnosis

Incipient nephropathy

Diagnosis of incipient nephropathy rests on the demonstration of persistent microalbuminuria without other urinary abnormalities or evidence of urinary tract infection or heart failure. Definition of microalbuminuria, however, was a matter of controversy until very recently. A general consensus is now emerging on defining as microalbuminuria asACR between 30µg/mg and 299µg/mg.

Overt nephropathy

It is a clinical syndrome characterized by persistent albuminuria (>300 mg/24 h orACR≥ 300 µg/mg), on at least two occasions separated by 3-6 months. This is equivalent to total proteinuria>500 mg/24 h which can be diagnosed clinically if the following additional criteria are fulfilled: presence of diabetic retinopathy and the absence of clinical or laboratory evidence of other kidney or renal tract disease[47].

Patients invariably develop associated hypertension, a progressive increase in proteinuria and a predictable and relentless decline in glomerular filtration rate (GFR).[48, 49]

NATURAL HISTORY OF NEPHROPATHY IN T2DM [50]

The natural history of diabetic nephropathy in patients with T2DM is less well understood than in patients with T1DM. This is because of the fact that T2DM is largely a disease of an older population, with associated obesity, hypertension, & dyslipidemia &high rates of cardiovascular disease that restrict the manifestation of diabetic renal disease. In addition approximately 7% of patients with T2DM already

have microalbuminuria at the time of diagnosis.[51] This may be partly related to the fact that most of these patients have had untreated diabetes for 10 years (on average) before diagnosis. Within 5 years of a diagnosis of T2DM, up to 18% of patients have microalbuminuria, especially those with poor metabolic control and high BP levels.[52]

Table1.Stages of diabetic nephropathy

Stage	Designation	Glomerular filtration	Albuminuria	Blood Pressure	Years after diagnosis
1	Hyper function / Hypertrophy	Elevated	Absent	Normal	At diagnosis
2	The Silent Stage	High normal	Absent	Normal	At diagnosis
3	Microalbuminuria / Incipient Nephropathy	Within the normal range	20-200mcg/min (30-300mg/day)	Increasing-3mmhg/yr	5-15
4	Macroalbuminuria/ overt nephropathy	Decreasing	>200mcg/min (>300mg/day)	Usually Frank	10-15
5	End stage renal Disease	Diminished	Macroalbuminuria, decreasing Due to glomerular occlusion	Hypertension	15-30

Stage 1: Hyperfiltration

The initial phase has been termed the hyper filtration phase. It is associated with an elevation of glomerular filtration rate (GFR) and an increase in capillary glomerular pressure. Hyperfiltration is considered to occur as a result of concomitant renal hypertrophy as well as being partly due to a range of intrarenal

Hemodynamic abnormalities that occur in the diabetic milieu that contributes to glomerular hypertension, which is thought to be an important factor in the development and progression of diabetic nephropathy. Hyperfiltration is less common in T2DM compared to T1DM. In newly diagnosed patients with type 2 diabetes, 30-40% has an elevated GFR.[53] This observation must be interpreted with caution, because GFR normally declines with age, and hyperfiltration can still exist although the GFR remains in the normal adult range. Institution of effective therapy and the consequent improvement in glycemic control leads to a decrease in GFR. These changes are seen predominantly in the younger patients.[54, 55, and 56]

Stage 2: The Silent Stage

The next stage is known as the silent stage, where, from, clinical point of view, there is no overt evidence of any form of renal dysfunction, Patients usually have normal GFR with no evidence of albuminuria. However, this phase is associated with significant structural changes including basement membrane thickening and mesangial expansion. The hyperfiltration is related to the degree of hyperglycemia up to 250 mg/ dl (14mmol/L). Levels of glycaemia higher than this are associated with a reduction in GFR.

Stage 3: Microalbuminuria

Stage 3 is microalbuminuria or incipient nephropathy and it usually occurs after 6-15 years of diabetes. It is present in 20-30% of type 2 diabetics at initial diagnosis.[53] The GFR may still be elevated or may be reduced into the normal range. Urine albumin excretion rate (UAER) during this stage is 20-200 micrograms/min (30-300 mg/24 hrs). The development of micro albuminuria is associated with a small but detectable increase in blood pressure although, at this stage, it usually remains within the conventional age-corrected normal ranges. Impairment of the normal nocturnal 'dipping' of blood pressure on 24-h ambulatory blood pressure monitoring has also been reported. There is also further progression of the histological changes, with increase in basement membrane thickness and fractional mesangial volumes within the glomerulus, which will ultimately impinge on the filtration surfaces. In adolescents who develop microalbuminuria, those with initial hyperfiltration have a greater subsequent rate of decline in GFR (1.1 mL/min/year) compared without (0.8 mL/min/year).[54] The rate of fall in GFR is positively correlated with glomerular basement membrane thickness, fractional mesangial volume and interstitial volume fraction. However finding of microalbuminuria may not be specific for diabetic renal disease as in T1DM.

Stage 4: Macroalbuminuria

The next stage is the macroalbuminuria phase or overt nephropathy. This stage represents the phase that has been previously described as diabetic nephropathy and is highly predictive of subsequent renal failure if left untreated. It is characterized by a urinary albumin excretion rate greater than 300 mg/24 hours (200micro grams/min). This phase usually occurs after 10 to 15 years of diabetes. Proteinuria increases at the rate of 15% to 40% per annum. The GFR commences an inexorable decline, By

comparison with type 1, the rate of decline in GFR in type 2 diabetics with established nephropathy has a wider range, with a mean of 5-10 ml/min/year but with a range of 1-20 ml/min/year. The rate of decline of GFR is still generally predictable in a given patient, unless a superimposed illness develops. The rate of decline correlates with blood pressure levels; in patients with type 2 diabetes, this correlation is particularly evident for the systolic blood pressure. Progressors are depicted by poorer glycemic control, albumin excretion rates, hypercholesterolemia, and smoking.

Stage 5: ESRD

Stage 5, the development of ESRD, ensues within a median of 7 years from the development of persistent proteinuria, if therapeutic interventions are not undertaken.

PATHOLOGY

Pathologic abnormalities are noted in patients with long-standing diabetes mellitus before the onset of microalbuminuria. There are three major histologic changes in the glomeruli in diabetic nephropathy, mesangial expansion, glomerular basement membrane thickening, and glomerular sclerosis. The last abnormality, which may have a nodular appearance (the Kimmelstiel-Wilson lesion), is often associated with hyaline deposits in the glomerular arterioles.[57] These different histologic patterns appear to have similar prognostic significance.[58] The mesangial expansion and glomerulosclerosis do not always develop in parallel, suggesting that they may have somewhat different underlying pathogenesis.[59]

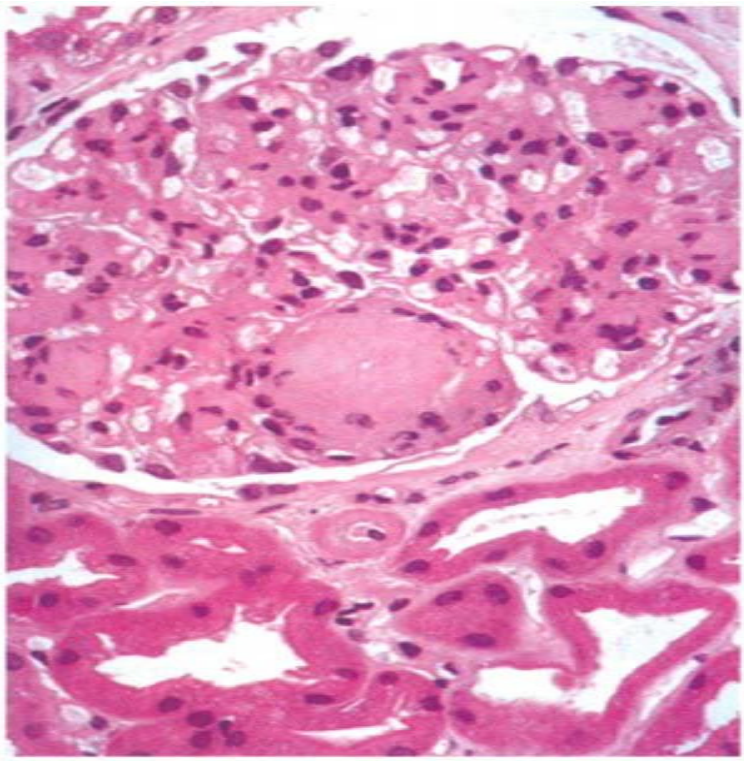


Figure 1: Glomerular and tubular manifestations of diabetic nephropathy. GBM, glomerular basement membrane; TBM, tubular basement membrane

RISK FACTORS:

In Studies, in patients who have or do not have clinically evident diabetic nephropathy, a number of factors as being associated with increased risk of renal involvement are identified.[60]

1. Genetic and environmental factors [61]

10-20% of those with type 2 diabetes develop nephropathy. Nephropathy may develop in some patients despite good glycemic control and it may fail to develop in others despite years of severe hyperglycemia. These findings suggest that non-metabolic influences such as familial, ethnic and environmental factors may also contribute to the development of diabetic nephropathy.

The prevalence of nephropathy among diabetic patients varies between different racial and ethnic groups such that it is relatively increased in African Americans, Native Americans and urbanized Indo-Asian when compared with Caucasians. Familial clustering of diabetic nephropathy has been reported in both type 1 and in type 2 diabetes. However, linkage between specific genes and the development of diabetic renal disease system has not been conclusively demonstrated although several studies have suggested a detrimental effect of the double deletion (DD) polymorphism of the ACE genotype on disease progression.[62] In addition shared environmental influences like tobacco smoking & intrauterine malnutrition may also contribute to this familial clustering. It is hypothesized that this may, in turn, lead to compensatory glomerular hypertrophy with the acceleration of glomerular injury in the setting of further renal insult, such as diabetes.

2. Race

The incidence and severity of diabetic nephropathy are increased in blacks (3- to 6-fold compared to Caucasians), Mexican-Americans, and Pima Indians with type 2 diabetes.[63] This observation in such genetically disparate populations suggests a primary role for socioeconomic factors, such as diet, poor control of hyperglycemia, hypertension, and obesity.

3. Obesity

A high body mass index (BMI) has been associated with an increased risk of chronic kidney disease among patients with diabetes.[64] In addition, diet and weight loss may reduce proteinuria and improve kidney function among patients with diabetes.[65, 66]

4. Smoking

Smoking is associated with a variety of adverse effects in patients with diabetes. This includes evidence of increases in albuminuria and the risk of end-stage renal disease and of decreased survival once dialysis is begun.[67]

5. Blood pressure

Prospective studies have noted an association between the subsequent development of nephropathy in type 2diabetes and higher systemic pressures.[68]

6. Glomerular filtration rate

Those patients with glomerular hyperfiltration appear to be at increased risk for diabetic renal disease.[69] This is particularly true for overt nephropathy if the initial GFR is above 150 ml/min; in comparison, lesser degrees of hyperfiltration may have a slower course, with a lesser risk for microalbuminuria.[70]

The potential importance of intraglomerular hypertension in the pathogenesis of diabetic nephropathy may explain why systemic hypertension is an important risk factor for the development of diabetic nephropathy. Studies in experimental animals suggest that the diabetic state is associated with impaired renal autoregulation. As a result, raising the systemic pressure does not produce the expected afferent arteriolar vasoconstriction that would minimize transmission of the elevated pressure to the glomerulus.[71]

7. Glycemic control

Diabetic nephropathy is more likely to develop in patients with worse glycemic control (higher HbA1c levels).[72] The mechanisms by which hyperglycemia induces DN are complex and may involve not only effects of elevated glucose levels

per se but also the generation from the hyperglycemic state of glycated proteins and alcohol sugars (polyols).

PATHOGENESIS

There appear to be different pathogenetic processes leading to the pathologic mechanisms in diabetic nephropathy.

Hemodynamic changes [73]

Hyperfiltration is common in early diabetes. The mechanism for the increased GFR is not completely elucidated, but may involve glucose-dependent effects on afferent arteriolar dilation, mediated by a range of vasoactive hormones and cytokines including insulin-like growth factor 1 (IGF-1), nitric oxide, prostaglandins and/or glucagon.

Renal hypertrophy

The early hyperfiltration response is associated with both glomerular and tubulointerstitial proliferation and hypertrophy. Kidney size may increase by several centimeters. Glomeruli enlarge with both an increase in capillary loop number and surface area. Most of the changes in the glomeruli are due to hypertrophy, whereas the tubular epithelial cells undergo both proliferation and an increase in cellular size. The mechanisms by which elevated plasma glucose levels cause hypertrophy appears to be due to the stimulation of a variety of growth factors within the kidney, including IGF-l, epidermal growth factor (EGF), platelet-derived growth factor, and transforming growth factor(TGF-beta). Proliferation and hypertrophy may also be initiated by the fall within the kidney of certain anti proliferative factors, such as SPARC, the secreted protein, acidic and rich in cysteine. 'The role of TGF-beta in diabetic renal hypertrophy has been most studied. Both glucose and glucose generated advanced

glycation end products (AGEs) stimulate production of TGF-beta in a variety of cell types. Although TGF-beta is secreted in a latent form, hyperglycemia also induces the expression of thrombospondin, a potent activator of TGF-beta. In turn, TGF-beta stimulates protein synthesis (hypertrophy) in numerous cell types, but prevents cell proliferation and division due to the stimulation of proteins (cyclin kinase inhibitors) that block progression through the cell cycle. TGF-beta is also expressed in glomeruli and the tubulointerstitium of both experimental and human DN.

Microalbuminuria and proteinuria

Early in the course of diabetes the GBM widens, and may reach three- to four-fold normal thickness. Glycation of the GBM appears to make it less prone to degradation, and there is also some evidence for increased type IV collagen synthesis. Structurally, the increased GBM thickness is associated with a loss of heparan sulfate proteoglycans, the principal negatively charged constituents in the GBM that provide the charge barrier to prevent proteins from escaping into Bowman's space. There is loss of the overlying glomerular epithelial cell (podocyte), a cell type important in restricting protein permeability. A potential link between proteinuria and a family of newly described slit pore proteins including nephrin is also being studied.

Mesangial expansion and nodule formation

The hallmark of DN is mesangial expansion, eventually culminating in the development of the Kimmelstiel-Wilson nodule. The mesangial expansion is mediated by both direct effects of glucose and glucose induced advance glycation end products (AGEs). Effects may be mediated by the prosclerotic cytokine, TGF-beta, given its known propensity to stimulate mesangial matrix production. A direct effect of glucose to stimulate protein kinase C is also likely.

Tubulointerstitial fibrosis

The potential mechanisms for the development of tubulointerstitial fibrosis include release of growth factors and cytokines from the glomerulus and direct or indirect effects of proteinuria. An important mechanism may relate to renal ischemia induced by the progressive hyalinosis of the afferent and efferent arteriole.

Role of protein kinase C (PKC)

A direct role for glucose in DN has been suggested by cell culture studies that have shown that glucose can induce cell hypertrophy, extracellular matrix synthesis & TGF-beta production in a variety of cell types. Many of the adverse effects of hyperglycemia are attributed to activation of PKC, a family of serine-threonine kinases that regulates diverse vascular functions, including contractility, blood flow, cellular proliferation,& vascular permeability.

Effect of Advanced glycation end products (AGEs)

Chronic hyperglycemia can lead to the nonenzymatic glycation of amino acids and proteins (Maillard or Browning reaction). Glucose nonenzymatically binds to amino residues to become glycated Schiff bases, with later 'rearrangement' to form a more stable but still reversible 'Amadori' products.[48] Over time these products undergo rearrangement including 'cross linking to become irreversible advanced glycation end products (AGEs). Both circulating and tissue proteins as well as lipids and nucleic acids may be glycated. AGEs have been shown to be increased in the sera of diabetic patients with nephropathy and have also been localized to glomeruli by immunohistochemistry. AGEs bind to a variety of cell types, including the macrophage and mesangial cell. AGEs mediate a variety of cellular actions, including expression of adhesion molecules involved in mononuclear cell recruitment, cell

hypertrophy, extracellular matrix synthesis, epithelial to mesenchymal transdifferentiation of tubular cells, and the inhibition of nitric oxide synthesis

Effect of sorbitol (polyol pathway)

Hyperglycemia-induced generation of polyols has also been suggested to mediate some of the complications of diabetes. In tissues where glucose uptake is independent of insulin, such as in the lens, retina and kidney, chronic hyperglycemia results in increased tissue levels of glucose. The excess glucose is subsequently reduced to sorbitol by the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzyme aldose reductase, the first enzyme in the polyol pathway. Accumulation of sorbitol is accompanied by an increase in intracellular osmolality, a depletion of free myoinositol, loss of Na+ K+ ATPase activity, and increased consumption of the enzyme cofactors NADPH and NAD+, leading to changes in cellular redox potential. The role of polyols in diabetic complications has been assessed primarily by studies using aldose reductase inhibitors, such as sorbinil, tolrestat and ponalrestat. Aldose reductase inhibitors also blunt hyperfiltration and have a mild effect on reducing albuminuria in both experimental and human diabetes.

DIABETES AN INFLAMMATORY CONDITION [74]

Although diabetic nephropathy is traditionally considered a non-immune disease, accumulating evidence now indicates that Chronic low grade inflammation &activation of innate immunity are involved in pathogenesis of type2 DM. Studies in nondiabetic patients ,with impaired glucose tolerance or impaired fasting glucose & diabetic patients have shown that inflammatory & pro-inflammatory markers such as CRP, sialic acid, tumor necrosis factor-alpha,interleukin-6 are positively correlated with measures of insulin resistance ,further more diabetic patients had high levels of

inflammatory parameters compared to non-diabetics. More over various inflammatorymarkers, adhesion molecules& proinflammmatory molecules may be critical in development of microvascular diabetic complications including nephropathy. Additional evidence is derived from the fact that agents with anti-inflammatory activity are able to reduce the acute phase response & may reduce the risk of developing type 2 diabetics.

Immunologic and inflammatory mechanisms play a significant role in development and progression of diabetic nephropathy.[75, 76] Therefore, diverse cells, including leukocytes, monocytes, and macrophages,[77,78,79] as well as other molecules, such as chemokines (monocyte chemoattractant protein-1),[80,81] adhesion molecules (intercellular adhesion molecule-1 (ICAM-1),[82,83] enzymes (cyclooxygenase-2, nitric oxide synthase),[84-87] growth factors (vascular endothelial growth factor, growth hormone, IGF, TGF-β),[88-91] and nuclear factors (NF-κB),[92,83] are implicated in processes related to diabetic nephropathy. Less is known, however, about the role of inflammatory cytokines in diabetic renal injury.

A number of experimental and clinical studies have demonstrated that DN exhibits signs of inflammation. Three very recent papers illustrate the idea that inflammation plays a significant role in the pathogenesis of DN. Dalla Vestra and colleagues[79] showed that patients with type 2 diabetes and overt nephropathy exhibit the highest levels of diverse acute-phase markers of inflammation, including C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen and IL-6. Furthermore, levels of CRP, SAA and IL-6 were higher in subjects with increased glomerular basement membrane (GBM) width. More important, the authors demonstrated the association between GBM thickening, a crucial lesion of diabetic glomerulopathy, andacute phase markers (fibrinogen and IL-6). In addition, two experimental studies

provide insights into the way in which renal damage in diabetes is linked to inflammation. Chow et al.[66] showed that db/db mice, a model of type 2 diabetes and DN, exhibited an increased expression of intracellular adhesion molecule-1 (ICAM-1), which promotes inflammation by increasing leukocyte infiltration and adherence, in glomeruli and tubules, along with a marked increase in macrophage infiltration. These findings strongly involve ICAM-1-induced inflammation in the development of renal injury in diabetes. In the second study, Kelly et al.[81] demonstrated in a model of diabetes and hypertension that, despite hyperglycemia and elevated blood pressure, albuminuria was reduced and renal function was preserved in rats treated with ruboxistaurin, an inhibitor of protein-kinase C-ß. Protein-kinase C has various isoforms, which are activated in diabetes and signal a number of cellular responses, including activation and expression of inflammatory mediators, such as pro-inflammatory cytokines.

Cytokines

Over the last 2 decades, an ample amount of scientific evidence has been generated and testifies to the role of cytokines in diabetic nephropathy,[93-99] specifically stimulated by this cytokine. Activation of cytokines, profibrotic elements, inflammation, and vascular growth factors (vascular endothelial growth factor, VEGF) may be involved in the matrix accumulation in diabetic nephropathy.[100] Hyperglycemia stimulates increased VEGF expression (a mediator of endothelial injury in human diabetes).[101] A potentially pathogenic role for VEGF in diabetic nephropathy is supported by the observation that VEGF blockade improves albuminuria in an experimental model of diabetic nephropathy.[102]

Specifically, hemodynamically- induced activation of transforming growth factor β-1 (TGFβ-1) appears to play a major role in mesangial expansion;[103-105] in concert with the induction of ECM production.[104,106-111] Several biochemical mechanisms have been identified to explain the adverse effects of hyperglycemia on the kidney, including protein kinase C (PKC), the (mitogen activate protein) MAP kinase pathway, in addition to activation of the polyol pathway, increased accumulation of advanced glycation products, and oxidative stress.[112-118] Despite the strides that we have made in understanding the factors that contribute to the evolution and the progression of diabetic kidney disease, this growing knowledge has yet to culminate in new therapeutic approaches. This is partially due to the extreme complexity of the underlying process. But also, some potent mediators of diabetic kidney disease are not viable or safe therapeutic targets. For example, as enticing as it has been to target TGFβ-1 for the treatment of diabetic nephropathy, TGFβ-1 carries out multiple vital biologic functions.[119-120] Importantly, it is a primary regulator of the immune system[121-122] and mice with targeted disruption of TGFβ-1 gene die within weeks of birth due to a generalized wasting syndrome characterized by multifocal mixed inflammatory cellular response and tissue necrosis.[123] This explains the apprehension towards inhibiting TGFβ-1 in humans and illustrates the need for other potentially modifiable factors in DN. One such factor that has made it onto the scene in recent years is uric acid.

URIC ACID (UA)-

UA Chemistry:

UA is a weak acid with 2 dissociation constants. Two factors contribute to UA solubility: UA concentration and solution ph. However, the solubility of UA in urine is primarily determined by the urinary ph. The first pKa of UA is at a pH of 5.5, resulting in the loss of 1 proton from UA and the formation of anionic urate (Finlayson, 1974). The second pKa is 10.3, which has no physiological significance in humans. The supersaturation of urine with UA occurs when urinary pH is less than 5.5. In contrast, at a pH of more than 6.5 the majority of UA is in the form of anionic urate. The solubility of urate salts is affected by the relative concentrations of cations present in the urine. Increased urinary sodium concentrations promote formation of the monosodium urate complex, which is more soluble than undissociated UA. Urine is frequently supersaturated with sodium urate but stones of this type are infrequent. However, supersaturation with sodium urate may contribute to calcium oxalate stone formation via heterogeneous nucleation.[124]

URIC ACID METABOLISM [124]

UA is the end-product of purine nucleotide metabolism in humans. In contrast to many lower vertebrates, human lack UA oxidase (uricase), an enzyme which further catalyses UA to allantoin, more soluble end product (Sautin & Johnson, 2008). Humans have higher serum UA levels when compared to other mammals due to the lack of uricase (Johnson etal, 2003). UA is primarily excreted via urine. The balance between dietary intake, endogenous metabolism of purines and the urinary excretion rate of UA determines plasma UA levels (Kutzing & Firestein, 2008).

Almost all serum UA is present in the ionized form, monosodium urate, and only about 5% of urate is protein bound at physiological pH. The definition of hyperuricemia is currently arbitrary and varies from >6 mg/dl (360μmol/l) in women and >7 mg/dl (416μmol/l) in men, to ≥6.5 mg/dl (387μmol/l), or to >8.3 mg/dl (494 μmol/l), regardless of gender. UA levels physiologically and gradually rise during the human lifetime; in female individuals, UA levels additionally rise after menopause (Hak et al., 2008)

UA Pool:

UA may be derived by endogenous or exogenous routes. Uric acid (Urate) is synthesized in the liver from purine compounds provided by the diet or by the endogenous pathway of purine synthesis de novo. Some uric acid is also produced in peripheral tissues, especially the intestine and kidney. The endogenous production of UA from purine synthesis, and tissue catabolism under normal circumstances, is relatively constant at 300 to 400mg per day. However, the exogenous pool varies significantly with diet. A diet rich in animal protein contributes significantly to the purine pool and subsequent UA formation by a series of enzymatic reaction involving xanthine oxidase as the final step.

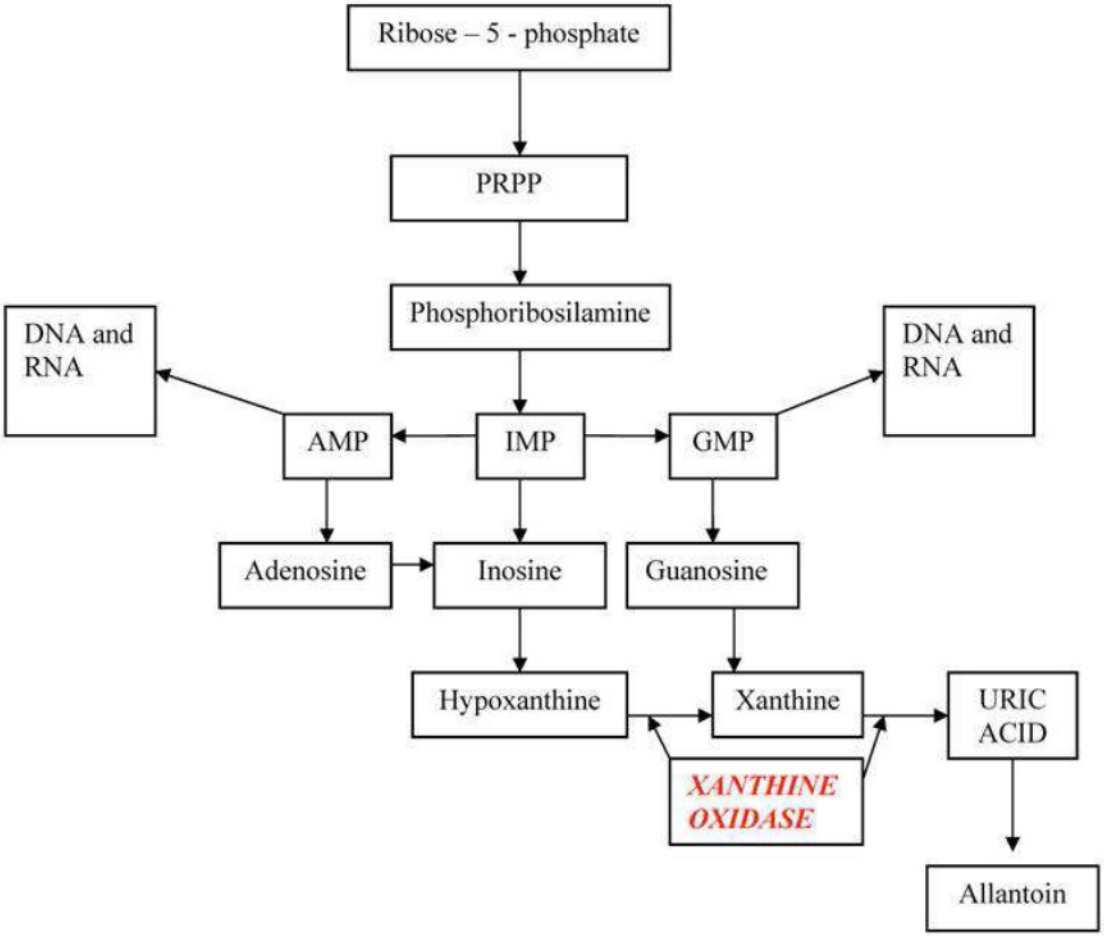


Fig.2. Schematic representation of the metabolism of Uric Acid

Uric acid that is produced in the liver is released into the circulation in its soluble form (monosodium urate), which is readily filtered by the glomerulus.

Excretion:

Renal excretion is the primary mode of UA clearance, accounting for two-third of its elimination. Intestine, skin, hair and nails account for the remaining one-third of UA excretion. In the intestine bacteria catabolise UA into carbon dioxide and ammonia, which are then eliminated as intestinal air or absorbed and excreted in the urine.

Urate is filtered completely at the renal glomerulus. The proximal tubular cells of the kidney reabsorb most of the uric acid resulting in a normal fractional excretion

of approximately 10%.[125] However, the normal fractional excretion of UA is only 8% to 12%. Therefore, postglomerular reabsorption and secretion are the ultimate factors regulating the amount of UA excretion (Gutman et al., 1968; Steele et al., 1973). The proximal tubule is the site of UA reabsorption and secretion. Almost complete reabsorption of urate occurs at the S1 segment of the proximal tubule. However, in the S2 segment of the proximal tubule urate is secreted at a range greater than reabsorption via transporters URAT1, OAT1, -2, and -3, -4, and -10, the multi-drug resistance proteins ABCC4 and ABCG2, and the glucose transporters GLUT 9a and b, and others. Finally, post-secretory resorption occurs at a more distal site of the proximal tubule. Approximately 10% of the filtered urate appears in the urine (Shekarriz et al. 2002).

Uric acid accumulation beyond its solubility point (6.8 mg/dl) in water defines hyperuricemia. In general, hyperuricemia develops due to uric acid overproduction, undersecretion, or both.[125] It is widely accepted that when uric acid levels are chronically elevated beyond their physiological levels, uric acid deposits in the joints and soft tissues leading to inflammatory arthritis and tophi (gout). Lowering uric acid levels is the key to preventing recurrent acute gout attacks.[126] Serum uric acid levels have also increased in Western populations where they have been found to predict the development of insulin resistance and diabetes.[127-128] The potential causal relationship between uric acid and other conditions such as chronic kidney disease, however, remains controversial.

Some authors indicate that uric acid is a potent antioxidant, and in a few studies when uric acid was administered acutely it appeared to improve endothelial function.[129-131] Other experimental evidence however suggests that uric acid may induce oxidative stress once it enters cells, and as such it may be a mediator of

disease.[132] Consistent with this latter observation, data demonstrates that even mild hyperuricemia, induced by the administration of an uricase inhibitor, causes endothelial dysfunction[133] that resolves once uric acid levels are lowered. These findings when viewed in light of the importance of endothelial dysfunction in the progression of DN hypothesize that uric acid plays a role in diabetic induced kidney disease.

Potential Mechanisms by which Uric Acid could Mediate DN-

Uric acid has several reported effects by which, it may cause DN illustrated in following figure. 3 , including endothelial dysfunction, increased activity of the RAAS, and induction of inflammatory cascades, in addition to profibrotic cytokine activation all of which have been demonstrated to contribute to progression of microvascular disease and thereby renal injury in DN.

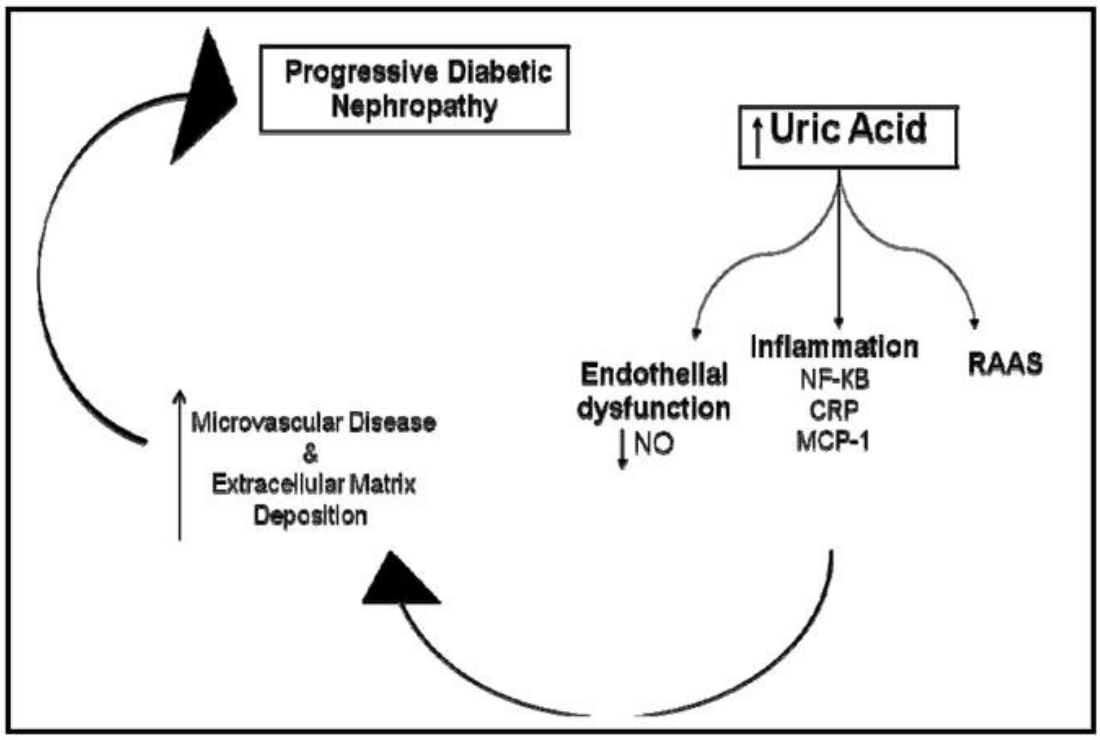


Figure 3: Mechanism by which uric acid may cause DN.

The effects of uric acid on the endothelium are subject of a contentious debate. On the one hand, uric acid has been shown to decrease nitric oxide (NO) production by endothelial cells *in vitro* [132] and it does so in association with increased CRP expression.[134] Uric acid can also react with NO irreversibly leading to the formation of 6-aminouracil and may thus lead to NO depletion.[135] Furthermore, hyperuricemic rats develop endothelial dysfunction (as noted by reduced urinary nitrites), and if given early, L-arginine supplementation can prevent both the systemic and glomerular hypertension in experimental hyperuricemia.[136, 137] These data by our group and others suggest that uric acid leads to endothelial dysfunction. On the other hand however, some studies suggest that oxidative stress due to increased xanthine oxidase activity rather than uric acid is the major factor contributing to endothelial dysfunction. An example of such findings can be found in 2 double- blind placebo-controlled studies by George et al.[138] In this study, it remains unclear if the favourable outcomes noted with allopurinol treatment are secondary to xanthine oxidase inhibition, lowering uric acid, or perhaps both.

In the kidney, experimental hyperuricemia causes an afferent renal arteriolopathy and tubulointerstitial fibrosis. This effect is largely mediated by activating the RAAS, as the renal injury was reversed with angiotensin converting enzyme inhibitors or angiotensin II receptor blockers but not with thiazide therapy despite all treatments lowering blood pressure.[139] In this study, uric acid was shown to induce vascular smooth muscle proliferation in vitro as well, and similar to the findings in the animal kidneys, the effects of uric acid on vascular smooth muscles was reversible with the use of losartan. In addition to a direct role for uric acid in the vasculature, such data suggest uric acid effects are mediated at least partially by activation of the RAAS.

On the inflammatory front, uric acid induces the interstitial inflammation and the local expression of chemokines such as MCP-1 in the kidney,[140, 141] as well as COX-2 in the blood vessels.[141] A direct role for uric acid in inducing inflammation is further supported by the findings that when infused into mice, uric acid increases cytokine production (TNF-α).[142] In humans with CKD, withdrawal of uric acid lowering therapy has been reported to increase urinary TGFβ-1 suggesting that hyperuricemia may contribute to the fibrotic process in patients with kidney disease.[143] In addition to stimulating TGF β-1 production, hyperuricemia may activate its downstream targets. Although the transcriptional effects of TGFβ-1 are generally mediated by a group of proteins; the Smads, [144] the expression of certain TGFβ-1-induced genes is mediated via the mitogen activate protein (MAP) kinase pathway.[145]

This pathway has also been reported to mediate uric acid effects in cell culture.[146] Although the results of these studies need confirmation, such findings raise the possibility that treatment of hyperuricemia may provide a safe venue for alleviating cytokine- mediated kidney disease progression.

Uric Acid in Animal Models of Diabetic Nephropathy

Despite the wealth of evidence linking uric acid to inflammation and endothelial dysfunction, animal studies evaluating the role of uric acid in DN are sparse. This is interesting considering the critical role that endothelial dysfunction is known to play in diabetic kidney disease.[147] Kosugi et al. explored the involvement of uric acid in DN in a recent study.[10] In this study, allopurinol at the dose used (30 mg/kg/day) did not reduce oxidative stress in the kidney, but rather it reduced intercellular adhesion molecule 1 (ICAM-1) expression by the tubular epithelial cells. In vitro, uric acid directly induced ICAM expression in proximal tubular cells. The findings of this study strongly suggest uric acid is a mediator of inflammation and

tubular injury in DN and that therapies aimed at lowering uric acid levels may be of benefit in human disease.

Other groups have also reported findings consistent with our results including evidence that uric acid inhibits proximal tubular cellular proliferation in vitro.[148] This inhibitory effect of uric acid appears to be mediated by signaling pathways ultimately affecting cytoplasmic phospholipase A_2 and the inflammatory transcription factor nuclear factor κ B (NF-κB).

Uric Acid as a Predictor of Human Disease

Understanding the relationship between uric acid and kidney disease in humans has been complicated by the fact that uric acid levels are elevated in patients with chronic kidney disease (CKD) due to a variety of factors including reduced GFR and diuretics use in patients with CKD.[149] Even early decline in renal function is associated with an increase in serum uric acid levels[150] creating a major confounder in the interpretation of many observational studies. Another major limitation of observational studies lies with uric acid being a product of xanthine oxidase activity. Xanthine oxidase, in addition to generating uric acid, generates reactive oxidative species. Uric acid in such instances may be a marker of oxidative stress. Further complicating the debate, although many studies have shown increased uric acid levels are predictive of incident CKD and of CKD progression, [33, 151, 153-155] some studies have not.[156-158]

Notwithstanding the limitations of observational studies, several have examined uric acid as a risk factor for D. N. Hovind et al[159]at the Steno Diabetes Center explored the association between uric acid levels and micro- and macro-albuminuria in patients with type 1 DM.

MATERIALS AND METHODS

SOURCE OF DATA:

A cross sectional study is conducted on type 2 diabetic patients attending the outpatient department and being admitted to B.L.D.E.U's Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, from December 2013 to July 2015.

SAMPLE SIZE:

With the prevalence rate of overt nephropathy in Diabetes Mellitus 2.2%[160] at 99% confidence interval and at ± 5 margin of error the sample size is 56.

$N = (2.56)^2 * P * Q / d^2$

Hence 56 cases of type 2 Diabetes Mellitus are included in the study to associate serum uric acid level with albuminuria.

STATISTICAL ANALYSIS:

Data is analyzed using

- Diagrammatic Presentation.
- Mean ± SD.
- Correlation Coefficient.
- Linear and multiple regression analysis.

METHOD OF COLLECTION OF DATA

A detailed history, physical examination including Blood pressure, BMI, current medications, insulin doses, habits like tobacco and alcohol consumption and family medical history is obtained from all the patients coming to the hospital with type 2 diabetes mellitus.

The biochemical investigations like examination of urine, serum uric acid, serum creatinine, HbA1C is estimated and Urine albumin and urine creatinine are measured.

INCLUSION CRITERIA:

Patients with known case of type 2 diabetes mellitus

EXCLUSION CRITERIA:

1. Treatment with uric acid lowering agents.
2. Treatment with diuretics.
3. History of gout.
4. Acute febrile illness
5. Urinary tract infection
6. Chronic Myeloid Leukemia
7. Cancer

METHOD OF TEST

Sample collection

Oral and written consent is taken from the subjects prior to the collection of specimens. Venous blood is collected from each subject and transported to the laboratory. Random spot urine sample is collected at the same time.

BIOCHEMICAL ANALYSIS:

ESTIMATION OF ALBUMINURIA:

Urine Albumin concentration (UAC) is measured by turbidimetric immunoassay and is expressed as urine albumin creatinine ratio (ACR).Urinary ACR is calculated by dividing UAC in micrograms by urinary creatinine concentration in milligrams.

1. ACR =30.0 µg/mg or lower is considered as "normal,"
2. ACR between 30µg/mg and 299µg/mg is considered as "microalbuminuria".
3. Very high ratio (ACR ≥ 300 µg/mg) is defined as "overt albuminuria."

ESTIMATION OF SERUM URIC ACID:

Serum uric acid level is measured by uricase peroxidase method (Liqui CHEK, AGAPPE). Enzymatic determination is done according to following reaction.

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{uricase}} \text{Allontoine} + CO_2 + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{EHSPT} \xrightarrow{\text{peroxidase}} \text{Red quinone.}$$

EHSPT=N-Ethyl N-(2-Hydroxy-3-Sulfoproyl) n-Toluidine.

INVESTIGATIONS

All the subjects are subjected to the following investigations

1. Urine Examination
2. Serum Creatinine
3. Serum Uric Acid
4. HbA1C
5. Urine Albumin
6. Urine Creatinine
7. Random Blood Sugar (RBS) Level
8. Fasting Blood Sugar (FBS) Level
9. Post Prandial Blood Sugar (PPBS) Level
10. HIV Rapid Test

OBSERVATIONS AND RESULTS

Clinical characteristics of 56 patients with type2 DM enrolled in this study are shown in Table 2.

Table 2: Characteristics of the study population

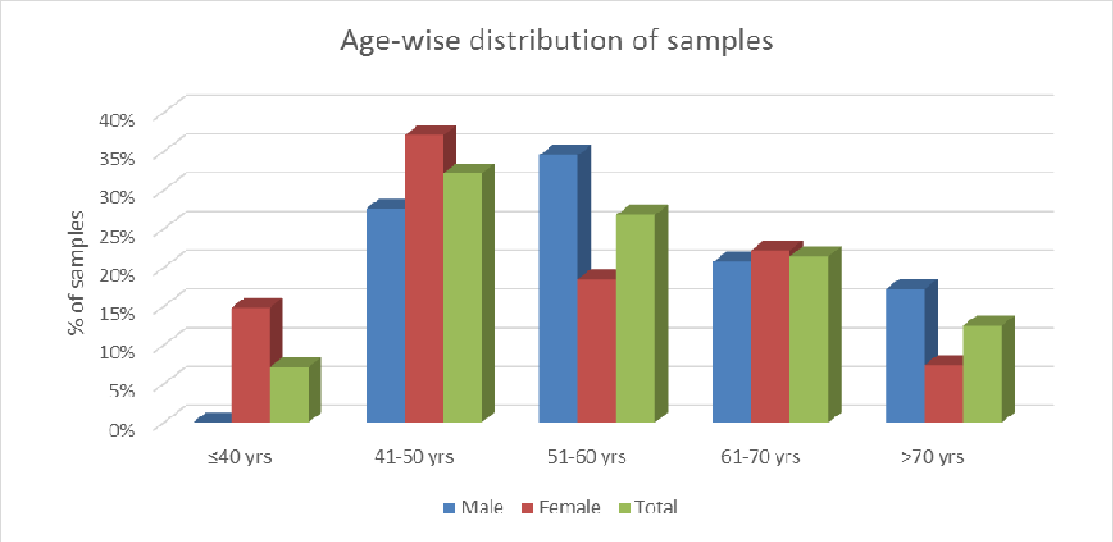
Sr. No.	Parameters	Value (Mean±SD)
1	Age(years)	56.61±12.11
2	Gender I. Male II. Female	29 (52%) 27 (48%)
3	BMI(kg/m^2)	23.82±3.43
4	SBP(mmHg)	137.86±14.36
5	DBP(mmHg)	81.39±6.86
6	Duration of duration(years)	6.63±4.03
7	SUA(mg/dl)	5.11±1.70
8	Sr. Creatinine (mg/dl)	1.05±0.40
9	HbA1C (%)	7.30±2.34
10	ACR(µg/mg)	96.05±91.56

Out of 56 patients, males and females were 29(52%) and 27(48%), respectively. The mean age of the patients included in this study was 56.61±12.11years and mean duration of diabetes of the patients was 6.63±4.03years. The mean body mass index of study population was 23.82±3.43kg/m^2, while the mean serum uric acid concentration observed in study population was 5.11±1.70 mg/dl.

Table 3: Age distribution of study population

Age(in years)	Male(N)	Female(N)	Total(N)
<40	(0)0%	(4)15%	(4)7%
41-50	(8)28%	(10)37%	(18)32%
51-60	(10)34%	(5)19%	(15)27%
61-70	(6)21%	(5)22%	(11)21%
>70	(5)17%	(3)7%	(8)13%

Graph 1: Age distribution of patients

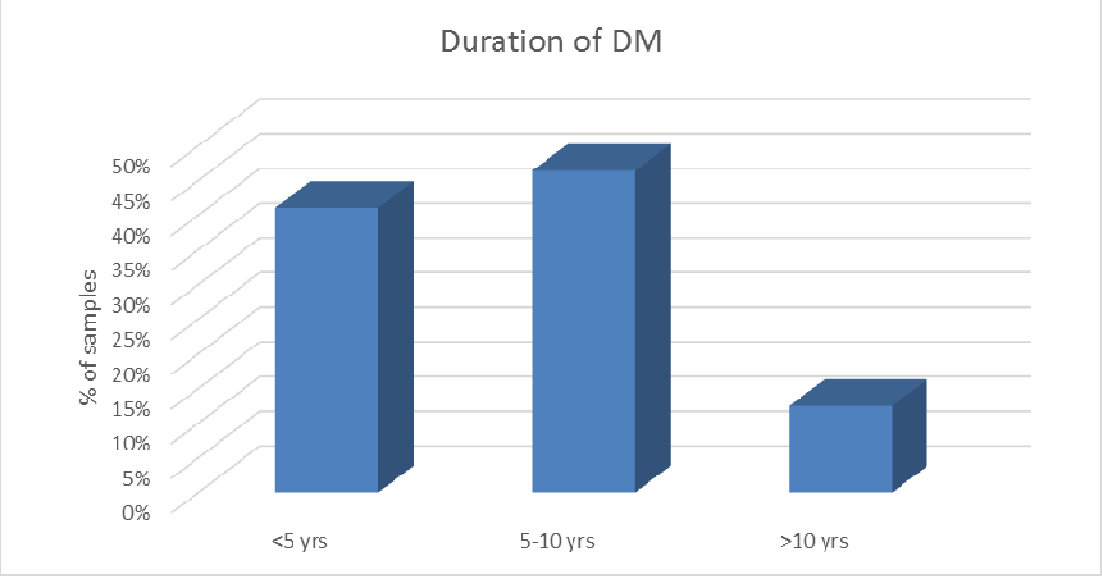


As shown in diagram No.1, majority of the patients were in age group of 41-50 years (N = 18) (32%) which included (N = 8) 28% males and (N = 10) 37% females.

Table 4: **Duration of Diabetes Mellitus in study population**

Duration of DM	**N**	**%**
<5 years	23	41%
5-10 years	26	46%
>10 years	7	13%
Total	**56**	**100%**

Graph 2: Duration of diabetes in study population



Majority (46%) of patients of study population had diabetes of duration between 5-10 years of age.

Table 5: Comparative levels of serum uric acid in mg/dl, in males and females

Gender	N	Mean (mg/dl)	SD	SE of Mean	Mean Difference	T	P-Value
Male	29	5.12	1.50	0.28	0.035	0.077	0.939
Female	27	5.09	1.92	0.37			

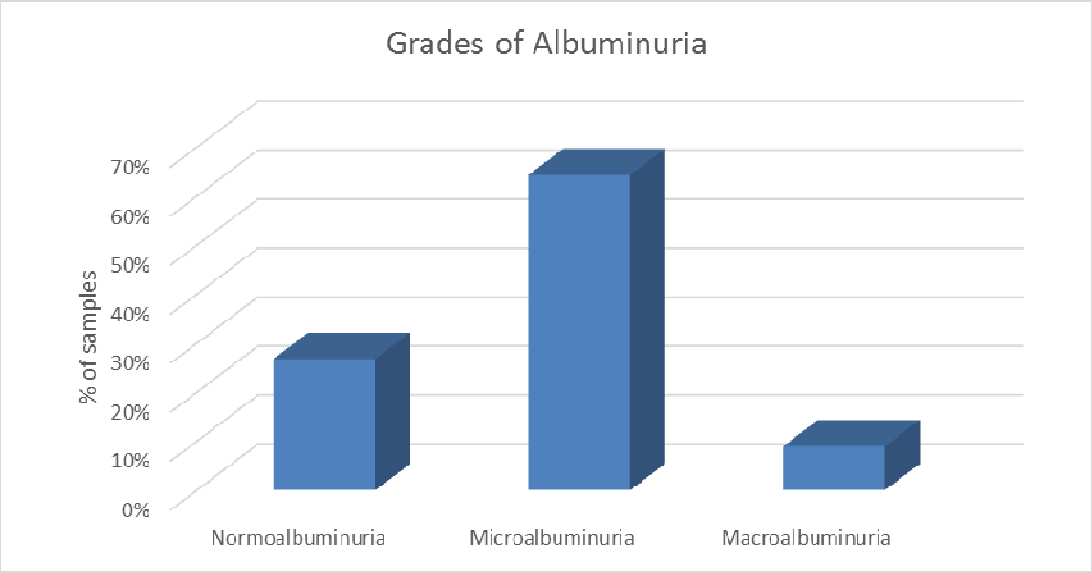
The mean uric acid concentration was 5.11±1.70 mg/dl, which was higher in men than in women (5.12 ± 1.50 mg/dl versus 5.09 ±1.92 mg/dl, respectively) But there was no statistically significant difference in serum uric acid concentration between males and females (*P=0.939*).

Table 6: Grades of albuminuria

ACR	N	%	Mean ACR	SD
Normoalbuminuria	15	26.79%	17.54	5.53
Microalbuminuria	36	64.29%	96.53	52.43
Macroalbuminuria	5	8.93%	327.43	32.68
Total	**56**	**100%**	**96.05**	**91.56**

The mean value of Normoalbuminuria, Microalbuminuria and Macroalbuminuria in the enrolled patients was 17.54 ± 5.53, 96.53±52.4 and 327.43±32.6, respectively.

Graph 3: Grades of albuminuria



Out of 56 patients 36(64.29%) patients were showing Microalbuminuria. While 15(26.79%) patients had normoalbuminuria and only 5(8.93%) patients had macroalbuminuria.

Table 7: All parameters compared with ACR classification

Parameter	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
Age	54.53±12.96	56.69±12.10	62.20±9.63
Male	10 (67%)	16 (44%)	3 (60%)
Female	5 (33%)	20 (55%)	2 (40%)
BMI	23.41±2.46	24.17±3.84	22.60±2.81
HTN	0 (0%)	9 (25%)	4 (80%)
SBP	133.87±10.89	137.28±13.32	154.00±21.91
DBP	80.27±5.60	80.94±6.82	88.00±8.37
HbA1C	5.81±1.50	7.76±2.37	8.40±2.60
Duration of DM	4.93±2.55	6.44±3.67	13.00±4.70
Serum Creatinine	0.95±0.41	1.06±0.36	1.30±0.58
SUA	4.09±1.36	5.21±1.60	7.38±0.87

As showed in above table, in this study it is found that, 9 (25%) of microalbuminuric and 4 (80%) of macroalbuminuric patients were having hypertension.

The mean HbA1C was more in macroalbuminuric patients (8.40±2.60) than in microalbuminuric patients (7.76±2.37), while in patients with normoalbuminuria, it was found to be 5.81±1.50.

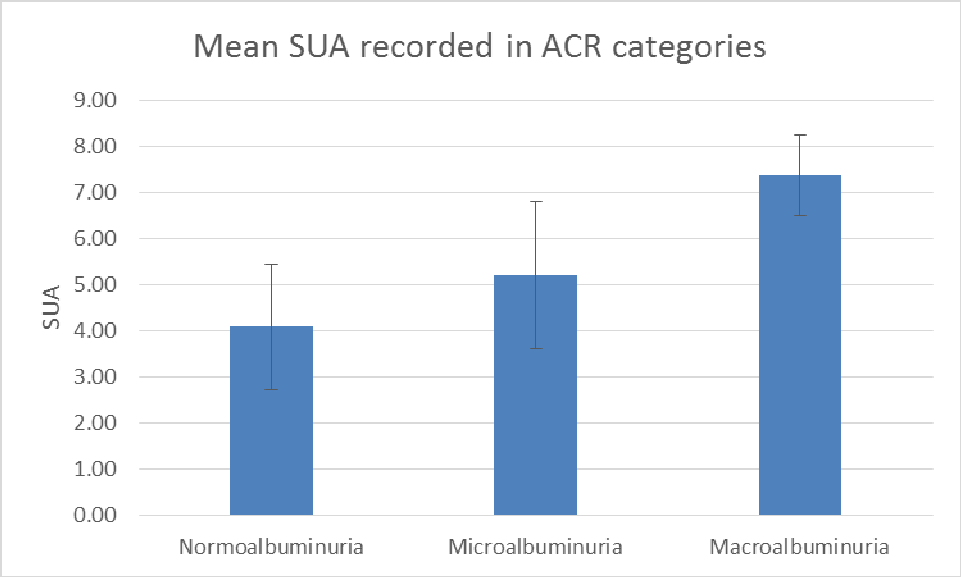
When albuminuria is categorized in two categories of normoalbuminuria and abnormal albuminuria (microalbuminuria + macroalbuminuria), clinical and blood parameters of the patients showed following results, as shown in table No.8

Table 8: Parameters compared with normoalbuminuria and abnormal albuminuria:

Parameter	**Normoalbuminuria** (mean±SD)	**Abnormal albuminuria** (mean±SD)
Age	54.53±12.96	57.37±11.86
Male	10 (67%)	19 (46%)
Female	5 (33%)	22 (54%)
BMI	23.41±2.46	23.98±3.73
HTN	0 (0%)	13 (32%)
SBP	133.87±10.89	139.32±15.29
DBP	80.27±5.60	81.80±7.29
HbA1C	5.81±1.50	7.84±2.37
Duration of DM	4.93±2.55	7.24±4.31
Serum Creatinine	0.95±0.41	1.09±0.39
SUA	4.09±1.6	5.48±1.68

It is found that, BMI was 23.41±2.46 in normoalbuminuria and it was 23.98±3.73 in abnormal albuminuria, without significant difference between two groups.

Graph 4: Mean Serum uric acid (mg/dl) in ACR categories



It is found that, concentration of serum uric was 4.09±1.36, 5.21±1.60 and 7.38±0.87 in patients with normoalbuminuria, microalbuminuria and macroalbuminuria, respectively. While considering normoalbuminuria and abnormal albuminuria, serum uric acid concentration was 4.09±1.6 and 5.48±1.68, respectively.

Table 9: Correlation between Albuminuria (ACR) and other parameters

Sl. No.	Parameters	Correlation coefficient 'r'	'p' value
1	Age	0.214	0.113
2	BMI	-0.001	0.994
3	SBP	0.431	0.001*
4	DBP	0.254	0.059
5	Duration of Diabetes (years)	0.526	<0.001*
6	Serum Uric Acid	0.559	<0.001*
7	Serum Creatinine	0.310	0.020*
8	HbA1C	0.429	0.001*

* denotes significant correlation

It was found that, there was statistically significant and positive correlation between serum uric acid concentration and albumin creatinine ratio ($r = 0.559$, $p < 0.001$) in the enrolled type 2 diabetic patients.

Duration of diabetes was also positively correlated with albumin creatinine ratio. It was also statistically significant ($r = 0.526$, $P < 0.001$).

Also, it was found positive and statistically significant correlation between HbA1C and albumin creatinine ratio ($r = 0.429$, $p = 0.001$).

The correlation between BMI and ACR was found to be almost nil (r = -0.001) and was not statistically significant (P>0.05), while SBP showed statistically significant correlation with albumin creatinine ratio (p = 0.001)

Graph 5: Scatter Plot (correlation graph) showing relation between ACR and blood parameters

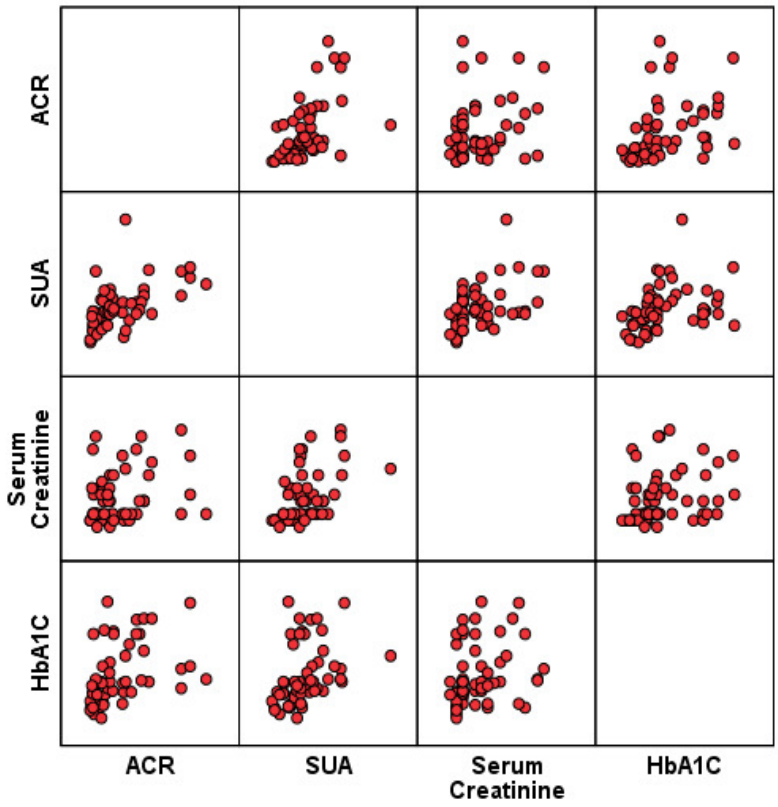


Table 10: Correlation between SUA and other parameters

Sl. No.	Parameters	Correlation Coefficient 'r'	'p' value
1	Age	0.322	0.015*
2	BMI	0.088	0.519
3	SBP	0.412	0.002*
4	DBP	0.253	0.060
5	Duration of DM (years)	0.362	0.006*
6	Serum Creatinine	0.483	<0.001*
7	HbA1C	0.347	0.009*
8	ACR	0.559	<0.001*

* denotes significant correlation

Apart from ACR, Serum Uric Acid also showed statistically significant and positive correlation with Systolic blood pressure ($r = 0.412$, $p = 0.002$)

Also, HbA1C ($r = 0.347$, $p = 0.009$) and Serum Creatinine ($r = 0.483$, $p < 0.001$) were correlated positively with Serum Uric Acid

In this study, Serum Uric Acid was not found to be significantly correlated with BMI. ($p = 0.519$).

Table 11: Simple Linear Regression-Predicting ACR using significantly correlated variables individually

Parameter	β	R^2	P-Value	95% CI for β	
				Lower Bound	Upper Bound
SBP	2.75	0.186	0.001*	1.18	4.32
Duration of DM (years)	11.93	0.276	<0.001*	6.67	17.20
SUA	30.04	0.312	<0.001*	17.88	42.20
Serum Creatinine	71.80	0.096	0.020*	11.82	131.78
HbA1C	16.77	0.184	0.001*	7.14	26.41

*denotes a significant factor:

Only those parameters which were found to be significantly correlated with ACR are used in regression**.** By linear regression, variations in ACR are estimated using different parameters individually.

The value of β (Regression coefficient) for Serum uric acid is 30.04. This means 1mg/dl change in Serum uric acid cause 30.04 µg/mg variation in albumin creatinine ratio. The β value for HbA1C is 16.77, which means 1% change in HbA1C cause 16.77 µg/mg variation in albumin creatinine ratio.

Considering the R^2 (coefficient of determination) value, serum uric acid is found to be a significant factor which could predict only 31.2% ($R^2 = 0.312$) variation in albumin creatinine ratio.

Similarly, Duration of DM and HbA1C were found to be significant factors which could predict only 27.6% (R^2 = 0.276) and 18.4% (R^2 = 0.184) of variations in albumin creatinine ratio.

Graph 6: Scattered plot of predicting ACR using SUA:

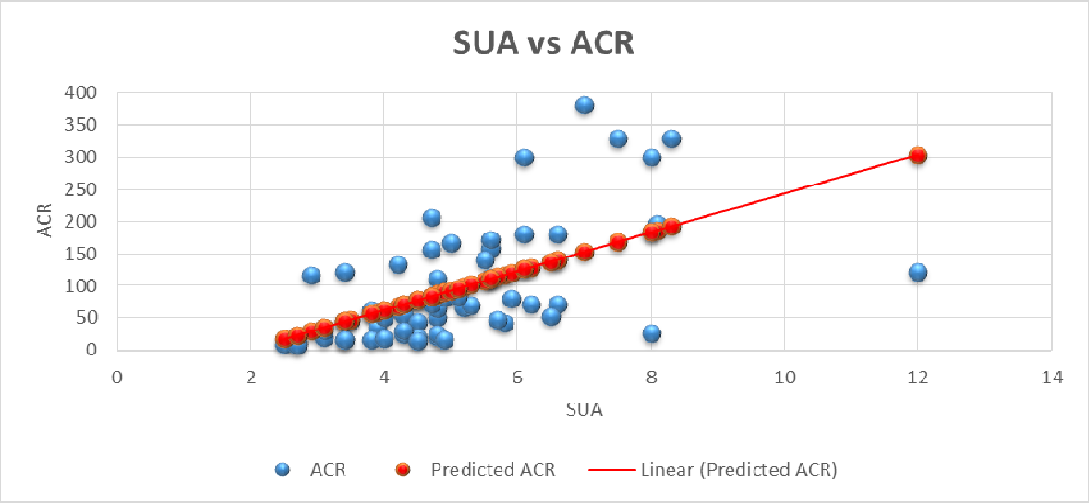


Table 12: Multiple Linear Regression- Predicting ACR using significantly correlated variables collectively:

Parameter	β	SE of β	P-Value		95% CI for β	
					Lower Bound	Upper Bound
SBP	1.08	0.70	0.129	R^2(Adj) 0.472	-0.33	2.48
Duration of DM (years)	8.15	2.40	0.001*		3.32	12.98
SUA	14.60	6.84	0.038*		0.86	28.34
Serum Creatinine	1.41	26.10	0.957		-51.02	53.83
HbA1C	9.51	4.16	0.026*		1.16	17.87

*denotes a significant factor

With the simple (univariate) linear regression analysis, R^2 value was unadjusted. Multiple linear regression analysis was done, with adjusted R^2 value to nullify the confounding effect of other variables. Thus the independent effect of different variables on albumin creatinine ration was found.

By multiple regression, considering all variables together, they predict 47.2% (R^2 = 0.472) variation in albumin creatinine ratio.

The value of β for serum uric acid was 14.60, which suggest that 1mg/dl change in serum uric acid cause 14.60 μg/mg variation in albumin creatinine ratio.

Thus, it showed that serum uric acid was an independent predictor of albumin creatinine ratio, after using adjusted R^2 value.

DISCUSSION

A cross sectional study was done in 56 type 2 diabetic patients attending the outpatient department and being admitted to B.L.D.E.U's Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, from December 2013 to July 2015. And the relationship between serum uric acid concentration and degree of urinary albumin excretion in type 2 diabetic patients was evaluated.

In this study, a positive correlation was found between serum uric acid and albumin creatinine ratio. In the study conducted by Fukui *et al*., positive correlation of serum uric acid and urinary albumin excretion in 343 men with type 2 diabetes mellitus was shown.[18] They concluded that serum uric acid concentration is associated with microalbuminuria and subclinical atherosclerosis in men with type 2 diabetes mellitus.

Similarly, Fu *et al*, in a study on Chinese diabetic patients, found that hyperuricemia was significantly associated with abnormal albuminuria in patients without diuretics or use of uricosuric agents or alcohol. They concluded that hyperuricemia was significantly related to the presence of albuminuria in patients with diabetes.[152]

Siu and colleagues reported lowering serum uric acid level in patients with hyperuricemia was associated with regression of kidney disease.[16] Since appearance of albuminuria is the first sign of kidney damage and onset of diabetic nephropathy in patients with DM,[40] the association between ACR and hyperuricemia confirmed the effect of hyperuricemia on diabetic nephropathy.

The results also showed that when these patients were divided into normoalbuminuria and abnormal albuminuria groups, diabetic duration, SBP, serum creatinine and SUA higher in abnormal albuminuria group as compared to normoalbuminuria group

In this study, it was found that, duration of diabetes and systolic blood pressure was significantly associated with albumin creatinine ratio. Mogensen[165] had concluded that age, diabetes duration, glycemic control, blood pressure, and metabolic syndrome were all associated with albuminuria and decline of GFR.

Bo and coworkers reported that hyperuricemia is associated with insulin resistance and onset or progression of nephropathy in type 2 diabetic patients.[40] Metabolic syndrome components include hypertension, hypertriglyceridemia, hyperinsulinemia, insulin resistance and obesity.[18, 161] In this study, Serum Uric Acid was significantly correlated with HbA1c. Also, a positive association was found between Serum Uric Acid and Systolic blood pressure. A study by Johnson and colleagues reported hyperuricemia to be a risk factor for hypertension.[149]

But, in this study, Serum Uric Acid and BMI were not significantly correlated. Similar results were found in a study by Bonakdaran et al[17] in which, in spite of significant relationship between Hyperuricemia and Metabolic Syndrome, such link was not found between Serum Uric Acid concentration and BMI. Lohsoonthorn and colleagues found different results showing positive correlation between BMI, metabolic syndrome, and serum uric acid.[162]

In this study, serum uric acid was found to be an independent predictor of albumin creatinine ratio, after multiple linear regression analysis. Similarly, in a study conducted by Obermayr *et al*., serum uric acid was found to be a predictor of new onset kidney disease, independent of other risk factors.[33]

Studies in rats showed that the renal changes can be prevented by maintaining SUA levels in the normal range by allopurinol, [163, 164] but only partially prevented by the treatment of hypertension with enalapril or lorsartan, [164, 136, 139] These observations suggested that a pathogenic role of uric acid in the renal abnormalities independent of blood pressure and imply a possible efficacy to lower urinary albumin-to-creatinine ratio in diabetic patients by bringing down the uric acid levels.

A recent human study did prove that allopurinol treatment could normalize endothelial dysfunction in type 2diabetic patients with mild hypertension. [166] Theoretically, it is also possible that both elevated uric acid and urinary albumin excretion rate are manifestations of a common underlying pathogenesis of insulin resistance. In humans, uric acid is the final breakdown product of adenosine, which plays an important role in the pathophysiology of insulin resistance.[167] Adenosine can also cause increased renal uric acid retention.[167] Moreover, hyperinsulinemia resulting from insulin resistance can decrease the renal excretion, increase the renal reabsorption, and increase the production of uric acid.[168] On the other hand, microalbuminuria is also an integral component of the metabolic syndrome characterized by insulin resistance.[169]

In clinical practice today, serum uric acid should be taken into consideration as a risk factor for abnormal albuminuria, in type 2 diabetic patients. This study suggested the importance of uric acid as a predictor of albuminuria in type 2 diabetic

patients. The pathogenic role of serum uric acid in renal injury and in increasing urinary albumin excretion rate is worthy of further investigation.

This study had some limitations. The sample size was small. The validity to extrapolate the relationship between uric acid and urinary albumin excretion rate to nondiabetic subjects requires confirmation. As this was a cross sectional study, further prospective studies should be made to evaluate the relationship between the serum uric acid and albuminuria in type 2 diabetic patients.

CONCLUSION

This study showed that, the serum uric acid concentration was significantly and positively associated with albuminuria in patients with type 2 Diabetes Mellitus. As hyperuricemia is a common finding in this group of patients, and its treatment is easy and available, early diagnosis and treatment may be helpful to prevent or decrease the rate of development of overt kidney disease in this patients.

Given this fact, it was hypothesized by this study that, serum uric acid may play a pathological role in the development of albuminuria in type 2 diabetes mellitus. It is of clinical importance to clarify the pathogenic role of uric acid in renal disease and to evaluate whether the increased urinary albumin excretion rate can be prevented by lowering uric acid levels with medications.

To approve this hypothesis, study of the effect of lowering uric acid with allopurinol on renal function in diabetic nephropathy patients is suggested in future.

SUMMARY

56 type2 diabetes mellitus patients were enrolled in the study. In all patients, blood parameters like serum uric acid, serum creatinine, HbA1C were measured. Albumin creatinine ration is calculated and patients are divided in normoalbuminuria, microalbuminuria and macroalbuminuria according to albumin creatinine ratio.

It was found that, concentration of serum uric was 4.09±1.36, 5.21±1.60 and 7.38±0.87 in patients with normoalbuminuria, microalbuminuria and macroalbuminuria, respectively.

Significant positive association was found between Serum Uric Acid and albumin creatinine ratio ($r = 0.559$, $p < 0.001$). Also, albumin creatinine ratio was positively correlated with duration of diabetes ($p = <0.001$) and HbA1C ($p = 0.001$)

Duration of Diabetes Mellitus and HbA1C were found to be significant factors which could predict only 27.6% and 18.4% of variations in albumin creatinine ratio. Serum uric acid was found to be a significant factor which could predict 31.2% of variation in albumin creatinine ratio.

By using multiple regression analysis, it was found that serum uric acid was an independent predictor of albumin creatinine ratio and also it was positively correlated with albumin creatinine ratio.

Statistical analysis was done as per research guidelines. Vancouver format was used in writing bibliography. Master chart reveals all patient data.

BIBLIOGRAPHY

1. Atlas of End-Stage Renal Disease. Bethesda: National Institutes of Health, NIDDK; 2005. USRDS 2005 Annual Data Report.
2. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis 2007; 49:12-154.
3. Stenvinkel P. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. J Intern Med 2010; 268:456-467.
4. Keen H. The Diabetes Control and Complications Trial (DCCT). Health Trends 1994; 26:41–43.
5. Schrier RW, Estacio RO, Mehler PS, Hiatt WR. Appropriate blood pressure control in hypertensive and normotensive type 2 diabetes mellitus: a summary of the ABCD trial. Nat Clin Pract Nephrol 2007; 3:428–438.
6. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med 1993; 329:1456–1462.
7. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 2001; 345:851-860.
8. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 2001; 345:861-869.
9. Momeni A.Serum uric acid and diabetic nephropathy.J Ren Inj Prev 2012; 1:37-38.

10. Kosugi T, Nakayama T, Heinig M, Zhang L, Yuzawa Y, Sanchez-Lozada LG, et al. The Effect of Lowering Uric Acid on Renal Disease in the Type 2 Diabetic db/db Mice. Am J Physiol Renal Physiol 2009; 297:481- 488.
11. Lal SS, Sukla Y, Singh A, Andriyas EA, Lall AM. Hyperuricemia, high serum urea and Hypoproteinemia are the risk factor for diabetes. Asian J Med Sci 2009; 1: 33-4.
12. Ilto H, Abe M, Mifune M, Oshikiri K, Antoku S, Takeuchi Y, Togane M. (2011) Hyperuricemia is independently associated with coronary heart disease and renal dysfunction in the patients of type 2 diabetes mellitus. PLoS ONE 6(11): e27817. Doi: 10. 1371/journal pone.0027817.
13. Safi A J, Mahmood R, Khan M A, Amin-ul-Haq. Association of serum uric acid with type 2 diabetes mellitus; JPMI Vol. 18(1) 2011 p 59-63.
14. Li-ying Chen, Wen-hua Zhu, Zhou-wen Chen, Hong-lei Dai, Jing-jing Reen, Lei-gian Chen, and Li-zheng Fang. Relationship between hyperuricemia and metabolic syndrome. Journal of Zhejiang University Science B Aug 2007 8(8): 593-98.
15. Edwards, N Lawrence; The role of hyperuricemia in vascular disorders; Current opinion in Rheumatology Vol 21 issue 2 Mar 2009, 132-137.
16. Siu YP, Leung KT, Tong MK, Kwan TH; Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level; American Journal of kidney diseases Vol 47 (1) Jan 2006 p 51-59
17. Bonakdaran S, Hami M, Shakeri MT. Hyperuricemia and Albuminuria in Patients With Type 2 Diabetes Mellitus. Iran J Kidney Dis 2011; 5:21-24.

18. Fukui M, Tanaka M, Shiraishi E, Harusato I, Hosoda H, Asano M, et al; Serum uric acid is associated with microalbuminuria and subclinical atherosclerosis in men with type 2 diabetes mellitus. Metabolism 2008; 57: 625-629.

19. Humans M, Geeraet B, De Keyzer D, Mertens A, Lanoo M, Vanaudenaerde B, Hoylaert M, Benhabiles N, Tsatsanic C, Mathieu C, Holvoet P. Interleukin-1 receptor-associated kinase-3 is a key inhibitor of inflammation in obesity and metabolic syndrome. Plos one 2012; 7: e30414.

20. Packham DK, Alves TP, Dwyer JP, Atkins R, de Zeeuw D, Cooper M, Shahinfar S, Lewis JB and Lambers Heerspink HJ. Relative incidence of ESRD versus cardiovascular mortality in proteinuric type 2 diabetes and nephropathy. Am J Kidney Dis 2012; 59: 75-83.

21. Wang Y, Yuan A and Yu C. Correlation between microalbuminuria and cardiovascular events. Int J Clin Exp Med 2013; 6: 973-978.

22. Fox CS, Matsushita K, Woodward M, Bilo HJ, Chalmers J, Heerspink HJ, Lee BJ, Perkins RM, Rossing P, Sairenchi T, Tonelli M, Vassalotti JA, Yamagishi K, Coresh J, de Jong PE, Wen CP, Nelson RG; Chronic Kidney Disease Prognosis Consortium. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet 2012; 380: 1662-1673.

23. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. J Clin Endocrinol Metab 2009; 94: 3171-3182.

24. Lim AK and Tesch GH. Inflammation in diabetic nephropathy. Mediators Inflamm 2012; 2012: 146154.

25. Akbas EM, Demirtas L, Ozcicek A, Timuroglu A, Bakirci EM, Hamur H, Ozcicek F and Turkmen K. Association of epicardial adipose tissue, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio with diabetic nephropathy. Int J Clin Exp Med 2014; 7: 1794-1801.

26. Valle M, Martos R, Canete MD, Valle R, van Donkelaar EL, Bermudo F and Canete R. Association of serum uric acid levels to inflammation biomarkers and endothelial dysfunction in obese prepubertal children. Pediatr Diabetes 2014.

27. Hung CC, Tsai JC, Kuo HT, Chang JM, Hwang SJ and Chen HC. Dyslipoproteinemia and impairment of renal function in diabetic kidney disease: an analysis of animal studies, observational studies, and clinical trials. Rev Diabet Stud 2013; 10: 110-120.

28. Mishra R, Emancipator SN, Kern T, Simonson MS. High glucose evokes an intrinsic proapoptotic signaling pathway in mesangial cells. *Kidney Int* 2005; 67: 82-93.

29. Hohenstein B, Hausknecht B, Boehmer K, Riess R, Brekken RA, Hugo CP. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. Kidney Int2006; 69: 1654-61.

30. Nagai Y, Yao L, Kobori H, Miyata K, Ozawa Y, Miyatake A, et al. Temporary angiotensin II blockade at the prediabetic stage attenuates the development of renal injury in type 2 diabetic rats. J Am Soc Nephrol2005; 16: 703-11.

31. Jalal DI, Rivard CJ, Johnson RJ, Maahs DM, McFann K, Rewers M, et al. Serum uric acid levels predict the development of albuminuria over 6 years in patients with type 1 diabetes: findings from the Coronary Artery Calcification in Type 1 Diabetes study. Nephrol Dial Transplant2010; 25: 1865-9.

32. Modan M, Halkin H, Karasik A, Lusky A. Elevated serum uric acid--a facet of hyperinsulinaemia. Diabetologia1987; 30: 713-8.

33. Obermayr RP, Temml C, Gutjahr G, Knechtelsdorfer M, Oberbauer R, Klauser-Braun R. Elevated uric acid increases the risk for kidney disease. J Am Soc Nephrol2008; 19: 2407-13.

34. Momeni A, Shahidi S, Seirafian S, Taheri S, Kheiri S. Effect of allopurinol in decreasing proteinuria in type 2 diabetic patients. Iran J Kidney Dis2010; 4: 128-32.

35. Eberhard Ritz, Danilo Flisa, Michael Siebers: "Diabetic Nephropathy" Chapter-47in Massry and Glassck's Text book of nephrology, Vol.I, III Edn, ShaulG.Massry, Richard Glossock Williams & Wilkins (Eds), 1995.

36. Keen H, Chlouvervakis C: "An immunoassay method of estimating urinary albumin at low concentration" Lancet, 1963; 2: 913-916.

37. Hasegawa G, Nakano K, Sawada M, Uno K, Shibayama Y, Ienaga K, Kondo M: Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. Kidney Int 40: 1007 –1012, 1991.

38. Hasegawa G, Nakano K, Kondo M: Role of TNF and IL-1 in the development of diabetic nephropathy. Nefrologia 15: 1 –4, 1995.

39. Iseki K, Oshiro S, Tozawa M, Iseki C, Ikemiya Y, Takishita S. Significance of hyperuricemia on the early detection of renal failure in a cohort of screened subjects. Hypertens Res 2001; 24: 691-697.

40. Bo S, Cavallo-Perin P, Gentile L, Repetti E, Pagano G. Hypouricemia and hyperuricemia in type 2 diabetes: two different phenotypes. Eur J Clin Invest 2001; 31: 318-321.

41. Tseng CH. Correlation of uric acid and urinary albumin excretion rate in patients with type 2 diabetes mellitus in Taiwan. Kidney Int 2005; 68: 796-801.

42. Cai X, Han X, Ji L. High-normal serum uric acid is associated with albuminuria and impaired glomerular filtration rate in Chinese type 2 diabetic patients. Chinese Medical Journal 2011; 124:3629-3634.

43. Kim ES, Kwon HS, Ahn CW, Lim DJ, Shin JA, Lee SH, et al. Serum uric acid level is associated with metabolic syndrome and microalbuminuria in Korean patients with type 2 diabetes mellitus.J Diabetes Complications 2011;25:309-313.

44. Behradmanesh S, Horestani MK, Baradaran A, Nasri H. Association of serum uric acid with proteinuria in type 2 diabetic patients. J Res Med Sci 2013; 18:44-46.

45. Shah N, Amanullah, Jamal S, Marwat MA. Association of hyperuricemia with diabetic nephropathy in type 2 diabetes mellitus. K J Med Sci 2014; :2.

46. Akbas EM, Timuroglu A, Ozcicek A, Ozcicek F, Demirtas L, Gungor L, et al. Association of uric acid, atherogenic index of plasma and albuminuria in diabetes mellitus. Int J Clin Exp Med 2014;7(12):5737-5743.

47. Ritz.E,Orth SR. Nephtopathy in patients with type2diabetes mellitus. N Eng J Med.1999; 341:1127-1133.

48. Jiten P Vora & Hisham A A Ibrahim. Clinical manifestations & Natural History of Diabetic nephropathy, "Comprehensive Clinical Nephrology", Mosby Elsevier.Rude2003, 2nd edition, Page1277.

49. Mc Cance DR, Hanson RL, Pettitt DJ, Diabetic nephropathy:a risk factor for diabetes mellitus in off spring.diabetololologia.1995:38:221-6.

50. Jiten P Vom and I-Iisham AA Ibrahim. Clinical Manifestations and .Natural History of Diabetic Nephropathy ,chapter-32,section 6 in Comprehensive clinical Nephrology,2nd ed, Richard J.Johnson & John Feehaly; Mosby elsevier science Publications;2003.p425-427.

51. Chan JCN, Cheung C.K, Cheung MYF. Abnormal albuminuria as a predictor of mortality & renal impairment in Chinese patients with NIDDM. Diabetes 1996; 45:51-5.

52. Mogensen CE. How to protect the kidney in diabetic patients with special reference to IDDM.Diabetes.1997; 46:104-11.

53. Rudberg S, osterby R. Decreasing glomerular filteration rate-an in indicator of more advanced glomerulopathy in early stages of microalbuminuria in IDDM adolescents. Nephrol Dial transplant.1977; 12: 1149-54.

54. Zatz R, Dunn BR, Meyer TW, Brenner B. Prevention of diabetic glomerulopathy by pharmacological amelioreation of glomerular capillary hypertension. J Clin Invest.1986; 77:1925.

55. Ishii H, Jirousek MR, Koya D, Amelioration of vascular dysfunction sin diabetic rats by an oral PKC beta inhibitor.science.1996;272:728-31.

56. Gilbert RE, Cooper ME. The tubuolointerstitium in progressive diabetic kidney disease: More than an aftermath of glomerular injury? .Kidney Int.1999; 56:1627-37.

57. Adler S. Diabetic nephropathy: Linking histology, cell biology and genetics. Kidney Int 2004; 66:2095.

58. Ruggenenti P, Gambara V, Perna A, et al. The nephropathy of non-insulin-dependent diabetes: Predictors of outcome relative to diverse patterns of renal injury. J Am Soc Nephrol 1998; 9:2336.

59. Chan JC, Tomlinson B, Nicholls MG, Swaminathan R, Cheung CK, Woo J, et al: albuminuria, insulin resistance and dyslipidemia in Chinese patients with non-insulin-dependent diabetes (NDDM). Diabet Med 1996; 13:150–155.

60. Tapp RJ, Shaw JE, Zimmet PZ, et al. Albuminuria is evident in the early stages of diabetes onset: results from the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). Am J Kidney Dis 2004; 44:792.

61. Ritz E, Ogata H, Orth SR. Smoking: a factor promoting onset and progression of diabetic nephropathy. Diabetes Metab. 2000; 26 Suppl 4: 54-63.

62. Schmidt S, Ritz E. Angiotensin I converting enzyme gene poly-morphism and diabetic nephropathy in type II diabetes. Nephrol Dial Transp. 1997; 12:37-41.

63. Smith SR, Svetkey LP, Dennis VW. Racial differences in the incidence and progression of renal diseases. Kidney Int 1991; 40:815.

64. Boer IH, Sibley SD, Kestenbaum B, et al. Central obesity, incident microalbuminuria, and change in creatinine clearance in the epidemiology of diabetes interventions and complications study. J Am Soc Nephrol 2007; 18:235.

65. Morales E, Valero MA, Leon M, et al. Beneficial effects of weight loss in overweight patients with chronic proteinuric nephropathies. Am J Kidney Dis 2003; 41:319.

66. Saiki A, Nagayama D, Ohhira M, et al. Effect of weight loss using formula diet on renal function in obese patients with diabetic nephropathy. Int J Obes (Lond) 2005; 29:1115.

67. Chase HP, Garg SK, Marshall G, et al. Cigarette smoking increases the risk of albuminuria among subjects with type 1 diabetes. JAMA 1991; 265:614.

68. Vijan S, Hayward RA. Treatment of hypertension in type 2 diabetes mellitus: Blood pressure goals, choice of agents, and setting priorities in diabetes care. Ann Intern Med 2003; 138:593.

69. Rudberg S, Persson B, Dahlquist G. Increased glomerular filtration rate as a predictor of diabetic nephropathy An 8-year prospective study. Kidney Int 1992; 41:822.

70. Nowack R, Raum E, Blum W, Ritz E. Renal hemodynamics in recent-onset type 2 diabetes. Am J Kidney Dis 1992; 20:342.

71. Hayashi K, Epstein M, Loutzenheiser R, Forster H. Impaired myogenic responsiveness of afferent arteriole in streptozotocin induced diabetic rats: Role of eicosanoid derangements. J Am Soc Nephrol 1992; 2:1578.

72. Gilbert RE, Tsalamandris C, Bach LA, et al. Long-term glycemic control and the rate of progression of early diabetic renal disease. Kidney Int 1993; 44:855.

73. Alvin C.Powers: "Diabetes mellitus" in Chapter-338 in Harrison's Principles of Internal Medicine, Fauci ,Braunwald, , Kasper, Hauser, Longo, Jameson, Loscalzo, 17th Edn, New York, McGraw Hill Publication,2008, 2287-2290.

74. Juan F. Navarro and Carmen Mora, Role of inflammation in diabetic complications; Nephrology Dialysis Transplantation; 2005 20(12):2601-2604.

75. Pickup JC. Inflammation and activated innate immune system in the pathogenesis of type 2 diabetes. Diabetes Care 2004; 27: 813–823.

76. Satoh J, Yagihashi S, Toyota T. The possible role of tumor necrosis factor-alpha in diabetic polyneuropathy. Exp Diabesity Res 2003; 4: 65–71.

77. Joussen AM, Poulaki V, Mitsiades N,shosen R,Ronald S,Fehale G *et al*. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF- suppression. FASEB J. 2002; 10.

78. Joussen AM, Poulaki V, Le ML.A central role for inflammation in the pathogenesis of diabetic retinopathy. *FASEB* J 2004; 18: 1450–1452.

79. Dalla Vestra M, Mussap M, Gallina P, Doni A, van Hinsbergh VW, Stehouwer CD et al. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. J Am Soc Nephrol. 2005; 16: 78-82.

80. Chow FY, Nikolic-Paterson DJ, Ozols E, Atkins RC, Tesch GH. Intracellular adhesion molecule-1 deficiency is protective against nephropathy in type 2 diabetic db/db mice. J Am Soc Nephrol 2005; 16: 1711–1722.

81. Kelly DJ, Chanty A, Gow RM, Zhang Y, Gilbert RE. Protein kinase C beta inhibition attenuates osteopontin expression, macrophage recruitment, and tubulointerstitial injury in advanced experimental nephropathy. J Am SocNephrol2005; 16: 1654–1660.

82. Hasegawa G, Nakano K, Sawada M,. Koga M, Fukuchi M, Hada T,et al. Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. Kidney Int 1991; 40: 1007–1012.

83. Navarro JF, Mora C, Rivero A. Urinary protein excretion and serum tumor necrosis factor in diabetic patients with advanced renal failure: effects of pentoxifylline administration. Am J Kidney Dis.1999; 33: 458-63.

84. Navarro JF, Mora C, Macía M, García J. Inflammatory parameters are independently associated with urinary albumin excretion in type 2 diabetes mellitus. Am J Kidney Dis 2003; 42:53–61.

85. Royall JA, Berkow RL, Beckman JS, Cunningham MK, Matalon S, Freeman BA. Tumor necrosis factor and interleukin 1 increase vascular endothelial permeability. Am J Physiol 1989; 257: L339–L410.

86. Melcion C, Lachman L, Killen D, Morel-Maroger L, Striker GE. Mesangial cells, effect of monocyte products on proliferation, and matrix synthesis. Transplant Proc1982; 14: 559–564.

87. Hirano T, Akira S, Taga T, Kishimoto T. Biological and clinical aspect of interleukin 6.ImmunolToday 1990; 11: 443–449.

88. Nakamura T, Fukui M, Ebihara I ,sholin S, Figiama H, Galen K et al. mRNA expression of growth factors in glomeruli from diabetic rats. Diabetes1993; 42: 450–456.

89. Navarro JF, Milena FJ, Mora C, Schrier RW, Edelstein CL Okamura H, et al. Tumor necrosis factor- gene express on in diabetic nephrophathy: relationship with urinary albumin excretion and effect of angiotensin-converting enzyme inhibition. Kidney Int 2005.

90. Ortiz A, Gonzalez-Cuadrado S, Bustos C, Donald,Vobalen, Mogen C, et al. Tumor necrosis factor as a mediator of glomerular damage. J Nephrol 1995; 8: 27–34.

91. MaCarthy E, Sharma R, Sharma M .TNF- increases albumin permeability of isolated rat glomeruli through the generation of superoxide. J Am Soc Nephrol 1998; 9: 433–438.

92. Dipetrillo K, Coutermarsh B, Gesek FA. Urinary tumor necrosis factor contributes to sodium retention and renal hypertrophy during diabetes. Am JPhysiol Renal Physiol 2003; 284: F113–F121.

93. .Border WA, Noble NA. Evidence that TGF-beta should be a therapeutic target in diabetic nephropathy. Kidney international. 1998; 54(4):1390–1.

94. Chen S, Hong SW, Iglesias-de la Cruz MC, Isono M, Casaretto A, Ziyadeh FN. The key role of the transforming growth factor-beta system in the pathogenesis of diabetic nephropathy. Renal failure. 2001; 23(3–4):471–81.

95. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic

mice. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97: 8015–20.

96. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA. Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. Proc Natl Acad Sci U S A. 1993; 90:1814–8.

97. Nakamura T, Fukui M, Ebihara I, Osada S, Nagaoka I, Tomino Y, et al. mRNA expression of growth factors in glomeruli from diabetic rats. Diabetes. 1993; 42: 450–6.

98. Sharma K, Jin Y, Guo J, Ziyadeh FN. Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. Diabetes. 1996; 45:522–30.

99. Sharma K, Ziyadeh FN. Renal hypertrophy is associated with upregulation of TGF-beta 1 gene expression in diabetic BB rat and NOD mouse. Am J Physiol. 1994; 267:1094–01.

100. . Burns WC, Twigg SM, Forbes JM, et al. Connective tissue growth factor plays an important role in advanced glycation end product-induced tubular epithelial-to-mesenchymal transition: implications for diabetic renal disease. J Am Soc Nephrol 2006; 17:2484.

101. Hohenstein B, Hausknecht B, Boehmer K, et al. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. Kidney Int 2006; 69:1654.

102. Sung SH, Ziyadeh FN, Wang A, et al. Blockade of vascular endothelial growth factor signaling ameliorates diabetic albuminuria in mice. J Am Soc Nephrol 2006; 17:3093.

103. Riser BL, Cortes P, Yee J, Sharba AK, Asano K, Rodriguez-Barbero A, et al. Mechanical strain- and high glucose-induced alterations in mesangial cell collagen metabolism: role of TGF-beta. J Am Soc Nephrol. 1998; 9:827–36.

104. Riser BL, Ladson-Wofford S, Sharba A, Cortes P, Drake K, Guerin CJ, et al. TGF-beta receptor expression and binding in rat mesangial cells: modulation by glucose and cyclic mechanical strain. Kidney international. 1999 ; 56 :428–39.

105. Ziyadeh FN, Sharma K, Ericksen M, Wolf G. Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-beta. J Clin Invest. 1994; 93:536–42.

106. Ayo SH, Radnik RA, Garoni JA, Glass WF, 2nd, Kreisberg JI. High glucose causes an increase in extracellular matrix proteins in cultured mesangial cells. Am J Pathol. 1990; 136:1339–48.

107. Haneda M, Kikkawa R, Horide N, Togawa M, Koya D, Kajiwara N, et al. Glucose enhances type IV collagen production in cultured rat glomerular mesangial cells. Diabetologia. 1991; 34:198–200.

108. Hayashi K, Epstein M, Loutzenhiser R, Forster H. Impaired myogenic responsiveness of the afferent arteriole in streptozotocin-induced diabetic rats: role of eicosanoid derangements. J Am Soc Nephrol. 1992; 2:1578–86.

109. Bidani AK, Griffin KA, Picken M, Lansky DM. Continuous telemetric blood pressure monitoring and glomerular injury in the rat remnant kidney model. Am J Physiol. 1993; 265:391–8.

110. Riser BL, Cortes P, Zhao X, Bernstein J, Dumler F, Narins RG. Intraglomerular pressure and mesangial stretching stimulate extracellular matrix formation in the rat. J Clin Invest. 1992; 90:1932–43.

111. Riser BL, Cortes P, Heilig C, Grondin J, Ladson-Wofford S, Patterson D, et al. Cyclic stretching force selectively up-regulates transforming growth factor-beta isoforms in cultured rat mesangial cells. Am J Pathol. 1996; 148:1915–23.

112. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. Diabetes. 1998; 47:859–66.

113. Haneda M, Araki S, Togawa M, Sugimoto T, Isono M, Kikkawa R. Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. Diabetes. 1997; 46:847–53.

114. Haneda M, Kikkawa R, Arimura T, Ebata K, Togawa M, Maeda S, et al. Glucose inhibits myo-inositol uptake and reduces myo-inositol content in cultured rat glomerular mesangial cells. Metabolism: clinical and experimental. 1990; 39:40–5.

115. Suzuki D, Miyata T, Saotome N, Horie K, Inagi R, Yasuda Y, et al. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. J Am Soc Nephrol. 1999; 10:822–32.

116. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. The New England journal of medicine. 1988; 318:1315–21.

117. Koya D, Haneda M, Nakagawa H, Isshiki K, Sato H, Maeda S, et al. Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. Faseb J. 2000; 14:439–47.

118. Derubertis FR, Craven PA. Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. Diabetes. 1994; 43:1–8.

119. Barnard JA, Lyons RM, Moses HL. The cell biology of transforming growth factor beta. Biochimica et biophysica acta. 1990; 1032:79–87.

120. Massague J. The transforming growth factor-beta family. Annual review of cell biology. 1990; 6:597–641.

121. Prud'homme GJ, Piccirillo CA. The inhibitory effects of transforming growth factor-beta-1 (TGF-beta1) in autoimmune diseases. J Autoimmun. 2000; 14:23–42.

122. McCartney-Francis NL, Frazier-Jessen M, Wahl SM. TGF-beta: a balancing act. Int Rev Immunol. 1998; 16:553–80.

123. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature. 1992; 359:693–9.

124. Albertoni GA, Borges FT, Schor N (2012). Uric Acid and Renal Function. In: Sahay M editor. Diseases of Renal Parenchyma. Croatia: In Tech; 2012.

125. Edwards NL. The role of hyperuricemia and gout in kidney and cardiovascular disease. Cleve Clin J Med. 2008; 75:S13–6.

126. Dalbeth N, Stamp L. Allopurinol dosing in renal impairment: walking the tightrope between adequate urate lowering and adverse events. Semin Dial. 2007; 20:391–5.

127. Kodama S, Saito K, Yachi Y, Asumi M, Sugawara A, Totsuka K, et al. Association between serum uric acid and development of type 2 diabetes. Diabetes care. 2009; 32:1737–42.

128. Johnson RJ, Perez-Pozo SE, Sautin YY, Manitius J, Sanchez-Lozada LG, Feig DI, et al. Hypothesis: could excessive fructose intake and uric acid cause type 2 diabetes? Endocr Rev. 2009; 30:96–116.

129. Waring WS, McKnight JA, Webb DJ, Maxwell SR. Uric acid restores endothelial function in patients with type1 diabetes and regular smokers. Diabetes. 2006; 55:3127–32.

130. Scott GS, Cuzzocrea S, Genovese T, Koprowski H, Hooper DC. Uric acid protects against secondary damage after spinal cord injury. Proc Natl Acad Sci U S A. 2005; 102:3483–8.

131. Waring WS, Adwani SH, Breukels O, Webb DJ, Maxwell SR. Hyperuricaemia does not impair cardiovascular function in healthy adults. Heart. 2004; 90:155–9.

132. Zharikov S, Krotova K, Hu H, Baylis C, Johnson RJ, Block ER, et al. Uric acid decreases NO production and increases arginase activity in cultured pulmonary artery endothelial cells. Am J Physiol Cell Physiol. 2008; 295:1183–90.

133. Khosla UM, Zharikov S, Finch JL, Nakagawa T, Roncal C, Mu W, et al. Hyperuricemia induces endothelial dysfunction. Kidney Int. 2005; 67: 1739–42.

134. Kang DH, Park SK, Lee IK, Johnson RJ. Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. J Am Soc Nephrol. 2005; 16:3553–62.

135. Gersch C, Palii SP, Kim KM, Angerhofer A, Johnson RJ, Henderson GN. Inactivation of nitric oxide by uric acid. Nucleosides Nucleotides Nucleic Acids. 2008; 27:967–78.

136. Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension. 2001; 38:1101–6.

137. Sanchez-Lozada LG, Tapia E, Lopez-Molina R, Nepomuceno T, Soto V, Avila-Casado C, et al. Effects of acute and chronic L-arginine treatment in experimental hyperuricemia. American journal of physiology. 2007; 292:1238–44.

138. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. Circulation. 2006; 114:2508–16.

139. Mazzali M, Kanellis J, Han L, Feng L, Xia YY, Chen Q, et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. Am J Physiol Renal Physiol. 2002; 282:991–7.

140. Roncal CA, Mu W, Croker B, Reungjui S, Ouyang X, Tabah-Fisch I, et al. Effect of elevated serum uric acid on cisplatin-induced acute renal failure. American journal of physiology. 2007; 292:116–22.

141. Kang DH, Nakagawa T, Feng L, Watanabe S, Han L, Mazzali M, et al. A role for uric acid in the progression of renal disease. J Am Soc Nephrol. 2002; 13:2888–97.

142. Netea MG, Kullberg BJ, Blok WL, Netea RT, van der Meer JW. The role of hyperuricemia in the increased cytokine production after lipopolysaccharide challenge in neutropenic mice. Blood. 1997; 89:577–82.

143. Talaat KM, el-Sheikh AR. The effect of mild hyperuricemia on urinary transforming growth factor beta and the progression of chronic kidney disease. Am J Nephrol. 2007; 27:435–40.

144. Massague J. How cells read TGF-beta signals. Nat Rev Mol Cell Biol. 2000; 1:169–78.

145. Choi ME. Mechanism of transforming growth factor-beta1 signaling. Kidney Int Suppl. 2000; 77:53–8.

146. Kanellis J, Watanabe S, Li JH, Kang DH, Li P, Nakagawa T, et al. Uric acid stimulates monocyte chemoattractant protein-1 production in vascular smooth muscle cells via mitogen-activated protein kinase and cyclooxygenase-2. Hypertension. 2003; 41:1287–93.

147. Nakagawa T, Sato W, Glushakova O, Heinig M, Clarke T, Campbell-Thompson M, et al. Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. J Am Soc Nephrol. 2007; 18:539–50.

148. Han HJ, Lim MJ, Lee YJ, Lee JH, Yang IS, Taub M. Uric acid inhibits renal proximal tubule cell proliferation via at least two signaling pathways involving PKC, MAPK, cPLA2, and NF-kappaB. Am J Physiol Renal Physiol. 2007; 292:373– 81.

149. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, et al. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? Hypertension. 2003; 41:1183–90.

150. Rosolowsky ET, Ficociello LH, Maselli NJ, Niewczas MA, Binns AL, Roshan B, et al. High-normal serum uric acid is associated with impaired glomerular filtration rate in nonproteinuric patients with type 1 diabetes. Clin J Am Soc Nephrol. 2008;3:706–13.

151. Hsu CY, Iribarren C, McCulloch CE, Darbinian J, Go AS. Risk factors for end-stage renal disease: 25 -year follow-up. Arch Intern Med. 2009; 169:342–50.

152. Fu CC, Wu DA, Wang JH, Yang WC, Tseng CH. Association of Creactive protein and hyperuricemia with diabetic nephropathy in Chinese type 2 diabetic patients. Acta Diabetol. 2009;46:127–34.

153. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS. Uric acid and incident kidney disease in the community. J Am Soc Nephrol. 2008; 19:1204–11.

154. Borges RL, Hirota AH, Quinto BM, Ribeiro AB, Zanella MT, Batista MC. Uric acid as a marker for renal dysfunction in hypertensive women on diuretic and nondiuretic therapy. J Clin Hypertens (Greenwich) 2009; 11:253–9.

155. Park JT, Kim DK, Chang TI, Kim HW, Chang JH, Park SY, et al. Uric acid is associated with the rate of residual renal function decline in peritoneal dialysis patients. Nephrol Dial Transplant. 2009; 24:3520–5.

156. Chonchol M, Shlipak MG, Katz R, Sarnak MJ, Newman AB, Siscovick DS, et al. Relationship of uric acid with progression of kidney disease. Am J Kidney Dis. 2007; 50:239–47.

157. Sturm G, Kollerits B, Neyer U, Ritz E, Kronenberg F. Uric acid as a risk factor for progression of non-diabetic chronic kidney disease? The Mild to Moderate Kidney Disease (MMKD) Study. Exp Gerontol. 2008; 43:347–52.

158. Madero M, Sarnak MJ, Wang X, Greene T, Beck GJ, Kusek JW, et al. Uric acid and long-term outcomes in CKD. Am J Kidney Dis. 2009; 53:796–803.

159. Hovind P, Rossing P, Tarnow L, Johnson RJ, Parving HH. Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes - an inception cohort study. Diabetes. 2009.

160. Ramachandran A, Snehalatha C. Epidemiology and Basic Considerations of Diabetes. In: Munjal YP, Sharma SK, Agarwal AK, Gupta P, Kamath SA, Nadkar MY, et al, editors. API Textbook of Medicine, 9th ed. Mumbai: Jaypee Brothers; 2012. P.-321-323.

161. Nakagawa T, Hu H, Zharikov S, et al. A causal role for uric acid in fructose-induced metabolic syndrome. Am J Physiol Renal Physiol. 2006; 290: F 625-31.

162. Lohsoonthorn V, Dhanamun B, Williams MA. Prevalence of hyperuricemia and its relationship with metabolic syndrome in Thai adults receiving annual health exams. Arch Med Res. 2006; 37:883-9.

163. Sanchez-Lozada LG, Tapia E, Avila-Casado C, Soto V, Franco M, Santamaría J, et al. Mild hyperuricemia induces glomerular hypertension in normal rats. Am J Physiol Renal Physiol 2002; 283: F1105-F1110.

164. Nakagawa T, Mazzali M, Kand DH, Kanellis J, Watanabe S, Sanchez-Lozada LG, et al. Hyperuricemia causes glomerular hypertrophy in the rat. Am J Nephrol 2003; 23: 2-7.

165. Mogensen CE. Microalbuminuria, blood pressure and diabetic renal disease: Origin and development of ideas. Diabetologia 1999; 42: 263-285.

166. Butler R, Morris AD, Belch JJ, et al: Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. Hypertension 2000; 35:746–751.

167. Bakker SJ, Gans RO, Maaten JC, et al: The potential role of adenosine in the pathophysiology of the insulin resistance syndrome. Atherosclerosis 2001;155:283–290.

168. Modan M, Halkin H, Karasik A, Lusky A: Elevated serum uric acid: a facet of hyperinsulinemia. Diabetologia 1987;30:713–718.

169. Mcfarlane SI, Banerji M, Sowers JR: Insulin resistance and cardiovascular disease. J Clin Endocrinol Metab 2001;86:713–718.

ANNUXURES

ETHICAL CLEARANCE CERTIFICATE

Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. SD/13
Date 14-11-13
B.L.D.E.U's Shri B.M. Patil Medical College

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Study of association of Serum uric acid with albuminuria in type-II Diabetes Mellitus," —x—x—

Name of P.G. student Dr Priti. S. Langote. Department of Medicine

Name of Guide/Co-investigator Dr Mallanna S. Mulimani Prof & HOD of Medicine.

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

RESEARCH INFORMED CONSENT FORM

TITLE OF RESEARCH : "STUDY OF ASSOCIATION OF SERUM URIC ACID WITH ALBUMINURIA IN TYPE 2 DIABETES MELLITUS"

GUIDE : DR. MALLANNA S MULIMANI

P.G. STUDENT : DR. PRITI S. LANGOTE

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to find out whether there is association of serum uric acid level with proteinuria in type 2 diabetic patients.

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved and I may experience mild pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to find out whether there is an association of serum uric acid level with proteinuria in type 2 diabetes mellitus.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of hospital. If the data is used for publications the identity of the patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

(Signature of Guardian) (Signature of patient)

(If the patient is conscious, well oriented and fully aware)

II. PROFORMA

Name: IP. No:

Age: Address

Sex: Date of Admission:

Occupation: Date of Discharge:

Religion: Status at Discharge:

Unit:

Chief complaints:

Present history:

Past history:

History of gout

H/O drug intake:

Allopurinol

Current medications:

Insulin: OHA: Diuretics:

Personal history:

Diet: Appetite: Sleep:

Bladder and bowel habits:

Tobacco use: Alcoholic Beverages:

Family history:

GENERAL PHYSICAL EXAMINATION

Pallor:

Icterus:

Cyanosis:

Clubbing:

Lymphadenopathy:

Edema:

VITAL SIGNS:

Pulse rate:

Blood pressure:

Temperature:

Respiration rate:

BMI Estimation:

Weight:

Height:

BMI:

SYSTEMIC EXAMINATION:

PER ABDOMEN:

RESPIRATORY SYSTEM:

CARDIOVASCULAR SYSTEM:

CENTRAL NERVOUS SYSTEM:

INVESTTIGATIONS:

1.Serum Uric Acid	
2.Serum Creatinine	
3.HbA1C	
4.RBS	
5.FBS	
6.PPBS	
7.HIV Rapid Test	

Urine Routine: **Albumin:** **Pus Cells:**	

Urine Albumin:	
Urine Creatinine:	
Urine ACR:	

KEY TO MASTER CHART

BMI	–	Body Mass Index
SBP	–	Systolic Blood Pressure
DBP	–	Diastolic Blood Pressure
SUA	–	Serum Uric Acid
U. Albumin	–	Urine Albumin
U. Creatinine	–	Urine Creatinine
ACR	–	Albumin Creatinine Ratio
HTN	–	Hypertension
1	–	Male
2	–	Female

MSTER CHART

Sl No.	Age	Sex	Weight (kg)	Height (meter)	Height (m2)	BMI	SBP	DBP	Duration of DM (years)	SUA	Serum Creatinine	HbA 1C	U.Albumin (mg/L)	U.Albumin (ug/dl)	U.Creat (mg%)	ACR	HTN YES=1, NO=0
1	67	1	50	1.6	2.56	19.53125	160	90	10	8	2.1	7.8	210	21000	70	300	1
2	58	1	60	1.7	2.89	20.76125	130	80	3	3.9	1.2	4.8	18	1800	50	36	0
3	77	1	64	1.56	2.4336	26.29849	140	90	6	6.6	1	6.1	36	3600	50	72	0
4	41	1	69	1.68	2.8224	24.44728	130	80	4	4	1.2	5.1	30	3000	60	50	0
5	50	1	66	1.58	2.4964	26.43807	140	90	2	4.3	0.7	5.8	33	3300	60	55	0
6	65	2	60	1.56	2.4336	24.65483	160	90	8	5.6	0.8	11.6	110	11000	70	157.142857	1
7	50	2	62	1.6	2.56	24.21875	180	80	12	7	0.8	7	190	19000	50	380	1
8	35	2	45	1.57	2.4649	18.25632	110	70	1	2.9	0.7	6	81	8100	70	115.714286	0
9	55	1	65	1.59	2.5281	25.71101	120	80	20	7.5	1.1	8	230	23000	70	328.571429	0
10	74	1	61	1.6	2.56	23.82813	150	100	15	6.1	0.8	6.3	180	18000	60	300	1
11	40	2	62	1.48	2.1904	28.30533	130	90	3	3.1	0.7	4.3	10	1000	50	20	0
12	61	2	61	1.6	2.56	23.82813	150	90	8	4.5	0.7	4	24	2400	55	43.6363636	1
13	62	1	67	1.7	2.89	23.18339	154	80	10	5.6	0.8	6.7	80	8000	71	112.676056	0
14	58	2	44	1.54	2.3716	18.55288	146	80	8	4.8	1	5	30	3000	60	50	0
15	55	2	57	1.52	2.3104	24.67105	140	80	3	4.2	0.7	9.7	120	12000	90	133.333333	1
16	47	2	54	1.53	2.3409	23.06805	130	80	5	6.6	1	9.2	90	9000	50	180	0

17	65	2	62	1.47	2.1609	28.69175	150	90	8	5.5	0.8	6	70	7000	50	140	0
18	45	1	65	1.68	2.8224	23.03005	136	86	4	4.3	0.8	5.6	15	1500	60	25	0
19	50	2	56	1.58	2.4964	22.4323	130	80	6	3.4	0.7	5.9	10	1000	60	16.6666667	0
20	49	2	64	1.57	2.4649	25.96454	146	80	4	4.8	0.8	5.1	15	1500	70	21.4285714	0
21	40	2	58	1.54	2.3716	24.45606	130	80	3	3.8	0.7	4.9	10	1000	60	16.6666667	0
22	54	1	60	1.7	2.89	20.76125	140	80	6	5.2	1	6.6	40	4000	60	66.6666667	0
23	50	2	56	1.56	2.4336	23.01118	130	70	4	2.7	0.7	4.8	8	800	70	11.4285714	0
24	50	1	56	1.64	2.6896	20.82094	136	80	6	4.5	1.1	6.1	15	1500	80	18.75	0
25	46	1	54	1.65	2.7225	19.83471	120	80	3	2.5	0.7	5.3	6	600	70	8.57142857	0
26	52	1	56	1.59	2.5281	22.15102	130	80	8	5.8	1.1	6.8	30	3000	70	42.8571429	0
27	60	1	63	1.68	2.8224	22.32143	140	90	10	4.8	0.8	5.9	20	2000	80	25	0
28	48	1	67	1.57	2.4649	27.18163	126	78	4	2.7	0.7	4.6	5	500	60	8.33333333	0
29	57	1	61	1.48	2.1904	27.84879	130	78	12	4.8	1.1	6.3	45	4500	70	64.2857143	0
30	49	1	54	1.6	2.56	21.09375	120	80	4	3.4	0.8	5.9	10	1000	60	16.6666667	0
31	65	1	64	1.66	2.7556	23.22543	120	70	8	4.9	1.8	4.8	10	1000	60	16.6666667	0
32	46	2	42	1.54	2.3716	17.70956	130	80	4	5.9	0.8	6.1	24	2400	30	80	0
33	44	2	75	1.62	2.6244	28.57796	136	80	2	3.1	0.6	5.8	15	1500	50	30	0
34	50	2	58	1.57	2.4649	23.53037	136	84	4	4.7	0.6	6.8	57	5700	80	71.25	0
35	80	2	50	1.5	2.25	22.22222	150	90	3	6.2	1.4	8.3	57	5700	80	71.25	0
36	73	1	56	1.68	2.8224	19.84127	120	80	15	4.8	1.7	5.1	67	6700	60	111.666667	0

37	32	2	70	1.58	2.4964	28.04038	110	70	4	5.7	0.8	8	24	2400	50	48	0
38	52	1	65	1.61	2.5921	25.07619	130	70	13	8.1	1.4	6.8	98	9800	50	196	0
39	70	2	56	1.54	2.3716	23.61275	116	78	1	4.3	0.8	6.8	27	2700	90	30	0
40	60	2	50	1.48	2.1904	22.82688	130	76	10	3.5	1.3	6.7	17	1700	37	45.9459459	0
41	65	1	64	1.77	3.1329	20.42836	146	80	3	4	0.8	10.5	24	2400	131	18.3206107	0
42	81	1	65	1.62	2.6244	24.76757	158	80	2	8	2	7	13	1300	50	26	0
43	52	2	60	1.58	2.4964	24.03461	150	80	4	5.3	1.2	7.5	35	3500	50	70	1
44	65	2	48	1.56	2.4336	19.72387	160	90	8	8.3	1.7	12.9	230	23000	70	328.571429	1
45	65	1	72	1.68	2.8224	25.5102	158	90	10	6.1	1	11.7	126	12600	70	180	1
46	52	1	62	1.72	2.9584	20.95727	140	80	15	4.9	1.4	10.7	56	5600	68	82.3529412	0
47	65	2	90	1.62	2.6244	34.29355	166	96	8	5.6	2	6.9	86	8600	50	172	1
48	80	1	70	1.7	2.89	24.22145	140	80	10	4.5	1.2	6.5	19	1900	140	13.5714286	0
49	55	1	66	1.74	3.0276	21.79945	130	70	6	3.4	0.8	6.8	110	11000	90	122.222222	0
50	90	2	62	1.58	2.4964	24.83576	146	80	6	5	1	10.5	83	8300	50	166	1
51	50	2	58	1.6	2.56	22.65625	140	80	6	5.1	0.7	10.5	10	1000	12	83.3333333	0
52	58	1	94	1.7	2.89	32.52595	150	90	10	6.5	0.8	10.8	53	5300	100	53	1
53	45	1	66	1.45	2.1025	31.3912	118	72	3	4.7	1.8	10.5	78	7800	50	156	0
54	65	1	55	1.7	2.89	19.03114	140	80	5	4.7	1.6	11.7	35	3500	17	205.882353	0
55	45	2	60	1.61	2.5921	23.14726	130	70	6	3.8	1.1	13	38	3800	60	63.3333333	0
56	55	2	65	1.62	2.6244	24.76757	136	80	5	12	1.5	8.8	46	4600	38	121.052632	1