# ROLE OF URICACID & hs-CRP AS RENAL AND CARDIOVASCULAR RISK MARKERS IN TYPE 1 DIABETIC PATIENTS WITH LOW GFR.

*Dissertation submitted for*

**M.D. BIOCHEMISTRY BRANCH – XIII**

**DEGREE EXAMINATION**


**THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY**

**CHENNAI – 600 032**

**TAMILNADU**

**APRIL 2016**

# BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "**ROLE OF URICACID & hs-CRP AS RENAL & CARDIOVASCULAR RISK MARKERS IN TYPE 1 DIABETIC PATIENTS WITH LOW GFR"** is the original bonafide work done by **DR.S.ANANDHI**, Post Graduate Student, Institute of Biochemistry, Madras Medical College, Chennai under our direct supervision and guidance.


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# DECLARATION

I, **Dr. S.ANANDHI** , Post Graduate , Institute of Biochemistry, Madras Medical College, solemnly declare that the dissertation titled "**ROLE OF URICACID & hs-CRP AS RENAL & CARDIOVASCULAR RISK MARKERS IN TYPE 1 DIABETIC PATIENTS WITH LOW GFR"** is the bonafide work done by me at Institute of Biochemistry, Madras Medical College under the expert guidance and supervision of **Prof. Dr. V.AMUTHAVALLI,** M.D., Professor, Institute of Biochemistry, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch XIII) in Biochemistry.

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## SPECIAL ACKNOWLEDGEMENT

The author gratefully acknowledges and sincerely thanks Professor **Dr. R. Vimala, M.D.,** Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, for granting her permission to utilize the facilities of this Institution for the study.

# ACKNOWLEDGEMENT

The author expresses her warmest respects and profound gratitude to **Dr.K.Ramadevi, M.D**., Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for her academic enthusiasm and for facilitating her research work in the institute.

The author expresses her heartfelt gratitude to her guide and supervisor **Dr.V.AMUTHAVALLI, M.D**., Professor, Institute of Biochemistry, Madras Medical College, Chennai, for his intellectual and valuable guidance, unfailing support, encouragement and continuous inspiration throughout the period of her study.

The author in particular, is extremely thankful to **Dr.DHARMARAJAN.P.,MD,Dip(Diab**), Director and Professor ,Institute of Diabetology, Rajiv Gandhi Government General Hospital, Chennai, for granting permission to obtain blood samples from the patients.

The author expresses her thanks to the **Professors Dr.I.Periyandavar M.D, Dr.R.ChitraaM.D**, **Dr.V.K.Ramadesikan M.D ,Dr.K.Pramila M.D and Dr.Sumathy.S. M.D** Institute of biochemistry, Madras Medical College, for their guidance, encouragement, insightful comments and suggestions.

The author expresses her warm respects and sincere thanks to her co-guide. **Dr.Mythili.C. M.D** Assistant Professor, Institute of biochemistry,

Madras Medical College for her guidance and support. The author expresses her warm respects and sincere thanks to other **Assistant Professors, Dr.Karpagavalli.V.C**, **Dr.C.Shanmugapriya, Dr.V.Ananthan, Dr.S.Siva, Dr.B.SudhaPresanna,** Institute of biochemistry, Madras Medical College, for their valuable suggestions regarding the practical issues of research which is something beyond the textbooks.

The author expresses warm respects to the members of the Institutional Ethical committee for approving the study.

The author expresses her special thanks to Diabetology Lab technicians, and Biochemistry Laboratory Staff , for their timely help and cooperation during sample collection.

The author is indebted to the patients from whom blood samples were collected for conducting the study.

The author expresses her special thanks to her co-PGs Dr.Michael RajamGeetha.S., Dr.Nirmaladevi.P., Dr.Divya.M. for their cooperation and genuine support. The author expresses her thanks to all her colleagues in the institute, for their constant encouragement throughout the study period.

The author gratefully acknowledges the help rendered by her father in law **Dr.V.Elangovan ,** for the statistical analysis of the study.

The author expresses her special thanks to her husband Dr.E.Aravindan, her daughter Nethra Aravindan, her Mother in law, her

parents and other family members for the moral support and encouragement extended by them which gave fulfillment to the dissertation work.

Above all, the author is grateful to the Almighty for providing this opportunity, without whose grace nothing could be accomplished.

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## ABBREVIATIONS

| | | | |
|---|---|---|---|
| 1. | IPEX | - | Immuno dysregulation polyendocrinopathy enteropathy X-linked **.** |
| 2. | FOXP3 | - | Forkhead box protein 3. |
| 3. | HLA | - | Human Leucocyte Antigen. |
| 4. | TNF α | - | Tumour necrosis factor alpha. |
| 5. | VNTR | - | Variable number of Tandem repeats. |
| 6. | INF γ | - | Interferon gamma |
| 7. | IL | - | Interleukins |
| 8. | PTPN | - | Protein Tyrosine Phosphatase non receptor Type. |
| 9. | IL-2Rα | - | Interleukin-2 Receptor alpha. |
| 10. | T regs | - | Regulator T cells. |
| 11. | CTLA4 | - | Cytotoxic T Lymphocyte associated protein-4. |
| 12. | IFIH | - | Interferon induced helicase. |
| 13. | ICA | - | Islet cell cytoplasmic Antibodies. |
| 14. | GAD | - | Glutamic acid decarboxylase. |
| 15. | VEGF | - | Vascular Endothelial Growth factor. |
| 16. | IAA | - | Insulin auto antibodies. |
| 17. | Zn T | - | Zinc Transporter. |
| 18. | CXCL | - | Chemokine Ligand. |
| 19. | SLC | - | Solute carrier |
| 20. | TGF β | - | Transforming growth factor beta. |

| | | | |
|---|---|---|---|
| 21. | GAG | - | Glycosaminoglycan.. |
| 22. | GBM | - | Glomerular basement membrane. |
| 23. | PDGF | - | Platelet derived growth factor.. |
| 24. | IGF | - | Insulin like Growth factor |
| 25. | NFK | - | Nuclear factor kappa. |
| 26. | SRB | - | Scavenger rceptor classB. |
| 27. | VCAM | - | Vascular cell adhesion molecule. |
| 28. | ICAM | - | Intercellular adhesion molecule. |
| 29. | PG | - | Prostaglandin |
| 30. | CRP | - | C Reactive protein. |
| 31. | hs-CRP | - | High sensitive C Reactive protein. |
| 32. | PRPP | - | Phosphoribosyl pyro phosphate |
| 33. | HGPRT | - | Hypoxanthine guanine phospho ribosyl transferase.. |
| 34. | IMP | - | Inosine mono phosphate. |
| 35. | GMP | - | Guanosine mono phosphate. |
| 36. | URAT | - | Urate anion Transporter. |
| 37. | OAT | - | Organic anion Transporter. |
| 38. | GLUT | - | Glucose transporter. |
| 39. | NO | - | Nitricoxide. |
| 40. | MAP | - | Mitogen activated protein kinase. |
| 41. | COX | - | Cyclooxygenase. |

| | | | |
|---|---|---|---|
| 42. | NOS | - | Nitric oxide synthase. |
| 43. | MSU | - | Mononsodium urate. |
| 44. | NLRS | - | Nucleotide binding oligomerisation domain like receptors. |
| 45. | CLR | - | C type Lectin receptors. |
| 46. | NALP | - | NACHT,LRR & PYD domain containing protein. |
| 47. | VWF | - | Von willebrand factor. |
| 48. | DIC | - | Disseminated intra vascular coagulation. |
| 49. | eGFR | - | Estimated Glomerular filtration rate. |
| 50. | CKD | - | Chronic kidney disease. |
| 51. | IFCC | - | International federation of clinical chemistry. |
| 52. | ADA | - | American Diabetes association. |
| 53. | HPLC | - | High performance liquid chromatography. |
| 54. | NGSP | - | National Glyco hemoglobin standardisation programme. |

# ROLE OF URICACID & hs-CRP AS RENAL AND CARDIOVASCULAR RISK MARKERS IN TYPE 1 DIABETIC PATIENTS WITH LOW GFR


## ABSTRACT:

**AIMS AND OBJECTIVES** :

Recent studies have reported that high serum levels of uric acid and hs-CRP are strongly associated with impaired renal function and cardio vascular disease. This study aims to find out the role of uric acid & hs-CRP in detection of early renal function decline before the onset of proteinuria and detection of cardiovascular risk in type 1 Diabetic patients .

**METHODOLOGY:**

A total of 130 type 1 Diabetic patients (65 normoalbuminuric & 65microalbuminuric) were recruited in the cross sectional study. Uric acid & hs-CRP were measured by enzymatic colorimetric method & ELISA method respectively. Other metabolic parameters blood pressure, bodymassindex,HbA1c, Fasting plasma glucose, Plasma lipid profile, serum creatinine, urine albumin creatinine ratio were assessed. Estimated Glomerular filtration rate was calculated by CKD-EPI equation.

**RESULTS**:

The respective mean uric acid levels in normoalbuminuria & microalbuminuria were 3.92+/-1.59 & 4.99+/-2.48mg/dL(p-0.004) .Mean hs-CRP levels in normoalbuminuria was 1.93+/-2.14mg/L and in microalbuminuria it was 4.47+/-3.05mg/L($p<0.001$). Urinary albumin creatinine ratio in normoalbuminuria

was 13.64+/-7.66 and in Microalbuminuria it was 127.98+/-81.23(p <0.001).In normoalbuminuria mean GFR levels were 110.40+/-20.30ml/min and in microalbuminurics it was 100.25+/-27.12ml/min.(p-0.02). eGFR >90ml/min was present in 54.5% of normoalbuminurics & 45.5% of microalbuminurics. eGFR <90ml/min was present in 34.5% of normoalbuminurics and 65.5 % of microalbuminurics. Lower GFR was strongly & independently associated with elevated serum uric acid & increased urinary albumin creatinine ratio. hs-CRP was positively correlated with uric acid (p-0.04) and Uric acid was negatively correlated with e GFR (p-<0.001).

**CONCLUSION**:

Serum uric acid in high normal range & elevated hs-CRP levels were associated with impaired renal function in patients with type1 diabetes. Therefore it can be used as markers for detecting cardiovascular risk & early renal function decline in patients with type 1 diabetes in Indian clinical setting , where detection rate is lower & complications are higher.

**KEY WORDS:**

Type 1 Diabetes, Serum Uric acid, hs-CRP, GFR.

# INTRODUCTION

Diabetes Mellitus represents a heterogenous group of disorders, which is characterised by Insulin insensitivity and /or hyposecretion.

Prevalence of diabetes is increasing globally and it is one of the major health problems of the 21st century.

**EPIDEMIOLOGY:**

The Estimated number of People with Diabetes Worldwide was 366 Million in 2013 and it is expected to rise around 522 Million in 2030[(1)].

About 62.4 Million people currently have Diabetes in India [(2)] and it is expected to rise around 101.2 Million by 2030[(3)].

The burden of Diabetes is primarily due to its many complications. Diabetic Nephropathy & Coronary artery disease are the common Micro vascular & Macrovascular complications of Diabetes.

Diabetic Nephropathy affects 20-30% of both Type1 and Type 2 Diabetic patients [(4).] ESRD develops in 50% of Type1 diabetic individuals compared to 20% in Type 2 diabetic individuals.[(5)] Prevalence of coronary artery disease among Diabetic Indians ranges from 9% to 14%.and it increases with age and duration of Diabetes.[(6)]

Microalbuminuria is considered as the earliest clinically detectable stage of Diabetic Nephropathy, at which appropriate interventions can reverse or

retard the progression of Diabetic kidney disease [7]. It is also considered as the marker of cardiovascular morbidity & Mortality in Type 1 Diabetes.

Recent studies have reported that, Uric acid &hs-CRP are well known markers for detecting Renal & Cardiovascular damage at an earlier stage before the onset of Microalbuminuria.

Increased serum concentrations of Uric acid induce endothelial dysfunction, glomerular hypertrophy, afferent arteriolar wall thickening, inhibit the production of nitric oxide & promote the development of Microalbuminuria. Thus this study was conducted with an aim to find the role of uric acid and hs-CRP in normoalbuminuric and Microalbuminuric Type 1 Diabetic patients with low GFR.

# *Review of Literature*

# REVIEW OF LITERATURE:

## DIABETES MELLITUS:

Diabetes Mellitus is a metabolic disorder of multiple etiologies due to defect in insulin secretion, action or both. It causes disturbances of carbohydrates, fat & protein metabolism [8].Incidence of type 1 Diabetes is 5-10% worldwide. [9] It causes a long-term damage & impaired function of various organs especially Eyes, Heart, Blood Vessels, Nerves and Kidney.

## TYPE 1 DIABETES:

Type 1 diabetes is characterised by hyper glycaemia due to destruction of beta cell of pancreas resulting in absolute deficiency of insulin. It is of three types.

**Type1a**:

- Polygenic & the most common form.
- 80-90% of the cases fall in this category
- Autoimmune mediated , characterised by the presence of Glutamic acid decarboxylase, Islet cell cytoplasmic antibodies and Insulin auto antibodies that causes beta cell destruction.
- Type 1a subtypes :

  1) LADA (Late onset autoimmune diabetes of adults):

  It occur in >30 years of age, associated with other autoimmune conditions[10].

2) Monogenic type 1 diabetes:

It corresponds to Type1 diabetes of autoimmune poly glandular syndrome type 1A [11] & IPEX SYNDROME. (Immune dysfunction poly endocrinopathy, enteropathy, X linked.) [12]

**Type1b**:

- Idiopathic.
- No evidence of autoimmunity.
- It has all the clinical features of type 1a.

Type 1b subtype:

- Fulminant diabetes, mostly found in Asians.
- No autoimmune etiology.
- Impairment of α & β cells of pancreatic islets.
- It is most often found in childhood (Juvenile onset diabetes)

**Mixed or Double : ( Type1 + Type2)**:

Type 1a autoimmunity & Type 2 (obesity, insulin resistance, dyslipidemia) diabetes characteristics in the same individual.

EPIDEMIOLOGY OF TYPE 1 DIABETES:

- Incidence of Type 1 Diabetes is 5-10% worldwide.
- In India, the incidence is 6.4/lakh population.
- Majority of Type 1 cases are less than 20 years of age with highest incidence among children of 0-4 years [13, 14, 15].

- In young populations, boys& girls are equally affected.[17]

**PATHOGENESIS OF TYPE1 DIABETES MELLITUS:**

**GENETIC FACTORS:**

**1) MONOGENIC FORMS:**

- Type1a is rarely caused by mutation defect in single gene.
- Other autoimmune conditions also accompany this due to disruptions of common regulatory pathway.
- In IPEX syndrome (Immune dysregulation polyendocrinopathy, enteropathy X linked.) , the mutation in FOXP3 transcription factor leads to dysfunction of T cell regulation[18,19,20].
- 80% of affected children develop autoimmune diabetes early.

**GENETIC STUDIES AMONG TWINS HAVING TYPE 1 DIABETES:**

- About 10% of Type 1 Diabetes has a parent or sibling with the disease.
- Monozygotic twins have 50%concordance for type 1Diabetes&

  Di zygotic twins have 10% concordance. Even though the concordance rate is higher in monozygotic twins, there are divergences in terms of time to develop type1 diabetes. i.e.)environmental factors also play a role in diabetic development.[21,22]

**2) HLA GENES:**

- HLA complex located in chromosome 6 carries more than 200 genes and contributes 50% genetic susceptibility to type1 diabetes.

- It is located in cell membrane and present as processed antigen , which is recognised by T cell receptor.
- In HLA complex, I-III classes are identified. HLA Class II genes are the strongest genetic contributor of type 1 diabetes. Dominant influence of class II alleles has been attributed to HLA-B & HLA-A genes.
- Two HLA class II haplotypes DR3-DQ2 & DR4-DQ8 carries high risk (5%) & early onset disease.
- Genotype having DR15-DQ6 haplotype is protective, present in 1% of children with Diabetes even in the presence of Type 1 Diabetes associated antibodies [23].

**3) INSULIN GENE:**

- The promoter region of insulin gene on chromosome 11 gene carries a genetic susceptibility of 10 %[24].
- Susceptibility is proportional to the variable number of Tandem repeat polymorphisms in insulin gene promoter region.
- VNTR Type1 (shorter repeats) homozygous individuals is the highest risk category compared to VNTR Type 3(longer repeats), which protects against type 1 diabetes.
- Increased insulin gene expression with class III allele confers greater tolerance to insulin precursor molecules [25].

**Other susceptibility genes:**

More than 40 genes make minor contributions to type 1 Diabetes. Some of them are:

**1) PTPN22 (Lymphoid protein Tyrosine phosphatase).**

New member of Type1 susceptibility gene is PTPN22 which encodes lymphoid protein tyrosine phosphatase located on chromosome 1.It is a negative regulator of T-cell receptor signalling.

**INTERLEUKIN -2 RECEPTOR -α (IL2RA)**

Alpha chain of IL-2 receptor complex located on chromosome 10, expressed on T cells upon activation & on natural regulator T cells at base line. Increased level of soluble IL2Rα in other autoimmune conditions has the potential to neutralise IL-2 & impairs Tregs functionality. [26, 27]

**CYTOTOXIC T LYMPHOCYTE ASSOCIATED PROTEIN-4 (CTLA4)**

It is located on chromosome 2q33, expressed only on activated T lymphocytes; it is a negative regulator of immune response. Mutation in CTLA-4 leads to persistence of lymphocyte activation & failure of immune tolerance [28].

**INTERFERON INDUCED HELICASE -1(IFIH1)**

IFIHL polymorphism links genetic with environmental factors. It interferes with proper detection & clearance of viral infection and leads to abnormal diabetogenic immune response. [29]

**IMMUNOLOGICAL FACTORS:**

Type 1 Diabetes is characterised by the presence of humoral antibody & T cell responses to islet cell proteins. (Antigen).

**Major Beta cell auto antibodies:**

1) ICA (Islet cell cytoplasmic antibodies):

- It is present in 75-85% of Type 1 Diabetes.
- It reacts with a sialoglyco conjugate antigen present in the cytoplasm of all endocrine cells of pancreatic Islet.

2) Insulin & Proinsulin Auto antibodies:

- It is present in more than 90% of children who develop type 1 Diabetes before age five and in less than 40% of individuals who develop Diabetes after age 12.
- It also develops in persons who use human insulin.

3) GAD (Glutamic acid decarboxylase):

- It is present in 65 K Da form in human islet.
- It have been found up to 10 years before the onset of clinical type 1 Diabetes.
- It is present in 60% of patients with newly diagnosed Diabetes.'
- It is used to identify patients with apparent type2 Diabetes who may progress to type 1 diabetes.

4) Insulinoma associated antigens (IA-2A and IA-2βA):

- It is directed against two tyrosine phosphatases.
- It is detected in more than 50% newly diagnosed type 1 Diabetes.

5) Zinc Transporter (ZnT8):

- It is present in 60-80% of Type 1 Diabetes.

**METABOLIC DYSREGULATION FOUND IN CHILDREN WHO LATER DEVELOP TYPE 1 DIABETES:**

Oresic et al states that "Metabolic dysregulation precedes autoimmunity" [30] Increased serum concentration of lysophosphatidyl choline, precedes the appearance of each islet auto antibody. Lysophosphatidylcholine is generated by hydrolysis of phosphatidylcholine by phospholipase A2. It increases the susceptibility of β cells to oxidative damage.

Changes in serum metabolites such as

- Decrease in succinate
- Decrease in Phospholipid
- Decrease in keto leucine
- Increased Branched chain aminoacids
- Increased glutamic acid is found in children who later develop type 1 diabetes. These by products are capable of producing reactive oxygen species and causes β cell destruction.[31]

## 2) PRECIPITATING CAUSES FOR TYPE 1 DIABETES:

### VIRAL INFECTIONS:

Enteroviruses specifically coxsackie viruses B up regulate chemokine CXCL10 in pancreatic beta cells [32] .Congenital rubella syndrome is associated with HLA-A1-B8 susceptibility haplotype.

Viruses may damage the beta cell by direct invasion or by triggering an autoimmune response. They may also persist within beta cells and cause long term interference with metabolic and secretory function.

### BACTERIA:

Autoimmunity ensues whenever the microbial balance in the intestine is disturbed. "Leaky gut phenotype" exists in type 1 diabetes & it enhances the exposure of bacterial antigen in the intestine to immune system.

Mutant forms of SLC11A1 alter the process and leads to diabetogenic responses [33].

### ENVIRONMENTAL FACTORS:

### 1) DIET:

- Prolonged cow's milk consumption promotes islet autoimmunity.
- The bovine serum albumin (BSA) an antigen may enter in an intact form through the gut of neonates and stimulate an immune response directed against β cells.[34]

- PTPN22 polymorphisms affect islet autoimmunity only if children are exposed to cow's milk during early infancy [35].

**2) WHEAT PROTEINS:**

Immunity to oral proteins is altered in Type 1 Diabetes. Increased peripheral blood T cell reactivity to wheat Gluten was found[36].But the cell reactivity to Gluten is not related to HLA DQB1*0201,a risk allele for Type1 Diabetes.

**VITAMIN-D:**

Interactions between Vitamin D Receptor & HLA alleles are mediated by Vitamin D responsive element present in HLA-DRB1-0301 allele.

Complete deficiency of vitamin D results in type1 Diabetes due to poor expression of DRB1-0301 in Thymus. [37]

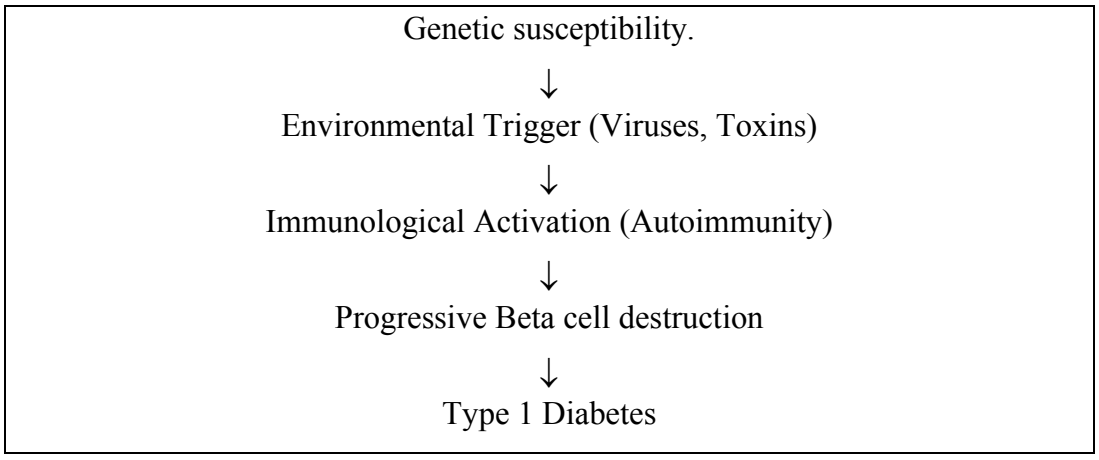



**Fig-1: Sequence of Events in the Development of Type 1 DM**.

**DIABETIC NEPHROPATHY:**

Diabetic Nephropathy is the most common cause of glomerulosclerosis &End stage renal disease. It occurs in 20-40% of uncontrolled Type 1 Diabetic patients. It is a syndrome characterised by

- Declining Glomerular filtration rate.
- Albuminuria
- Arterial hyper tension.

It increases the cardiovascular risk, affecting 20-40% of Diabetic patients. [38] It starts in the first decade of life in Type 1 diabetes. Pure Diabetic Glomerulopathy is commonly observed in patients with earlier onset of Diabetes & it is evaluated at the stage of Incipient Nephropathy. [39]

Tubulointerstitial damages are seen at the stage of Overt Nephropathy with arterial hypertension in older patients [40].

**Natural history of Diabetic Nephropathy**:

It differs based on the Type of Diabetes & the presence of microalbuminuria. If untreated, 80% of Type 1 Diabetes will progress to Overt Nephropathy compared to 20-40% in Type 2 Diabetes over a period of 15 years.

Mauer& colleagues [41] states that, in Type 1 Diabetes with microalbuminuria, histological alteration were detected such as

- Glomerular hyper filtration

- Hyper perfusion
- Thickness of Glomerular basement membrane
- Mesangial expansion and they progress to Overt Nephropathy.

It is highly variable & it is influenced by other factors such as Glycemic control& Blood pressure.

Hyper glycaemia

↓

Increased Glomerular filtration Rate.

↓

Microalbuminuria

↓

Frank Proteinuria

↓

Decreased Glomerular filtration Rate.

↓

Possible development of Nephrotic syndrome.

↓

End stage renal disease.

**Fig2: Natural History of Diabetic Nephropathy**.

**RISK FACTORS OF DIABETIC NEPHROPATHY:**

**1) Genetic:**

Cell membrane cation transport system namely the red cell (Na-Li counter transport ) is genetically determined and it is involved in the renal resorption of sodium & regulation of B.P.

In susceptible individuals, environmental changes induce cell hypertrophy & hyperplasia leading to glomerular hypertrophy & mesangial expansion.

The altered sodium balance leads to glomerular hypertension, increased glomerular filtration rate causing proteinuria.

**2) Duration of Diabetes:**

During first 5 years, Nephropathy is rare. The incidence reaches a peak by $15^{th}$ year and rarely develops after 25 years of Diabetes.

**3) Hypertension:**

Presence of Hypertension indicates renal involvement. Incidence of hypertension increases with progression of renal disease.

**4) Hyper glycaemia:**

GFR is positively correlated with HbA1c. Progression of Nephropathy is arrested with adequate metabolic control.

**5) Smoking:**

Smoking causes Vasoconstriction, coagulation abnormalities & platelet dysfunction which can accelerate vascular damage.

**STAGES OF DIABETIC NEPHROPATHY**:

There are five stages of Diabetic Nephropathy based on

1) Changes in Glomerular filtration rate.
2) Albuminuria & proteinuria.
3) Changes in arterial pressure.
4) Structural changes.

**Stage 1 :( Stage of Hyper function& Hypertrophy**)

1) GFR is>150 ml/min.
2) Normal Blood pressure.
3) Urinary albumin excretion may be increased.
4) Large Kidneys with Glomerular hyper filtration &Hypertrophy

**Stage 2 :( silent stage**)

1) GFR is normal or increased.
2) Normal Blood pressure.
3) Urinary albumin excretion is normal.
4) Increased basement membrane thickening & mesangial expansion may be present.

**Stage 3 :( Stage of Incipient Diabetic Nephropathy)**

1) GFR is normal or increased.
2) Blood pressure increasing by 3mmHg/year.
3) Persistent microalbuminuria (20-200µg/min.)
4) Increased basement membrane thickness with mesangial expansion.

**Stage 4 :( Stage of overt Diabetic Nephropathy**)

1) Fall in GFR
2) Hyper tension.
3) Macroalbuminuria (>200µg/min).
4) Increasing Glomerular occlusion& severe mesangial expansion.

**Stage 4 :( stage of ESRD**)

1) GFR<20ml/min.
2) Frank hyper tension.
3) Macroalbuminuria often decreasing, due to Glomerular occlusion.
4) Uraemia with Glomerular occlusion & mesangial expansion.

**TABLE: 1: ALBUMIN EXCRETION AND ALBUMIN CREATININE RATIO.**

| CONDITION | 24hr URINARY ALBUMIN EXCRETION RATE | URINARY ALBUMIN EXCRETION RATE | ALBUMIN CREATININE RATIO |
|---|---|---|---|
| MACROALBUMINURIA | >300mg/day. | >200µg/min. | >25mg/mmol. |
| MICROALBUMINURIA | 30-300mg/day. | 20-200µg/min. | 2.5-25mg/mmol (FOR MEN) 3.5-25mg/mmol (FOR WOMEN) |
| NORMOALBUMINURIA | <30mg/day | <20µg/min. | <2.5mg/mmol (FOR MEN) <3.5mg/mmol (FOR WOMEN) |

**PATHOGENESIS OF DIABETIC NEPHROPATHY:**

The main pathophysiological mechanisms causing Diabetic Nephropathy are

**1) Hemodynamic pathway**.

**2) Metabolic pathway**.

i) Non enzymatic Glycosylation.

ii) Polyol pathway.

iii) Protein kinase c pathway.

iv) Biochemical abnormalities of Extracellular matrix.

**3) Intracellular factors/Growth factors& cytokines**.

**HEMODYNAMIC PATHWAY**:

The early signs of Glomerular hyper filtration & hyperperfusion are due to decreased efferent& afferent arteriolar resistance. The factors such as

1) Nitric oxide
2) Vascular endothelial growth factor
3) Transforming growth factor -β1
4) Prostanoids
5) Renin angiotensin system particularly angiotensin II are responsible for defective auto regulation.

These changes causes thickening of Glomerular Basement membrane, Mesangial matrix expansion, injury to podocytes & release of cytokines &Growth factor [42].

Blockade of Angiotensin II by the use of Angiotensin & aldosterone blockers will preserve kidney function. [43].

**2) METABOLIC PATHWAY:**

Hyperglycaemia is an important factor in the development of Diabetic Nephropathy. It causes Mesangial cell expansion & Basement membrane thickening.

Mesangial cell modulated Glomerular filtration via smooth muscle activity [44].

Mesangial cell expansion is mediated by increased Glucose concentration in mesangial cells or by increase in Glucose transporters. (GLUT1&GLUT4)[45].

It increases the vascular permeability by upregulation of VEGF expression in podocytes. [46]

Three mechanisms have been postulated to explain the effect of hyper glycaemia in tissue damage.

**1) Advanced Glycated end products:**

Glucotoxicity leads to the formation of advanced glycation end products by non enzymatic glycosylation of intracellular proteins where Glucose & other glycating compounds attach to aminogroup& other molecule such as nucleic acid without the aid of enzyme, producing reversible early glycated end products (schiff base) and later, irreversible (Amadori products).

These products are slowly converted in to advanced glycated end products.

It accumulates in tissue (arterial wall& Glomerular basement membrane) by cross linking with the collagen causing atherosclerosis & Glomerular dysfunction.

It alters the signal transduction by altering the level of cytokines, hormones & free radicals and contributes to renal & microvascular complications. [47].

**2) Protein kinase C pathway:**

Hyper glycaemia activates protein kinase C by denovo formation of diacylglycerol & oxidative stress[48] leading to secretion of vasodilatory prostanoids,TGF-β1 which contributes to Glomerular hyper filtration & mesangial cell expansion.[49] .

**3) Polyol pathway:**

When intracellular Glucose concentration is raised, Glucose metabolism takes place via sorbitol pathway by the enzyme aldose reductase. It is present in mesangial cells, distal tubular cell, and glomerular epithelial cell of normal kidney. It generates sorbitol in response to high salinity in medullary interstitium. Sorbitol causes

a) Defect in inositol signalling.
b) Depletion of Myo inositol.
c) Decreased NaK ATPase activity.
d) Decreased intracellular NADPH leading on to Oxidative injury.

**4) BIOCHEMICAL ABNORMALITIES OF EXTRA CELLULAR MATRIX:**

- In Diabetic Nephropathy, thickness of Glomerular basement membrane & mesangial matrix increases.
- Synthesis of collagen in Extracellular membrane is also increased. The lysyl hydroxylase enzyme involved in hydroxylation of peptide bound lysine during collagen biosynthesis is increased.

- In Diabetes, denovo synthesis of GAG content of GBM is decreased leading to loss of negative charge which results in fusion of foot process & obliteration of slit & diaphragm and ends in albuminuria.
- Glycosylation of GBM leads to increased cross linking of disulfide bridges between collagen via increased oxidation of sulfhydryl groups which affects the architecture of GBM due to the presence of reactive carbonyl group of Glucose attached to these structures .It results in linear deposition of albumin & IgG along the Glomerular & Tubular membrane.
- Membrane bound receptor which selectively scavenges the removal of cross linked denatured proteins induces the synthesis of Macrophages &Monokines (IL-1,TNFα).These Monokines initiate a cascade of stimuli resulting in increased cellular proliferation ,protein synthesis & Vascular permeability leading to albuminuria.

**3) GROWTH FACTORS & INFLAMMATORY CYTOKINES:**

**a) GROWTH FACTORS:**

**1) Transforming Growth factor -β:**

Endogenous TGF-β under hyperglycaemic conditions is able to enhance Glucose transport by over expression of mRNA &protein GLUT-1.i.e) it accelerates the Glucose induced abnormalities of mesangial cells.

**2) Platelet derived growth factor:**

Hyper glycaemia induces an early stimulation of PDGF which causes an increased TGF-β1 expression results in mesangial cell expansion.

**3) Connective Tissue Growth Factor:**

It increases fibronectin, collagen types I & IV, Transient actin cytoskeleton disassembly in mesangial cell leading to mesangial cell hypertrophy.

**4) Growth Hormone & Insulin Like Growth Factors:**

Normally Growth hormone secreted from pituitary induces the synthesis of Insulin like Growth factor through activation of Growth hormone Receptor. Growth hormone is involved in the pathogenesis of Glomerulosclerosis independent of IGF-1.

It increases laminin, Type IV collagen, Fibronectin & proteoglycan.

**5) Vascular Endothelial Growth Factor:**

Hyperglycaemia stimulates VEGF that mediates endothelial injury in Diabetes. [50]

It stimulates α3 chain of collagen IV and contributes to thickening of GBM.

**INFLAMMATORY CYTOKINES:**

Inflammatory cytokines (IL-1, IL-6, IL-18, and TNF) are involved in the progression of Diabetic nephropathy.

- IL-1 alters the expression of chemotactic factor, intraglomerular hemodynamics, increases the permeability of endothelial cell, and increases hyaluron production, which in turn increases Glomerular cellularity. [51].
- IL-6: Involved in GBM thickening & increases Glomerular permeability.
- IL-18: Induces the production of other cytokines such as IL-1, IF-γ& TNF.
- TNF: Causes early hypertrophy & hyper function of Diabetic Nephropathy.[52]

**OXIDATIVE STRESS:**

The metabolism of Glucose via harmful alternate pathway such as Advanced Glycated end product formation and Protein kinase C activation are partly dependent on reactive oxygen species. Concentration of markers of DNA damage produced by reactive oxygen species is higher in patients with severe Nephropathy. [53]

**CORONARY ARTERY DISEASE:**

Cardiovascular disease is about twice as frequent in diabetic Men and four times as frequent in diabetic women after menopause. The risk is proportional to increased HbA1c level [54]. 7-10% of Type1 Diabetic patients develop coronary artery disease.

In Type 1 Diabetic patients Coronary vascular disease events are generally not expected to occur during childhood. But, the atherosclerotic process begins during child hood. The relationship of hyperglycaemia with microangiopathy &macroangiopathy is more significant in type1 compared to type2 Diabetes. [55, 56]

**RISK FACTORS:**

**1) Poor glycemic control:**

Atherosclerosis is the cause of major cardiovascular events. Cardio vascular risk is increased in patients with metabolic syndrome &Type 1 Diabetes [57].

Hyperglycaemia causes activation of nuclear factor Kappa B [58, 59] which results in expression of several adhesion molecules that facilitate monocyte adhesion to endothelial cells [58].

Monocyte is then converted into macrophages taken up by lipids forming foam cells & deposit in the arterial wall leading to fatty streak formation. With time, it causes smooth cell proliferation & necrotic core formation. Some of these lesions become unstable &rupture leading to clinical symptoms of coronary vascular disease.

**2) Hypertension:**

Hypertension & Diabetes increases 10-30% of risk of developing macrovascular complications. [60]. Blood pressure tends to increase 3 years after the development of microalbuminuria [61].

**3)Dyslipidemia:**

- Auto oxidation of Glucose leads to the formation of super oxide anion that facilitates LDL oxidation [(62)].
- Scavenger receptors present on arterial macrophages taken up these modified Lipoproteins. AGE modified albumin inhibit SRB-1 mediated efflux of cholesterol to HDL [(63)] and interfere with reverse cholesterol transport.
- High glucose concentration alters the delivery & removal of lipid from macrophages by modification of lipoproteins. It leads to lipid accumulation & foam cell formation.

It worsens the cardiovascular risk due to increased VLDL, Triglycerides & modification of Lipoproteins by oxidation, glycosylation and there is a reduction in vascular compliance predisposing to early atherosclerosis [(64)].

In Type 1 Diabetes, atherogenesis is not caused exclusively by increase in lipid levels, Hyper glycaemia is also pivotal in this process. [(65)]

**PATHOGENESIS**:

**1) OXIDATIVE STRESS:**

Oxidative stress results from imbalance between production of reactive oxygen species & antioxidant defence mechanism. Reactive oxygen species are highly reactive & chemically unstable and function as second messengers regulating the expression of redox signal sensitive genes. (Nuclear factor kappa-B) & in the production of inflammatory mediators [66].

They are generated from the enzyme that uses oxygen as the electron acceptor including NADPH oxidase, Cyp450, lipo-oxygenase &cyclooxygenase.

The active form of NADPH oxidase reduces the molecular oxygen resulting in the formation of superoxide anion.

$$NADPH+2O_2 \rightarrow NAD\ (p+) + H^+ + 2O_2^{-} \quad [67]$$

Reactive oxygen species production are involved in apoptosis, hypertrophy, extracellular protein modification, protein synthesis &changes in gene transcription [68] In addition superoxide overproduction can divert the production of Nitric oxide to generate peroxynitrite [69].Which damage the cellular structures such as DNA & proteins [70].In Type1 Diabetic with overt Nephropathy, Hyperglycaemia causes increase lipid peroxidation without increase in compensatory antioxidant enzymes [71].

Patients without chronic complications & exposure to shorter duration of diabetes have less antioxidant capacity suggesting that Oxidative stress occurs early in the disease [72].

**EPIGENETICS:**

Epigenetic factors by different types of reactions mediate interplay between genes & environment resulting in activation or repression of genetic transcription.

The most important reactions are acetylation &methylation .Brownlee et al [(73)] have demonstrated that" Reactive oxygen species resulting from hyperglycaemia, induce mono methylation of Lysine from histone 3 & increase the expression of Nuclear factor Kappa-B."

It increases the transcription of VCAM-1, Monocyte chemo attractant protein-1, IL-6&, ICAM-1.

**INFLAMMATION:**

Increase in inflammatory cytokines such as TNF-α,IL-1,IL-6, Monocyte chemo attractant protein, Platelet activating inhibitor, C-reactive protein, Visfatin & adiponectin cause plaque instability[(74)],endothelial injury & abnormalities in coagulation ,resulting in increased risk of coronary events.[(74)]

**Hypercoagulability:**

Diabetes causes glycation & oxidation of clotting factors, and increases the thrombotic risk. It increases the plasma levels of procoagulant factors [(75)] and decreases the fibrinolytic capacity resulting in hypercoagulability.

**HYPOGLYCEMIA:**

In Hypoglycaemia, there is an increase in myocardial contractility, stroke volume & cardiac output. In healthy people, arteries have been reported to become more elastic with decrease in wall stiffness [76].

In long term type 1 Diabetes with hypoglycaemia, increased arterial stiffness, with fewer falls in CAP [76, 77] increases the workload of the heart. It causes abnormal electrical activity in the heart and provokes sudden death. [77]

**HYPERGLYCEMIA:**

Intense Glycemic control decreases the cardiovascular complications. It is evident from the results of Diabetes complications &control trial (DCCT)/Epidemiology of Diabetes interventions &complications trial(EDIC) [78].

It is because of "Metabolic memory". i.e. Effect of early glycaemic exposure is remembered later in target organs resulting in protective or deleterious effects. [79]

**ENDOTHELIAL DYSFUNCTION:**

Healthy endothelium is anti atherogenic. It inhibits platelet aggregation, smooth muscle cell proliferation & leucocyte adhesion. It synthesizes & releases nitric oxide, PGI2 (vasodilatory substance) &Vasoconstrictor substances (endothelin-1, ThromboxaneA2&PGH2).It maintains the balance between vasodilatory & vasoconstrictory substances to preserve sufficient vascular diameter.

Endothelial dysfunction results in reduction of relaxing factors& increase in contracting factors. It increases vascular endothelial permeability, leukocyte adhesion and impairment in endothelial signal transduction.

It is an important determinant of inflammatory activity & it can be considered as an early marker for coronary vascular disease [80] regardless of the presence or absence of complications.

Apart from hyperglycaemia, other factors such as genetic &environmental factors are also play a role in endothelial dysfunction [81, 82].

**CARDIAC AUTONOMIC NEUROPATHY:**

Cardiac autonomic neuropathy is a common complication of Type 1 Diabetes. Its prevalence is around 20% and it increases with age & duration of Diabetes. It is an important predictor of cardiovascular mortality & morbidity in Type 1Diabetes [83].

Dysfunction of the autonomic nervous system mediates the development of arterial stiffness, Left ventricular hypertrophy & Ventricular diastolic dysfunction.

This type of cardiac sympathetic dysinnervation was identified in long term than in newly diagnosed Type 1 Diabetes.[84, 85].

Neuronal abnormalities progression correlates with the duration of Diabetes[86].Various immunological factors against sympathetic ganglia are also

associated with sympathetic dysfunction.[87,88] Auto antibodies against sympathetic ganglia are present in 20-35% of Type 1Diabetes[89,90].

**URICACID:**

Uric acid (2, 6, and 8- Tri hydroxy purine) is a heterocyclic organic compound. Its molecular weight is 168 Daltons. It is a weak organic acid with a P Ka of 5.75. At physiological pH, it exists principally as urate which is more soluble than uric acid [91] .At a urine pH below 5.75, uric acid is the predominant form.

The daily synthesis rate of uric acid is 400 mg approximately and from dietary source is 300 mg.

In purine free diet, the total body pool of exchangeable urate is 1200mg in men and it is about 600mg in women. It is the end product of purine metabolism.

**SYNTHESIS OF URICACID:**

Catabolism of purine nucleotides begins with removal of ribose linked phosphate catalysed by purine 5'-Nucleotidase.Removal of Ribose moiety of inosine &guanosine by purine nucleoside phosphorylase forms Hypoxanthine& Guanine, both are converted into xanthine. Xanthine is converted to uric acid by the action of xanthine oxidase. Renal handling of uric acid is complex and it involves

1)Glomerular filtration

2)Tubular re absorption of 98-100% of filtered uric acid is by the proximal convoluted tubule.

3)Secretion of uric acid in to distal portion of proximal convoluted tubule

4)Further reabsorption in the distal tubule.

6-12% of filtered uric acid is excreted. Uric acid is degraded in most mammals by hepatic enzyme uricase to allantoin& it is freely excreted in the urine. Distinct mutational silencing occurs early &makes the uricase gene non-functional in humans &apes[92] and that's why humans & apes have increased uric acid levels than most mammals

Uric acid is higher in men & post menopausal women & lower in Females because estrogens are uricosuric. Uric acid levels are altered due to various factors that increase the production or decrease its excretion.

**Normal Reference range**:

Male 3to7 mg/dl,

Female 2.5-6.5 mg/dl and

Daily excretion is about 250-700 mg/dl.

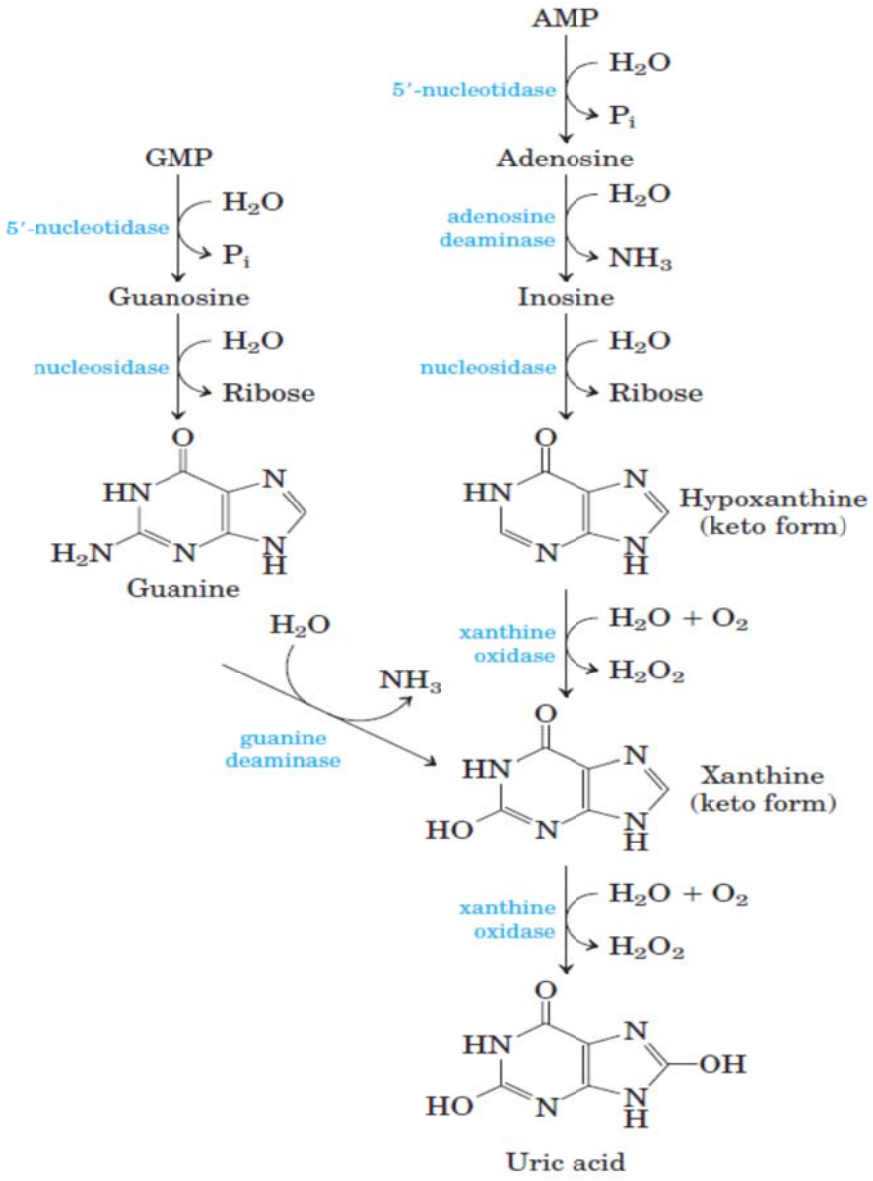



**FIG: 3 SYNTHESIS OF URIC ACID**

(Adapted from Lehninger's principles of Biochemistry).

**CAUSES OF HYPERURICEMIA:**

Plasma concentration of uric acid is >7.0mg/dl in men and >6.0mg/dl in Women.

**INCREASED FORMATION:**

**PRIMARY:**

Idiopathic

Inherited metabolic disorder

**SECONDARY:**

Excess Dietary protein intake

Increased nucleic acid turnover. (Leukaemia, Chemotherapy, Radiotherapy, Trauma)

Psoriasis

Alcohol

Tissue Hypoxia

Preeclampsia

**DECREASED EXCRETION:**

**PRIMARY :( Idiopathic)**

**SECONDARY:**

Acute or chronic kidney disease

Increased renal absorption, reduced secretion

Lead poisoning

Organic acids(Lactate, acetoacetate)

Preeclampsia

Thiazide diuretics, Salicylates (low doses).

**GOUT:**

When Monosodium urate precipitates from supersaturated body fluids, the deposits of urate are responsible for the clinical signs & symptoms.

- Gouty arthritis may be associated with urate crystals in joint fluid and deposit of crystals (Tophi) in tissue surrounding the joint.
- Urate crystals deposited in renal parenchyma results in Gouty Nephropathy.

**CAUSES OF HYPOURICEMIA:**

Plasma urate concentration is<2mg/dl.

1) Severe hepatocellular damage with reduced purine synthesis or xanthine oxidase activity.
2) Defective renal tubular reabsorption of uric acid. It may be congenital(ex:Fanconi syndrome) or acquired( ex: Injection of radioopaque contrast media/chronic exposure to toxic agents or over treatment with allopurinol/ uricosuric drugs ) will produce hypouricemia.
3) Molybdenum cofactor deficiency.
4) Purine nucleoside phosphorylase deficiency.

## HYPERURICEMIA IN CARBOHYDRATE METABOLISM

- Glucose 6 Phosphatase enzyme catalyses the release of Glucose. Deficiency of Glucose 6phosphatase in Von Gierke disease causes increased Glucose 6 Phosphate which stimulates pentose phosphate pathway and synthesis excess purine& ultimately uric acid.
- Organic acidemias- Lactic acidosis, Diabetic keto acidosis (Interferes with tubular secretion of urate.)

## GENETICS OF URATE TRANSPORTER:

Uric acid homeostasis depends on the balance between production, reabsorption, secretion & excretion. 30% of uric acid excretion is by the intestine & the remaining 70% is by the kidney. Some of the transporters are

### 1) Organic anion transporter (SLC22Afamily)

URAT-1- is the urate/anion exchanger. It is a 12 trans membrane domain containing protein, transports urate in exchange for $Cl^-$ or organic anions. It is found in apical membrane of proximal tubular epithelial cells.

### OAT-4&OAT10:

OAT4 (SLC22A11 gene) is a multi specific anion transporter present in proximal tubular epithelial cell, activated by intracellular dicarboxylates and it is involved in luminal urate reabsorption. [93, 94, 95]

OAT10 (SLC22A13) is a urate transporter expressed in brush border of proximal tubule &collecting ducts [96]

**OAT1&OAT3:**

OAT1(SLC22A6)&OAT3(SLC22A8) function as urate/ dicarboxylate exchanger found on baso lateral side[97,98,99,100].OAT3 is found in all segments, from proximal convoluted tubule to collecting duct. Through organic anion transporter, uric acid enters into smooth muscle cell and activates mitogen activated protein kinases, cyclooxygenase-2, causes upregulation of PDGF, thromboxane formation and increases the cell proliferation.

**OTHER TRANSPORTERS IDENTIFIED BY GENETIC ASSOCIATION STUDIES:**

**GLUCOSE TRANSPORTER FAMILY MEMBER SLC2A9:**

GLUT9 (SLC2A9) has a sequence similar to GLUT family of Glucose transporter. (GLUTII isoform)[101].It exists as two alternatively spliced variants (GLUT9A, GLUT9B) and encodes different amino terminal tails. [102] GLUT9A has wide tissue distribution.GLUT9B is present in Liver& kidney. This kind of urate transport is inhibited by uricosuric drugs & Glucose transporter inhibitor.

**MONOGENIC MUTATIONS IN SLC2A9;**

Hypouricemia is linked with mutation. Recent genetic studies show that single mutation of a highly conserved cysteine in transmembrane 5 of GLUT9 causes both liver urate uptake & renal reabsorption defects. [102]

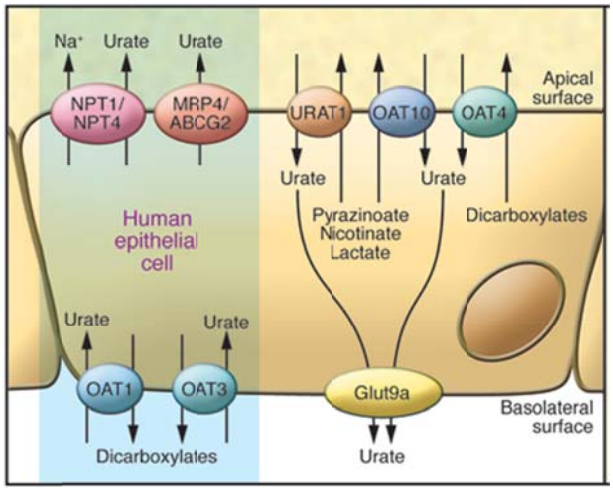
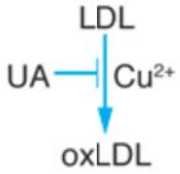



**FIG: 4 URATE TRANSPORTER IN KIDNEY EPITHELIAL CELLS:**

**ROLE OF URIC ACID AS AN ANTIOXIDANT:**

Urate the soluble form of uric acid in blood chelates metals and scavenges super oxide &hydroxy radical. Peroxy nitrite formed by the reaction of superoxide anion with nitric oxide can injure the cell by nitrosylating the tyrosine residues. Uric acid can block this reaction. [103]

Hink et al [104] states that" Uric acid prevents the degradation of extracellular superoxide dismutase". It is important for maintaining endothelial vascular function because, superoxide dismutase removes the superoxide anion ($0_2^-$ ) and converts it into hydrogen peroxide and maintains the nitric oxide levels for normal endothelial function. Hydrogen peroxide inactivates superoxide dismutase by negative feedback mechanism. In the presence of ascorbate, uric acid blocks this inactivation and regenerates super oxide dismutase with the production of urate radical and is converted back to urate[105].It protects the LDL from $cu^{2+}$mediated oxidation and shows protection against cardiovascular disease, ageing and cancer.




Ames et al [106] states that “Uricase mutation has provided an evolutionary advantage. The antioxidant role of uric acid may account for greater longevity in humans".

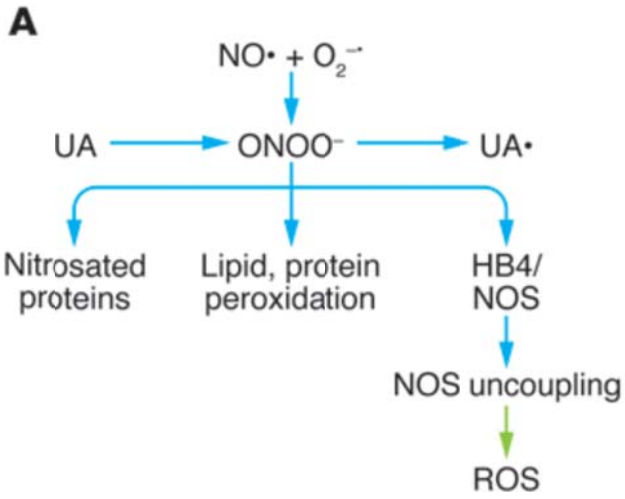



**FIG: 5 ANTIOXIDANT ROLE OF URIC ACID**

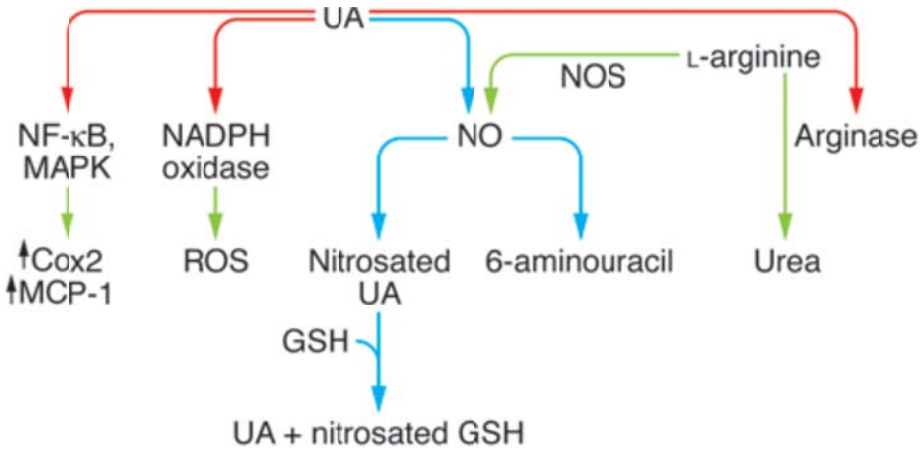



**FIG: 6 PROOXIDANT ROLE OF URIC ACID**

**Pro-oxidant role of uric acid**:

1) Uric acid also acts as a prooxidant. It behaves differently inside & outside the cell.
2) Inside the cell, it acts as a pro oxidant & outside the cell it acts as an antioxidant.
3) It oxidises the already oxidised LDL, which contains lipid peroxidation products and this type of oxidation depends upon the presence of transition metals [107].
4) Uric acid is oxidised by per oxynitrite and produce urate radicals, which acts as a pro oxidant. Under aerobic conditions, Urate reacts with NO to form an unstable nitrosated uricacid product & transfers NO to glutathione.
5) Urate is converted in to stable 6-aminouracil in the presence of NO under anaerobic conditions. [108]So, it increases the bioavailability of urate,

decreases the NO availability & also decreases the VEGF stimulated NO production. [109]

6) During adipo gene differentiation of 3T3-L1 cells, addition of uric acid increases the reactive oxygen species production by NADPH oxidase and plays a role in inflammation. [110]

**BIOLOGICAL ROLE OF URIC ACID**:

**CARDIOVASCULAR DISEASE:**

- Uric acid plays a role in endothelial function & vascular remodelling [111].
- The enzyme xanthine oxidoreductase has 2 forms. It exists as xanthine dehydrogenase in normal physiological conditions that has greater affinity towards $NAD^+$ than $o_2$.
- In ischemic conditions, xanthine dehydrogenase is converted in to xanthine oxidase. Here it uses $o_2$ instead of $NAD^+$ as an electron acceptor resulting in the formation of uric acid, hydrogen peroxide, &superoxide anion. The inflammation & arterial wall damage are related to the free radicals produced during the reactions or by uric acid itself.[112]
- It acts as a pro oxidant, increases the reactive oxygen species, prevents the reverse cholesterol transport and increases the cardiovascular risk. It increases the production of Monocyte chemo attractant protein-1 via, MAP kinase&cyclooxygenase-2 results in increased production of c- Reactive protein & cell proliferation[113]

**RENAL DISEASES:**

- Uric acid induces endothelial dysfunction & local inflammation in kidney.
- It induces phenotypic transition of renal tubules from epithelial to mesenchymal (EMT).It increases α smooth muscle actin expression & decreases the expression of E-cadherin & Zonaoccludens protein. Hypoxia, increased glucose concentration and inflammatory cytokines activate the transcription factor which down regulates the expression of epithelial cell markers in tubular epithelial cell and induce phenotypic transformation.[114]
- It is one of the earliest changes which lead to renal fibrosis. Thus uric acid induced epithelial mesenchymal transition of renal tubular cell explains the association of hyper uricemia & the progression of renal disease.

**HYPERTENSION:**

- Hypertension due to hyper uricemia is because of increased renin & decreased neuronal NOS-1 in juxta glomerular apparatus [115].
- It increases the salt sensitivity. The mechanism behind is, preglomerular vascular disease produces renal ischemia and generates local oxidants by infiltration of Leucocytes and altering the balance of vasoregulatory factors resulting in vasoconstriction. This decreases the sodium excretion and increases the B.P.

- It causes thickening and hypercellularity of glomerulus with vascular remodelling resulting in increased medial thickness and decreased luminal diameter[116].It damages small renal vessels and leads to irreversible salt sensitive Hypertension

**INFLAMMATION:**

- DiGovine et all reports that" urate crystals stimulate the production of TNF-α from synovial cells& human blood monocytes".
- MSU induced inflammation are Toll like receptors dependent. This Toll like receptors work in synergy with cytosolic receptor families NLRS (NOD-Nucleotide binding & oligomerization domain like receptors.), and CLR(C type Lectin receptors).MSU activates NALP3, a type of NLR leading to the production of protein complex called inflammosome.
- It is involved in the secretion of IL-1β, IL-18, by the activation of caspases and induce inflammation.
- The generation of reactive oxygen species is essential for NALP3 inflammosome activation.
- Studies by Liu -Bryan et al demonstrated that, "MSU stimulates the dendritic cells and promotes the release of cytokines associated with TH17 polarization ".
- Presence of NF-KB signalling suggests the modulation of adaptive immunity in addition to early innate responses.[117]

**URICACID AND PARKINSONISM:**

The antioxidant role of uric acid serves as a therapeutic benefit in Parkinson's disease. because oxidative stress plays a role in degeneration of dopaminergic neurons in the substantia nigra .So, lowering the level of uric acid is associated with Parkinsonism.

**PRE ECLAMPTIC TOXEMIA:**

Increased plasma uric acid concentration is caused by utero placental breakdown and decreased kidney perfusion. It correlates with the severity of preeclampsia.

Redman et al states that "concentration in excess of 6mg/dl at 32 weeks of gestation is associated with a high perinatal mortality rate".

**hs-CRP:**

hs-CRP was first described by Tillet & France in 1930 in the sera of acutely ill patients. This substance was identified as a protein and has the ability to bind the cell wall polysaccharide of most bacteria, fungi, parasites & poly cations (histones) and it was named as C-Reactive protein.

It is an acute phase reactant and it is monitored routinely to detect infection and autoimmune diseases.

Numerous epidemiological data show that increased concentration of hs-CRP predicts future coronary events, peripheral arterial disease and cerebrovascular disease [118-120] Traditional assays have the detection limit of 3-8

mg/l. To predict cardiovascular risk hs-CRP assays with detection limit of <0.3mg/l was developed.

**BIOCHEMISTRY OF hs-CRP:**

C-Reactive protein consists of five identical, non glycosylated polypeptide subunits, none covalently linked to form a disk shaped cyclic polymer with a molecular weight of 115 k Da. It is synthesized in the Liver, and binds to polysaccharide of other organisms. Its production is controlled by IL-6.

**QUALITY OF hs-CRP as a Biomarker:**

Recent epidemiological evidence show that, half of the incidence of Myocardial infarction occurs among individuals without hyperlipidemia. Based on hemostatic & fibrinolytic function, wide variety of non-lipid biochemical markers were developed .ex: VWF, D dimer, Homocysteine, hs-CRP, Tissue plasminogen activator antigen. But most of the above markers, except hs-CRP cannot satisfy the criteria to be accepted as a biomarker for predicting the cardiovascular risk in primary prevention setting [121].

**TABLE 2:RECOMMENDATIONS BY CENTRE FOR DISEASE CONTROL&PREVENTION (CDC) ANDAMERICAN HEART ASSOCIATION (AHA) FOR CLINICAL MEASUREMENT OF hs-CRP:**

1) hs-CRP can be measured both in Fasting & Non fasting state.
2) When the Test values are >10 mg/l, the result should be discarded and test has to be repeated after two weeks.

3) For a reliable estimate, an average of two assays performed two weeks apart should be considered.
4) When hs-CRP levels are >10mg/l on two consecutive tests, search for other infections and inflammation has to be performed.

**TABLE: 3**

**Categorization of patients using standard hs-CRP assays to different risk strata:**

| Concentration of hs-CRP (mg/l) | Risk stratification |
|---|---|
| <1.0 mg/l | Low Risk |
| 1.0-3.0mg/l | Average risk |
| >3.0mg/l | High risk. |

There are three groups of inflammatory markers.

1) Cytokines &chemokines.

2) Soluble adhesion molecule

3) Acute phase reactants. [122]

C-reactive protein is an acute phase reactant, increases in response to inflammation & tissue damage [123]. Level of CRP increases from 6 hrs and reaches a peak with in 48 hrs and have a half life of 19 hrs. The magnitude of underlying pathology is detected from its concentration.

**STABILITY OF hs-CRP:**

Based on large number of trials ,it was observed that the concentration of hs-CRP remained stable and it can be used as the marker of future cardio vascular disease in community based screening.[124]

**FACTORS THAT INCREASE THE CONCENTRATION OF hs-CRP:**

1) Obesity
2) Smoking
3) Metabolic syndrome
4) Dys lipidemia
5) Hormones
6) Hypertension.

**FACTORS DECREASING THE CONCENTRATION OF hs-CRP:**

1) Weight loss
2) Medications:-Statins, Fibrates, Niacin, Aspirin, NSAIDs, Moderate alcohol consumption.

**SEASONAL VARIATION:**

Concentration of hs-CRP decreases in summer (lowest found in June), and increases in Winter. (November-December). [125, 126]

**DIURNAL VARIATION;**

There is no diurnal variation .It can be measured during any period of time .But IL-6, a cytokine, that synthesizes CRP has diurnal variation.

**AGE & GENDER INFLUENCE:**

hs-CRP increases with age & in Women. NHANES111 (Third National health and Nutritional evaluation survey) shows the relationship of hs-CRP and age. They derived the equation to estimate the hs-CRP based on age.

**hs-CRP concentration:**

Males :Age/50

Females : Age/50+0.6.

**RACIAL DIFFERENCE:**

Based on a systematic review of population studies, south asians, & Hispanics have increased hs-CRP concentrations. Average concentration of hs-CRP in healthy individuals is 2.19mg/L and it is more in people with metabolic syndrome.

**VARIABILITY AMONG INDIVIDUALS:**

In individuals with 1) CRP genetic polymorphisms-Elevations of CRP concentration without Coronary heart disease risk.

2) Influence of genetic loci mediating CRP response.

3) Life style factors.

4) Ethnic factors.

5) sex.

There is significant base line variation due to Diabetes ,Hypertension, Dietary patterns, Physical exercise, smoking, Periodontal disease ,Oral contraceptive pills & Environmental pollution.

**MECHANISM OF ACTION:**

IL-6 is released from the activated Leukocytes in response to trauma or infection. CRP released from IL-6, binds directly with oxidised LDL and is present within Lipid laden Plaques [127].

CRP catalyses the early step in atherosclerosis. It causes Monocyte adhesion &trans migration in to the vessel wall. It causes polarisation of Macrophages, which is a pro inflammatory trigger in plaque deposition in atherosclerotic lesions and in adipose tissue.

In addition, it inhibits endothelial Nitric oxide synthases, and impaired Vasoreactivity [128,129].

**hs-CRP AND GLOBAL CARDIOVASCULAR RISK TOOLS:**

Based on three scores,

- Framingham CHD risk score (FRS)
- Reynolds Risk score (RSS)
- Systematic coronary risk evaluation. (SCORE)

Individual cardiovascular risk is assessed for ten years based on traditional risk factors. i.e.) Age, Gender, Diabetes, Smoking, Dyslipidemia& Hypertension and categorise the patients in to low, intermediate, and high risk. CRP is a potential risk factor; it decreases the NO synthesis, promotes chemotaxis, & increases the expression of tissue Factor which acts as a procoagulant resulting in DIC. Production of Plasminogen activating inhibitor

inhibits tissue plasminogen activator, decreases fibrinolysis and results in atherogenesis [130,131].

**hs-CRP AND CARDIOVASCULAR DISEASE:**

Chronic inflammation plays an important role in Atherogenesis. Net reclassification improvement(NRI).- states that hs-CRP values and family history of cardio vascular disease in addition to Framingham risk components, accurately reclassify the proportion of intermediate individuals in to higher and lower risk categories. U.S preventive service task force recommends the use of hs-CRP for improving risk stratification.

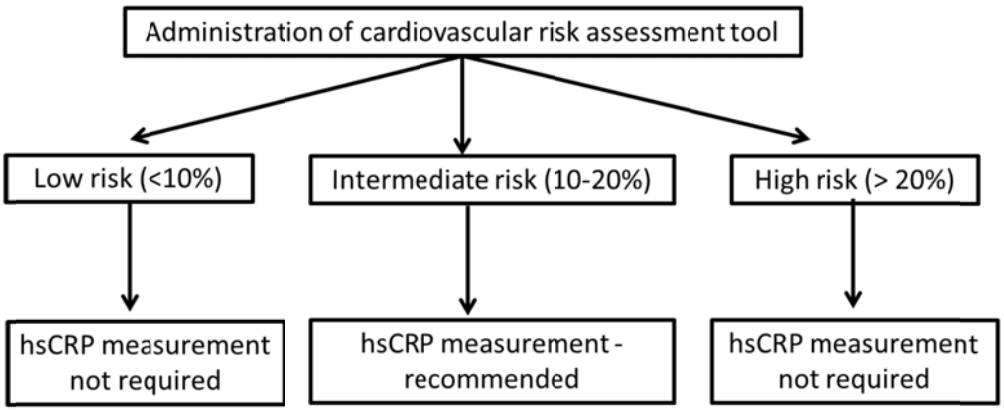



**FIG: 7 CARDIOVASCULAR RISK ASSESSMENT TOOL.**

**hs-CRP AND METABOLIC SYNDROME:**

hs-CRP is positively correlated with the components of Metabolic syndrome and also with other components such as Fasting Insulin, Microalbuminuria & impaired Fibrinolysis. Data from World health survey (WHS) states that "Those with Metabolic syndrome &hs-CRP >3 mg/l had twice

the risk of future coronary events compared to Metabolic syndrome &hs-CRP <3 mg/l". [132]

**hs-CRP and RENAL DISEASE:**

- hs-CRP predicts the progression and cardiovascular mortality in renal disease patients.
- In Glomerular hyper filtration, the presence of increased inflammatory markers signals the worsening of renal function.
- It also induces Nuclear factor Kappa-B leading to release of vasoactive and inflammatory substances in interstitium. The release of cytokine & thereby growth factor causes fibroblast proliferation, renal scarring and decreases renal function. It indicates, significant inflammatory response in early renal dysfunction contributing to progressive deterioration of renal function.

**hs-CRP AND HYPERTENSION:**

- Increased CRP correlates with endothelial dysfunction. It activates Renin angiotensin system, increases angiotensin II, and up regulates angiotensin I receptor activity.

**hs-CRP AND TYPE I DIABETES MELLITUS:**

In hyperglycaemia, the concentration of Advanced Glycated end products increases. It activates Macrophages, increases the oxidative stress, up regulates the synthesis of IL-6, IL-1& TNF-α resulting in the production of CRP.

**MEASUREMENT OF GFR:**

National Kidney Foundation recommends Glomerular Filtration rate estimation is necessary for diagnosis, screening, classification and monitoring of Chronic Kidney disease[133,134].Without GFR measurements ,CKD may remain silent and the patient will land up in End stage Renal disease.

**GLOMERULAR FILTRATION RATE:**

"GFR is a measure of the rate at which water and dissolved substances (low molecular weight, ultra filterable compounds) are filtered out of the blood per unit time." It provides a measure of filtering capacity of the kidneys.

$$GFR = \frac{\text{Urine Concentration} * \text{Urine Volume}}{\text{Plasma Concentration}}$$

Normal GFR adjusted for Body surface area=100-130 ml/min/1.73$m^2$ in Men &Women.

In children, GFR measured by Inulin clearance =110ml/min/1.73$m^2$until 2yrs of age in both sexes. At >40 yrs of age, GFR decreases by about 0.4-1.2 ml/min/yr. In Indians, accurate estimation of CKD prevalence is limited due to lack of GFR estimated equation validated for the Indian population.[135].Indians have lower normal range of GFR than Western population.[136]

Mean GFR for Indians =95.5+/-11.6ml/min. [137].

**USES OF ESTIMATION OF GLOMERULAR FILTRATION RATE:**

1) It is an index of functioning renal mass.
2) Monitoring changes in GFR can delineate progression of Kidney disease.
3) It is a strong predictor of the time of onset of Kidney failure as well as the risk of complications of CKD.
4) It allows proper dosing of drugs excreted by Glomerular filtration to avoid potential drug toxicity.

**PROCEDURES FOR DETERMINING GFR:**

**IDEAL FILTRATION MARKER:**

1) Ideal filtration marker is one, which is not protein bound.
2) Physiologically inert.
3) Freely filtered by the Glomerulus.
4) There is no Tubular secretion.
5) It is not metabolised by the kidneys.

**EXOGENOUS SUBSTANCE:**

Insulin is one of the exogenous substances ,that fulfils the above criteria and it remains the Gold standard for estimation of GFR.. Other substances used are cr51-EDTA, 99m TC-DTPA and $I^{125-}$Iothalmate (Require estimates of body size.). They are expensive, labour intensive, time consuming, and carry some risk to the patient. So these substances are not routinely used in screening procedure for the detection of CKD.

**ENDOGENOUS SUBSTANCE:**

Creatinine is a naturally occurring endogenous substance and it was considered as the renal marker of choice earlier, because it is freely filtered at the glomerulus with minor absorption and secretion by the Renal tubules.

**TABLE: 4 LIMITATIONS OF CREATININE AS A MARKER FOR GFR:**

| LIMITATIONS | COMMENTS |
|---|---|
| **Non-Renal Factors** | • Gender<br>• Ethnicity<br>• Diet, muscle mass.<br>• Drugs which affect tubular secretion of Creatinine |
| **UTILITY** | • Poor sensitivity for CKD "Creatinine blind range."(Serum Creatinine remains in the normal range until 50% of renal function is lost.) Insensitive to loss of GFR in stage2 &stage 3 of CKD |
| **Analytical Problems** | • Non-specific bias frequently reported with the commonly used Jaffe assay(alkaline picrate)<br>• Use of enzymatic assays for creatinine can significantly improve test performance by eliminating many source of analytical error. |

**SERUM CREATININE:**

We use serum creatinine for the measurement of GFR because,

1) Creatinine is one of the filtration markers, whose clearance approximates the GFR.
2) Creatinine excretion is constant among individuals and over time.
3) Measurement of serum Creatinine is accurate & reproducible across clinical laboratories.

But, these assumptions are not true. Numerous errors occur in the estimation of GFR by using serum Creatinine concentration alone.

**CREATININE CLEARANCE:**

$$C_{cr} = \frac{U * V}{P}$$

U = Urine Creatinine Concentration

V= Volume of Urine

P = Plasma Creatinine Concentration

Creatinine is a breakdown product of creatine phosphate. Since it is actively secreted by peritubular capillaries it overestimates GFR by 10-20 %[138]. It varies with muscle mass. It is higher in younger than older people, Black than White, Men than women. It also requires 24 hrs urine Creatinine excretion rate; Hence we use Cockcroft Gault Formula

**COCKCROFT-GAULT FORMULA :( eCcr)**

A commonly used marker for estimating Creatinine clearance .It estimates GFR in ml/min[139].It requires Weight, Height, Age and Plasma

creatinine. It is used for guiding administration of many drugs. It does not require 24 hrs urine collection.

It provides useful information for

1) Estimates of GFR in individuals with specific dietary intake.(Vegetarian diet, Creatine supplements) or muscle mass (amputation, Malnutrition, muscle wasting).
2) Assessment of diet & Nutritional status.
3) Need to start dialysis.

**LIMITATIONS:**

1) The trial study based on this formula was done only in hospitalised CKD patients and only 9 females had participated in the cohort study.

2) It requires height & Weight .It is a cumbersome procedure So, it is not used in Lab.

$$eC_{Cr} = \frac{(140\text{-age})*\text{Mass (Kg)}*0.85(\text{if female})}{72*\text{serum Creatinine (mg/dl)}}$$

**MDRD (Modification of diet in renal disease):**

It is based on Multicentre trial to evaluate the role of B.P control & dietary protein restriction on Renal disease progression in patients with CKD & prediction of GFR from plasma Creatinine.[140] .

MDRD eGFR = 186* (plasma Creatinine (μmol/l) * 0.0011312.)$^{-1.154}$ * age (years)$^{-0.203}$ * 0.742 if female * 1.212 if black.

MDRD (IDMS aligned) =175*(plasma Creatinine (µmol/l)*0.0011312)$^{-1.154}$*age (years)$^{-0.203}$*0.742 if female*1.212 if black.

**ADVANTAGES:**

1) GFR is measured directly by urinary clearance of $I^{125}$iothalamate.
2) A large sample population of >500 individuals with a wide range of kidney disease were studied
3) It provides eGFR standardised to Body surface area.
4) Lack of requirement of body weight/height.
5) Validated in Diabetic kidney disease &renal transplant patient.

**DISADVANTAGES:**

1) The formula is derived based on a group of CKD patients. Hence its use in healthy population is unclear.
2) It is not validated in children <18 yrs of age, pregnant Women, >70 yrs of age,& in extremes of body weight.
3) Limited data on the use of eGFR in other ethnic groups.
4) Calibration & measurement imprecision for plasma Creatinine, produce uncertainty in e GFR particularly when the plasma Creatinine values are low, i.e. Corresponding to higher renal function. (141)
5) It tends to under estimate renal function in those with an e GFR >90ml/min/1.73m$^2$.

Because of these limitations, we use CKD-EPI formula.

**CKD-EPI (CKD-epidemiology collaboration group):**

**ADVANTAGES:**

1) It met the accuracy of MDRD equation at GFR <60 ml/min/1.73m$^2$.
2) It has greater accuracy at higher GFR i.e. GFR >60ml/min./1.73m$^{2.}$ It decreases the over diagnosis of CKD with MDRD equation. [142]
3) It includes Gender, Age, Race & serum Creatinine.
4) It has different equations for Black, Male, Female, White or other Race with different serum creatinine concentrations.
5) High accuracy have important applications in public health as well as in clinical practice.
6) Based on CKD-EPI, prevalence of CKD is lower, when compared to MDRD, cardiovascular risk also decreases and the resources can be more appropriately targeted for those who are having CKD.

**CKD-EPI (IDMS CALIBRATED)**

eGFR=141*min (Serum Cr/K,1)$^{\alpha}$ *Max (Serum Cr/K,1)$^{-1.029}$ *0.993$^{age}$ *(1.018 if female)*1.159 if Black.

K-0.7 for female, &0.9 for Male.

α is -0.329 for female&-0.411 for Male, Min indicates minimum of serum Cr/K or 1,Max-Maximum of serum Cr/K or 1.

**WHITE OR OTHER RACE (Female)**

If serum Cr<=0.7

eGFR=144*(serum Cr/0.7)$^{-0.329}$x0.993$^{Age.}$

If serum Cr>0.7,

e GFR=144*(serum Cr/0.7)$^{-1.209}$x0.993$^{Age.}$

**WHITE OR OTHER RACE (MALE)**

If serum Cr<=0.9,

e GFR=141*(serum Cr/0.9)$^{-0.411}$xo.993$^{Age}$

If serum Cr>o.9,

eGFR=141*(serum Cr/0.9)$^{-1.209}$x0.993$^{Age.}$

**MAYO QUADRATIC FORMULA:**

It was developed by Rule et al, It is mainly used to estimate GFR in preserved kidney function.

eGFR=exp (1.911+5.249/serumCr-2.114)/serum Cr$^2$ -0.00686*age-(0.205 if female)

If serum Cr<0.8 mg/dl, use 0.8mg/dl for serum creatinine.

**SCHWARTZ FORMULA;**

It requires serum Creatinine (mg/dl) & height in cm.

e GFR=$\frac{K*ht}{\text{serum Cr}}$ K-constant depends on muscle mass and varies with child age.

In 1$^{st}$ year of life for preterm babies, k=0.33 & for full term infants k=0.45.

For infants & children of age (1-12 years) K=0.55.

**CYSTATIN C:**

Problems in the estimation of creatinine (varying muscle mass, protein ingestion), led to the evaluation of other agents for estimation of GFR. one of this is cystatin C, an ubiquitous protein, inhibitor of serine protease, secreted by most cells in the body.[143]It is freely filtered at the glomerulus. It is reabsorbed and catabolised by tubular epithelial cells with only small amount being excreted in the urine. It is measured in the blood stream.

Equations are developed linking estimated GFR to serum cystatin C levels.

**Disadvantages:**

1) Lack of international standardised calibrator limits its use.
2) Expensive.(Will not justify its use until sufficient evidence suggesting improved clinical decision making & better outcomes.)

**Table:5Cystatin C based GFR equation for adults [144].**

| | |
|---|---|
| Grubb et al | e GFR=84.69*cystatinC$^{-1.680}$(*0.948 if female) |
| Le Bricon et al | e GFR=78/cystatin C (mg/l)+4 |
| Hoek et al | eGFR=80.35/cystatin C (mg/l)-4.32. |
| Larsson et al | eGFR=77.239*cystatin C(mg/l)$^{-1.2623}$ |
| MacIsaac et al | eGFR=86.7/cystatin C(mg/l)-4.2 |
| Rule et al | eGFR=76.6*Cystatin C (mg/l). |

Clinician needs to be known about the limitations of various equations and to interpret the results. Thus by reviewing the literatures, the role of Uric acid and hs-CRP in Type 1 Diabetes with low GFR was understood .Hence this study was carried out.

# *Aims & Objectives*

# AIMS & OBJECTIVES

- To correlate uric acid level with glomerular filtration rate in normoalbuminuric & microalbuminuric patients with type 1 diabetes.

- To correlate the uric acid level with hs-CRP for predicting cardiovascular risk in normoalbuminuric & microalbuminuric patients with type 1 diabetes.

# *Materials & Methods*

# MATERIALS &METHODS

This is a cross sectional case control study and was conducted after getting ethical committee approval. The study composed of a total number of 130 subjects of Type 1 Diabetes mellitus patients attending Diabetology outpatient department in Rajiv Gandhi Government General Hospital, Chennai.

**Inclusion Criteria:**

**GROUP A&B : –**

- Type1 diabetic patients
- Duration of diabetes > 5 yrs
- Age 10-40 yrs
- Treatment with insulin.

**Exclusion Criteria:**

- Macroalbuminuric patients
- End stage renal disease patients
- Renal transplant patients
- Type2 Diabetic patients
- Patient with acute illness; inflammation
- Patients with cardiac ailments.

Patients were selected after doing urine dipstick to rule out proteinuria.

Cases were divided in to two groups based on Urine albumin creatinine ratio.(UACR).

**Group1:**

65 Type 1 Diabetic patients with normoalbuminuria.(UACR<30mg/g of creatinine).

**Group2:**

65 Type 1 Diabetic patients with Microalbuminuria.(UACR 30-300mg/g of creatinine.)

**Urine sample collection:**

Urine sample was collected after giving instructions to the patient.

- First voided sample was discarded.
- Early morning mid-stream urine was collected.
- Urine for albumin creatinine ratio was analysed on the same day.

**Blood sample collection:**

- Blood was collected after 8-12 hrs of overnight fasting.
- About 5mL of venous blood was collected from antecubital vein after aseptic precautions and transferred in to 2 tubes and the investigations performed as per the following table.

| Tubes | Anticoagulant | Amount of blood | Investigations |
|---|---|---|---|
| Tube1 | EDTA | 3mL | Fasting Plasma Glucose,HbA1c,Fasting Plasma Lipid profile. |
| Tube2 | --- | 2mL | Serum Creatinine, Protein, albumin, Uric acid ,hs-CRP. |

The blood samples were analysed with in 4 hrs of sample collection and were analysed by the following methodologies.

**Estimation of Urine albumin concentration**:

Method: Latex agglutination method/Immunoturbidimetry.

Kit from Bio systems Instruments &Reagents.

**Principle:**

Albumin in the urine sample causes agglutination of the latex particles coated with anti human albumin. The agglutination of the particles is directly proportional to the concentration of albumin and can be measured by turbidimetry.

**Composition:**

**Reagent A:**

Sodium azide 0.95g/L

Borate buffer 0.1mol/L,PH-10.0

**Reagent B:**

Sodium azide 0.95g/L

Suspension of latex particles coated with anti-human albumin antibodies.

**Preparation of Reagents:**

Smaller working reagent volumes were prepared by mixing 1 mL of Reagent B with 4mL of Reagent A.(1:4 ratio)and mixed thoroughly.

Albumin standard: Reconstitute with 1mL of distilled water.

**PROCEDURE:**

Instrument &Working reagent were brought to 37□c.After being standardised with the given microalbumin standard solution . 7µL of sample was added to 1mL of working reagent and mixed well .The absorbance was measured after 10 sec (A1) and 2mins (A2) at 540nm.

**Calculation:**

Albumin concentration in the sample was calculated by

$$\text{concentration of albumin(mg/L)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of std}} * \text{concentration of Std.}$$

Urine albumin mg/dL= albumin in mg/L*10.

**Reference value:**

Urine albumin=up to 15mg/L.

**Estimation of Creatinine concentration in the urine sample:**

Method: Modified Jaffe's method.

**Principle:**

Creatinine in the urine sample reacts with alkaline picrate to form an orange yellow colour creatinine picrate. The intensity of the colour is directly proportional to the creatinine concentration and is measured spectrophotometrically at 500-520nm.

**Working Reagent Preparation:**

Equal volumes of Reagent1 &Reagent 2 was mixed & kept aside for 15mts.

**Preparation of sample:**

Dilute urine samples with 1:10 dilution.

Reagent1 : Picric acid-25.8mmol/L

Reagent2 : Sodium hydroxide-95mmol/L.

**Procedure:**

Analyser was calibrated using the creatinine standard provided with the kit.50µL of diluted sample was added to 500µL of working reagent mixed well and the reading obtained from the semi automatic analyser was multiplied by the dilution factor to get correct concentration.

**Calculation:**

$$\text{Creatinine(mg/dL)} = \frac{\Delta A \text{ of Test}}{\Delta A \text{ of std}} * \text{Concentration of Std.}$$

Creatinine(g/dL)=Creatinine(mg/dL)/1000.

**Reference range:**

Adult Male-14 to26 mg/kg/day.

Adult Female:11-20mg/kg/day.

Urine albumin creatinine Ratio:

UACR=Urine albumin(mg/dL)

---

Urine Creatinine(g/dL)

UACR=mg/g of creatinine.

After classifying the patients based on UACR in to 2 groups, following analytes were estimated.

**Estimation of serum uric acid:**

Method: uricase, Endpoint assay.

**Principle:**

Uric acid in the presence of uricase is oxidised to allantoin. Peroxidase reacts with $H_2o_2$catalyses the oxidative coupling of 4AAP with TOOS to form Quinoneimine complex and the intensity of colour is directly proportional to the concentration of uric acid in the sample. The absorbance of final colour is measured at 546nm & 670nm.

$$\text{Uric acid} + o_2 + H_2o \xrightarrow{\text{Uricase}} \text{Allantoin} + co_2 + H_2o_2$$

$$\text{TOOS} + 4\text{AAP} + 2\,H_2o_2 \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4\,H_2o.$$

**Procedure:**

Analyser was calibrated using the given standard concentration of 6 mg/dL. To 1mL of reagent, 25 µL of sample was added and after 5mts the absorbance was measured spectrophotometrically at 546 &670nm.

**Calculation:**

$$\text{Uricacid(mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of std.}} \times \text{concentration of std.}$$

Reference Values:

| | | |
|---|---|---|
| Men | : | 3.6-7.7mg/dL. |
| Women | : | 2.5-6.8mg/dL. |

**Estimation of hs-CRP:**

Method: Quantitative sandwich enzyme immunoassay

Kit from Calbiotech.

**Principle:**

- A monoclonal antibody specific for CRP has been precoated on to a microplate.
- Samples and anti CRP -HRP conjugate were pipetted in to the wells & CRP present in the sample is bound by the immobilised antibody.
- Anti CRP second antibody then binds to CRP. After washing away any unbound substances & HRP conjugate ,a substrate solution is added to

the wells and colour develops which is proportional to the concentration of CRP in the samples.

- The colour development is stopped & intensity of colour is measured at 450nm
- .Reagent composition:

  1.CRP microplate-96 microwells coated with CRP monoclonal antibody.

  2.CRP enzyme conjugate -12mL of monoclonal antibody against CRP conjugated to HRP.

  3.CRP standard-0.25mL with preservatives.

  4.Sample diluent-50mL with preservatives.

  5.Wash buffer concentrate-25mL of 20 fold concentrated solution with preservatives.

  6.TMB subsrate-12mL

  7.Stop solution-12mL.

**Working reagent preparation:**

Wash buffer:(1x wash Buffer)-Wash Buffer 1 fold concentrate was prepared by diluting 25 mL of 20 fold concentrate to 475 mL of distilled water.

**Sample preparation:**

Samples were diluted using sample diluent in the ratio 1:100.(5µL of samples to 495µL of sample diluent.)

**Assay procedure:**

- Reagent & Sample preparation was done..
- Allow the reagent to stand at room temperature(18-25□c).
- Add 10µL of std, diluted sample & control per well.
- Add 100µL of enzyme conjugate to all wells. Mix well.
- Incubate for 60 mins at room temperature.
- Empty the wells. Wash well three times with 300 µL of wash buffer. Blot on absorbent paper towels.
- Add 100µL 0f TMB substrate to all wells.
- Incubate for 15 mins at room temperature.
- Add 50µL of stop solution to all wells.

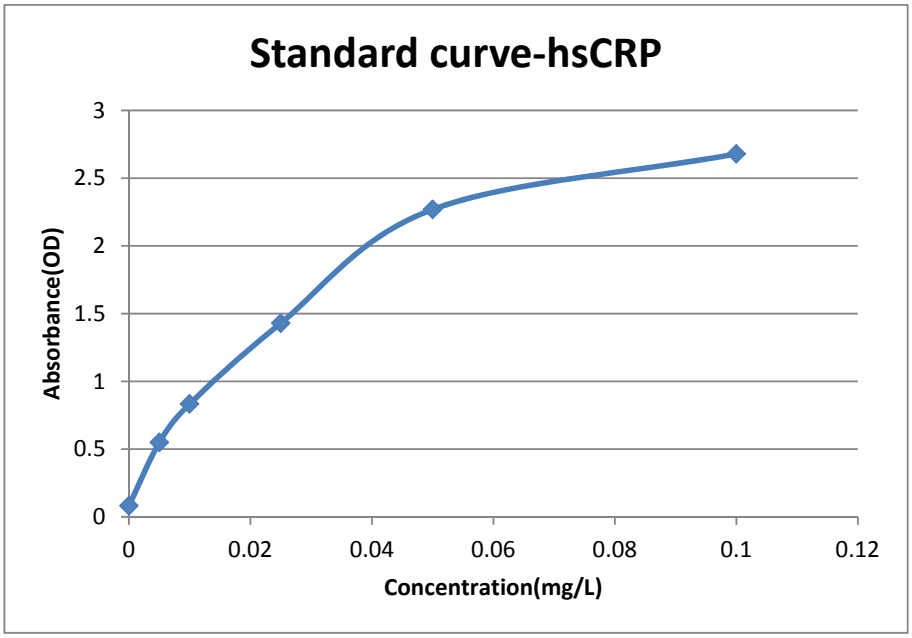
  OD of each well was determined with in 15 mins after adding the stop solution using microplate reader set at 450 nm.

**Calculation:**

A standard curve was constructed by plotting the concentration on the x-axis and the mean absorbance of each standard on the Y-axis and a best fit curve was drawn through points on the graph.

The following table shows the absorbance (OD) of each std and the concentration of CRP in the sample was obtained based on this absorbance. Final concentration of the sample was obtained by multiplying with the dilution factor.

| S.No | Concentration of std(mg/L) | Absorbance(OD) |
|---|---|---|
| 1. | 0 | 0.0818 |
| 2. | 0.005 | 0.5487 |
| 3. | 0.01 | 0.8336 |
| 4. | 0.025 | 1.4297 |
| 5. | 0.05 | 2.2694. |
| 6. | 0.1 | 2.6793. |




**Serum Creatinine**:

**Method:** Modified Jaffe's method.

**Principle:**

Creatinine reacts with alkaline picrate to form an orange yellow colour creatinine picrate. The intensity of the colour is directly proportional to the creatinine concentration and is measured spectrophotometrically at 500-520nm.

**Reagents:**

R1-Sodium hydroxide-240mmol/L

R2-Picric acid-26mmol/L

**Procedure:**

Automated chemistry analyser ERBA 640 was first calibrated using the creatinine calibrator traceable to Isotope dilution mass spectrometry(IDMS) . Results were calculated automatically by the instrument.

**Reference values:**

Males : 0.7-1.4mg/dL.

Females : 0.6-1.2mg/dL.

**Estimated GFR(eGFR)**

eGFR is calculated using serum creatinine by CKD-EPI formula as follows.

eGFR=141*min (Serum Cr/K,1)$^{\alpha}$ *Max (Serum Cr/K,1)$^{-1.029}$ *0.993$^{age}$ *(1.018 if female)*1.159 if Black.

(a) $\kappa = 0.7$ (female) or 0.9 (male);

(b) $\alpha = -0.329$ (female and SC $\leq$ 0.7mg/dL),

$\alpha = -1.209$ (female and SC >0.7mg/dL);

(c) $\alpha = -0.411$ (male and SC $\leq$ 0.9mg/dL),

$\alpha = -1.209$ (male and SC >0.9mg/Dl

**Estimation of Plasma Glucose:**

Method: Glucose oxidase -Peroxidase method(GOD-POD), End point assay.

**Principle:**

Glucose in the sample in the presence of Glucose oxidase ,oxidises to Gluconic acid & hydrogen peroxide, which in turn reacts with 4-aminoantipyrine in the presence of peroxidase enzyme to form a coloured quinoneimine complex. The intensity of colour is proportional to the concentration of glucose in the sample.

$$\text{Glucose} + o_2 + H_2O \xrightarrow[\text{Glucose oxidase}]{} \text{Gluconic acid} + H_2o_2.$$

$$H_2o_2 + \text{Phenol} + 4\text{AAP} \xrightarrow[\text{Peroxidase}]{} \text{Quinoneimine dye} + 2H_2O.$$

**Procedure:**

After being standardised with standard Glucose solution of 100 mg/dL,10µL of plasma was added to1 mL of working reagent & incubated at 37°c for 15 mts and the absorbance was measured at 505nm.

**Calculation:**

$$\text{Glucose mg/dL} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard.}} \times \text{Concentration of standard.}$$

Reference interval: Fasting plasma Glucose=70-100 mg/dL.

**Estimation of Urea:**

Method: UV-SLR, Fixed time Kinetic assay.

**Principle:**

Urea is hydrolysed in the presence of water & urease to produce ammonia & $co_2$. The ammonia produced combines with α-oxoglutarate& NADH in the presence of glutamate dehydrogenase to yield glutamate & NAD.

**Reagent composition:**

Reagent1 : Buffer reagent.

Reagent 2 : Enzyme reagent.

Urea standard: 50mg/dL.

**Procedure:**

After being standardised with 50mg/dL of urea standard solution, Serum samples were analysed. To 1mL of working reagent 10µL of serum. was added. Mixed well & the absorbance was read after 30 sec and 60 secs at 340nm.

$$\text{Concentration of Urea(mg/dL)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times 50$$

Reference range: 15-50 mg/dL.

Reference interval:0.2-10 mg/L.

**Estimation of serum protein**:

Method: Biuret method, Endpoint assay.

**Principle:**

Protein in serum sample reacts with copper salts in an alkaline medium forming a purple coloured complex. The intensity of colour is directly proportional to total protein concentration that can be measured spectrophotometrically at 540nm.

**Procedure:**

Analyser was calibrated using the given protein standard of 6g/dL concentration. To 500µL of reagent 10µL of sample was added and incubated for 10 mts at 15-30$^{o}$c. The readings were measured against reagent blank at 540nm.

**Calculation:**

$$\text{Concentration of Protein(g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \text{concentration of std}$$

Reference interval:adults:6-8g/dL.

**Estimation of serum albumin:**

Method: Bromocresol green, End point assay.

**Principle:**

Albumin acts as a cation ,binds to anionic dye bromocresol green at pH 3.68 forming a green coloured complex. The colour intensity is directly proportional to concentration of albumin in sample. The absorbance of final colour is measured at 578nm.

**Procedure:**

Analyser was calibrated using the given std concentration of 4g/dL.

To 1 mL of reagent 10µL 0f sample was added .Mixed well & after 5 mins the absorbance was measured spectrophotometrically at 578nm.

**Calculation**

$$\text{Albumin(g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of std.}} \times \text{concentration of std.}$$

Reference interval: Adult:3.5-5g/dL.

**Estimation of HbA1c:(Bio Rad D-10 Analyser).**

Method: Ion exchange high performance liquid chromatography.

**Principle**:

In HPLC, the samples are automatically diluted on D-10 and injected in to the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flowcell of the filter photometer, where changes in the absorbance at 415 nm are measured.

Two level calibration is used for quantitation of HbA1c values. A sample report & chromatogram are generated for each sample. The A1c peak is shaded. This area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes labile A1c & Carbomylated peak areas from the A1c peak area.

ADA & IFCC issued a consensus statement on the world wide standardisation of HbA1c measurement. They recommended the use of IFCC SI units(mmol/mol).

Master equation for designated comparison method (DCM)

NGSP% =(0.09148x IFCC )+2.152.

Reportable range:

NGSP% :3.8 -18.5

IFCC(mmol/mol):18-179.

**Estimation of Total Cholesterol:**

Method: Cholesterol esterase-Cholesterol Oxidase, End point assay.

**Principle:**

Cholesterol esterase hydrolyses Cholesterol ester to Cholesterol .It is acted upon by Oxidase to form cholestenone and hydrogen peroxide. It reacts with aminoantipyrine and phenol to give pink coloured complex and its optical density measured at 540nm.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol} + \text{Fatty acids.}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + 4AAP + \xrightarrow{\text{Peroxidase}} \text{PhenolQuinoneimine dye} + 4\ H_2O.$$

**Procedure:**

The semiautoanalyser was first calibrated using Cholesterol standard of 200 mg/dL. 10µL of Plasma was pipetted in to 1mL of working reagent,& incubated at 37$^{o}$c for 5 minutes. The absorbance was measured at 540nm.

**Calculation:**

$$\text{Cholesterol(mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std}} \times \text{Concentration of Standard.(mg/dL)}$$

Reference interval:150-260mg/dL.

**Estimation of Triglycerides:**

Method: Glycerophosphate oxidase -chlorophenol Amino phenazone(GPO-PAP) method, End point assay.

**Principle:**

Triglycerides in the sample are hydrolysed by Lipoprotein Lipase to give glycerol and Fatty acid. Glycerol is then acted upon by glycerol kinase to form glycerol-3-phosphate.Which on action by oxidase converted in to DHAP & hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine(AAP) to give pink coloured complex. The intensity of colour is related to concentration of triglyceride in the sample.

$$\text{TGL} + H_2o \xrightarrow[\text{Lipoprotein Lipase}]{} \text{Glycerol+ Free Fatty acids.}$$

$$\text{Glycerol+ ATP} \xrightarrow[\text{Glycerol Kinase}]{} \text{Glycerol-3-Phosphate +ADP}$$

Glycerol-3-phosphate oxidase

Glycerol-3-phosphate+$o_2$ ⟶ DAP+ $H_2o_2$

Peroxidase

$H_2o_2$ +4AAP+ TOOS ⟶ Quinoneimine dye+2 $H_2o$

**Procedure:**

The analyser was first calibrated with Triglyceride standard(200mg/dL). 10 µL of sample was mixed with 1 mL of reagent and incubated for 10 minutes at 37$^o$c. the absorbance was measured at 540nm.

**Calculation:**

$$\text{Triglycerides(mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{concentration of Std(mg/dL)}$$

**Reference Values:**

Males:60-165mg/dL

Females:40-140mg/dL.

**Estimation of HDL-Cholesterol**:

Method: Phosphotungstic acid method, Endpoint assay

**Principle:**

Phosphotungstate precipitates Chylomicrons, LDL and VLDL in the presence of divalent cations .The supernatant ,which contains HDL cholesterol remains unaffected and is estimated using cholesterol reagent.

Phosphotungstate

Plasma ⟶ HDL+(Chylomicrons, LDL, VLDL precipitate)

Precipitating reagent:

Phosphotungstic acid-2.4mmol/L.

Magnesium chloride-40mmol/l.

**Procedure:**

**1)Precipitation:**

Precipitation of LDL, VLDL, Chylomicrons.

500µL of precipitating reagent & 250µL of sample was mixed well and allow to stand for 10 mts at room temperature. Centrifuged for 10 minutes at 4000 r.p.m to get a clear supernatant. Concentration of HDL cholesterol was determined by using cholesterol reagent.

**2)Estimation with Cholesterol reagent:**

50µL of Supernatant was pipetted in to 1000µL of Cholesterol working reagent and incubated for 10 minutes at $37^{o}$c. Read the absorbance at 505 nm.

**Calculation:**

HDL Cholesterol=Absorbance of test

----------x concentration of Std x 2(mg/dL)

Absorbance of Std

(2 is the dilution factor due to deproteinisation step).

**Reference Values:**

In Male:35-55mg/dL

In Females:45-65mg/dL.

# *Statistical Analysis*

# STATISTICAL ANALYSIS:

- Data was analysed using SPSS software version 16.0 and p value less than 0.05 was considered as statistically significant.
- Age, Duration of Diabetes, BMI, Total cholesterol, HbA1c, Uric acid, eGFR, hs-CRP were compared between study groups by Student t-test.
- Gender was analysed by Chi square test
- One way ANOVA was done to compare more than two variables in the same group & between two groups . It was carried out to compare eGFR between Normoalbuminuric & Microalbuminuric type 1 Diabetic patients.
- Correlation of Parameters namely hs-CRP, eGFR, with uric acid were found out by Pearson correlation analysis.
- To find out the correlation between uric acid &eGFR in the presence of HbA1c, partial correlation was used.
- Multiple regression analysis was performed to evaluate the mathematical relationship between eGFR and other variables (Age, duration, Gender, HbA1c, Cholesterol, BMI & Uric acid) in Normoalbuminuric & Microalbuminuric Type 1 Diabetes.

# *Results*

## MASTER CHART OF NORMOALBUMINURIC TYPE 1 DIABETIC PATIENTS

| CASES | AGE | GENDER | DURATION | HT | WT | BMI | HYPER T | fasting PG | Urea | creatinine | uricacid | cholester | TGL | HDL | Hba1c | S.Protein | S.albumin | U.Albumin | U.Creatinine | urine ACR | hsCRP | eGFR (CKD-EPI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (Kg/m^2) | | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | % | (g/dL) | (g/dL) | (mg/L) | (mg/dL) | (mg/g) | (mg/L) | (ml/min) |
| 1 | 24 | F | 12 | 139 | 49 | 25.361006 | N | 159.4 | 15 | 0.6 | 1.2 | 143 | 45 | 36 | 8.9 | 5.6 | 3.3 | 1.8 | 75.2 | 2.39 | 0.6 | 128 |
| 2 | 28 | M | 5 | 142 | 49 | 24.300734 | N | 98 | 40 | 1.3 | 6.8 | 236 | 118 | 45 | 6 | 6.7 | 4.5 | 1.8 | 66.6 | 2.7 | 0.2 | 74.3 |
| 3 | 22 | F | 5 | 154 | 50 | 21.082813 | N | 105 | 26 | 0.7 | 2.35 | 79 | 74 | 15 | 7.1 | 6.8 | 3.9 | 0.9 | 29.2 | 3.08 | 0.3 | 123.4 |
| 4 | 21 | M | 5 | 149 | 47 | 21.170218 | N | 68 | 18 | 0.8 | 5.6 | 140 | 73 | 44 | 6.3 | 6.3 | 2.8 | 1.5 | 42.3 | 3.54 | 0.2 | 127.7 |
| 5 | 26 | F | 8 | 151 | 51 | 22.36744 | N | 160.2 | 33 | 0.7 | 2.6 | 264 | 149 | 42 | 10.3 | 6.3 | 3.4 | 5.4 | 138.8 | 3.89 | 0.8 | 120 |
| 6 | 31 | F | 6 | 160 | 45 | 17.578125 | Y | 151 | 13 | 0.6 | 2.4 | 87 | 59 | 45 | 7.6 | 5.7 | 3.7 | 0.9 | 22 | 4.09 | 4 | 121.8 |
| 7 | 40 | M | 18 | 169 | 61 | 21.357796 | Y | 144 | 29 | 0.9 | 4.5 | 156 | 84 | 47 | 7.7 | 6.5 | 4 | 0.9 | 22 | 4.09 | 0.4 | 111.7 |
| 8 | 16 | M | 5 | 140 | 38 | 19.387755 | N | 80.8 | 28 | 0.7 | 3.8 | 100 | 98 | 38 | 15.3 | 6 | 4 | 1 | 23.8 | 4.2 | 1.35 | 139.7 |
| 9 | 25 | F | 9 | 155 | 63 | 26.222685 | Y | 183.4 | 32 | 0.8 | 2.58 | 133 | 115 | 40 | 8 | 6 | 4.6 | 2 | 45 | 4.44 | 0.5 | 102.8 |
| 10 | 17 | F | 10 | 144 | 35 | 16.878858 | N | 229 | 30 | 0.8 | 4.32 | 231 | 98 | 26 | 7.1 | 6.4 | 4.4 | 1.01 | 20 | 5.05 | 1.8 | 108.7 |
| 11 | 24 | M | 16 | 157 | 60 | 24.341758 | N | 184.9 | 29 | 0.8 | 4.3 | 168 | 80 | 51 | 10.1 | 6.5 | 4.2 | 6 | 118.4 | 5.06 | 1.91 | 125 |
| 12 | 40 | F | 25 | 140 | 37 | 18.877551 | N | 231 | 30 | 0.9 | 6.7 | 167 | 135 | 66 | 9 | 5.9 | 3.9 | 1.2 | 20.8 | 5.76 | 1.4 | 80.2 |
| 13 | 19 | M | 10 | 152 | 54 | 23.372576 | N | 70 | 30 | 0.9 | 7.7 | 201 | 83 | 21 | 9.2 | 6.8 | 4.4 | 8 | 122.2 | 6.54 | 2.8 | 118.3 |
| 14 | 40 | M | 15 | 168 | 61 | 21.612812 | N | 121 | 23 | 0.9 | 2.9 | 164 | 150 | 34 | 10.3 | 6.9 | 3.8 | 6.1 | 92.8 | 6.57 | 15.41 | 106.5 |
| 15 | 23 | M | 6 | 159 | 60 | 23.733238 | N | 135.2 | 26 | 0.7 | 5 | 127 | 39 | 40 | 9.5 | 6.1 | 4.3 | 5.2 | 76.6 | ~~6.78~~ | 2.75 | 130.2 |
| 16 | 16 | F | 2 | 135 | 40 | 21.947874 | N | 229.2 | 27 | 0.8 | 5.9 | 217 | 78 | 51 | 10 | 6.5 | 4.4 | 4.3 | 62.8 | 6.84 | 2.2 | 109.5 |
| 17 | 34 | F | 14 | 151 | 49 | 21.490286 | N | 86 | 14 | 0.8 | 3.8 | 119 | 53 | 45 | 7 | 5.9 | 3.3 | 5.4 | 63.6 | 8.4 | 1 | 96.5 |
| 18 | 34 | F | 19 | 155 | 54 | 22.476587 | N | 55.5 | 27 | 0.6 | 3.3 | 174 | 82 | 37 | 6.9 | 6.4 | 4.1 | 4 | 47.2 | 8.47 | 1 | 119.3 |
| 19 | 30 | M | 12 | 148 | 54 | 24.653031 | Y | 280 | 37 | 0.8 | 3.4 | 86 | 238 | 34 | 7.7 | 6.4 | 4 | 9.1 | 107 | 8.5 | 1.3 | 119.9 |
| 20 | 26 | M | 5 | 159 | 74 | 29.270994 | Y | 71 | 37 | 0.9 | 3.82 | 150 | 74 | 50 | 7.4 | 5.9 | 4 | 2.1 | 24.4 | 8.6 | 0.6 | 117.5 |
| 21 | 37 | F | 20 | 156 | 62 | 25.47666 | N | 66.8 | 16 | 0.7 | 2.1 | 227 | 127 | 55 | 7.9 | 5.8 | 4 | 1.9 | 22 | 8.63 | 4 | 111 |
| 22 | 34 | F | 15 | 153 | 41 | 17.514631 | N | 176 | 24 | 0.5 | 3.3 | 124 | 47 | 35 | 7.1 | 6.3 | 4.6 | 3.1 | 34.6 | 8.95 | 1.6 | 126.7 |
| 23 | 35 | F | 14 | 154 | 65 | 27.407657 | N | 107 | 23 | 0.5 | 3.2 | 100 | 135 | 29 | 8.8 | 6.3 | 4.4 | 2.3 | 25 | 9.2 | 0.1 | 125.8 |
| 24 | 40 | M | 15 | 155 | 51 | 21.227888 | N | 119 | 25 | 1.3 | 8.4 | 142 | 126 | 37 | 7.3 | 6.2 | 4 | 6.5 | 65.5 | 9.92 | 12.7 | 68.3 |
| 25 | 16 | F | 9 | 132 | 43 | 24.678604 | N | 338 | 33 | 0.8 | 2.64 | 68 | 125 | 35 | 10.7 | 6.7 | 5.4 | 6.9 | 69.1 | 9.98 | 1 | 109.5 |
| 26 | 17 | F | 5 | 161 | 46 | 17.746229 | N | 68 | 34 | 0.7 | 3.8 | 218 | 97 | 53 | 10.4 | 6.5 | 4 | 2.5 | 24.3 | 10.28 | 3 | 127.8 |
| 27 | 40 | F | 10 | 155 | 56 | 23.309053 | N | 93.5 | 24 | 0.6 | 3.1 | 135 | 40 | 148 | 7.1 | 6.4 | 4.2 | 2 | 18.8 | 10.6 | 2.4 | 114.4 |
| 28 | 40 | M | 25 | 151 | 45 | 19.735976 | Y | 72.5 | 33 | 0.8 | 4.9 | 119 | 88 | 17 | 6.9 | 5.8 | 2.7 | 4.14 | 30.8 | 10.89 | 8.3 | 111.7 |
| 29 | 29 | F | 5 | 154 | 48 | 20.239501 | N | 93.3 | 28 | 0.6 | 3.6 | 134 | 57 | 36 | 7 | 6.5 | 3.7 | 13.6 | 122.3 | 11.12 | 0.8 | 123.6 |
| 30 | 37 | M | 23 | 154 | 64 | 26.986001 | N | 414 | 11 | 0.8 | 1 | 107 | 94 | 19 | 9.3 | 7.4 | 4.4 | 2.62 | 23.4 | 11.19 | 5.9 | 114.1 |
| 31 | 39 | F | 15 | 158 | 53 | 21.230572 | Y | 98 | 41 | 0.9 | 7.8 | 132 | 98 | 40 | 6.5 | 6.3 | 3.5 | 2.3 | 19.9 | 11.5 | 13.8 | 80.8 |
| 32 | 40 | M | 26 | 161 | 59 | 22.761468 | Y | 185 | 50 | 1.1 | 5.25 | 94 | 210 | 25 | 7.5 | 6 | 2.62 | 2.62 | 22.6 | 11.59 | 8.2 | 83.5 |
| 33 | 33 | F | 20 | 146 | 55 | 25.802214 | N | 212.8 | 18 | 0.8 | 3.4 | 130 | 55 | 54 | 7 | 6.3 | 2.6 | 2.62 | 22.5 | 11.64 | 0.5 | 97.2 |

| CASES | AGE | GENDER | DURATION | HT | WT | BMI | HYPER T | fasting PG | Urea | creatinine | uricacid | cholester | TGL | HDL | Hba1c | S.Protein | S.albumin | U.Albumin | U.Creatinine | urine ACR | hsCRP | eGFR (CKD-EPI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (Kg/m^2) | | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | % | (g/dL) | (g/dL) | (mg/L) | (mg/dL) | (mg/g) | (mg/L) | (ml/min) |
| 34 | 25 | F | 8 | 160 | 55 | 21.484375 | Y | 113.9 | 30 | 0.7 | 3.54 | 177 | 134 | 34 | 9.6 | 6.3 | 3.5 | 5.9 | 50.4 | 11.7 | 0.9 | 120.8 |
| 35 | 18 | F | 10 | 135 | 42 | 23.045267 | N | 90.6 | 29 | 0.5 | 2.68 | 207 | 123 | 37 | 9.7 | 6.3 | 4.4 | 3.01 | 23.6 | 12.75 | 24.8 | 141.8 |
| 36 | 18 | F | 5 | 151 | 51 | 22.36744 | N | 86 | 34 | 0.6 | 3 | 102 | 125 | 40 | 6.7 | 5.6 | 4 | 6.9 | 52.8 | 13.06 | 0.8 | 133.5 |
| 37 | 30 | M | 15 | 160 | 59 | 23.046875 | N | 64 | 32 | 1 | 6 | 188 | 137 | 38 | 6.7 | 6.6 | 4 | 6.5 | 50 | 13.2 | 0.3 | 100.5 |
| 38 | 37 | F | 25 | 151 | 51 | 22.36744 | N | 77.4 | 43 | 0.6 | 3.94 | 188 | 83 | 34 | 9.4 | 5.7 | 3.2 | 4.4 | 32 | 13.75 | 1.1 | 116.8 |
| 39 | 35 | M | 25 | 159 | 58 | 22.94213 | Y | 193 | 43 | 1 | 3.8 | 128 | 130 | 29 | 9 | 5.7 | 4.2 | 13 | 90 | 14.44 | 0.3 | 97.1 |
| 40 | 21 | F | 8 | 148 | 51 | 23.283419 | N | 225 | 40 | 0.8 | 2.8 | 63 | 25 | 46 | 6.3 | 6.1 | 3.4 | 4.8 | 31.8 | 15.09 | 0.9 | 106 |
| 41 | 16 | M | 6 | 169 | 38 | 13.304856 | N | 87.8 | 31 | 0.8 | 4.5 | 96 | 110 | 38 | 8.6 | 5.8 | 3 | 19.8 | 128.4 | 15.42 | 1.5 | 132.3 |
| 42 | 15 | F | 5 | 152 | 46 | 19.909972 | N | 109 | 38 | 0.8 | 2.28 | 147 | 82 | 30 | 9.5 | 5.9 | 4 | 3.06 | 19.6 | 15.61 | 1.3 | 110 |
| 43 | 40 | F | 15 | 156 | 61 | 25.065746 | N | 83 | 31 | 0.8 | 4 | 138 | 88 | 22 | 7.4 | 6.8 | 3.5 | 6.6 | 40.5 | 16.29 | 1.2 | 92.5 |
| 44 | 26 | M | 17 | 163 | 47 | 17.689789 | Y | 50 | 64 | 1.6 | 1.6 | 139 | 92 | 40 | 6.2 | 6.5 | 4 | 4.24 | 25.7 | 16.49 | 9.3 | 58.6 |
| 45 | 40 | M | 16 | 146 | 50 | 23.456558 | Y | 105 | 19 | 1 | 4.3 | 233 | 68 | 59 | 6.2 | 6.5 | 4.2 | 5.54 | 32.85 | 16.86 | 5 | 93.7 |
| 46 | 24 | M | 17 | 170 | 51 | 17.647059 | N | 297.4 | 26 | 0.8 | 3.6 | 147 | 55 | 46 | 9.4 | 6.5 | 5.3 | 3.6 | 21.3 | 16.9 | 4.8 | 125 |
| 47 | 29 | M | 9 | 174 | 58 | 19.157088 | N | 132 | 57 | 0.7 | 3.5 | 98 | 100 | 30 | 8 | 5.2 | 3 | 3.94 | 21.9 | 17.99 | 0.2 | 127.5 |
| 48 | 30 | F | 14 | 156 | 65 | 26.709402 | N | 116 | 101 | 0.5 | 1.7 | 140 | 78 | 41 | 8 | 7.5 | 4.1 | 8.4 | 44.7 | 18.79 | 0.4 | 130.3 |
| 49 | 35 | F | 13 | 154 | 74 | 31.202564 | N | 283 | 31 | 0.8 | 2.39 | 227 | 66 | 41 | 10.7 | 6.2 | 4 | 11 | 54 | 20.37 | 0.2 | 95.8 |
| 50 | 36 | F | 10 | 154 | 62 | 26.142688 | N | 83 | 23 | 0.6 | 2.7 | 112 | 100 | 25 | 10.1 | 5.7 | 4 | 9.6 | 46.8 | 20.51 | 4.7 | 117.6 |
| 51 | 23 | F | 14 | 155 | 64 | 26.638918 | N | 89 | 43 | 0.8 | 2.9 | 121 | 79 | 15 | 8.7 | 5.6 | 2.9 | 15 | 70.5 | 21.27 | 0.6 | 104.3 |
| 52 | 23 | M | 5 | 140 | 58 | 29.591837 | N | 87 | 43 | 0.9 | 4.14 | 107 | 77 | 55 | 7 | 6.5 | 4 | 9.8 | 45.6 | 21.49 | 0.3 | 120 |
| 53 | 28 | M | 12 | 164 | 50 | 18.590125 | N | 226.5 | 40 | 1 | 4.72 | 68 | 71 | 24 | 7.8 | 6 | 3.5 | 8.2 | 37.2 | 22.04 | 0.4 | 102 |
| 54 | 17 | F | 6 | 140 | 45 | 22.959184 | N | 191.3 | 19 | 0.6 | 2.8 | 117 | 47 | 53 | 8.5 | 5.9 | 4.4 | 18.2 | 81.6 | 22.3 | 4.5 | 134.4 |
| 55 | 24 | M | 5 | 140 | 55 | 28.061224 | N | 298.5 | 29 | 0.8 | 3.3 | 115 | 64 | 46 | 8.7 | 5.9 | 3.9 | 4.62 | 19.9 | 23.21 | 0.3 | 125 |
| 56 | 17 | F | 10 | 150 | 40 | 17.777778 | N | 184 | 36 | 0.7 | 1.88 | 102 | 239 | 23 | 10.6 | 5.9 | 4 | 11 | 46 | 23.91 | 1.8 | 127.8 |
| 57 | 14 | M | 5 | 138 | 30 | 15.752993 | Y | 126 | 42 | 0.6 | 4.43 | 185 | 95 | 49 | 11.2 | 8.4 | 4.3 | 4.14 | 17.3 | 23.93 | 2 | 151 |
| 58 | 21 | F | 11 | 159 | 55 | 21.755469 | Y | 163.2 | 36 | 1.1 | 3.31 | 170 | 132 | 46 | 8.9 | 6.3 | 4.4 | 10.6 | 43.9 | 24.1 | 11.8 | 71.9 |
| 59 | 40 | M | 18 | 148 | 46 | 21.00073 | Y | 60 | 27 | 0.8 | 4.6 | 219 | 145 | 36 | 7 | 6.5 | 4.2 | 5.4 | 22.2 | 24.32 | 1.3 | 111.7 |
| 60 | 32 | F | 7 | 144 | 44 | 21.219136 | Y | 136.8 | 34 | 1 | 4.9 | 162 | 69 | 53 | 5.2 | 6.5 | 3.6 | 21.3 | 83.7 | 25.44 | 0.62 | 74.7 |
| 61 | 23 | M | 5 | 172 | 57 | 19.267171 | N | 189.8 | 45 | 1.1 | 3.5 | 227 | 167 | 28 | 12.2 | 5.6 | 4.2 | 6.04 | 21.9 | 27.59 | 18 | 94.1 |
| 62 | 22 | F | 11 | 147 | 49 | 22.675737 | Y | 254.7 | 18 | 1.2 | 4.5 | 215 | 160 | 39 | 9.3 | 6.6 | 4 | 12.1 | 43.8 | 27.62 | 4.48 | 64.3 |
| 63 | 34 | M | 7 | 169 | 64 | 22.408179 | N | 157.6 | 33 | 0.9 | 6.1 | 226 | 85 | 60 | 8.6 | 6.5 | 4.2 | 13.4 | 48 | 27.91 | 4.34 | 111 |
| 64 | 24 | F | 7 | 147 | 37 | 17.122495 | N | 123.2 | 15 | 0.7 | 7.1 | 136 | 50 | 52 | 9.3 | 5.1 | 3 | 6.23 | 21.6 | 28.84 | 2.7 | 132.1 |
| 65 | 37 | M | 10 | 156 | 54 | 22.189349 | N | 333 | 24 | 1.2 | 5 | 182 | 37 | 35 | 9.6 | 6.5 | 4.1 | 9.7 | 33.1 | 29.3 | 0.49 | 76.8 |

## MASTER CHART OF MICROALBUMINURIC TYPE 1 DIABETIC PATIENTS

| CASES | AGE | GENDER | DURATION | HT | WT | BMI | HYPER T | fasting PG | Urea | creatinine | uricacid | cholester | TGL | HDL | Hba1c | S.Protein | S.albumin | U.Albumin | U.Creatinine | urine ACR | hsCRP | eGFR (CKD-EPI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (Kg/m^2) | | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | % | (g/dL) | (g/dL) | (mg/L) | (mg/dL) | (mg/g) | (mg/L) | (ml/min) |
| 1 | 15 | M | 10 | 139 | 49 | 25.36101 | N | 191.3 | 18 | 0.7 | 1 | 104 | 62 | 29 | 7.9 | 5.9 | 3 | 38.4 | 126.7 | 30.3 | 0.3 | 140.7 |
| 2 | 38 | F | 10 | 155 | 57 | 23.72529 | Y | 50 | 31 | 1.1 | 1.6 | 139 | 65 | 48 | 8 | 6.4 | 3.7 | 7.8 | 24.2 | 32.23 | 1.2 | 63.8 |
| 3 | 18 | F | 12 | 140 | 39 | 19.89796 | N | 513 | 23 | 0.7 | 2.4 | 263 | 126 | 68 | 11.9 | 6.2 | 3.5 | 6.3 | 19.2 | 32.8 | 2.4 | 126.9 |
| 4 | 40 | F | 15 | 139 | 54 | 27.94886 | N | 206 | 26 | 0.8 | 3.4 | 206 | 114 | 56 | 9.2 | 6.5 | 4.3 | 13.18 | 39 | 33.79 | 8 | 92.5 |
| 5 | 38 | F | 6 | 148 | 52 | 23.73996 | N | 60 | 33 | 0.9 | 3.28 | 174 | 87 | 30 | 5.9 | 5.7 | 3.4 | 9.3 | 24.7 | 37.65 | 21.9 | 81.4 |
| 6 | 27 | M | 9 | 156 | 45 | 18.49112 | Y | 93.6 | 19 | 0.8 | 5.3 | 183 | 88 | 29 | 7.5 | 6.7 | 4.1 | 33.9 | 87.3 | 38.83 | 4.65 | 122.4 |
| 7 | 30 | M | 5 | 166 | 52 | 18.87066 | N | 158.7 | 48 | 0.9 | 4.41 | 210 | 199 | 20 | 13 | 5.2 | 3.4 | 15 | 36 | 41.66 | 7.1 | 114.2 |
| 8 | 40 | M | 25 | 149 | 57 | 25.67452 | Y | 185 | 43 | 1 | 5 | 200 | 135 | 35 | 9.4 | 5 | 3.2 | 11.06 | 23.8 | 46.47 | 2.8 | 93.7 |
| 9 | 26 | F | 8 | 142 | 49 | 24.30073 | N | 122 | 32 | 0.8 | 3.4 | 87 | 65 | 35 | 7.1 | 5.3 | 3.3 | 9.2 | 19.4 | 47.42 | 1.4 | 102.1 |
| 10 | 24 | F | 5 | 151 | 45 | 19.73598 | N | 68 | 28 | 0.9 | 3.1 | 188 | 50 | 52 | 10.9 | 6 | 4.3 | 11.4 | 24 | 47.5 | 1.3 | 89.8 |
| 11 | 15 | M | 5 | 159 | 36 | 14.23994 | N | 350 | 34 | 0.8 | 2.5 | 179 | 186 | 51 | 12.6 | 6.7 | 4.5 | 11.9 | 24.9 | 47.79 | 0.5 | 133.2 |
| 12 | 14 | F | 5 | 132 | 30 | 17.21763 | N | 92 | 26 | 0.4 | 4.07 | 96 | 102 | 20 | 9 | 6.1 | 3.4 | 12.3 | 23.9 | 51.46 | 2 | 156.9 |
| 13 | 34 | M | 12 | 170 | 70 | 24.22145 | Y | 68 | 49 | 1 | 7.54 | 98 | 245 | 25 | 6 | 5.4 | 3.7 | 11 | 21.3 | 51.64 | 9.3 | 97.8 |
| 14 | 23 | M | 10 | 156 | 59 | 24.24392 | Y | 138.7 | 33 | 1.3 | 6.5 | 294 | 130 | 38 | 9.1 | 4.9 | 3 | 52.14 | 95.4 | 54.65 | 8.7 | 76.9 |
| 15 | 35 | F | 11 | 149 | 59 | 26.57538 | N | 66 | 28 | 0.6 | 3 | 153 | 96 | 20 | 8.3 | 5 | 3.5 | 15.73 | 28.7 | 54.8 | 27.9 | 119 |
| 16 | 32 | M | 23 | 160 | 55 | 21.48438 | N | 84 | 36 | 1.2 | 4.8 | 205 | 88 | 39 | 9.2 | 7.2 | 4.1 | 22.6 | 40 | 56.5 | 5.2 | 81.8 |
| 17 | 40 | M | 20 | 156 | 60 | 24.65483 | N | 108 | 60 | 1.6 | 6.3 | 129 | 99 | 45 | 7.9 | 6 | 4.6 | 48.54 | 85.2 | 56.97 | 6.8 | 53.1 |
| 18 | 17 | F | 7 | 139 | 35 | 18.115 | Y | 77.6 | 12 | 0.7 | 5.5 | 158 | 168 | 36 | 5.8 | 6.4 | 3.3 | 10.5 | 18 | 58.33 | 17.5 | 127.8 |
| 19 | 30 | F | 9 | 149 | 75 | 33.78226 | N | 206.8 | 20 | 0.7 | 1 | 187 | 63 | 30 | 9.8 | 5.9 | 4 | 13.3 | 21.5 | 61.86 | 3.3 | 116.6 |
| 20 | 30 | M | 10 | 154 | 50 | 21.08281 | Y | 117 | 47 | 1 | 5.3 | 138 | 125 | 40 | 10 | 5.6 | 2.9 | 185.4 | 299.1 | 61.98 | 2.4 | 100.5 |
| 21 | 40 | M | 24 | 150 | 55 | 24.44444 | Y | 64 | 47 | 0.8 | 5.8 | 172 | 132 | 39 | 7.4 | 4.7 | 3.2 | 16.7 | 26.5 | 63.01 | 5.9 | 111.7 |
| 22 | 21 | M | 5 | 170 | 85 | 29.41176 | N | 265.7 | 24 | 0.7 | 5.5 | 183 | 110 | 80 | 9.8 | 8.2 | 3.8 | 59.39 | 91 | 65.26 | 3.8 | 134.9 |
| 23 | 27 | M | 7 | 161 | 60 | 23.14726 | N | 64.8 | 17 | 0.9 | 4.1 | 165 | 76 | 58 | 7.4 | 6.5 | 3.6 | 14.4 | 21.8 | 66.05 | 0.6 | 116.6 |
| 24 | 39 | M | 24 | 171 | 64 | 21.88708 | Y | 151.5 | 45 | 1 | 5 | 170 | 100 | 37 | 8.2 | 6.6 | 4.3 | 94.4 | 138.8 | 68.01 | 1.61 | 94.4 |
| 25 | 22 | F | 5 | 150 | 50 | 22.22222 | N | 67.9 | 36 | 0.7 | 3.8 | 199 | 145 | 36 | 12.1 | 5.5 | 3.7 | 77.7 | 104.1 | 74.63 | 6.9 | 123.4 |
| 26 | 32 | M | 17 | 167 | 58 | 20.79673 | N | 197 | 41 | 0.8 | 5 | 173 | 56 | 34 | 8.4 | 5.6 | 4.3 | 40.5 | 53.2 | 76.12 | 17.4 | 118 |
| 27 | 28 | F | 10 | 140 | 34 | 17.34694 | N | 83.3 | 29 | 0.9 | 3.2 | 152 | 52 | 52 | 8.1 | 5.9 | 3 | 15.63 | 20 | 78.15 | 17.2 | 87.3 |
| 28 | 29 | M | 7 | 175 | 106 | 34.61224 | Y | 198.9 | 23 | 1.3 | 4.3 | 130 | 81 | 35 | 8.7 | 6.8 | 4.1 | 102.4 | 112.2 | 91.26 | 11.8 | 73.7 |
| 29 | 23 | F | 12 | 158 | 58 | 23.23346 | N | 169 | 25 | 0.7 | 4.58 | 138 | 85 | 49 | 6.6 | 5.7 | 4.2 | 20 | 21.8 | 91.74 | 19.9 | 122.5 |
| 30 | 35 | M | 16 | 160 | 57 | 22.26563 | Y | 92.5 | 28 | 0.7 | 3.1 | 176 | 155 | 26 | 9.1 | 6 | 4.6 | 32.96 | 35 | 94.17 | 14.7 | 122.3 |
| 31 | 26 | F | 20 | 154 | 55 | 23.19109 | N | 227.5 | 33 | 0.6 | 3.97 | 165 | 78 | 33 | 8.1 | 5.6 | 4.2 | 26.24 | 26.8 | 97.91 | 1.7 | 126.2 |
| 32 | 40 | M | 15 | 152 | 50 | 21.64127 | Y | 137 | 50 | 1 | 5.1 | 125 | 65 | 42 | 8.4 | 5.3 | 3 | 78.3 | 78.6 | 99.61 | 3.1 | 93.7 |

| CASES | AGE | GENDER | DURATION | HT | WT | BMI | HYPER T | fasting PG | Urea | creatinine | uricacid | cholester | TGL | HDL | Hba1c | S.Protein | S.albumin | U.Albumin | U.Creatinine | urine ACR | hsCRP | eGFR (CKD-EPI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (Kg/m^2) | | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | (mg/dL) | % | (g/dL) | (g/dL) | (mg/L) | (mg/dL) | (mg/g) | (mg/L) | (ml/min) |
| 33 | 40 | M | 20 | 160 | 76 | 29.6875 | Y | 81 | 28 | 1.1 | 6.6 | 177 | 123 | 53 | 8.5 | 5.3 | 3.4 | 53.96 | 52.8 | 102.19 | 2.61 | 83.5 |
| 34 | 33 | F | 20 | 148 | 47 | 21.45727 | N | 183.8 | 73 | 0.9 | 4.1 | 191 | 200 | 35 | 10.2 | 6.2 | 3 | 25 | 22 | 113.6 | 23.1 | 84.3 |
| 35 | 22 | F | 13 | 162 | 65 | 24.76757 | Y | 173.8 | 49 | 0.9 | 6.1 | 150 | 150 | 40 | 7.5 | 5.2 | 3.5 | 51.3 | 40.2 | 127.6 | 10.4 | 91.1 |
| 36 | 40 | F | 17 | 150 | 43 | 19.11111 | N | 81 | 16 | 0.5 | 2.6 | 202 | 88 | 40 | 9 | 6.2 | 3.6 | 47.1 | 36.3 | 129.75 | 22.9 | 121.5 |
| 37 | 40 | F | 20 | 154 | 59 | 24.87772 | N | 74.6 | 32 | 0.8 | 4.2 | 178 | 73 | 47 | 7.8 | 6.3 | 3.7 | 29 | 22 | 131.81 | 17.11 | 97.2 |
| 38 | 32 | F | 7 | 160 | 60 | 23.4375 | N | 281.9 | 20 | 0.8 | 5 | 153 | 95 | 32 | 7.8 | 5.9 | 3.4 | 39 | 29.5 | 132.2 | 25.2 | 97.9 |
| 39 | 27 | M | 5 | 168 | 58 | 20.54989 | N | 203 | 42 | 1 | 5.28 | 243 | 75 | 35 | 8.6 | 4.3 | 3.6 | 37.9 | 28.6 | 132.5 | 0.9 | 102.7 |
| 40 | 40 | M | 26 | 170 | 55 | 19.03114 | Y | 273 | 60 | 1.3 | 5.28 | 184 | 260 | 19 | 9.4 | 5.7 | 4.2 | 48 | 32 | 137.14 | 7.6 | 68.3 |
| 41 | 20 | M | 4 | 133 | 45 | 25.43954 | N | 83 | 57 | 0.9 | 2.78 | 166 | 245 | 25 | 9.2 | 5.8 | 3.7 | 42.6 | 30.7 | 138.76 | 8.7 | 123 |
| 42 | 28 | M | 7 | 160 | 54 | 21.09375 | N | 76 | 52 | 1.3 | 4.9 | 86 | 102 | 42 | 10 | 5 | 3.2 | 74.2 | 53.2 | 139 | 9.1 | 74.3 |
| 43 | 39 | F | 5 | 149 | 56 | 25.22409 | Y | 254.7 | 35 | 0.9 | 1.89 | 87 | 38 | 20 | 8.1 | 5.8 | 2.7 | 97.8 | 68.8 | 142.15 | 27.5 | 80.8 |
| 44 | 37 | M | 24 | 147 | 49 | 22.67574 | N | 74 | 46 | 0.9 | 5.1 | 175 | 230 | 29 | 11.9 | 5 | 3.5 | 82.58 | 55 | 150.15 | 23.6 | 108.7 |
| 45 | 34 | F | 16 | 160 | 74 | 28.90625 | Y | 164 | 10 | 0.6 | 11.7 | 148 | 112 | 58 | 9.1 | 6.4 | 3.6 | 109.1 | 65.6 | 166.31 | 7.68 | 119.3 |
| 46 | 24 | F | 5 | 152 | 40 | 17.31302 | N | 336 | 37 | 0.7 | 4.53 | 129 | 144 | 40 | 10.1 | 5.3 | 3.5 | 39.1 | 23.5 | 166.38 | 1 | 121.7 |
| 47 | 38 | M | 15 | 170 | 64 | 22.14533 | N | 79.9 | 21 | 0.9 | 7.5 | 124 | 77 | 32 | 5.1 | 5.5 | 3.7 | 37.5 | 22 | 170.45 | 12.4 | 108 |
| 48 | 18 | M | 5 | 135 | 45 | 24.69136 | N | 212.5 | 42 | 0.8 | 4.7 | 191 | 134 | 46 | 9.3 | 6 | 3.5 | 122.4 | 69.3 | 176.62 | 7.8 | 130.4 |
| 49 | 40 | F | 18 | 151 | 57 | 24.9989 | Y | 167 | 29 | 0.6 | 4.75 | 200 | 104 | 60 | 10 | 5 | 3.3 | 41.9 | 23.3 | 179.82 | 3.1 | 114.4 |
| 50 | 23 | F | 13 | 138 | 50 | 26.25499 | N | 174.4 | 70 | 0.8 | 4.64 | 183 | 156 | 30 | 6.7 | 5.6 | 2.9 | 51.5 | 26.5 | 194.3 | 10.1 | 104.3 |
| 51 | 25 | M | 15 | 154 | 56 | 23.61275 | Y | 165.7 | 40 | 0.9 | 14 | 111 | 65 | 45 | 5.9 | 6.8 | 3.3 | 94.9 | 48 | 197.7 | 1.1 | 118.3 |
| 52 | 24 | F | 14 | 154 | 44 | 18.55288 | N | 324 | 21 | 0.6 | 6.24 | 187 | 96 | 25 | 8.3 | 4.3 | 3.6 | 41.9 | 19.8 | 211.6 | 19.4 | 128 |
| 53 | 30 | M | 20 | 157 | 60 | 24.34176 | N | 526 | 87 | 2.5 | 10.3 | 151 | 48 | 25 | 6.9 | 5.6 | 4.2 | 64.22 | 30.1 | 213.35 | 14.9 | 33.2 |
| 54 | 30 | M | 17 | 160 | 64 | 25 | Y | 387.7 | 25 | 1 | 4.3 | 280 | 121 | 40 | 9.6 | 7.2 | 3.8 | 432.9 | 192.3 | 225.11 | 18.8 | 100.5 |
| 55 | 27 | M | 20 | 161 | 64 | 24.69041 | Y | 61 | 58 | 1.6 | 11 | 213 | 117 | 57 | 11.3 | 6 | 3.7 | 52.47 | 22.8 | 230.13 | 27 | 58.2 |
| 56 | 40 | M | 12 | 163 | 70 | 26.34649 | Y | 52 | 20 | 1.1 | 3.2 | 168 | 104 | 39 | 10.3 | 6.3 | 3 | 50.7 | 21.3 | 238.02 | 5.6 | 83.5 |
| 57 | 40 | M | 25 | 159 | 58 | 22.94213 | Y | 401 | 28 | 1 | 6.3 | 194 | 306 | 27 | 9.1 | 7.3 | 3.5 | 89.6 | 37.2 | 240.8 | 14.76 | 93.7 |
| 58 | 18 | F | 5 | 152 | 43 | 18.6115 | N | 242.2 | 34 | 0.8 | 3.3 | 161 | 319 | 23 | 7.5 | 5.5 | 3.7 | 73.2 | 29.1 | 251.54 | 18.2 | 108 |
| 59 | 35 | F | 20 | 150 | 49 | 21.77778 | Y | 135 | 381 | >20 | 12.4 | 35 | 32 | 47 | 7.1 | 6.7 | 5.3 | 149 | 58.1 | 256.45 | 12.1 | 1.8 |
| 60 | 26 | F | 10 | 165 | 48 | 17.63085 | N | 223 | 20 | 0.6 | 4.04 | 252 | 134 | 29 | 8.5 | 5.2 | 3.1 | 60.42 | 21.9 | 275.89 | 9.6 | 126.2 |
| 61 | 40 | F | 27 | 136 | 34 | 18.38235 | y | 148 | 77 | 1.4 | 6.7 | 117 | 58 | 34 | 7.7 | 4 | 2.9 | 62.3 | 22.3 | 279.37 | 8.2 | 47 |
| 62 | 26 | F | 17 | 150 | 43 | 19.11111 | N | 183.4 | 38 | 1 | 6.13 | 162 | 75 | 38 | 8.6 | 5.66 | 3.4 | 94.68 | 32.9 | 287.79 | 14.1 | 77.9 |
| 63 | 28 | M | 15 | 164 | 49 | 18.21832 | N | 183.8 | 29 | 0.8 | 3.9 | 144 | 54 | 40 | 11 | 7 | 3.8 | 62.36 | 21.6 | 288.7 | 6.2 | 121.6 |
| 64 | 30 | F | 5 | 145 | 39 | 18.54935 | N | 70 | 41 | 1 | 5.1 | 88 | 123 | 35 | 5.7 | 5.2 | 3.3 | 58 | 20 | 290 | 24.6 | 75.8 |
| 65 | 36 | F | 20 | 155 | 52 | 21.64412 | N | 427.7 | 32 | 0.8 | 3.2 | 123 | 128 | 30 | 8.4 | 4.3 | 3.6 | 51.3 | 17.6 | 291.4 | 10.8 | 95.2 |

# RESULTS

## TABLE1: COMPARISION OF CHARACTERISTICS OF TYPE1 DIABETICS WITH NORMOALBUMINURIA AND MICROALBUMINURIA

| | Albuminuria | N | Mean | Std. Deviation | p value |
|---|---|---|---|---|---|
| Age | Normal | 65 | 28.10 | 8.32 | 0.16-NS |
| | Micro | 65 | 30.12 | 7.78 | |
| BMI | Normal | 65 | 22.26 | 3.50 | 0.46-NS |
| | Micro | 65 | 22.74 | 3.85 | |
| FBS(mg/dL) | Normal | 65 | 149.28 | 80.50 | 0.19-NS |
| | Micro | 65 | 171.98 | 111.39 | |
| HbA1C(%) | Normal | 65 | 8.44 | 1.723 | 0.41-NS |
| | Micro | 65 | 8.69 | 1.69 | |
| Total Cholesterol(mg/dL) | Normal | 65 | 150.06 | 49.34 | 0.09-NS |
| | Micro | 65 | 164.49 | 47.39 | |
| TGL(mg/dL) | Normal | 65 | 97.49 | 44.28 | 0.04* |
| | Micro | 65 | 117.13 | 61.79 | |
| HDL(mg/dL) | Normal | 65 | 40.52 | 17.71 | 0.39-NS |
| | Micro | 65 | 38.21 | 12.25 | |
| Creatinine(mg/dL) | Normal | 65 | .81 | .21 | 0.21-NS |
| | Micro | 65 | 1.28 | 3.00 | |
| Albumin(g/dL) | Normal | 65 | 3.90 | .56 | 0.003** |
| | Micro | 65 | 3.62 | .50 | |
| Total Protein(g/dL) | Normal | 65 | 6.25 | .52 | <0.001** |
| | Micro | 65 | 5.81 | .79 | |
| UrineACR | Normal | 65 | 13.64 | 7.66 | <0.001** |
| | Micro | 65 | 127.98 | 81.23 | |

NS-NOT SIGNIFICANT

*-SIGNIFICANT.

**- HIGHLY SIGNIFICANT.

Table-1 shows the data of Age, Body mass index, Fasting plasma glucose, HbaA1c, Total Cholesterol, Triglycerides, HDL-c, Serum creatinine, Serum albumin, Total protein, Urine Albumin creatinine Ratio among Normoalbuminuric & Microalbuminuric Type 1 Diabetic patients.

An insignificant p value was obtained with respect to variables like Age, Fasting Plasma Glucose,HbA1c,Total cholesterol, HDLc& serum Creatinine.

A significant p value was obtained for

i. Triglycerides in Normoalbuminurics (97.49 ± 44.2) &Microalbuminurics (117.13 ± 61.79) with p value of 0.04.
ii. Serum albumin in Normoalbuminurics (3.90±0.56) &Microalbuminurics (3.62±0.50) with p value of 0.003.
iii. Serum Total protein in Normoalbuminurics (6.25±0.52) &Microalbuminurics (5.81±0.79) with p value of<0.001.
iv. Urine albumin creatinine ratio in Normoalbuminurics (13.64+/7.66)&Microalbuminurics (127.98+/81.23) with p value of <0.001.

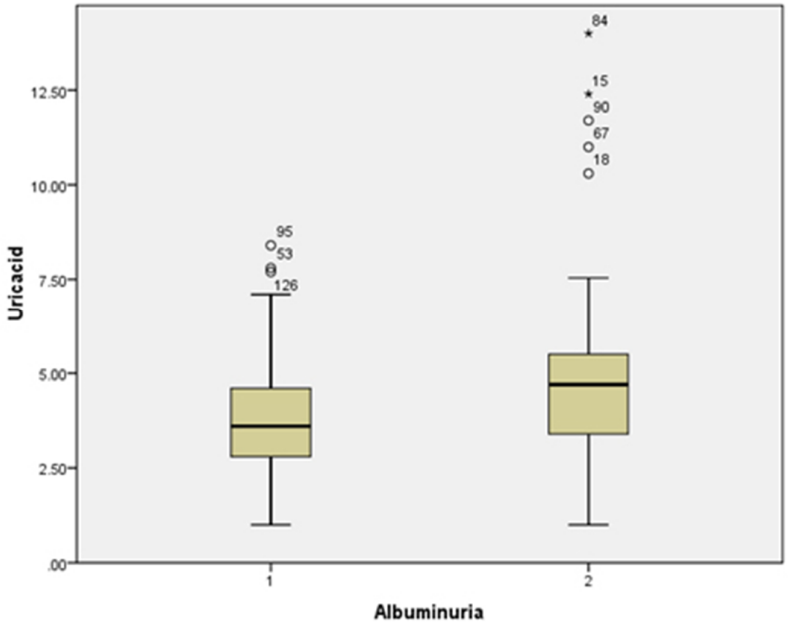
**TABLE 2 : COMPARISION OF URIC ACID WITH NORMOALBUMINURIA & MICROALBUMINURIA**

| | Albuminuria | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Uricacid | Normal | 65 | 3.92 | 1.59 | 0.20 |
| | Micro | 65 | 4.99 | 2.48 | 0.31 |

p = 0.004 (highly significant)

Table 2 Shows the comparison of uric acid between study groups. The Mean Uric acid in Diabetics with normo albuminuria was (3.92+/-1.59) and Microalbuminuria was (4.99+/-2.48).A highly significant p value of 0.004 was obtained.

**BOX & WHISKER PLOT SHOWS THE DISTRIBUTION OF URIC ACID IN NORMOALBUMINURIA &MICROALBUMINURIA.**




1=Normoalbuminuria, 2=Microalbuminuria,
o-Outliers, *-Extreme values.

.

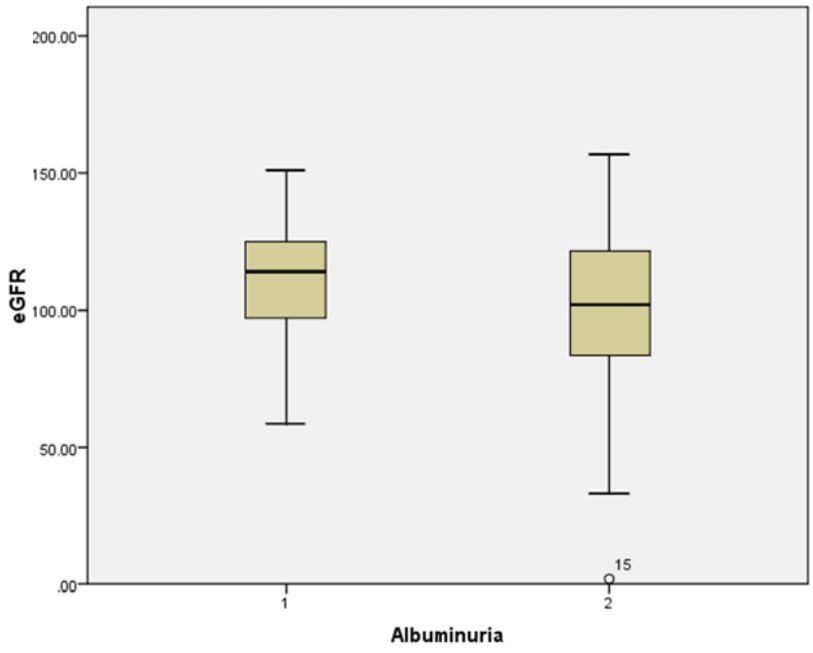
**TABLE:3 COMPARISION OF eGFR BETWEEN NORMOALBUMINURIA & MICROALBUMINURIA**

| | Albuminuria | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| eGFR | normal | 65 | 110.40 | 20.30 | 2.52 |
| | micro | 65 | 100.25 | 27.12 | 3.36 |

p= 0.02(Significant)

Table 3 shows the comparison of e GFR between study groups. The Mean eGFR in normoalbuminuria was ( 110.40+/-20.30) and Microalbuminuria was (100.25+/-27.12)Significant P value 0f 0.02 was obtained.

**BOX &WHISKER PLOT SHOWS THE DISTRIBUTION OF eGFR IN NORMOALBUMINURIA & MICROALBUMINURIA**




1=Normoalbuminuria, 2=Microalbuminuria, o-Outliers

**Table:4 : ANOVA**

| eGFR | | | | |
|---|---|---|---|---|
| | **N** | **Mean** | **Std. Deviation** | **P value** |
| 1 | 29 | 114.58 | 20.03 | <0.001 |
| 2 | 29 | 109.55 | 17.69 | |
| 3 | 28 | 107.80 | 22.72 | |
| 4 | 19 | 104.12 | 19.72 | |
| 5 | 23 | 84.93 | 32.00 | |
| Total | 128 | 105.07 | 24.50 | |

P<0.001(highly significant)

**Uric acid concentrations**:

< 3 mg = 1

3 to 3.9 = 2

4 to 4.9 = 3

5 to 5.9 =4

> 6 mg = 5

Table 4 : ANOVA was used to compare the Mean GFR between 2 groups. The Mean GFR was higher in Normoalbuminuria than Microalbuminuria and the difference in Mean was statistically significant with a P value <0.001.

**TABLE:5**

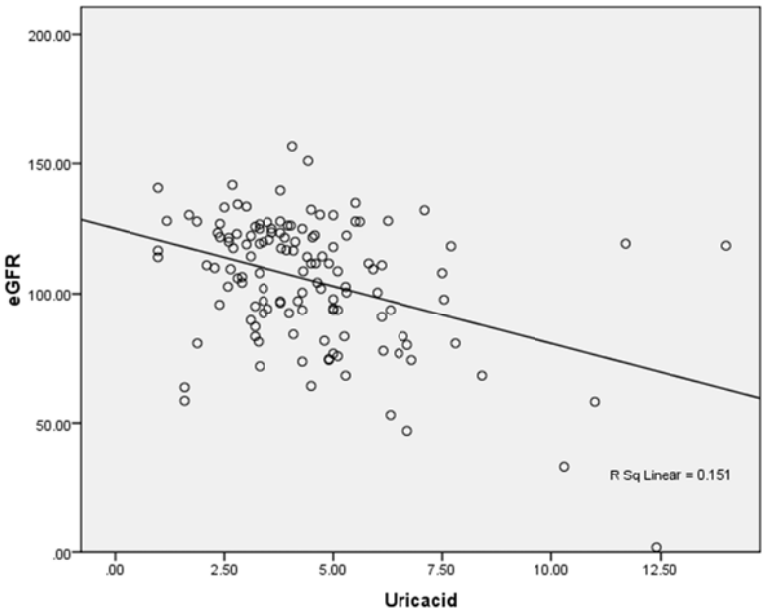
**CORRELATION OF URICACID WITH eGFR IN STUDY GROUPS**

| | | Uricacid | eGFR |
|---|---|---|---|
| **Uricacid** | Pearson Correlation | 1 | -.389** |
| | Sig. (2-tailed) | | .000 |
| | N | 130 | 130 |
| **eGFR** | Pearson Correlation | -.389** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 130 | 130 |
| **. Correlation is significant at 0.01 level (2-tailed). | | | |

r = -0.39 p = <0.001

Table 5: Shows the Pearson coefficient correlation to find the relationship between uric acid &eGFR level in the study groups. It is observed that Uric acid increases, eGFR decreases with a significant negative correlation of r=-0.39,P=<0.001.

**CORRELATION OF eGFR WITH URIC ACID**



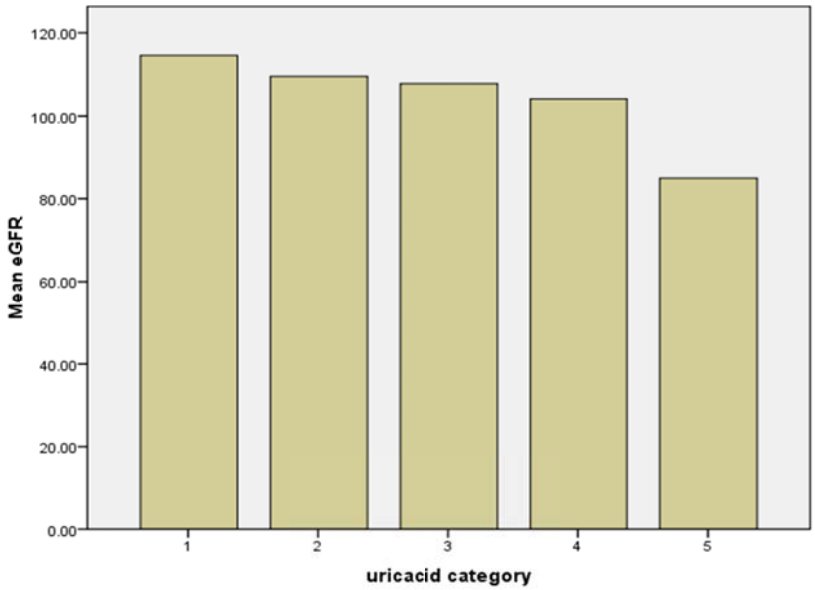
## TABLE 6 : PARTIAL CORRELATIONS

| Control Variables | | | Uric acid | eGFR |
|---|---|---|---|---|
| HbA1C | Uric acid | Correlation | 1.000 | -.261 |
| | | Significance (2-tailed) | . | .038 |
| | | Df | 0 | 62 |
| | eGFR | Correlation | -.261 | 1.000 |
| | | Significance (2-tailed) | .038 | . |
| | | Df | 62 | 0 |

P<0.05(significant).

Table 6: Shows the strength of relationship between uric acid & eGFR after eliminating Hba1c. Correlation is significant with a P value<0.05.

## MEAN eGFR AMONG VARIOUS URICACID CATEGORIES




< 3 mg = 1
3 to 3.9 = 2
4 to 4.9 = 3
5 to 5.9 = 4
> 6 mg = 5

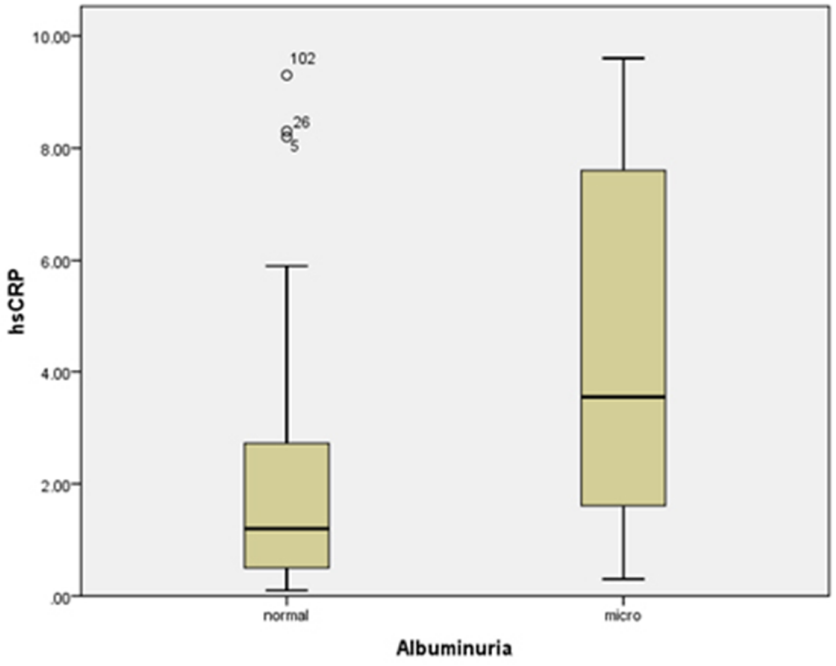
**TABLE7 :COMPARISION OF hs-CRP BETWEEN NORMOALBUMINNURIA & MICROALBUMINURIA**

| | Albuminuria | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| hsCRP | normal | 59 | 1.9939 | 2.14449 | .27919 |
| | micro | 38 | 4.4776 | 3.05950 | .49632 |

p< 0.001(highly significant).

Table 7 shows the comparison of hsCRP between normoalbuminuric& Microalbuminuric Type 1 Diabetic patients..The mean hsCRP in Normoalbuminuria was (1.99+/-2.14),Whereas Microalbuminuria was (4.47+/-3.05) highly Significant P value of <0.001 was obtained.

**BOX & WHISKER PLOT SHOWS THE DISTRIBUTION OF hs-CRP IN NORMO & MICROALBUMINURIA**




o-Outliers.

**TABLE7 :CORRELATION OF URICACID WITH hs-CRP IN STUDY GROUPS:**

| | | Uric acid | hsCRP |
|---|---|---|---|
| **Uric acid** | Pearson Correlation | 1 | .205* |
| | Sig. (2-tailed) | | .044 |
| | N | 97 | 97 |
| **hsCRP** | Pearson Correlation | .205* | 1 |
| | Sig. (2-tailed) | .044 | |
| | N | 97 | 97 |
| *. Correlation is significant at the 0.05 level (2-tailed). | | | |

r = 0.21P = 0.04

Table 7: Shows the Pearson coefficient correlation to find the relationship between uric acid& hs-CRP level in study groups. It is observed that as Uric acid increases, hs-CRP also increases with a significant positive correlation of r=0.21,P=0.04

**CORRELATION BETWEEN hs-CRP AND URICACID**

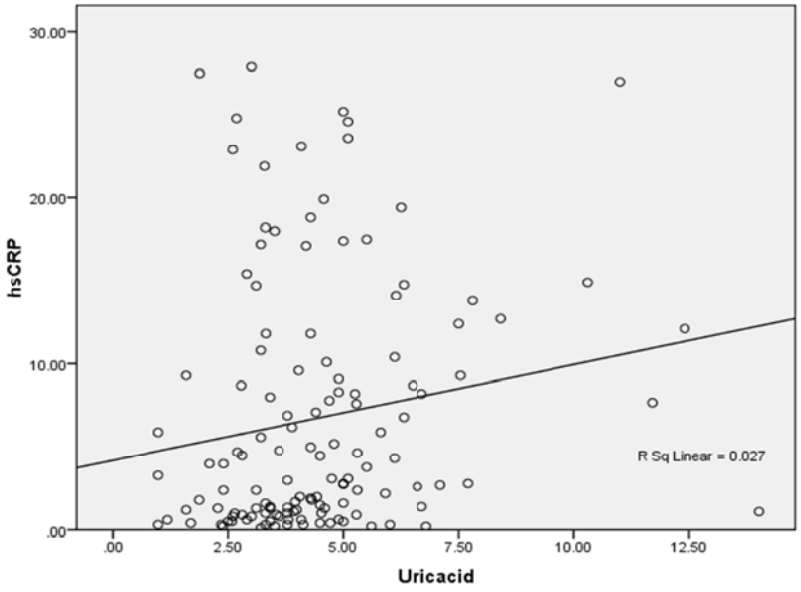


**TABLE:8**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Tolerance | VIF |
| 1 | (Constant) | 162.04 | 19.74 | | 8.20 | .000 | | |
| | Age | -1.01 | .31 | -.34 | -3.24 | .002 | .50 | 1.97 |
| | Gender | -4.61 | 3.85 | -.09 | -1.19 | .233 | .85 | 1.16 |
| | Duration | -.082 | .38 | -.02 | -.21 | .829 | .54 | 1.84 |
| | BMI | -.26 | .50 | -.04 | -.51 | .607 | .92 | 1.08 |
| | Hypertension | -7.10 | 4.22 | -.13 | -1.68 | .095 | .82 | 1.21 |
| | Uric acid | -3.43 | .92 | -.30 | -3.70 | .000 | .81 | 1.22 |
| | HbA1C | .58 | 1.17 | .04 | .49 | .621 | .79 | 1.26 |
| | Cholesterol | -.00 | .03 | -.00 | -.07 | .942 | .90 | 1.11 |
| a. Dependent Variable: eGFR | | | | | | | | |

Age $p < 0.01$

Uric acid $p< 0.001$

Other variables $p> 0.05$

Table 8: shows the Multiple regression analysis of eGFR with other risk factors in Normoalbuminuric &Microalbuminuric patients with Type 1 Diabetes.

- Uric acid remains significantly higher in Microalbuminurics compared to normoalbuminurics with a p-value of <0.001.
- There was a significant difference in age groups with a p value of <0.01.

# *Discussion*

# DISCUSSION

Diabetes is the most common disease causing End stage renal disease[145]. About 20-30% of Type1 diabetic patients develop nephropathy and 7-10% develop coronary artery disease. Generally increase in albumin excretion rate is widely accepted as the first clinical sign of Diabetic Nephropathy.

Recent studies show that reduced Glomerular filtration rate manifests as the primary clinical abnormality of diabetic nephropathy even before microalbuminuric stage and progresses to overt nephropathy. Dyslipidaemia, hyperglycemia and systemic hypertension have additive effects on the progression of microalbuminuria.

Serum uricacid is a potentially important mediator of renal disease. Hyperuricemia increases systemic bloodpressure, proteinuria, renal dysfunction & progressive renal scarring via renin angiotensin and COX-2 dependent pathway.It is an important risk factor for development of microalbuminuria and this risk was independent of age,gender and other risk factors. Hence estimation of serum uric acid may help to identify individuals with increased risk for developing microalbuminuria & CKD. Our study aims to correlate uricacid with glomerular filtration rate in non proteinuric type 1 Diabetes.

Uricacid also induces endothelial dysfunction by inhibiting nitricoxide production and it is a common finding in patients with both cardiovascular &renal disease. Diabetes increases advanced glycated end products which

activates macrophages & upregulates the synthesis of IL-6 &TNF α resulting in production of hs-CRP[146].Several studies demonstrated that hs-CRP predicts future coronary events.

Hence in our study we measured uricacid & hs-CRP for predicting cardiovascular & renal risk in type1 diabetes,so that we would be able to treat the patients at an earlier stage and slow down the progression of renal & cardiovascular disease in type1 diabetes

In the present study ,we recruited people in to two groups

Group-I : 65 Normoalbuminurics and

Group-II: 65Microalbuminuric type 1 diabetic patients.

In group -1: Mean serum uricacid level was found to be 3.92+/-1.59.mg/dL which is in normal range.

In group-II: Mean serum uric acid level was found to be 4.99+/-2.48 mg/dL which is in high normal range. This result was similar to the study by Krolewski et al[147], in which they had showed that majority of concentration of uricacid was in the normal range. but it is higher in microalbuminurics than normoalbuminurics .

Commmunity based study of Japanese adults[148] showed that hyperuricemia has strong predictive capacity of the risk of renal failure than proteinuria.Our study also reflects the same findings.

Similar result have been found in experimental study in Rats.[149].

Mean GFR was higher in Normoalbuminuric individuals and was in the range of 110.40+/-20.30 ml/min and it was lower in Microalbuminuric individuals which is in the range of 100.25+/-27.12ml/min.

Although mean GFR in both groups were in the normal range, HyperfiltrationGFR>130ml/min was less frequently seen in Microalbuminurics compared to Normoalbuminuric diabetic patients.

Conversely moderately impaired renal function with GFR 30-59ml/min & mildly impaired renal function with GFR 60-89 ml/min were more in Microalbuminurics than Normoalbuminuric diabetic patients..

Variables like Age, duration, BMI, BP, HbA1c that were associated with eGFR in Microalbuminuria & normoalbuminuria were examined by multiple regression equations to evaluate their independent contributions.HbA1c, BP and BMI measurements did not show independent effects on eGFR .

Increasing age &duration of Diabetes is associated with significant Glomerular lesions even in the absence of elevated albumin excretion rate. In our study,there is a statistically significant difference in age groups with p value of <0.01 was noted ,but the duration was not significant.The possible reason for this,may be that Diabetes had manifested in a much younger age & the duration of Diabetes was longer ,but it was detected later. As a result of this,there is a decrease in eGFR.

Normal range of CRP level in human serum is 0.2-10mg/L. hs-CRP ELISA assay kit has the analytical sensitivity of 0.005µg/mL.Values of hs-CRP >10mg/L on two consecutive tests were excluded,as they were more likely to be due to infections or inflammation[150].

In our study, the Mean of hs-CRP in Normoalbuminuria was 1.93+/-2.14mg/L. which was much lower compared toMicroalbuminuria 4.47+/-3.05mg/mL. In Oxford Regional Prospective study[151], hs-CRP in Microalbuminuric Type 1 Diabetic subjects were significantly higher than Normoalbuminuric Type 1 Diabetics.Which is similar to the results of our study.

Mean of urinary albumin creatinine ratio in Normoalbuminuria was 13.64+/-7.66.It is lower than the mean of Microalbuminuria 127.98+/-81.23. Similarly mean value of serum albumin in Normoalbuminuria, 3.90+/-0.56g/dL was lower compared to Microalbuminuria mean value of 3.62+/-0.50g/dL.

Microalbuminuria is asssociated with the state of subclinical inflammation and endothelial dysfunction[152,153] . It activates the Macrophages and upregulates the synthesis of IL-1,IL-6, & TNF and increases the production of hs-CRP. It explains the association between Microalbuminuria and cardiovascular disease.

In this study, Correlation was done to measure the linear relation ship between

- e GFR & Uricacid,
- Uric acid &hs-cRP.

**URIC ACID &eGFR:**

Strong negative Correlation was observed . It indicates that when the concentration of Uric acid increases, eGFR decreases which implies impaired renal function.

Uric acid causes thickening &hypercellularity of Glomerulus & induces epithelial mesenchymal transition of renal tubular cell resulting in increased medial thickness and decreased luminal diameter & it contributes to the decrease in Glomerular filtration rate[154,155].

**URICACID&hs-CRP:**

Strong Positive correlation was observed. It indicates when the concentration of uricacid increases, hs-CRP also increases.

Uricacid acts as a prooxidant. It increases the production of Monocyte chemoattractant protein -1 through cyclooxygenase-2 & MAP kinase pathway resulting in increased production of hs-CRP[156-159].

hs-CRP directly binds oxidised LDL and present with in lipid laden plaques.It is a proinflammatory trigger in plaque deposition, leading to macrophage infiltration in atherosclerotic lesions. It increases the expression of tissue factor which can lead to thrombosis during inflammatory states.

Mean TGL of microalbuminuria was 117.13+/-61.79mg/dL which is higher than the mean TGL of Normoalbuminuria 97.49+/-44.28 mg/dL.The

results of our study matches EURODIAB IDDM Complication study, where Microalbuminuria was associated with increased plasma triglyceride level.[160]

Some Quantitative lipid modifications may occur in Type 1 Diabetic patients with Microalbuminuria. Hypertriglyceridemia occurs mainly due to decreased lipoprotein lipase activity secondary to insulin deficiency.

# *Conclusion*

# CONCLUSION

The present study was done with an aim to find out the correlation of serum uric acid with GFR in normoalbuminuric & microalbuminuric Type 1 Diabetic patients and its correlation with hs-CRP for predicting cardiovascular risk.

From this study, we conclude that,

- Serum uric acid is an independent determinant of GFR in Type 1 Diabetic patients.
- There is a significant negative correlation of uric acid with GFR in Type 1 Diabetic patients.
- There is a significant positive correlation between hs-CRP and uric acid levels.
- Serum uric acid &hs-CRP can be used to assess the renal & cardiovascular risk in Type 1 Diabetic patients particularly in patients with low GFR.
- It is also cost effective to measure serum Uric acid & hs-CRP in Type 1 Diabetes even in Normoalbuminuria, to assess the renal & cardiovascular risk earlier particularly in Indian clinical settings, where the early detection rate of Diabetes was lower and complications were higher.

# *Limitations of the study*

# LIMITATIONS OF THE STUDY

- Even though there is a significant association between elevated uric acid concentration and decline in renal function, randomized controlled trials are essential to evaluate the role of uric acid lowering drugs in the prevention of further impairment of renal function .

- Prospective cohort studies are needed to confirm elevated hs-CRP levels in type 1 Diabetic patients are associated with Cardiovascular disease.
- Being a tertiary care hospital based study , a large community based study in Indian population is needed to verify the findings in general population

- Our study was carried out only in Type 1 Diabetic patients, and whether the result can be extrapolated to patients with Type 2 Diabetes is uncertain.

# *Future prospects of the study*

# FUTURE PROSPECTS OF THE STUDY

- Uric acid may be in future used as a marker of
  - Oxidant damage in ischemic liver injury .
  - Ischemic reperfusion injury

- To our knowledge this is the first study to be conducted in India evaluating hs CRP and Uric Acid in type 1 diabetes. In India renal replacement therapy is a great burden to the health system. Larger studies if confirm our results may help in identifying and risk stratifying patients who might end up in End stage renal disease later. Earlier institution of renoprotective measures would reduce the overall burden on the system.

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# *Annexures*

# INSTITUTIONAL ETHICS COMMITTEE
# MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

## CERTIFICATE OF APPROVAL

To
Dr. S.Anandhi,
Postgraduate M.D.(Biochemistry),
Madras Medical College,
Chennai - 600 003

Dear Dr.S.Anandhi,

The Institutional Ethics Committee has considered your request and approved your study titled **"Role of uric acid and hs-CRP as renal and cardiovascular risk markers in Type 1 diabetic patients with low GFR". No.16102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

| | | |
|---|---|---|
| 1. | Dr.C.Rajendran, M.D., | : Chairperson |
| 2. | Dr.R.Vimala, M.D., Dean, MMC, Ch-3 | : Deputy Chairperson |
| 3. | Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3 | : Member Secretary |
| 4. | Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC | : Member |
| 5. | Dr.Raghumani, Director Incharge, Inst.of Surgery | : Member |
| 6. | Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC | : Member |
| 7. | Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3 | : Member |
| 8. | Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC | : Member |
| 9. | Thiru S.Rameshkumar, Administrative Officer | : Lay Person |
| 10. | Thiru S.Govindasamy, B.A., B.L., | : Lawyer |
| 11. | Tmt.Arnold Saulina, M.A., MSW., | : Social Scientist |

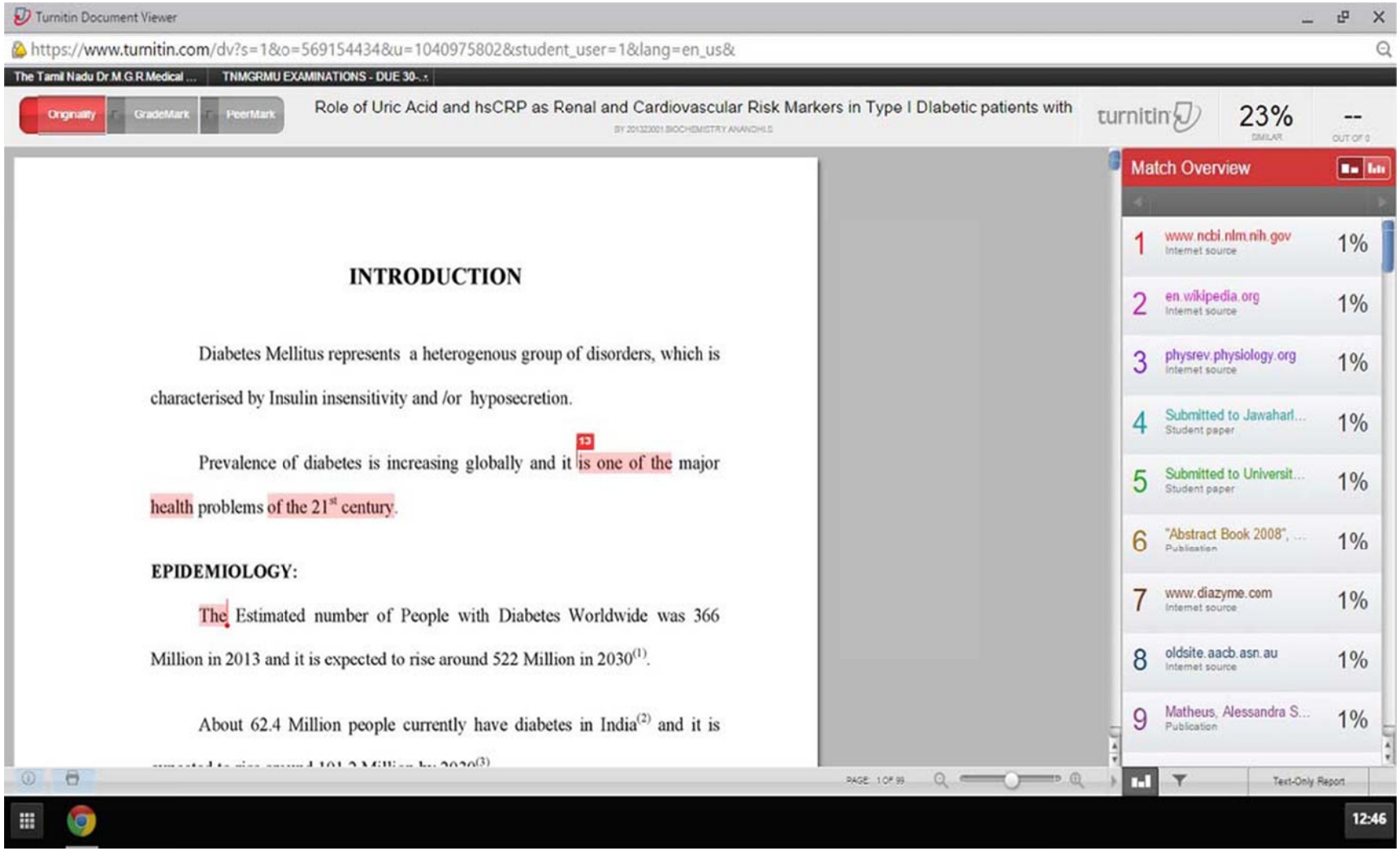
We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

9/10/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003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Role of Uric Acid and hsCRP as Renal and Cardiovascular Risk Markers in Type I Diabetic patients with
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INTRODUCTION
Diabetes Mellitus represents a heterogenous group of disorders, which is characterised by Insulin insensitivity and /or hyposecretion.
Prevalence of diabetes is increasing globally and it is one of the major health problems of the 21st century.
EPIDEMIOLOGY:
The Estimated number of People with Diabetes Worldwide was 366 Million in 2013 and it is expected to rise around 522 Million in 2030(1).
About 62.4 Million people currently have diabetes in India(2) and it is
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Text-Only Report
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## INFORMATION SHEET

- Your blood sample has been accepted.
- We are conducting a study on type1 diabetic patients among patients attending Government General Hospital, Chennai and for that your blood sample may be valuable to us.
- The purpose of this study is to find out the role of uricacid&hs-CRP as a renal& cardiovascular risk marker in type 1 diabetic patients with low GFR. with the help of certain special tests.
- We are selecting certain cases and if your blood sample is found eligible, we may be using your blood sample to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

# PATIENT CONSENT FORM

Title of the study **:" ROLE OF URICACID AND hs-CRP AS RENAL AND CARDIOVASCULAR RISK MARKERS IN TYPE 1 DIABETIC PATIENTS WITH LOW GFR" .**

Name : Date :

Age : OP No :

Sex : Project Patient No
:

**Documentation of the informed consent**

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in "**ROLE OF URICACID AND hs-CRP AS RENAL AND CARDIOVASCULAR RISK MARKERS IN TYPE 1 DIABETIC PATIENTS WITH LOW GFR.".**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____ month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.

11. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
12. I have understand that my identity will be kept confidential if my data are publicly presented.
13. I have had my questions answered to my satisfaction.
14. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

**For participants:**

Name and signature / thumb impression of the participant (or legal representative if participant in competent/For age 10-17 yrs-Name& signature of the parent/guardian.)

Name _____ Signature_____Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

# ஆராய்ச்சி தகவல் தாள்

**தலைப்பு:**

**வகை 1 நீரிழிவு மற்றும் குறைந்த குளோமருலர் வடிகட்டுதல் விகிதம் உள்ள நோயாளிகளுக்கு சிறுநீரக மற்றும் இருதய ஆபத்து கணிப்பிற்கு யூரிக் அமிலம் மற்றும் எச்.எஸ்.சி.ஆர். புரதத்தின் பங்கு.**


ஆராய்ச்சியாளர் : **மரு. சொ. ஆனந்தி,**
பட்ட மேற்படிப்பு மருத்துவ மாணவி,
உயிர்வேதியியல் உயர்நிலைத்துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.

ஆராய்ச்சி மேற்பார்வையாளர் : **மரு. வி. அமுதவள்ளி,**
பேராசிரியர்,
உயிர்வேதியியல் உயர்நிலைத்துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.


சர்க்கரை நோயினால் ஏற்படும் சிறுநீரக பாதிப்பு 20-30% மற்றும் இருதய பாதிப்பு 7-10% வகை 1 சர்க்கரை நோயாளிகளில் காணப்படுகின்றது. சிறுநீரில் அதிகமாக புரதம் வெளியேறுவதே சிறுநீரக பாதிப்பின் முதல் அடையாளமாக கண்டறியப்படுள்ளது. சில சர்க்கரை நோயாளிகளில் குறைந்த குளோமருலர் வடிகட்டுதல் விகிதம்; சிறுநீரில் புரதம் வெளியேறுவதற்கு முன்னரே ஏற்பட்டு, சிறுநீரக பாதிப்பை அதிகப்படுத்துகின்றது. அப்பொழுதே இதயமும் பாதிப்படைகிறது.

யூரிக் அமிலம் மற்றும் எச்.எஸ்.சி.ஆர். புரதத்தின் அளவும் சிறுநீரக மற்றும் இதய பாதிப்பின் போது அதிகமாகக் காணப்படுகின்றது என்று கண்டறிந்துள்ளனர். இதன் மூலம் சிறுநீரக மற்றும் இதய பாதிப்பை முன்னரே கண்டறிந்து நோயின் வீரியத்தை கட்டுப்படுத்தலாம் எனவும் ஆராய்ச்சிகள் தெரிவிக்கின்றன. எனவே, சென்னை இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் வகை 1 நீரிழிவு நோயாளிகளின் இரத்தத்தில் யூரிக் அமிலம் மற்றும் ஹெச்.எஸ்.சி.ஆர். புரதத்தின் அளவையும் சிறுநீரில் புரதத்தின் அளவையும், கண்டறியும் ஆராய்ச்சியில் ஈடுபட்டுள்ளேன்.

இதற்கு சிறுநீரில் புரதம் () வெளியேறும் அறுபத்தைந்து நோயாளிகளிடமும், அவர்களின் வயதிற்கு ஏற்றார் போலுள்ள, சிறுநீரில் புரதம் (30-299 mg/day)

வெளியேறும் அறுபத்தைந்து நோயாளிகளிடமும், 5மி.லி. இரத்தமும் எடுத்து ஆராய்ச்சிக்கு உட்படுத்த உள்ளேன்.

தங்களிடமிருந்து ஊசியின் மூலம் 5 மி.லி. இரத்தம் எடுப்பதனால் எந்த விதமான பக்க விளைவுகளும் ஏற்படாது என உறுதி அளிக்கின்றேன்.

தாங்கள் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதில் பங்கு பெறுவதினால் நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் கூடுதல் செலவீனம் ஏற்படாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயர் மற்றும் அடையாளங்கள் வெளியிடப்படாது என்பதை தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களின் விருப்பத்தின் பேரில் தான் இருக்கிறது. எந்நேரமும் இதிலிருந்து பின்வாங்கம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியின் முடிவுகளை தங்களுக்கு அறிவிப்போம் என்பதை தெரிவித்துக் கொள்கிறோம்.

நீங்கள் இந்த ஆராய்ச்சியில் பங்குபெற விருப்பம் இருப்பின் இந்த தகவல்தாள் மற்றும் ஆராய்ச்சி ஒப்புதல் படிவத்திலும் கையொப்பம் இடுமாறு கேட்டுக் கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம்

பங்கேற்பாளர் கையொப்பம்
/இடது கைவிரல் ரேகை

இடம் :

தேதி :

# நோயாளியின் ஒப்புதல் படிவம்

**தலைப்பு:**

**வகை 1 நீரிழிவு மற்றும் குறைந்த குளோமரூலர் வடிகட்டுதல் விகிதம் உள்ள நோயாளிகளுக்கு சிறுநீரக மற்றும் இருதய ஆபத்து கணிப்பிற்கு யூரிக் அமிலம் மற்றும் எச்.எஸ்.சி.ஆர். புரதத்தின் பங்கு.**

பங்கேற்பாளர் பெயர் : புற / உள் நோயாளி எண்:

வயது : பால் : கைபேசி/தொலைபேசி எண் :

முகவரி : ஆராய்ச்சி சேர்க்கை எண் :

.....ஆகிய நான் மரு.சொ.ஆனந்தி, பட்டமேற்படிப்பு, மருத்துவ மாணவி, உயிர்பேதியியல் உயர்நிலைத் துறை, சென்னை மருத்துவக் கல்லூரி, சென்னை. மேற்கொள்ளும் ஆராய்ச்சியில் பங்கேற்க எந்தவித நிர்பந்தமின்றி, முழு சுதந்திரத்துடன் சுய நினைவுடன் முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

இந்த ஆராய்ச்சி பற்றிய தகவல் தாளை பெற்றுக் கொண்டு அதன் முழு விரவங்களையும்ன, நோக்கங்களையும் மருத்துவரின் மூலம் புரிந்துக்கொண்டு எனது மருத்துவ குறிப்புகள், பரிசோதனை முடிவுகள் மற்றும் இரத்தத்தை பயன்படுத்திக் கொள்ளவும் மேலும் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் எனவும் அச்செயலினால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்துக் கொண்டு முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

எனது பரிசோதனை முடிவுகளை அறிவியல் சார்ந்த அமைப்புகள் மற்றும் மருத்துவ இதழ்களில் வெளியிடுவதற்கு முழு மனதுடன் சம்மதிக்கின்றனே.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம் /இடது கைவிரல் ரேகை

இடம் :

தேதி :

# PROFORMA

| | | | | |
|---|---|---|---|---|
| Name | : | | | Patient ID No. : |
| Age / Sex | : | | | |
| Height | : | | | |
| Weight | : | | | |
| Ethnicity | : | | | |
| Type of Diabetes | : | | | |
| Duratio of Diabetes | : | | | |
| Smoking Status | : | Current - | Ex - | Never-smoker |
| Body Mass Index | : | | | |
| Hypertension | : | | | |
| Waist Hip Ratio | : | | | |
| Renal Failure / Transplant | : | | | |
| Cardiac Failure | : | | | |
| Any other serious | : | | | |
| Medical Illness | : | | | |
| Family H/o. Diabetes | : | | | |

**Drug History:**

| | | |
|---|---|---|
| ACE Inhibitors | : | ☐ |
| ATI Antogonist | : | ☐ |
| Diuretics | : | ☐ |
| Calcium channel blockers: | | ☐ |
| Insulin | : | ☐ ☐ ☐ |

Any other medications : _____

**Investigations:**

| | | | | |
|---|---|---|---|---|
| Serum fasting glucose | : | | | |
| Lipid Profile | : | TGL | HDL | LDL |
| Serum Creatinine | : | | | |
| Serum Urea | : | | | |
| Serum Protein | : | | | |
| Serum Albumin | : | | | |
| eGFR (CKD-EPI) | : | | | |
| Spot Urine Albumin | : | | | |
| Spot Urine Creatinine | : | | | |
| Albumin Creatinine Ratio | : | | | |
| Serum hs-CRP | : | | | |
| Serum HbA1C | : | | | |
| Serum Uric Acid | : | | | |