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Comparison of inflammatory markers (Interleukin-1β, Interleukin-6 and hs-CRP) in Type 2 Diabetes Mellitus patients with and without Nephropathy: A hospital based cross-sectional study in Mumbai

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ABSTRACT

Background

Diabetic nephropathy is a microvascular diabetic complication secondary to longstanding diabetes due to angiopathy of capillaries in glomeruli now recognized as long standing chronic inflammatory disease. The study was done with an objective to compare the levels of inflammatory biomarkers (hs-CRP,IL-1β,IL-6) in patients of type 2 diabetes mellitus with nephropathy and without nephropathy.

Material and Method

After permission of Institutional Ethics Committee patients satisfying inclusion and exclusion criteria were included. Study was conducted in a tertiary care medical hospital. 100 patients of type 2 diabetes mellitus were divided into three groups of Normoalbuminuria, Microalbuminuria and Macroalbuminuria depending on urine albumin excretion levels. Serum hs-CRP, IL-1β and IL-6 estimation was done on Fully Automated IMMULITE 1000 analyzer. eGFR was calculated based on CKDEPI formula. Plasma glucose, Serum Urea levels, serum Creatinine and HbA1c were estimated.

Results

Interleukin-1β in Macroalbuminuria (Group III) 9.98 ± 2.42 (pg/ml) was higher and statistically significant ($p<0.05$) than Microalbuminuria (Group II) 8.19 ± 1.59 and Normoalbuminuria 6.89 ± 1.21 group (Group I). Interleukin 6 levels in Macroalbuminuria 16.17 ± 3.07 was higher and statistically significant ($p<0.05$) than Microalbuminuria 13.85 ± 1.66 and Normoalbuminuria 11.26 ± 1.61 group. hs-CRP levels in Macroalbuminuria 7.61 ± 2.02 was higher and statistically significant ($p<0.05$) than Microalbuminuria 6.41 ± 1.25 and Normoalbuminuria 4.72 ± 0.81. Serum Creatinine in Macroalbuminuria 3.86 ± 1.60 was higher and statistically significant than Microalbuminuria 2.10 ± 0.89 and Normoalbuminuria 1.31 ± 0.43. IL 1β, IL6 and hs-CRP correlated positively with serum Creatinine, urine albumin levels, HbA1c and eGFR.

Conclusion:

hs-CRP, IL-1β and IL-6 were strongly associated with urine albumin levels and serum Creatinine levels in diabetic nephropathy. Thus, these inflammatory markers are potential indicators for diabetic nephropathy.

Key words Urine Albumin, Creatinine, Urea, eGFR, HbA1c

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INTRODUCTION

In the last century there is enormous increase in incidence of diabetes mellitus and also its complications, this has caused enervating increase in morbidity and mortality due to diabetes mellitus complications worldwide. [1] In India 77 million people are living with diabetes and will increase to 134.2 million by 2045.[2] In India diabetic nephropathy claims to have around 30% of the type 2 diabetes mellitus. [3]Hyperglycemia promotes gradual increase in renal parenchyma inflammation, increase in proinflammatory cytokines and progressive angiopathy of capillaries present in glomeruli. [4] hs-CRP (High Sensitivity C-Reactive Protein) an inflammatory marker related to many diseases from diabetes mellitus, renal failure, cardiovascular diseases etc. [5] Interleukin (IL)-1 causes increase in the production of adhesion molecule of intercellular cells-1 and vascular cellular adhesion molecule-1 by endothelial cells of glomeruli. [6] IL-6 has important role in stimulation of mesangial cell proliferation and endothelial permeability enhancement.[7] These findings indicate inflammatory origin as one of the important factor in development of kidney injury in diabetes Mellitus. Thus, the present study was undertaken to compare the levels of inflammatory biomarkers (hs-CRP, IL-1β, and IL-6) in patients of type 2 diabetes mellitus with nephropathy and without nephropathy.

Material and Methods

Permission of Institutional Ethics Committee (GGMC & Sir JJGH/IEC/Pharm/50/14) for Humans was taken before starting the study. Patients were screened and purpose of the study was explained. The study participants were screened from inpatient medicine ward from a Grant Government Medical College & Sir JJ Group of Hospital, Mumbai medical college and tertiary care level hospital. The inclusion criteria included patients of age 18-75 years and both gender patients diagnosed as type 2 diabetes mellitus and diabetic nephropathy by American Diabetes Association guidelines according to urine albumin excretion (UAE) as Normoalbuminuria UAE < 30 mg/24 h, Microalbuminuria UAE: 30–300 mg/24 h and Macroalbuminuria UAE ≥300 mg/24 h. [8] Type 2 DM was diagnosed according to the criteria set by the American Diabetes Association criteria 2017 having fasting plasma glucose levels >126 mg/dL or HbA1c >6.5% or random plasma glucose of >200 mg/dL. [9]

The exclusion criteria included patients having type 1 diabetes mellitus, pregnant women, smoking, history of urolithiasis, alcohol consumption, taking allopurinol, patients with history of acute febrile illness, ongoing urinary tract infection, Pyelonephritis, Urinary tract obstruction, history of patient on dialysis, history of cardiovascular disease. Patients screened from inpatient medicine ward that were satisfying inclusion and exclusion criteria and willing to sign written informed consent were enrolled for the study. Patients were stratified according to gender and randomized in groups depending on UAE rate.

Sample size was calculated with power analysis of $\alpha = 0.05$ and $\beta = 80\%$ showed 49 patients were needed per study group to detect a minimum increase of 1.25 mg/dl in hs-CRP as mentioned by Patil et al [10]. Sample size calculation was done by Epiinfo software version 3.1. [11]

Total 100 patients were divided into three groups, according to American Diabetes Association guidelines as per group I, included type 2 diabetes mellitus with normoalbuminuria (n = 50, UAE < 30 mg/24 h), group II included type 2 diabetes mellitus with microalbuminuria (n = 27, UAE: 30–300 mg/24 h) and group III included type 2 diabetes mellitus with macroalbuminuria (n = 23, UAE ≥300 mg/24 h).[8]The procedures performed in this study were followed according to Helsinki declaration, its amendments, institutional ethics committee's and the national guidelines. 10 ml blood sample was collected by venepuncture from antecubital vein, under all aseptic precautions and transferred in plain, fluoride and Ethylene Diamine Tetra Acetatae (EDTA) for HbA1c vacutainer tube, centrifuged at 1000xg for 10 minute. Serum was separated, aliquoted and stored for -80°C till further analysis. All the investigations in the study were done in Central Biochemistry Laboratory in tertiary care hospital.

24 hour urinary samples were collected in clean container and refrigerated till transported to laboratory. Properly mixed aliquot was used for estimation and cloudy specimens were latter thawed and centrifuged briefly to remove particulate matter before analysis. Midstream

Urine sample was collected in sterile plastic container. Urine samples were obtained from the patients and centrifuged at 1000 ×g for 5 min. Urine albumin concentrations were assessed using particle enhanced immuoturbidimetric method assay on automated analyzer ADVIA 800. Serum creatinine was done by Jaffe's method while Glucose and urea levels were determined by enzymatic methods on ADVIA 1800 autoanalyzer.[4] Glycosylated hemoglobin (HbA1c; %) was estimated by high-performance liquid chromatography.

Serum hs-CRP, IL-1β and IL-6 estimation was done on Fully Automated Enzyme Amplified Chemiluminescent Immunoassay based IMMULITE 1000 analyzer with kits of hs-CRP, IL-1β and IL-6 based on principle of chemiluminescence. Blood Pressure was measured by Sphygmomanometer according to National Institute for Health Care Excellence guidelines. [12]

The estimated glomerular filtration rate (eGFR; mL/min/1.73 m2) was calculated using the formula devised by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [13]. The CKD-EPI equation was calculated as eGFR (mL/min/1.73 m2) = 141 X min (serum creatinine/k,1)$^{\alpha}$ X max (serum creatinine/k, 1)$^{-1.209}$ X 0.993^{Age} X 1.018 (if female) X 1.159 (if black), where k is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, (min) indicates minimum serum creatinine/k or 1, and (max) indicates maximum serum creatinine/k or 1. [13]

Statistical Analysis

Data were expressed as mean ± standard deviation (SD), in the form of numbers and percentages, while for statistical significance P<0.05 was considered statistically significant. One way ANOVA test was applied to compare parameteric data between different groups along with post hoc Tuckey's test. Unpaired t test was used between two groups to find the significance of study parameters on continuous scale. Two way ANOVA test was applied to compare different parameters in between different groups along with post hoc Bonferroni test. Receiver operating characteristic (ROC) curve analyses were used to compare the different parameters used for diabetic nephropathy Data analysis was done using the "GraphpadInstat 6.2a, San Diego, California" software & SPSS 20.0 (Chicago, USA)

Results

The mean age of study subjects was 62.46 ± 8.17 years. The mean age in females was 61.64 ± 7.22 years and in males was 62.40 ± 8.47 years. There were 28 males and 22 females in group I, 15 males and 12 females in group II, and 13 males and 10 females in group III. In group I there were 18 patients in age group 60-70 yrs, 15 in 50-60 yrs and 17 patients were < 50 yrs. In group II there were 06 in 60-70 yrs of age group and 14 in 50-60 yrs and 07 were < 50 yrs. In group III there were 11 in 60-70 yrs of age group and 08 in 50-60 yrs and 04 were < 50 yrs. The distribution of age, blood glucose and duration of diabetes is given in table I

Table I: Distribution of Age, Blood Glucose and Duration of diabetes (N=100)

Parameters	Group I Normo (N=50)	Group II Micro (N=27)	Group III Macro (N=23)	t value	P value
Age (years)	61.71 ± 9.28	64.3 ± 8.76	66.47 ± 11.5	0.6448	0.138
FBG (mg/dl)	131.6 ± 32.65	142.06 ± 35.7	150.6 ± 55.35	2.574	0.156
PPBG (mg/dl)	203.8± 43.5	238.7± 52.1*	276 ± 83.9**	9.780	0.001
Duration of diabetes (years)	06.7 ± 2.59	09.1 ± 3.74*	11.9 ± 4.93**#	5.80	0.001
Systolic BP (mm of Hg)	132.4 ± 9.3	139.2 ± 12.03	148.7 ± 14.5	5.67	0.01
Diastolic BP (mm of Hg)	80.6 ± 6.4	86.50 ± 8.2**	89.30 ± 9.6**	1.178	0.06

*# Significant as compared to Group I: * Significant as compared to Group II:*

Distribution of comorbid illnesses in study groups is given in table II. Distribution of IL-1β and IL-6, hs-CRP, HbA1c, serum (Sr.).Creatinine & Sr. Urea in study groups is given in table III.

Table II: Distribution of comorbid illnesses in study groups (N=100)

	Group I (Normo) (N=50)		Group II (Micro) (N=27)		Group III (Macro) (N=23)	
	Number	Percentage	Number	Percentage	Number	Percentage
Hypertension	34	68	27	100	23	100
Cerebrovascular accident	5	10	10	37.03	14	60.86
Cardiovascular disease	7	14	14	51.85	16	69.57
Diabetic Neuropathy	0	0	3	11.11	10	43.48
Diabetic Retinopathy	0	0	0	0	5	21.74

Table III - Distribution of IL-1β and IL-6, Sr.Creatinine & Sr.Urea in study groups (N=100)

Parameter	Group I (Normo) (N=50)	Group II (Micro) (N=27)	Group III (Macro) (N=23)	t value	P value
HbA1c (%)	7.02 ±1.11	7.48 ± 1.64**	9.51 ± 2.49 **##	4.31	0.001
Sr.Urea (mg/dl)	30.57 ± 7.23	83.85 ± 20.70 **	139.09 ± 47.84 **##	10.82	0.001
Sr.Creatinine (mg/dl)	1.31 ± 0.43	2.10 ± 0.89**	3.86 ± 1.60 **##	10.75	0.001
hs-CRP (mg/L)	4.72 ± 0.81	6.41 ± 1.25**	7.61 ± 2.02 **##	8.86	0.001
IL-1β (pg/ml)	6.89 ± 1.21	8.19 ± 1.59**	9.98 ± 2.42 **##	7.42	0.001
IL-6 (pg/ml)	11.26 ± 1.61	13.85 ± 1.66***	16.17 ± 3.07 **##	9.52	0.001
UAE (mg/24 h)	18.75 ± 4.58	234.93 ± 78.18 **	518.04 ± 164.84 **##	22.42	0.0001
eGFR (mL/min/1.73 m2)	120.10 ± 90.06	30.58 ± 15.60**	21.35 ± 13.40 **	52.84	0.0001

#Significant as compared to Group I: * Significant as compared to Group II:

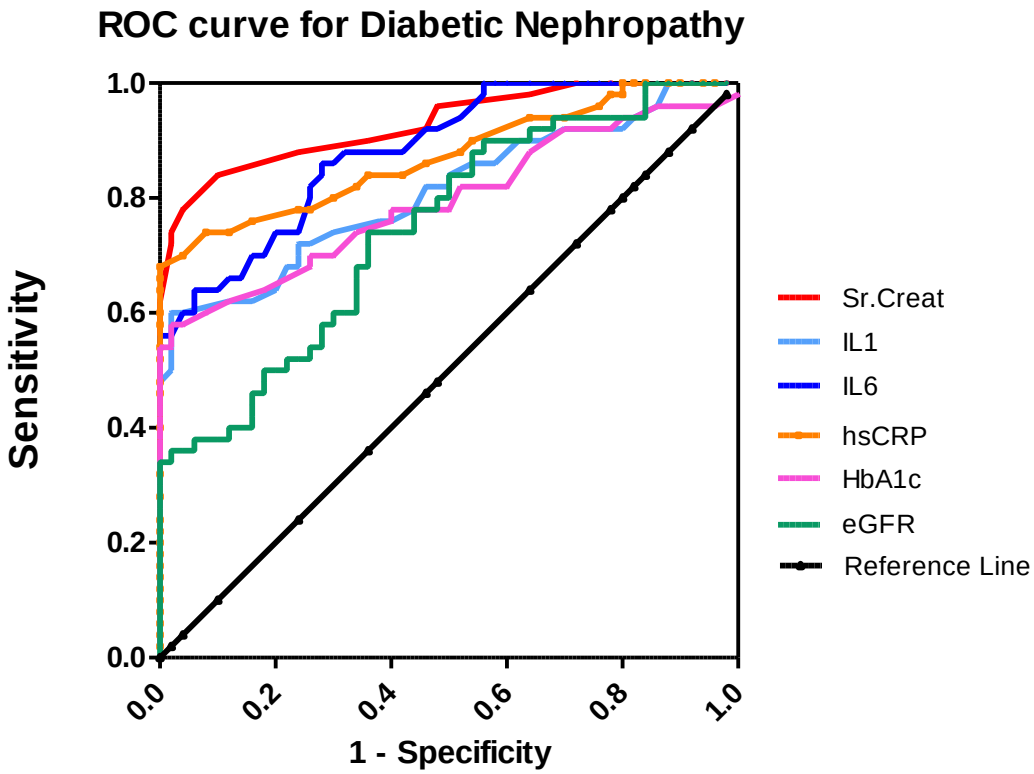
In group I there were 50 patients on oral antidiabetics. In group II there were 15 patients on oral antidiabetics, 12 patients were on both oral antidiabetic and Insulin therapy while in group III there were 14 patients on both oral antidiabetic and Insulin therapy and 09 patients were on only Insulin. Area under the curve for type 2 diabetes mellitus with nephropathy was given in table IV while in graph I Receiver operating characteristic curve was given for Diabetic Nephropathy.

Table IV: Area under the curve for type 2 diabetes mellitus with nephropathy (N=100)

Variables	AUC	SE	P value	95% CI	
				Lower bound	Upper bound
UAE	1.0	0.00	<0.0001	0.0	0.0
S.Urea	0.998	0.002	<0.0001	0.994	1.003
Sr.Creatinine	0.928	0.025	<0.0001	0.878	0.978
IL6	0.882	0.031	<0.0001	0.820	0.945
hs-CRP	0.872	0.036	<0.0001	0.802	0.943
IL1β	0.810	0.043	<0.0001	0.724	0.895
HbA1c	0.794	0.046	<0.0001	0.704	0.885
Egfr	0.746	0.048	<0.0001	0.652	0.841

AUC: Area Under Curve SE: Standard Error CI: Confidence Interval

Graph I: ROC (Receiver operating characteristic) Curve for Diabetic Nephropathy



DISCUSSION

Diabetic nephropathy is a chronic complication of Type 2 diabetes mellitus affecting almost 40% of all diabetic patients. It is a microvascular complication of diabetes mellitus that impairs the quality of life leading to increased morbidity and mortality.[14,15] In diabetes, inflammatory cytokines are involved in the development of chronic endothelial inflammation and microvascular diabetic complication leading to end-stage renal disease. [16]

In our study inflammatory markers IL1β, IL6 and Hs-CRP significantly correlated with Sr.Creatinine and urine albumin excretion. In study by Domingueti, IL6 levels were increased in patients with chronic kidney disease to 16 pg/ml, UAE rate of 65 (mg/g creatinine) and

eGFR of 75 (mL/min/1.73 m2).[12] Similar results were seen in study by Abid and Malenica. [15, 16]

In diabetes, hyperglycemia and Insulin resistance promote inflammation by increased oxidative stress, enhanced cyclic oxygenize-2 expression and prostaglandin E2 production. This induces proinflammatory cytokines like IL-6 expression in renal tubular epithelial cells that further contribute to the progress of glomerulosclerosis, and albuminuria. [11,18] In study by Berthier increased activation of key pathways involved in the transcriptional regulation of cytokine production was observed in the kidneys of patients with nephropathy and also in study by Sanchez-Nino in the mouse models of diabetic nephropathy.'[19,20] During early diabetic renal injury, development of microalbuminuria has been associated with circulating levels of C-reactive protein and inflammatory biomarkers like interleukins that are independently associated with diabetic nephropathy.'[21,22] In diabetic nephropathy renal inflammation plays a vital role in renal injury that progresses during diabetes.

In study by Abid, hs-CRP level of the diabetic and diabetic nephropathy patients were significantly high as compared to controls.'[15] Study by Devaraj found that CRP levels were elevated in Type 2 diabetic patients with the metabolic syndrome. [23] Study by Mahajan and Lima reported that hyperglycemia is a conducive factor for increased serum CRP levels in Type 2 diabetes mellitus subjects that are uncontrolled.'[24,25] In study by Mojahedi, microalbuminuria was accompanied by elevated hs-CRP levels and inflammatory pathways activation.'[26] The inflammatory process increase has been linked to progression of renal and cardiovascular disease in Type 2 diabetes mellitus patients.'[15] Other studies have also reported the relationship between inflammation, Type 2 diabetes mellitus and diabetic nephropathy.'[17]

IL-1, an important proinflammatory cytokine and has stimulating effects on mesangial cells proliferation and the production of extracellular matrix conjectures a key role in the progression of renal failure in diabetic patients. Interleukin-1β gene polymorphism is associated with diabetic nephropathy in type 2 diabetic patients. [27] Circulating IL-6 is now considered to be an independent marker to predict type 2 diabetes mellitus as it is involved in the development of insulin resistance and inflammation along with β-cell dysfunction. Diabetic Nephropathy patients had elevated levels of inflammatory cytokines, including IL-6, which positively correlated with the extent of proteinuria.'[28] IL-6 gene polymorphism is an independent risk factor for diabetic nephropathy in type 2 diabetic mellitus patients.'[22,30] Diabetic Nephropathy patients showed an elevated serum level of IL-6 that are generated by renal podocytes, mesangial cells, and tubules which contributes to local as well as systemic inflammatory process. Even Meta-analysis by Zhen had shown the association of IL6 polymorphism with the risk of type 2 diabetes mellitus.' [31] IL-6 neutralizing monoclonal antibody Tocilizumab has been approved for treatment in patients with autoimmune diseases, such as rheumatoid arthritis.' [22] Interleukins IL1β, IL6 and Hs-CRP are the markers of chronic inflammatory process that participates from early stages in the development of diabetic nephropathy.

The proinflammatory cytokines involved in the inflammatory process of diabetic nephropathy include IL-1β, IL-6, and IL-18. Out of these IL-6 and Hs-CRP are correlated positively with urinary albumin excretion in type 2 diabetes mellitus subjects. [16] Hs-CRP is consistently correlated and increased in diabetic nephropathy.'[32] Study by Abid had shown significant association between hs-CRP and eGFR in both diabetic and diabetic nephropathy subjects.'[15] In our study HsCRP, IL-1β and IL-6 were positively correlated with Serum creatinine in diabetic patients and it was more strongly correlated in group with diabetic nephropathy patients. These inflammatory markers are present before the beginning of diabetic nephropathy.

There are few available pharmacological therapies for treatment of renal disease. The molecular and metabolic mechanisms involved with development and progression of diabetic nephropathy will contribute to the development of new therapeutic strategies.'[32] The diabetic subgroups based on albuminuria levels, suggested that e-GFR may not be a reliable predictor of kidney injury.'[16] In our study among the various parameters eGFR was least reliable predictor of diabetic nephropathy.

Increased in albuminuria has been used for screening, but studies have suggested that renal

damage caused by diabetes mellitus starts even before the start of albuminuria. Currently there is paucity of biomarkers that can speculate the progress of underlying renal pathological changes in patients with diabetes mellitus. Current therapeutic strategies focus on stringent glucose and blood pressure control.[33, 34]

Use of new predictive biomarkers along with the urinary albumin excretion during the initial stages of diabetic renal disease would help to prevent or delay the onset of unavoidable long-term complications.[35] In our study ROC showed that IL6, Hs-CRP and IL1β had almost the same predictive values as serum creatinine and serum urea. These markers were more predictive than eGFR. Without interventions, 20–40% of patients with type 2 diabetes mellitus with microalbuminuria progress to overt nephropathy. Although microalbuminuria has been widely used for screening diabetic nephropathy, it is still not the earliest biomarker for diagnosis of diabetic nephropathy. When microalbuminuria appears, diabetic nephropathy has already progressed to the third stage.[30] The inflammatory marker levels of IL1β, IL6 and Hs-CRP together can be utilized for the early detection of diabetic nephropathy. Early detection of diabetic nephropathy can help in early halt of the disease and can improve the evaluation of patients with diabetic nephropathy. The limitation of our study is that this was a single center study with a small sample size.

CONCLUSION

Inflammatory markers (IL1β,IL6 and Hs-CRP) were increased in patients with diabetic nephropathy. Thus, inflammatory markers can help in evaluation of the progression and management of Diabetes Mellitus. We recommend larger studies to establish the role of inflammatory markers as potential indicators of nephropathy in Diabetes patients.

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