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A Study of Urinary Inflammatory Makers and Hba1c Levels in Type 2 Diabetic Nephropathy Patients

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KEYWORDS

Type 2 diabetic nephropathy, inflammatory marker, HbA1c

ABSTRACT:

Introduction: Diabetic nephropathy (DN), the largest single cause of end-stage renal disease, and a medical catastrophe of worldwide dimensions. There is growing evidence that activated innate immunity and inflammation are relevant factors in the pathogenesis of diabetes mellitus, with convincing data that DM2 includes an inflammatory component. Diabetic nephropathy (DN), defined as albuminuria (albumin excretion rate >300mg/24h) and declining renal function in patient with known diabetes in absence of urinary tract infection, or any other renal disease.

Material and methods: This study was a case control study carried out in department of Biochemistry, Index Medical College and Hospital in which the patients were selected as per the inclusion and exclusion criteria. 98 patients of Type 2 Diabetic Nephropathy admitted in Department of Medicine, Index medical collage & hospital, Indore and 98 healthy controls were assessed after taking ethical clearance from the college ethical committee. Written and informed consent was also taken.

Results & Conclusion: The mean FBS of the type 2 diabetic nephropathy subjects was higher (187.55±53.20) than for healthy controls (166.74±27.04) and the mean HbA1c of the type 2 diabetic nephropathy subjects was higher (9.27±2.33) than for healthy controls (8.13±1.48) Similarly, the mean PPBS of type 2 diabetic nephropathy subjects was higher 271.81±83.29 as compared 240.21±48.40 healthy controls.and urinary inflammatory marker also higher in case group compare to control. the mean of Microalbumin Creatinine Ratio is insignificantly higher in case 2.78±5.14 compared to control 1.52±4.14 (p>0.05).

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Introduction:

Diabetic nephropathy (DN), the largest single cause of end-stage renal disease, and a medical catastrophe of worldwide dimensions [1]. In the past few years, numerous studies have shown that low-grade inflammation is associated with the risk of developing type 2 diabetes mellitus (DM2). There is growing evidence that activated innate immunity and inflammationare relevant factors in the pathogenesis of diabetes mellitus, with convincing data that DM2 includes an inflammatory component [2]. Several studies have also shown that patients with type DM2 and DN exhibit high levels of diverse acute phase markers of inflammation, including C-reactive protein (CRP), serum amyloid A, fibrinogen and Interleukin-6 (IL6) [3]. Diabetic nephropathy (DN), defined as albuminuria (albumin excretion rate >300mg/24h) and declining renal function in patient with known diabetes in absence of urinary tract infection, or any other renal disease [4]. Urinary TNF-α excretion appears increased in diabetes with micro or macro-albuminuria than normo-albuminuric patients [5&6]. with one study reporting increase of 90.0% between normo and micro-albuminuric patients [6]. Thereis a correlation between the excretion of NAG, a measure of the severity of tubular injury, and the excretion of TNF-α in the urine [7]. Two different receptors, TNF receptor 1 (TNFR1) and TNFR2, which are both membranes bound and soluble in serum, are how TNF-α exerts its effects [8]. It has been demonstrated that, in diabetes individuals, serum levels of both of these receptors correlate with GFR regardless of the presence of albuminuria [9]. research, blood levels of TNFR1 and TNFR2 may be able to predict the development of renal disease in diabetics [10]. It has been shown that microalbuminuria is a marker of vascular damage and atherosclerosis [11]. Persistent microalbuminuria is a strong predictor of development of clinical DN, which is reversible, but may lead to kidney failure if neglected.Therefore, early diagnosis may help to prevent progression of kidney disease. Accordingly, annual screening of microalbuminuria is recommended by experts. It is reported that prevalence of microalbuminuria is about 12.6% to 25.3% in patients with type 2 diabetesmellitus [12].

MATERIAL AND METHODS

This study was a case control study carried out in department of Biochemistry, Index MedicalCollege and Hospital in which the patients were selected as per the inclusion and exclusion criteria. 98 patients of Type 2 Diabetic Nephropathy admitted in Department of Medicine, Index medical collage & hospital, Indore and 98 healthy controls were assessed after taking ethical clearance from the college ethical committee. Written and informed consent was alsotaken.

Inclusion Criteria:

- Subjects who are willing to participate.
- Patients aged between 35-60 years, irrespective of their gender diagnosed with T2DMand were suffering with Diabetic Nephropathy.
- Diabetic patients who have not been progressed to diabetic nephropathy will beincluded as subjects to control group.

Exclusion Criteria:

- Patients with DM but otherwise having normal kidney functions.
- Patients with any known renal anomaly.

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- Red blood cell wall defects.
- Hemoglobinopathies.
- Hypertension.
- Malignancies.
- Urinary tract infections.
- Acute febrile illness.
- Any other condition or medication which may interfere with the proteinuriaassessments in the patients will be excluded from the study.

Parameters to be Studied:

The following parameters were estimated: -

1. **Diabetes Profile**
   a. Fasting Blood Sugar
   b. Postprandial blood sugar
   c. Estimation of HbA1c
2. **24-hour urine biomarkers**
   a. Microalbumin
   b. Protein
   c. Creatinine
   d. Microalbumin Creatinine Ratio
   e. Transferrin
   f. Ceruloplasmin
   g. IgG
   h. TNF-a
   i. IL6

Statistical Analysis:

Microsoft Excel was used in creating the database and producing graphs, while the data were analysed using the Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows.

Mean and standard deviation (±SD) were used to describe quantitative data meeting normal distribution. Continuous two independent groups were compared by parametric independent Student's t test. Discrete (categorical) groups were compared by chi-square ($\chi 2$) test. Bivariate analysis (Karl's Pearson's correlation coefficient) was used to determine the correlation between blood sugar level, HbA1c, blood (serum) biomarkers and 24-hour biomarkers of early stage of diabetic nephropathy. p values less than 0.05 ($p<0.05$) was considered as statistically significant.

RESULTS

The present study included 196 subjects, out of which 98 were type 2 diabetic and 98 were healthy controls. The mean FBS of the type 2 diabetic nephropathy subjects was higher (187.55±53.20) than for healthy controls (166.74±27.04) and the mean HbA1c of the type 2 diabetic nephropathy subjects was higher (9.27±2.33) than for healthy controls (8.13±1.48) Similarly, the mean PPBS of type 2 diabetic nephropathy subjects was higher 271.81±83.29 as compared 240.21±48.40 healthy controls [Table 1]. The mean of Urine Microalbumin (mg/L) is higher compare to control (type 2 diabetic nephropathy 90.68±110.92) (healthy controls36.49±28.20), mean of 24-hour urinary protein quantity (mg/ L) is higher compare to control (263.74±28.73 case) (control 180.22±47.94). The mean of Urine Creatinine (umol/L) in case group is 61.85±46.80 higher compared to control is 45.34±37.54, the mean of Microalbumin

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Creatinine Ratio is insignificantly higher in case 2.78±5.14 compared to control 1.52±4.14 (p>0.05). the mean of Transferrin (mg/g) is higher in case group 5.65±1.25compare to control 2.35±0.61. Ceruloplasmin (mg/L) in case group is higher 235.61±18.59 compare to control. IgG (g/L) is higher in case 11.93±1.79 compare to control 5.46±1.01.the mean of TNF-α (pg/ml) is higher 17.45±3.32 compare to control 9.45±1.67 and IL-6 (pg/ml) is higher in case group 29.40±12.27 compare to control 23.92±14.85 [Table 2].

Table No. 1: Diabetes profiles of studied patients

Blood Sugar (mg/dl)	**Group**		**P value**
	Case (n=98)	**Control (n=98)**	
Fasting Blood Sugar (mg/dl)	187.55±53.20	166.74±27.04	**<0.001**
Post-Prandial Blood Sugar (mg/dl)	271.81±83.29	240.21±48.40	**<0.001**
HbA1c (%)	9.27±2.33	8.13±1.48	**<0.001**

Blood sugar profile (fasting, post-prandial, HbA1c level and duration of diabetes) was significantlyhigher in case group in compare to control group (p<0.05).

Table No. 2: Urine (24 hours) inflammatory makers in studied patients

In Urine (24Hrs)	**Group**		**P value**
	Case (n=98)	**Control (n=98)**	
Urine Microalbumin (mg/L)	90.68±110.92	36.49±28.20	**<0.001**
24-hour urinary protein quantity (mg/ L)	263.74±28.73	180.22±47.94	**<0.001**
Urine Creatinine (umol/L)	61.85±46.80	45.34±37.54	**0.007**
Microalbumin Creatinine Ratio	2.78±5.14	1.52±4.14	0.060
Transferrin (mg/g)	5.65±1.25	2.35±0.61	**<0.001**
Ceruloplasmin (mg/L)	235.61±18.59	180.07±42.87	**<0.001**
IgG (g/L)	11.93±1.79	5.46±1.01	**<0.001**
TNF-α (pg/ml)	17.45±3.32	9.45±1.67	**<0.001**
IL-6 (pg/ml)	29.40±12.27	23.92±14.85	**0.005**

Urine (24 hours) inflammatory makers (Microalbumin, Protein, Creatinine, Transferrin, Ceruloplasmin, IgG, TNF-a and IL-6) was significantly higher in case group p<0.05; but Microalbumin Creatinine Ratio was

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insignificantly higher in case group in compare to controlgroup (p>0.05).

Table No.3: Association between blood sugar profile and inflammatory makers

		FBS	PPBS	HbA1c
Microalbumin	Pearson Correlation	0.059	0.165*	0.051
	Sig. (2-tailed)	0.410	**0.021**	0.478
Protein	Pearson Correlation	0.187**	0.171*	0.163*
	Sig. (2-tailed)	**0.009**	**0.017**	**0.023**
Urine Creatinine	Pearson Correlation	0.062	0.121	0.014
	Sig. (2-tailed)	0.387	0.091	0.849
Microalbumin Creatinine Raio	Pearson Correlation	-0.055	0.031	-0.026
	Sig. (2-tailed)	0.443	0.662	0.722
Transferrin	Pearson Correlation	0.227**	0.144*	0.163*
	Sig. (2-tailed)	**<0.001**	**0.045**	**0.022**
Ceruloplasmin	Pearson Correlation	0.113	0.121	0.104
	Sig. (2-tailed)	0.115	0.090	0.148
IgG	Pearson Correlation	0.269**	0.231**	0.288**
	Sig. (2-tailed)	**<0.001**	**<0.001**	**<0.001**
TNF-α	Pearson Correlation	0.138	0.134	0.238**
	Sig. (2-tailed)	0.054	0.061	**<0.001**
IL6	Pearson Correlation	0.128	0.115	0.236**
	Sig. (2-tailed)	0.074	0.109	**<0.001**

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Above table show the positive significant correlation of diabetes profile level with urine (24 hours) several inflammatory makers (p<0.05). Only microalbumin creatinine ratio and urine creatinine shows insignificant associated with diabetes level (p>0.05). While in our study ceruloplasmin was significantly associated with diabetes duration and IL-6 was significantly associated with uncontrol diabetes level (HbA1c) (p<0.05). But TNF-α was significantly associated with uncontrol diabetes level (HbA1c).

Discussion

DM is a global problem with approximately 194 million diabetic patients worldwide; 85– 95% of them suffering from type 2 DM. [13] DM was the fourth or fifth leading cause of death in most developed countries. An early manifestation of DN is microalbuminuria, which was classically defined as urine albumin excretion (UAE) between 30 and 300 mg/day, or equivalent amounts when timed overnight or spot urine samples were used [14]. Prevalence of

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microalbuminuria was about 12.6% to 25.3% in patients with type 2 DM [15].

The present hospital based, cross sectional, analytical study was conducted to study of serum & urinary inflammatory biomarkers in diabetic patients with and without diabetic

nephropathy in population residing in Malwa Region. **Ismail MI et al [16].** reported the

inflammatory cytokines (IL-6 and Hs-CRP) are thought to be significant serum markers of

induction of microalbuminuria and renal injury in patients with type 2 diabetes mellitus. Theycame to this conclusion after conducting a case-control study of the serum and urine biomarkers for early detection of diabetic nephropathy in type 2 diabetes mellitus. **Soni S et al [17].** performed a case-control study to examine the pro-inflammatory marker IL-6 and
microalbuminuria in subjects with type-2 diabetic nephropathy. They found that the subjects with diabetic nephropathy had higher blood pressure, serum levels of FBS, HbA1c, serum urea, serum creatinine, TC, TG, LDL, VLDL, IL-6, and lower levels of HDL, serum albumin,and A/G ratio. **Gupta S et al [18]**

This hospital based, cross sectional, analytical study was carried out in the Department of Biochemistry Index Medical College, Hospital and Research Centre Indore (M.P.). Patients aged between 35-60 years, irrespective of their gender diagnosed with T2DM and were suffering with Diabetic Nephropathy and Diabetic patients who has not been progressed to diabetic nephropathy were included as subjects to control group. The following patients were excluded from the study: those with diabetes mellitus (DM) but otherwise normal kidney function; those with any known renal anomaly; those with red blood cell wall defects; hemoglobinopathies; hypertension; cancers; acute febrile illness; and those taking any medication that could affect the patients' assessments of proteinuria. The concentrations of fasting plasma glucose (FPG) and high sensitivity C-reactive protein (hsCRP) were measured. The urine albumin-to-creatinine ratio (UACR) was used to estimate the degree of albuminuria. Creatinine was measured and estimated glomerular filtration rate (eGFR) was calculated. **Ismail MI et al [16], Soni S et al [17], Gupta S et al [18], Sharma D et al [19]** and **Mondal K & Mukherjee D [20]**

Present study was noted that the blood sugar profile (fasting, post-prandial, HbA1c) wassignificantly higher in case group in compare to control group. Urine (24 hours)
inflammatory makers (Microalbumin, Protein, Creatinine, Transferrin, Ceruloplasmin, IgG, TNF-a and IL-6) was significantly higher in case group; but Microalbumin Creatinine Ratiol was insignificantly higher in case group in compare to control group. **Ismail MI et al [21]**

Conclusion

Blood sugar profile (fasting, post-prandial, HbA1c level) was significantly higher in case group in compare to control group. Urine (24 hours) inflammatory makers (Microalbumin, Protein, Creatinine, Transferrin, Ceruloplasmin, IgG, TNF-a and IL-6) was significantly higher in case group. Microalbumin Creatinine Ratiol was insignificantly higher in case group in compare to control group. The positive significant correlation of diabetes profile level with urine (24 hours) several inflammatory makers. Microalbumin creatinine ratio and urine creatinine shows insignificant associated with diabetes level and duration. Ceruloplasmin was significantly associated with diabetes duration. IL-6 was significantly associated with uncontrol diabetes level (HbA1c). TNF-α was significantly associated

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with both uncontrol diabetes level (HbA1c).

Diabetic nephropathy, which results in renal impairment, is the primary cause of death among patients with type 2 diabetes. Diabetes mellitus is primarily caused by independent risk factors such as fasting and postprandial plasma glucose, HbA1c (glycosylated haemoglobin), , and proinflammatory markers such as TNF-α, IgG, Transferrin, Ceruloplasmin, urine protein,IL-6. Patients with type-2 diabetes have demonstrated an increased excretion of albumin in their urine, which has been linked to these biomolecules. This study clearly shows that these inflammatory chemicals contribute to the development of diabetic nephropathy on their own.

Reference:

1. Yach D, Hawkes C, Gould C and Hofman K (2004): The global burden of chronic diseases: Overcoming impediments to prevention and control. JAMA; 291: 2616–2622.
2. Crook M (2004): Type 2 diabetes mellitus: A disease of the innate immune system?An update. Diabet Med; 21: 203–207.
3. Choudhary N and Ahlawat RS (2008) : Interleukin-6 and Creactive protein in pathogenesis of diabetic nephropathy: new evidence linking inflammation, glycaemic control, and microalbuminuria Iran J Kidney Dis; 2(2): 72-79.
4. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 2005; 28:164-176
5. Navarro JF, Mora C, Gómez M, Muros M, López-Aguilar C, García J. Influence of renal involvement on peripheral blood mononuclear cell expression behaviour oftumour
6. Navarro JF, Mora C, Muros M, García J. Urinary tumour necrosis factor-alpha excretion independently correlates with clinical markers of glomerular and tubulointerstitial injury in type 2 diabetic patients. Nephrol Dial Transplant 2006; 21: 3428-3434
7. Navarro JF, Mora C, Maca M, Garca J. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. Am J Kidney Dis 2003; 42: 53-61
8. Niewczas MA, Ficociello LH, Johnson AC, Walker W, Rosolowsky ET, Roshan B, et al. Serum concentrations of markers of TNF-α and Fas-mediated pathways and renal function in nonproteinuric patients with type 1 diabetes. Clin JAm Soc Nephrol 2009; 4: 62-70.
9. Niewczas MA, Ficociello LH, Johnson AC, Walker W, Rosolowsky ET, Roshan B, et al. Serum concentrations of markers of TNF-α and Fas-mediated pathways and renal function in nonproteinuric patients with type 1 diabetes. Clin JAm Soc Nephrol 2009; 4: 62-70
10. Gohda T, Niewczas MA, Ficociello LH, Walker WH, Skupien J, Rosetti F, et al.Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. J Am Soc Nephrol2012; 23: 516-524
11. Holm J, Ravn J and Ingemann Hansen S (2006): Urinary excretion of alpha 1- microglobulin and albumin in acute myocardial infarction. Correlation with plasma concentrations of troponin I and C reactive protein. Scand J Urol Nephrol ; 40: 339-344.
12. Mojahedi MJ, Bonakdaran S, Hami M, Sheikhian MR, Shakeri MT and Aiatollahi H (2009): Elevated serum C-reactive protein level and microalbuminuria in patients with type-2 diabetes mellitus. Iran J Kidney Dis; 3(1): 12-

16.

13. Yach D, Hawkes C, Gould C and Hofman K (2004): The global burden of chronic diseases: Overcoming impediments to prevention and control. JAMA; 291: 2616–2622.
14. Redon J and Williams B (2002): Microalbuminuria in essential hypertension. Redefining the threshold. J Hypertens; 20: 353–355.
15. . Viswanatha n V, Snehalatha C, Shina K and Ramachandran A (2002): Persistent microalbuminuria in type 1 diabetic subjects in South India. J Assoc Physicians India;50: 1259-1261
16. Jamshid R, Ahmad M, Farahnaz B, Raha A, Hooman Y. Significance of serum and urine neuraminidase activity and serum and urine level of sialic acid in diabetic nephropathy. International urology and nephrology 2011 January; 43(4).
17. Hojs R, Ekart R, Bevc S, Hojs N. Markers of Inflammation and Oxidative Stress in the Development and Progression of Renal Disease in Diabetic Patients. Nephron 2016; 133(3): 159–162.
18. Motawi TK, Shehata NI, El-Nokeety MM, El-Emady YF. Potential serum biomarkers for early detection of diabetic nephropathy. Diabetes Research and Clinical Practice February 2018; 136:150-158.
19. Uwaezuoke SN. The role of novel biomarkers in predicting diabetic nephropathy: a review. International Journal of Nephrology and Renovascular Disease 2017:10 221–231.
20. Shao M, Lu H, Yang M, Liu Y, Yin P, Li G, et al. Serum and urine metabolomics reveal potential biomarkers of T2DM patients with nephropathy. Ann Transl Med 2020; 8(5):199.
21. Jamshid R, Ahmad M, Farahnaz B, Raha A, Hooman Y. Significance of serum and urine neuraminidase activity and serum and urine level of sialic acid in diabetic nephropathy. International urology and nephrology 2011 January; 43(4).