Status of pro-inflammatory Marker IL-6 and Microalbuminuria in type- 2 Diabetic Nephropathy subjects

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Abstract

Introduction: Diabetic nephropathy is a progressive renal complication of type-2 diabetes. Inflammation is found as main cause of diabetic nephropathy pathogenesis. Cytokines influence inflammatory and immune responses by their effects on cells. Chronic low-grade inflammation together with innate immune system activation is closely associated in diabetes mellitus pathogenesis and its micro-vascular complications.

Aim: The aim of this study is to estimate the level of pro- inflammatory marker IL-6 and microalbuminuria in subjects diabetic nephropathy compared to non-diabetic healthy subjects.

Materials and Method: The study was conducted at Government Medical College, Banda as well as M.L.N. Medical College, Allahabad (UP).The study was made on a group of 150 selected subjects. From these, 50 were healthy subjects without Diabetes and100 were patients of diabetic nephropathy of type-2 DM. They were evaluated by measurement of various parameters such as BP, blood parameters as FBS, HbA1c, lipid profile level, serum urea, serum creatinine, Serum albumin, A:G ratio and inflammatory marker like IL-6.

Results: Elevated Blood Pressure and serum levels of FBS, HbA1c, serum urea, serum creatinine, TC, TG, LDL, VLDL, IL-6 level and reduction in HDL, Serum albumin, A/G ratio were observed in diabetic nephropathy subjects. These values of biochemical markers were found to be significant statically.

Conclusion: An increase in the levels of inflammatory marker like IL-6 in DN patients with contrast to normal levels of these parameters shows a significant role in development of DN.

Keywords: Diabetic nephropathy, Glycosylated hemoglobin, Interleukins-6.

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Introduction

Diabetes mellitus (DM) is the most frequent endocrine disease characterized by metabolic defects and by long term complications involving the retinopathy, nephropathy, neuropathy and blood vessels etc. Type-2 DM, considered a chronic inflammatory condition resulting from obesity induced dys regulation of adipocytes, produce elevated inflammatory cytokines levels such as IL-6, high sensitive- C-reactive protein and TNF – α.[1] This persistent inflammatory state further contributes to insulin resistance and abnormal endothelial vascular reactivity.[2,3] Chronic inflammation exacerbates renal damage through the mechanisms of vascular endothelial cell impairments. Population-based studies suggest that these inflammatory parameters are strong predictors of the development of Diabetic nephropathy (DR) in type-2 diabetic subjects.[4-6]

Approximately one third of all diabetic patients are affected by DN,[7] which produces significant social and economic burdens[8] and forms the most common cause of end stage renal disease (ESRD).[9,10] The important inflammatory cytokines such as IL-1, TNF-α and IL-6 are involved in the pathogenesis of diabetes mellitus.[11] Recent studies shows inflammation, specifically inflammatory cytokines are involved in development of microvascular diabetic complications like retinopathy, neuropathy, nephropathy and myopathy etc.[12-17]

This study is designed to explore the relationship between the commencement of DN and the various inflammatory factors. However no study has been performed that compared the inflammatory cytokines in type-2 diabetes mellitus with nephropathy. The aim of our study was to evaluate the inflammatory marker like IL-6 and microalbuminuria, their association with diabetic nephropathy in an eastern Uttar Pradesh of India.

Materials and Method

This study was done in Government Medical College, Banda as well as M. L. N. Medical College, Allahabad (UP) between November 2015 and February 2017. Subjects were selected from department of medicine and those who attending the outpatient camps at Allahabad region of UP. The study was conducted in 150 subjects, diagnosis being based on duration of type-2 DM.

The patients were divided into two groups-

Group 1: 50 were healthy individuals free from any diabetes mellitus complications.

Group 2: 100 were type-2 diabetic patients with diabetic complications such as diabetic nephropathy.

A written consent was obtained from the patients. This study was approved by institutional ethical committee. A questionnaire regarding the demographic data such as age, sex, height, body weight and duration of diabetes were measured while wearing light weight clothing but not shoes. Smoking habit, vegetarian and non-vegetarian, family history of diabetes, Blood pressure, hypertension, MI and renal disease were also recorded for each subject.

Presence of diabetic nephropathy has been assessed by measuring GFR and urinary excretion of albumin in morning urine sample.

Albuminuria: Significant albuminuria is defined as urinary protein excretion of albumin ≥30mg/dl albumin in 24hrs collection or ≥30 mcg/mg of creatinine in spot urine collection (morning urine sample).

Microalbuminuria: Urinary excretion of 30-300 mg/dl albumin in 24 hrs collection or 30-300 mcg/mg of creatinine in spot urine collection

Macroalbuminuria: Urinary excretion of ≥300 mg/dl albumin in 24 hrs collection ≥300 mcg/mg of creatinine in spot urine collection.

Diabetic patients suffering from any other medical problems were debarred from the study. The body mass index (BMI) was also calculated as weight (Kg) divided by height (m) squared. Venous blood was collected after 12 hours fasting condition (complete physical and mental) rest into test tubes with Ethylene Diamine Tetra Acetic Acid (EDTA) for HbA1c. Morning urine sample was collected in a container (without preservative) for analysis of urinary albumin.

The patients were further evaluated by the measurement of Blood Pressure, FBS, HbA1c, serum urea, serum creatinine, TC, TG, LDL, VLDL, HDL, Serum albumin, A:G ratio (we were done using MERCK microlab300 Semi autoanalyser for biochemical parameters) and IL-6 levels (Immunotech, Backman Coulter, France ELISA Kit, 96 wells -cat. # IM1120).

The data was analyzed by Statistical packages for social science (SPSS version 22.0). Mean and standard deviation were analyzed for quantitative variables. Independent sample t-test was done for compare mean of all the quantitative variables between the two groups of diabetic patients were considered significant (0.01 and 0.001 level). Correlation coefficient (r) analysis was done for risk factors of diabetic nephropathy of type-2 DM.

Results

Comparison of means of physiological and serum biochemical parameters between patients of type-2 DM with diabetic nephropathy and healthy control groups are presented in the Table 2. The values of all these biochemical parameters except HDL, Serum albumin and A/G ratio were increased in diabetic nephropathy patients with type-2 DM as compared to healthy control group.

A positive correlation was found between IL-6 and Diabetic Nephropathy in patients with type-2 DM. Pro-inflammatory cytokine like IL-6 (r= 0.656, p<.001) increased with increased urinary albumin excretion.

It was seen that after proper therapeutic methods of treatment to decrease the urinary albumin excretion, the level of IL-6 returned back to normal in Diabetic Nephropathy patients.

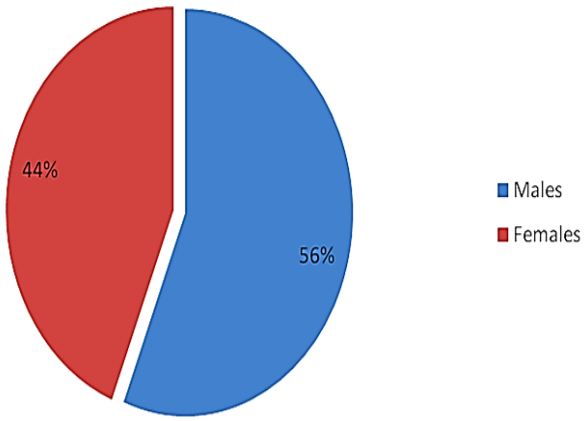


Fig. 1: A total 0f 100 diabetic patients with DN were selected for this cross sectional study. Out of which 56 (56%) were males and 44 (44%) females

Table 1: Distribution of DN patients with type-2 DM according to sex and age wise

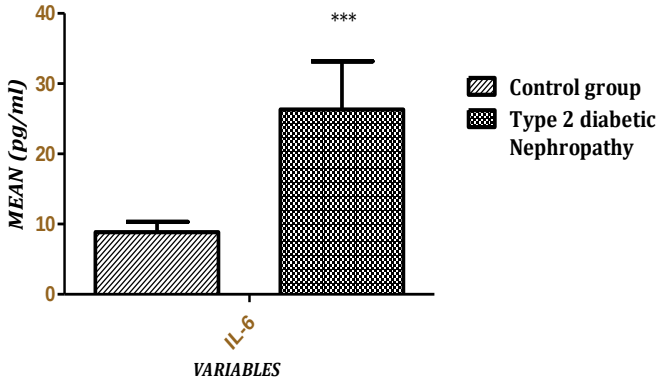
Age group (years)	Male	Female	Total
30-39	04	02	06
40-49	22	15	37
50-60	40	17	57
Total	56	44	100

Table 2: Comparison of laboratory abnormalities (physiological and biochemical parameters) between Healthy Control and Type-2 nephropathy subjects

Study Variables	Group 1=50 Healthy Controls	Group 2=100 Type-2Diabetic Nephropathy	t-Values
Age	48±8.80	52±8.24	3.246**
Duration of diabetes	-	12.5±4.32	-
BMI (kg/m²)	21.42± 1.56	27.62± 3.68	2.026*
BP Systolic (mm/Hg)	106.6±6.18	166.85±12.89	5. 408**

BP Diastolic (mm/Hg)	78.6±4.28	110.45±6.85	2.052*
FBS	86.22±12.26	188.0±37.7	6. 246**
HbA1c	5.10±0.46	11.2±2.26	5. 326**
Total Cholesterol	158.3±24.09	252.4±41.9	4.234**
TG (mg/dl)	108.04±19.49	186.7±45.05	5. 459**
HDL (mg/dl)	46.45±6.14	28.27±7.02	3. 627**
LDL (mg/dl)	110.1±16.20	158.7±39.3	6. 843**
VLDL (mg/dl)	29.38±4.09	45.89±11.3	2. 215*
Serum urea (mg/dl)	26.28±4.41	61.25±12.2	4.542**
Serum creatinine (mg/dl)	0.96±0.06	3.60±1.16	8. 482**
Serum Albumin(g/dl)	4.46±1.02	2.80±0.20	4.267**
A/G Ratio	1.24±0.06	3.25±0.22	7.902**
IL-6(pg/ml)	8.86±1.46	26.32±6.86	4. 426**

* Significant at P<0.01,
** Significant at P<0.001



Graph: Comparison of proinflammatory markers between Healthy Control and Type-2 Diabetic nephropathy subjects.

***** Highly Significant (P<0.001)**

Fig. 2: Comparison of pro-inflammatory marker IL-6 between Healthy subjects and Type-2 diabetic with Diabetic nephropathy subjects

Table 3: Correlation studied of inflammatory marker cytokine IL-6 with the other biochemical parameters in Type-2 diabetic with Diabetic nephropathy patients

Biochemical Parameters	R	P
FBS	0.264	P<0.001
HbA1c	0.547	P<0.001
Serum Urea	0.264	P<0.001
Serum Creatinine	0.432	P<0.001
Microalbuminuria	0.656	P<0.001

Discussion

Type-2 DM has increased drastically in few decades together with its microvascular complications like retinopathy, neuropathy, nephropathy and macrovascular complications like ischemic heart disease, peripheral vascular diseases etc. Although diabetic nephropathy is traditionally considered a non-immune disease, accumulating evidence now indicates that immunologic and inflammatory mechanisms play a significant role in its development and progression.[18,19] A potential participation of inflammatory cytokines in the pathogenesis of diabetic retinopathy was suggested for the first time in 1991 by Hasegawa et al.[20] Hypertension plays a key role in the development of DN. In our study the mean Systolic Blood Pressure values were 106.6±6.18 in controls and 166.85±12.89 in cases and mean Diastolic Blood Pressure values were 78.6±4.28 in controls and 110.45±6.85 in cases which is statistically significant. Our study is supported with several studies.[21,22]

Our study showed that fasting blood sugar levels in type-2 DN group (mean 188.0±37.7) were higher than healthy group (mean 86.22±12.26), which confirmed the dysglycemia is seen in these patients (P value <0.001). Hyperglycemia is the most common factor in

the pathogenesis of diabetic nephropathy. When glucose reacts with N-terminal valine of each β-chain of HbA (non-enzymatically) forming glycated compounds. Hyperglycemia causes toxic effects and results in kidney damage by directly altering many biochemical pathways and via intracellular signaling pathways.[23]

The mean of HbA1c were directly related to the risk of developing retinopathy and nephropathy. In our study the mean HbA1c values were 5.10±0.46 in controls and 11.2±2.26 in patients which is highly significant (P<0.001).[21,22]

In our study we observed that lipid profile- serum total cholesterol, triglyceride and low density lipoprotein were higher in DN patients and whereas high density lipoprotein lower in these diabetic patients than healthy control and the results were statistically significant (P value <0.001). Similar results were reported by others studies.[24]

Measurement of blood urea has been broadly used as an indicator of renal function.[25] In this study the mean values of blood urea were 26.28±4.41 in controls and 61.25±12.2 in DN subjects which is highly significant (P<0.001). Serum creatinine level is the useful indicator of renal function. In this study the mean values of serum creatinine were 0.96±0.06 in controls and 3.60±1.16 in patients which is statistically significant (P<0.001). Our study is supported by several studies.[26,27,28]

Microalbuminuria is defined as the excretion of 30 to 300 mg of albumin per day in urine. It is an earliest symptom of hypertensive renal damage in diabetic patients. It is due to common endothelial dysfunction due to effects of cytokines and other inflammatory mediators free during the intense inflammatory responses.[29] Our study is supported by Shivananda Nayak B and Geetha Bhaktha, who confirmed significantly increased urinary excretion of albumin in diabetic nephropathy patients compared to healthy subjects.[30] Approximately 40% of persons with diabetes develop diabetic nephropathy, established as albuminuria and/or reduced glomerular filtration rate (GFR). Even mild stage of albuminuria and decrease in glomerular filtration rate are associated with increased risks of end-stage renal disease (ESRD), cardiovascular disease (CVD) and premature deaths.

Our study showed that proinflammatory cytokine IL-6 level in type-2 diabetic nephropathy group (mean 26.32±6.86) were increase than healthy control group (mean 8.86±1.46), which is significant in these patients (P value <0.001). In 1991, Sekizuka et al.[31] reported that serum levels of IL-6 cytokine was statically significantly higher in diabetic nephropathy patients than the levels observed in diabetic patients without nephropathy, showing the relation of these substances with DN. Early after that report, Suzuki et al.[32] analyzed kidney biopsies in patients with diabetic nephropathy by high-resolution in-situ hybridization. They found that cells infiltrating the mesangium, interstitium, and renal tubules were positive for mRNA encoding IL-6. Moreover, they observed a relationship between the severity of diabetic glomerulopathy and expression of IL-6 mRNA in glomerular cells, which indicated that IL-6 may affect the dynamics of extra-cellular matrix. In this studies type-2 diabetic patients demonstrate a significant association between IL-6 and thickening of glomerular basement membrane, a very important lesion of diabetic nephropathy and a good predictor of renal development.[33,34] Elevated levels of**,** Pro-inflammatory cytokines IL-6, TNF-α and fasting insulin level are more commonly seen in type- 2 diabetic patients with several complications like retinopathy, nephropathy, cardiovascular problem, neuropathy etc.[35]

Correlation study between inflammatory marker IL-6 and biochemical parameters: There was a large positive correlation between serum inflammatory marker IL-6 and systolic BP in DN cases (r=0.596) whereas moderate positive correlation also found between serum inflammatory marker IL-6 and diastolic BP in cases (r=0.331).

A small positive correlation was observed between serum inflammatory marker IL-6 and FBS in diabetic nephropathy subjects (r=0.264), indicating the role of hyperglycemia for renal damage. There was a significantly large positive correlation between serum IL-6 and HbA1c (r=0.547) in diabetic nephropathy cases.

Correlation study was shown that a small positive correlation between serum inflammatory marker IL-6 and blood urea (r=0.264) whereas a moderate positive correlation was found between serum inflammatory marker IL-6 and serum creatinine in cases (r=0.432).

Our correlation study was also shown large positive correlation between serum inflammatory marker IL-6 and urinary albumin (r=0.656) in DN cases showing that increases pointing contributory role of serum inflammatory marker IL-6 towards renal damage. This supports our findings of involvement of IL-6 in cause of DN. Results from this study reveals that inflammatory molecules increase during the course of development of DN in type-2 diabetic patients. Treatments to reduce albumin excretion in urine have shown to reduce the levels of these inflammatory markers. Further study and research is required for the confirmation of the significance of role of anti-inflammatory drugs in the prevention and treatment of DN in the patients of type-2 DM.

Conclusion

The most important cause of mortality in type- 2 diabetic mellitus patients is diabetic nephropathy which result in renal damage. Our study has shown that there is a high prevalence of DN in type-2 Diabetic patients in India. Independent risk factors of DM to be blood

pressure, fasting plasma glucose, HbA1c (Glycosylated hemoglobin), lipid profile, along with the pro-inflammatory marker like IL-6 are responsible for development of diabetic nephropathy. These biomolecules have shown association with the increase in albumin excretion in urine of type-2 Diabetic patients. According to this study it is evident that these inflammatory molecules have independent role in the development of diabetic nephropathy.

Limitation

The limitation of the present study was that the sample size was less. More elaborative studies with large study population are needed. Some clinical trials regarding use of other inflammatory markers also prove valuable.

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Conflict of Interest

There has been no conflict of interest at any stage of the study.

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