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OXIDATIVE STRESS, INTERLEUKIN (IL-6) AND ATHEROGENIC INDEX OF PLASMA IN DIABETIC NEPHROPATHY

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Introduction: Diabetes mellitus (type2 DM) is a proinflammatory state with increased oxidative stress, which could tremendously increase the micro and macrovascular complications. This study was focused to explore the association between IL-6 and oxidative stress, and to assess the cardiovascular risk in type 2 diabetic patients of south India.

Materials and Methods: Sixty patients with type 2 diabetes were included in the study and were further divided into 3 groups based on urinary albumin excretion rate (UAE), and the results were compared with the age matched normal individuals as control. Group I: normal controls (n=10), Group II: Diabetic patients with normoalbuminuria (n=24), Group III: Diabetic patients with microalbuminuria (n=17),GroupIV: Diabetic patients with macroalbuminuria (n=19).

Result: Plasma IL-6 and serum MDA were significantly high in microalbuminuria and macroalbuminuria as compared to normal controls and normoalbuminuria. Plasma TAC was significantly decreased in all the groups of diabetic patients as compared to normal controls. Significant increase in lipid parameters and AIP were observed in macroalbuminuria and microalbuminuria as compared to normal controls. Plasma IL-6 showed negative correlation with HDL-c (r=-0.255) and significant positive correlation was observed between MDA and HbA1c (r=0.537) in diabetic patients.
Conclusion: Increased IL-6, MDA levels and decreased plasma TAC levels in diabetic patients revealed inflammation with increased oxidative stress, which may involve in the development of renal damage. The associated altered lipid profile and high risk AIP indicates the risk of developing cardiovascular complications in diabetic patients with macroalbuminuria.

Keywords: Hyperglycemia, Interleukin-6, Oxidative stress, Total antioxidant capacity, Atherogenic Index of Plasma.

INTRODUCTION

Diabetes is a chronic inflammatory state associated with insulin resistance (Festa A, et.al., 2000). Chronic hyperglycemia has been shown to be responsible for multiple micro and macrovascular complications as a result of hyperglycemic damage through four major biochemical processes, including advanced glycation end products (AGEs), the polyol pathway, the hexosamine pathway and activation of protein kinase C as described by (Brownlee MB, 2002).

Many in vitro studies and more recent cross sectional data suggest that interleukin-6 (IL-6) and C-reactive protein (CRP), the two sensitive physiological markers of subclinical systemic inflammation are associated with hyperglycemia, insulin resistance and overt type 2 DM (Tsigos C, et.al., 1997).Various possible explanations may underline the advantage of IL-6 over CRP as an outcome predictor. One possibility is that, being located upstream in the cascade of events which may lead to the synthesis of many acute-phase reactants, IL-6 is a better marker of the inflammatory burden affecting the development of cardiovascular disease (Castell JV, et.al., 1989).Another possibility is that levels of IL-6 vary less than those of CRP, leading to a more accurate classification of patients at risk when one single sample is taken (Vincenzo Panichi et.al., 2004). Finally, the toxic effects of IL-6 on the heart and peripheral vasculature might be stronger than those of CRP (Wollert KC, et.al., 2001).

Many invitro studies have shown that IL-6 affects extracellular matrix dynamics at mesangial and podocyte levels, stimulates mesangial cell proliferation, increases fibronectin expression, and enhances endothelial permeability (Vatesta DM, et.al., 2005). These mechanisms strongly involve in the development of kidney injury in patients with diabetes. Patients with diabetic nephropathy especially in the context of type 2 DM, have a high incidence of cardiovascular disease (CVD), which leads to increased mortality (Mora C, et.al, 2004).

The mechanism of elevation of serum IL-6 levels in type 2 DM remain unclear, although oxidative stress is a candidate. Oxidative stress might be implicated in promoting a low grade systemic inflammation in patients with type 2 DM (Amalich F, et.al., 2005). Activation of nuclear factor –kappa B (NF-κB) through oxidative stress induced by hyperglycemia increases the concentration of proinflammatory cytokines (Esposito K, et.al., 2002).

Therefore IL-6, oxidative stress, and the status of the antioxidant defense mechanism appear to be closely associated in their common outcome of both renal and cardiovascular disease in diabetic patients. Taking these observations into consideration, the present study was aimed to explore the relationship of IL-6, oxidative stress, Total Antioxidant capacity(TAC), Urinary Albumin Excretion (UAE) along with lipid profile in subjects with type 2 DM.

METHODS

The present study included 60 patients, who attended as outpatient to the Kidney Research Centre, Madurai from January-2009 to March-2009 and they were divided into 3 groups based on urinary albumin excretion rate (UAE).Group I: controls (n=10), Group II: Diabetic patients with normoalbuminuria(n=24),(UAE<20µg/ml), GroupIII: Diabetic patients with microalbuminuria(n=17),(UAE>20µg/ml), GroupIV: Diabetic patients with macroalbuminuria(n=19),(UAE >200µg/ml). Informed written consent was obtained from all the subjects. Exclusion criteria were: patients with renal insufficiency, treatment with angiotensin-converting enzyme inhibitors or hypolipidemic drugs, acute infectious diseases, and pregnancy.

Blood was collected by venipuncture into an EDTA venipuncture tube .Plasma was separated from the cells and stored at -20ºC. Urinary albumin was quantified by immunoturbidometric method (Hofmann W, et.al., 1989). Estimation of cytokine IL-6 (e- bioscience) was assayed by ELISA method. MDA level in serum was estimated by measuring the pink colored chromophore formed by the reaction of thiobarbituric acid with malondialdehyde according to the method of Satoh (Satoh K, 1978). TAC was determined by the modified method of Benzie and Strain (Benzie IFF, et.al., 1996). Basic biochemical parameters were assayed in automated analyzer using diagnostic kits.

STATISTICAL ANALYSIS

The results are expressed as Mean ± SD (standard deviation). Analysis of Variance (ANOVA) was used to compare the four groups and post-hoc Tukey test was applied to compare individual groups. The mean difference is considered significant at $p<0.05$. Pearson's correlation analysis was used to determine correlation between different parameters.

RESULTS & DISCUSSION

Table 1 shows the baseline characteristics and biochemical parameters of both control and diabetic groups. There were no significant difference between diabetic patients and controls with respect to age, sex distribution, and BMI. Statistical significance was seen for SBP in all diabetic patients when compared to normal controls. DBP was significantly high in microalbuminuria and macroalbuminuria as compared to controls and normoalbuminuria. Statistical significance was seen for serum FBS, serum creatinine, eGFR in all diabetic patients when compared to controls. $HbA1_c$ was statistically significant in macroalbuminuria when compared to control and normalbuminuria. Results of the present study showed that there was no association between BMI and IL-6, and BMI did not differ between the groups of diabetic patients. The considerable amount of IL-6 is synthesized by the adipose tissue (Mohamed-Ali, et.al.,1997), and the difference in IL-6 attributes to the differing severity of diabetic nephropathy (DN), and supported a relationship between diabetic nephropathy and low grade inflammation in patients with type 2 DM (Nikhil C, et.al., 2008).

Table 1: Characteristics of diabetic patients in relation to UAE (μg/ml).

Variables	Control	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
Number	10	24`	17	19
Age (years)	47±5.6	53±6.7	48±7.3	50±5.5
Duration (years)	------	5.96±1.93	7.8±2.4	8.14±2.52
BMI (kg/m^2)	23.9±2	25.7±4.33	25±3.5	26±4.79
SBP (mm/Hg)	113±9	137±13.4[a]	138±15.7[a]	144±22.7[a]
DBP (mm/Hg)	76±6.6	81.8±6.88	87.3±7.99[a]	89.8±14[a,b]
FBG(mg/dl)	76±6.6	145±29[a]	148±41[a]	150±32[a]
Urea(mg/dl)	25.4±6.1	42.01±17.03	46.3±29	71.71±46.27
Creatinine(mg/dl)	0.82±0.15	1.41±17.03[a]	1.51±0.62a	1.86±0.7[a]
eGFR(ml/min/1.73m2)	108.7±26.91	60±25.6[a]	55.3±26.8[a]	44.5±27.3[a]
HbA1c %	5.43±0.42	6.4±2	7.5±1.5[a]	8.7±2.1[a,b]
UAE(ug/ml)	-	11.8±4.18	74.47±39.92[b]	226±24.01[b,c]

Data are expressed as mean + SD. a: significantly different from control group at $p<0.05$;
b: significantly different from the diabetic patients with normoalbuminuria at $p<0.05$;
c: significantly different from the diabetic patients with microalbuminuria at $p<0.05$.

Plasma IL-6 concentrations were significantly higher in diabetic patients than in controls as shown in Table 2. The levels of IL-6, both secreted as well as intracellular IL-6, were significantly increased with high glucose. Under high glucose, monocytes secrete increased amounts of IL-6 via upregulation of PKC, p38MAPK and NF-κB activity, leading to increased IL-6 transcription and release (Sridevi D, et.al., 2005). The present study demonstrated that significant increase in plasma IL -6 was found in macroalbuminuria and microalbuminuria, when compared to normoalbuminuria. Several previous reports have suggested associations between different inflammatory markers and the severity of diabetic nephropathy, although with conflicting results (Stehouwer CD, et.al.,2002, Saraheimo M, et.al., 2003). Several acute-phase markers which were high in patients with overt nephropathy compared with patients with normal AER , and the acute-phase markers of inflammation are associated with nephropathy status and GBM thickening, suggesting a role for inflammation in the pathogenesis of diabetic glomerulopathy (Vestra DM, et.al.,2005). Oxidative stress did not influence this cytokine level in diabetic nephropathy, as there was no significant change in different nephropathy stages and diabetes may be associated with enhanced IL- 6 level but may not be an indicator for nephropathy (Alsancak, et.al., 2003). Table 4 shows significant association between UAE and IL-6 which supports the hypothesis of a link between inflammation and diabetic nephropathy. The expression of IL-6 mRNA in glomerular cells is related to the severity of diabetic glomerulopathy which contributes to both mesangial expansion and glomerular basement membrane thickening (Suzuki D, et.al., 1995).

Table 2: Parameters of study subjects in relation to UAE (μg/ml).

Parameter	Control	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
IL-6 (pg/ml)	1.7+1.41	3.2+1.1	5.3+2.6a,b	5.7+3a,b
MDA(nmol/ml)	0.94+0.2	1.2+0.8a	2.5+0.5a,b	3.8+0.9a,b,c
TAC(umol/l)	796+467	373+58.8a	363+57.4a	361+30.3a

Data are expressed as mean + SD.
a: significantly different from control group at $p<0.05$;
b: significantly different from the diabetic patients with normoalbuminuria at $p<0.05$;
c: significantly different from the diabetic patients with microalbuminuria at $p<0.05$.

Table 3: Lipid parameters of study subjects in relation to UAE (μg/ml).

Parameters	Control	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
T. Chol (mg/dl)	162±10	203±32.5[a]	222±31.3[a]	226±24[a]
TG (mg/dl)	145.2±10.07	156.2±21.8	166.3±26.63	195±28.54[a,b,c]
LDL (mg/dl)	77.86±12.28	123±32.6[a]	141±29.7[a]	141±27[a]
VLDL (mg/dl)	29.04±2.01	31.23±4.3	33.25±5.32	39.01±5.7[a,b,c]
HDL-c (mg/dl)	55±7.7	48.9±11.1	45±11.2	42.8±7.0[a]
LDL/HDL	1.44±0.36	2.76±1.0[a]	2.82±0.9[a]	3.43±1.0[a]
TC/HDL	2.9+0.42	4.5+1.2[a]	4.5+1.2[a]	5.36+1.1[a,b]
AIP	0.08±0.04	0.18±0.1	0.18±0.1	0.3±0.09[a,b,c]

Data are expressed as mean ± SD.
a: significantly different from control group at $p<0.05$;
b: significantly different from the diabetic patients with normoalbuminuria at $p<0.05$;
c: significantly different from the diabetic patients with microalbuminuria at $p<0.05$.

Table 4 : Correlation analysis between IL-6 and MDA with other biochemical parameters.

Parameters	r value	Significance
MDA & HbA1c	0.537	P<0.05
IL- 6 & HDL-c	-0.255	P<0.05
IL- 6 & UAE	0.402	P<0.01

Lipid peroxidation, one of the radical reactions *in vivo*, is an important index for oxidative stress (Baynes, 1991). In our study, lipid peroxide levels increased significantly in diabetic patients as compared to normal controls (Table 2). In addition, a significant increase in lipid peroxide levels was found among the groups of the diabetic patients with macroalbuminuria and microalbuminuria when compared to normoalbuminuria. An oxidative stress was increased in diabetes and the overproduction of ROS in diabetes was a direct consequence of hyperglycemia. Diabetes patients have more severe oxidative stress than normal persons. Oxidative stress is higher in diabetic nephropathy when compared to diabetic patients without complications
(Pan HZ, et.al., 2009,Aslan M et.al., 2007).

A significant decreased level of TAC was observed in all groups of diabetic patients irrespect of renal status (Table 2). Results of the present study indicate that the oxidative stress was increased and there was imbalance between oxidant – antioxidant defense according to the severity of the diabetic nephropathy. In patients at the acute phase of the disease decreased total antioxidant capacity may lead to abnormal lipid peroxidation, resulting in a high rate of glomerular injury. On the other hand prolonged lipid oxidation may lead to diminished antioxidant activity (Jyoti**,** et.al., 2010). Hyperglycemia induced oxidative stress, along with soluble advanced glycation end products and products of lipid peroxidation, possibly serves as a key activator of upstream kinases, leading to induction of inflammatory gene expression (Schmidt AM, et.al., 1999)

Elevated circulatory IL-6 concentrations predict cardiovascular morbidity and overall mortality in non-renal as well as renal patients (Ridker PM, et.al.,2000). Increased oxidative stress, with elevated plasma IL-6 in patients with nephropathy increases the risk of cardiovascular disease **(**Mattock MB, et.al., 1998, Iwasaki T, et.al., 2008,). According to the present study, all groups of diabetic patients showed significant dyslipidemia as compared to the controls (Table 3). There was significant increase in TG in macroalbuminuria when compared to microalbuminuria and normoalbuminuria, in addition negative association was found between IL-6 with HDL-c in diabetic patients (Table 4). Hypertriglyceridaemia usually accompanies decreased HDL-c which is also a prominent feature of plasma lipid abnormalities seen in diabetic subjects (Howard BV, et.al., 1987). An increased plasma concentration of TGs is associated with an increased incidence of coronary artery disease (CAD) (Hokanson JE**,** et.al., 1996). The Atherogenic Index of Plasma (AIP), defined as log(TG/HDL-c), has recently been proposed as a marker of plasma atherogenicity because it is increased in people at higher risk for coronary heart disease and is inversely correlated with LDL particle size (Tan MH, et.al.,2004). The results of the present study showed that statistically significant increase in AIP in macroalbuminuria and microalbuminuria as compared with controls and normoalbuminuria.

CONCLUSION

In summary, this study suggest an additional aspect of low grade inflammation, with increased oxidative stress and decline in TAC which was significantly correlated with the renal involvement and this association may contribute to the progression of nephropathy in type 2 diabetes. High risk AIP and altered lipid parameters suggests the increase in cardiovascular risk among diabetic nephropathy patients than diabetic patients without nephropathy.

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ABBREVATIONS

BMI - body mass index SBP - Systolic blood pressure DBP - Diastolic blood pressure.
eGFR – Estimated Glomerular Filteration Rate HbA1c – Glycosylated hemoglobin
UAE – Urinary Albumin Excretion IL- 6 – Interleukin 6 MDA – Malondialdehyde ROS – Reactive oxygen Species TAC – Total Antioxidant Capacity T. Chol - Total cholesterol TG – Triglycerides
LDL-c - Low density lipoprotein VLDL - Very low density lipoprotein HDL-c - High density lipoprotein.
AIP - Atherogenic Index of plasma

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