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Potential role of transforming growth factor β1 and interleukin-6 in elderly type 2 diabetes mellitus patients

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Abstract: *Background:* Studies suggest that various cytokines like TGF-β1 and IL-6 mediate development and progression of diabetic nephropathy. *Aim:* To determine the role of cytokines by evaluating the plasma levels of TGF-β1 & IL-6 in Type 2 DM elderly patients with different degree of renal function insufficiency. *Settings and Design:* Cross sectional. *Material and Methods:* This study comprised of seventy three type2 DM patients between 50-60 years and 44 age & sex matched controls were enrolled in the study were sampled for Serum (Creatinine and HbA1C) and Urine (Albumin and Creatinine). Type 2 DM patients were stratified into normoalbuminura, microalbuminuria and macroalbuminuria. Plasma TGF-β1 and IL-6 were analyzed by ELISA method. Serum TGF-β1 and IL-6 levels were increased in Type 2 DM patients compared with controls. *Statistical analysis:* Tukey Post-hoc test, Analysis of variance and Mann Whitney U test were used for statistical analysis of the results. *Results and Conclusion:* The mean TGF-β1 and IL-6 were significantly different among the groups with increased level in subjects with macroalbuminuria. This study confirms the role of cytokines in development of diabetic nephropathy.

Keyword: Cytokines; TGF-β 1; IL -6; Type2 DM; Nephropathy

Introduction

It has been predicted that worldwide the prevalence of diabetes in adults would rise from 135 million in 1995 to 380 million in the year 2025 and 418 million people with impaired glucose tolerance. Approximately 10% of patients with type 2 diabetes die of renal failure [1].

Diabetic nephropathy is one of the leading causes of chronic renal failure in India. It has been reported that among 4837 patients with chronic renal failure seen over a period of 10 years, the prevalence of diabetic nephropathy was 30.3% [2]. The manifestations of diabetic nephropathy may be a consequence of the actions of certain cytokines and growth factors.

TGF-β1 causes augmented extracellular matrix protein deposition at the glomerular level, thus inducing mesangial expansion and glomerular basement membrane thickening [3]. The role of these cytokines as aetiologic mediators in diabetic nephropathy was observed in many studies [4]. Joshi *et al,* depicted increased IL-6 levels in diabetics with glycaemic control revealed the presence of inflammation [5].

The aim of this study was to find out the association of cytokines TGF-β1 & IL-6 with albumin creatinine ratio (ACR) & HbA1c and identifying the cytokines role in risk evaluation for developing diabetic nephropathy.

Material and Methods

The total number of subjects enrolled in this study were 117, which is a cross-sectional study with type 2 DM (duration of disease being more than 10 years) aged 50 - 60 years were included as cases from Nephrology and

Endocrinology departments of M.S. Ramaiah Medical College and Teaching Hospital. Patients having the past history of concomitant hypoglycemic and antihypertensive medications were included. All the patients were tested clinically for vital parameters. Blood and urine samples were collected.

HbA1c, serum creatinine, urine creatinine and urine albumin were tested. Diabetic patients were stratified into 3 groups on the basis of ACR: Group I with 14 cases of type 2 DM with normoalbuminuria (ACR<30mg/g); Group 2 with 37 cases of type 2 DM with microalbuminuria (ACR: 30-299mg/g) and Group 3 – with 22 cases of type 2 DM with macroalbuminuria (ACR>300mg/g). Forty four age and sex matched healthy controls were included as Group 0. Informed consent was taken from all patients and guardians. Ethical clearance was obtained from the institutional ethics committee.

Plasma samples were stored at -20^0C till analysis. TGF-β1 and IL-6 were analyzed in plasma using commercially available kits (R&D systems) by ELISA method. The glycated hemoglobin (HbA1c) was measured by using high performance liquid chromatography (D10 Biorad kit, USA).

Results

The total number of subjects enrolled in this study were 117 of which 44 were age and sex matched healthy controls and included as Group 0. Group I consisted of 14 subjects of type 2 DM with normoalbuminuria, Group II consisted of 37 patients of type 2 DM with microalbuminuria and Group III consisted of 22 patients of type 2 DM with macroalbuminuria. The age range distributed across the groups was 53 to 56 years (p=0.011).

Systolic blood pressure increased as albuminuria increased across all groups. This increase was statistically significant (p<0.001). HbA1c parameters increased statistically (p<0.001) as the albuminuria increased. A statistically significant (p<0.001) increase in serum creatinine was observed as the albuminuria increased with higher levels seen in group III. Urine Albumin was higher in healthy controls compared to Group I. This result was statistically significant (p<0.001). However a linear increase was observed in the value in group II and group III. A statistically significant (p<0.001) linear increase in ACR was observed as albuminuria increased. HbA1c, Serum Creatinine, Urine Albumin and ACR increased significantly (p<0.001) in the Type 2 DM patients (Table-1& 2).

Table-1: Comparison of clinical parameters [Mean (SE)] between Non diabetics and Type 2 Diabetics

Clinical parameters	Non Diabetics	Diabetics
Age	53.5 (4.3) years *	55.8 (4.1) years *
HbA1C	5.4 (0.8) **	7.4 (0.9)**
S.Creatinine	0.8 (0.1) mg/dl **	1.5 (1.3) mg/dl**
U.Albumin	42.8 (21.1) mg/dl **	127.3 (137.2) mg/dl **
ACR	28.2 (1.8) **	370.0 (552.6) **

HbA1C – Hemoglobin A1C; ACR: Albumin Creatinine Ratio; SE = Standard Error ; *p= 0.004, ** p<0.001

Table-2: Comparison of Biochemical parameters among study participants across all groups

Clinical parameters (Mean ± SE)	Group 0	Group I	Group II	Group III
HbA1C	5.35 ± 0.78*	7.05 ± 0.86*	7.17 ± 0.66*	7.92 ± 1.13*
S. Creatinine (mg/dl)	0.84 ± 0.11*	0.96 ± 0.15*	0.99 ± 0.36*	2.73 ± 1.86*
U. Albumin (mg/dl)	42.84 ± 21.07*	21.79 ± 7*	68.35 ± 42*	293.45 ± 137*
ACR	28.19 ± 1.81*	33.18 ± 13*	130.05 ± 79*	987.94 ± 677*

HbA1C – Hemoglobin A1c; ACR: Albumin Creatinine Ratio; SE – Standard Error , *p<0.001

Table-3: Comparison of study parameters between Type 2 Diabetics and Non Diabetics		
Study parameters (pg/ml)	**Non Diabetics**	**Type 2 Diabetics**
TGF-β1 (Mean ± SE)	830.28 ± 60.64*	1131.31 ± 77.44*
IL-6 (Mean ± SE)	36.64 ± 0.68†	54.87 ± 10.90†
TGF- β : Transforming Growth Factor β1; IL- 6: Interleukin – 6; SE – Standard Error; *p=0.007, †p=0.027		

Table-4: Comparison of study parameters (pg/ml) of study participants across all groups				
Study parameters (pg/ml)	**Group 0**	**Group I**	**Group II**	**Group III**
TGF-Beta (Mean ± SE)	830.28 ± 60.64	1066.94± 4.61	1139.64 ± 117.9	1159.01 ± 122.81*
IL-6 (Mean ± SE)	36.64 ± 0.68	34.87 ± 1.19	47.35 ± 9.05	80.24 ± 32.69†
TGF- β : Transforming Growth Factor β1; IL 6:Interleukin – 6; SE – Standard Error;*p= 0.061, †p=0.128				

A statistically significant increase was observed in TGF-β1 (p=0.007) and IL-6 (p=0.027) levels in the Type 2 DM groups (Table-3). TGF-β1 levels were increased in Group III (p=0.061) and similarly IL-6 (p=0.128) levels were higher in Group III but not statistically significant (Table-4).

Statistical analysis: Statistical software namely SPSS 15.0 was used. ANOVA and Tukey's HSD test carried out to findout the siginificant difference. p value <0.05 was considered to be significant.

Discussion

With the global epidemic of type 2 diabetes mellitus, diabetes has become the leading cause of end stage renal failure (ESRF) in most Western countries. Approximately 20-30% of all diabetic subjects will develop evidence of diabetic nephropathy, which represents a continuum from microalbuminuria, to overt nephropathy or macroalbuminuria, and finally ESRF. While there have been significant breakthroughs in the last decade with regards to the prevention and treatment of diabetic kidney disease, in particular blockade of the renin angiotensin system, there is a vital need to identify and target novel pathophysiologic pathways which appear to be centrally involved in diabetic renal disease in order to reduce the rising burden of this disease [6]. TGF-β1 and IL-6 levels were estimated in control group and cases (group I, Group II and Group III). A statistically significant elevated levels of TGF-β1 (p=0.007) and IL-6 (p=0.027) were observed in the cases compared to controls. It was seen that TGF-β1 levels were highest in Group III (p=0.061) and similarly IL-6 (p=0.128) levels were higher in Group III. Statistically significant correlation was observed between serum TGF-β1 level and HbA1c, blood urea, serum creatinine and 24-hour urinary protein excretion (p<0.01) in a study conducted by Ibrahim et al [7].

Our results coincided with those observed with studies conducted in South Indian type 2 DM patients [8] and the other study by Metwally SS et al [9] have indicated a statistically significant higher serum TGF-β1 level in patients with diabetic nephropathy versus diabetic patients without nephropathy (p<0.001). Serum TGF-β1 was significantly positively correlated with albumin excretion rate, fasting and postprandial blood glucose levels, serum cholesterol and HbA1c, these correlations were only found in diabetic patients with nephropathy but not in those without nephropathy or the control group. In our study, we found that statistically significant elevation of TGF-β1 and IL-6 in type 2 diabetics.

In patients with type II diabetes mellitus both urine and serum TGF-β1 and IL-6 were elevated. After one-year-observation of patients with type II diabetes mellitus it was established that the increase of serum creatinine concentration values was higher in those patients, whose initial TGF-β1 levels exceeded normal values. A positive

correlation between urine TGF-β1 level and the progression of renal failure measured by the increase of serum creatinine level was observed [4]. However, in this study, there were no correlation between TGF-β1 and IL-6 with serum creatinine. TGF-β1 causes podocyte apoptosis and an increase in extracellular matrix deposition. As a consequence, the denuded glomerular basement membrane adheres to Bowman's capsule initiating the development of glomerulosclerosis. TGF-β1 upregulates GLUT-1, which induces an increased intracellular glucose transport and D-glucose uptake [10].

Genes play an important role in the development of diabetes mellitus. The most exciting and promising gene associated with type 2 diabetes is the TCF7L2 gene. Grant and colleagues searched for a T2DM susceptibility gene under the suggestive linkage peak on chromosome 10q in an Icelandic population. They identified a single nucleotide polymorphism in intron 3 of the transcription factor 7 like 2 gene (TCF7L2). By far, this gene has shown greatest promise as a strong candidate for type 2 diabetes risk since positive replication has been reported by virtually all the studies conducted so far, including in south Indian population and by Chandak et al in a western Indian population studied at Pune where the intronic SNP has been shown to be associated with type 2 diabetes. As Asian Indians have an increased susceptibility to diabetes and have increased insulin resistance. There appears to be certain genes which predispose Indians to diabetes while other genes (for example Pro 12 Ala polymorphism of PPAR gamma gene) which afford protection against diabetes and insulin resistance to Caucasians, do not appear to protect Indians [11].

Conclusion

Serum TGF-β1 and IL-6 levels were increased in Type 2 DM patients compared to healthy controls. TGF-β1 levels were increased with the severity of albuminuria. Thus it plays a role in evaluating risk of DN. There was no correlation of plasmaTGF-β1 and IL-6 with HbA1c, ACR and serum creatinine. It may be used as a marker of renal injury. Longitudinal studies are required to prove if TGF-β1 can be used as a surrogate marker of renal damage in Type 2 DM patients.

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