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**RESEARCH ARTICLE**

# STUDY OF OXIDATIVE STRESS AND TRANSFORMING GROWTH FACTOR β1 (TGFβ1) In Type 2 DIABETIC NEPHROPATHY OF SOUTH INDIAN POPULATION

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**ABSTRACT**

Diabetic nephropathy is one of the commonest causes for End stage renal disease. The aim of the present clinical study was to establish whether oxidative stress and TGF-β1 may be significant in evaluating its role in variable stages of nephropathy and to determine its association with other renal parameters in type 2 diabetes mellitus patients among the south Indian population. This study was conducted on sixty patients with Type 2 Diabetes Mellitus and compared with age matched control subjects. The patients were grouped as Group I: healthy controls and Group II: Diabetic patients without nephropathy and Group III: Diabetic patients with nephropathy. Plasma TGF-β1, MDA and TAC, were measured and correlated with different biochemical parameters. Our results showed significant statistical increase in TGF-β1, MDA and MDA/TAC ratio in all diabetic patients when compared to controls ($p<0.05$). In addition significant difference was observed in HbA1c, TGF- β 1 and MDA among the diabetic patients with nephropathy as compared to diabetic patients' without nephropathy. MDA showed positive correlation with TGF-β1, $HbA_1c$ and significant negative correlation with TAC among the diabetic patients. Our results suggest that diabetic patients with nephropathy and having poor glycemic control have increased level of TGF-β1 and oxidative stress. Hyperglycemia may trigger the oxidative stress in turn causes the depletion of antioxidants which up regulates the level of TGF-β1. The supplementation of antioxidants and having strict glycemic control may delay the development of renal disease in type 2 diabetes.



## INTRODUCTION

Diabetic nephropathy is the leading cause of end stage renal disease with characteristic morphological changes due to microvascular complications of diabetes mellitus (Yokoyama *et al*., 2000). Clinical studies have demonstrated that high blood glucose is the main determinant of initiation and progression of diabetic vascular complications including nephropathy (The Diabetes Control and Complications Trial Research Group, 2000). Oxidative stress is the state of imbalance between prooxidants and antioxidants. Diabetes, accelerate free radical generation and attenuate the antioxidant defense system creating oxidative stress (McCord, 2000). ROS (Reactive oxygen species) generated by high glucose levels activates signal transduction cascade and transcription factors and upregulate TGF-β1 (Transforming Growth Factor-β1) and fibronectin in renal cells and antioxidants effectively inhibit high glucose and $H_2O_2$-induced activation (Lee *et al*., 2003). TGF-β1 a multifunctional cytokine with fibrogenic properties, has been implicated in the pathogenesis as well as the progression of a variety of chronic renal diseases (Border, 1996). It plays a key role in the deposition of extracellular matrix via stimulation of synthesis of extracellular matrix protein and inhibition of extracellular matrix degradation proteinases (Chen *et al.,* 2000). The early manifestations of diabetic renal disease in mice can be prevented by systemic treatment with anti-TGF-β1 antibody or antisense TGF-β1 oligodeoxynucleotides (Sharma *et al*., 1996, Han *et al*.,2000).

Most of the early studies demonstrate the role of TGF-β1 in the development and the progression of diabetic nephropathy in type 2 diabetes mellitus among the western population. Because a relationship may be hypothesized between oxidative stress and TGF-β1, the present study was undertaken in the south Indian population to analyze the role of oxidative stress and TGF-β1 and to determine its correlation with the other biochemical parameters in the development of diabetic nephropathy in type II diabetic patients. The present study may reveal the effect of hyperglycemia on oxidative stress,

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total antioxidants status and the plasma level of TGF b1 in the development of diabetic nephropathy, in type II diabetes mellitus.

## MATERIALS AND METHODS

This study was conducted on 60 subjects including males [n = 32; age 30-60 years] and females [n = 28; age 30-60 years] who attended as outpatient to the Madurai Kidney centre and Transplantation Research Institute, Madurai from January-2009 to March-2009 (Table1), and the results were compared with age matched control group. Diabetic patients were diagnosed according to the criteria established by the American Diabetes Association (The Expert committee on the diagnosis and classification of Diabetes Mellitus, 1997). The patients were subdivided into two groups according to their urinary albumin excretion rate (UAE). Group I comprised 30 normal control subjects. Group II comprised 26 diabetic patients without nephropathy having UAE<20ug/ml. Group III comprised 34 diabetic patients with nephropathy having UAE>20ug/ml. A detailed medical history and drug treatment was collected for all subjects. The details of the study were explained and the written consent was obtained from all the study subjects and the study was approved by the institute ethical clearance committee. Exclusion criteria were: patients with renal insufficiency, treatment with angiotensin converting enzyme inhibitors or hypolipidemic drugs, patients on antioxidant therapy, acute infectious diseases and pregnancy,

Venous blood was drawn, and samples were kept at - 70$^{o}$ C for determination of TGF-β1 assays. Random urine samples were collected for urinary albumin determination. Urinary albumin excretion rate [UAE] was assayed by Immunoturbidity method. MDA level in serum was estimated by measuring the pink colored chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (Satoh 1978). Total Antioxidants capacity (TAC) was determined by FRAP(Benzie and Strain, 1996). TGF-b1 protein concentration was determined by ELISA (e -BIOSCIENCE USA), as described. The results are expressed as mean ± SD. Analysis of Variance [ANOVA] was used to compare the groups and post hoc test Tukey was used to compare the individual groups. The mean difference is considered significant at p<0.05. Pearson correlation was used to determine association between different parameters among the diabetic patients.

## RESULTS

Diabetic patients are grouped according to Urinary Albumin Excretion (UAE). The clinical data of the diabetic patients without and with nephropathy are presented in the table 1. Significant increase in SBP was seen in both the groups of diabetic patients over that of control, a significant increase in DBP was observed in only diabetic patients with nephropathy. FBS, HbA1c, Serum Creatinine, and eGFR were significantly higher in diabetic groups compared to the control, however no significant difference was seen among the diabetic patients without and with nephropathy. The level of TGF b1 showed significant increase in diabetic patients with nephropathy over that of control, and diabetic patients without nephropathy. The control subjects had significant high plasma level of TAC when compared to diabetic patients without and with nephropathy, but the level of TAC does not differ between the diabetic patients without and with nephropathy. MDA was significantly high in diabetic patients with nephropathy when compared to control group and diabetic patients without nephropathy. The MDA/TAC ratio was increased in diabetic patients with nephropathy as compared to control group. The results of the correlation studies were represented in Table 4.

**Table 1. Clinical data of study subjects**

| Variables | Group I | Group II | Group III |
|---|---|---|---|
| Number | 30 | 24 | 36 |
| Age (years) | 47±5.6 | 53±6.7 | 49.7±6.3 |
| Duration (years) | ------ | 5.9±1.9 | 8±2.4 |
| BMI (kg/m$^2$) | 23.9±2 | 26±4.3 | 26±4.2 |
| SBP (mm/Hg) | 113±9 | 135±14* | 142±20* |
| DBP (mm/Hg) | 76±6.6 | 81±6.7 | 89±11.8* |

Datas are expressed as (mean ± S.D).*p<0.05; BMI (Body mass index), SBP (Systolic blood pressure), DBP (Diastolic blood pressure).

**Table 2. Biochemical parameters of study subjects**

| Variables | Group I | Group II | Group III |
|---|---|---|---|
| FBS (mg/dl) | 92.3±7 | 219±49* | 223±60.5* |
| Urea(mg/dl) | 25.4±6.1 | 42.01±17.03 | 46.3±29 |
| Creatinine(mg/dl) | 0.82±0.15 | 1.41±17.03$^{*}$ | 1.73±0.71* |
| eGFR(ml/min/1.73m$^2$) | 108.7±26.91 | 60±25.6* | 48.66±27.81* |
| HbA1$_c$ % | 5.43±0.42 | 6.4±2* | 8.27±.5*$^{\#}$ |

Datas are expressed as (mean ± S.D). *: significantly different from control group at p<0.05; #: significantly different from the diabetic patients without nephropathy at p<0.05 FBS (Fasting blood sugar), eGFR (estimated glomerular filtration rate), HbA1c (Glycated hemoglobin).

**Table 3. The level of TGFβ1, MDA, TAC in study subjects**

| Variables | Group I | Group II | Group III |
|---|---|---|---|
| TGF-β1(pg/ml) | 214±44.3 | 354.4±150 | 508±201*$^{\#}$ |
| MDA(nmol/l) | 0.94±0.2 | 1.2±0.8 | 3.31±0.98*$^{\#}$ |
| TAC (umol/l) | 796±467.6 | 373±58.8* | 362.5±42.9* |

MDA/TAC 0.001±0.00, 0.003±0.002004±0.003; *: significantly different from control group at p<0.05; #: significantly different from the diabetic patients without nephropathy at p<0.05; TAC(Total antioxidant capacity),TGF-β1(Transforming growth factor),MDA (malondialdehyde).

**Table 4. Correlation studies of MDA with different parameters in diabetic patients**

| Parameter | r value | P level of significance |
|---|---|---|
| MDA & HbA1c | 0.537 | P<0.001 |
| MDA & TGFb1 | 0.331 | P<0.01 |
| MDA & TAC | -0.259 | P<0.05 |

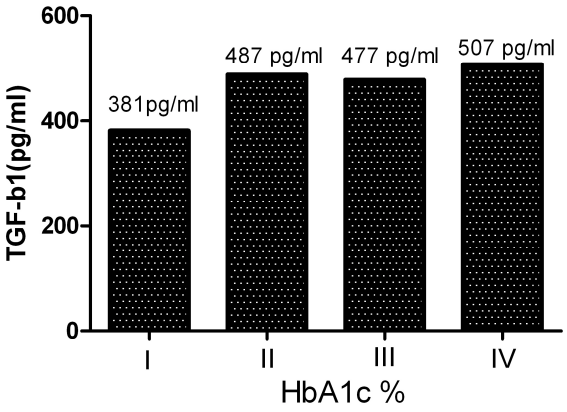


**Fig.1. Level of plasma TGF-β1 in diabetic patients with and without nephropathy, having good and poor glycemic control.**

I. Diabetic patients without nephropathy (<6 %), II. Diabetic patients without nephropathy (>6 %), III. Diabetic patients with nephropathy (<6 %), IV. Diabetic patients with nephropathy (>6 %).

MDA showed positive correlation with HbA1c and TGFb1 and showed significant negative correlation with TAC.

## DISCUSSION

Hyperglycemia is the major driving force behind renal injury in Diabetic nephropathy, via altered cell growth, gene expression, increased ECM accumulation and stimulated growth factor production(Lappin *et al*., 2002). Hence the present study was carried out to determine the effect of hyperglycemia on oxidant – antioxidant status and the level of TGF-β1 in type 2 diabetes. Our study found that renal parameters like creatinine, and eGFR (estimated Glomerular Filteration Rate) were not significantly altered in diabetic nephropathy as compared to diabetic patients without nephropathy. Another study showed that serum creatinine and eGFR were within the normal range in type II diabetic patients with renal insufficiency (Chowta *et al*., 2009).

In our study all the diabetic patients had increased level of TGFb1, this could be explained on the basis that hyperglycemia causes over expression of TGF-β1 in diabetes. Our study found that diabetic patients with nephropathy have significant increase in TGF-β1 than those of diabetic patients without nephropathy. This may due and the deleterious effects of proteinuria in the progression of renal disease may be mediated via TGF- β1. Similar observation was noted in a previous study which showed marked increase in circulating level of TGF- β1 level in patients having type II DM with DN compared to those without renal involvement (Hellmich *et al*., 2000). On contrary to this, another study showed that Serum TGFb1 level was increased at the onset of type II diabetes and remained elevated throughout the disease even in normoalbuminuric patients(Azar *et al*., 2000).

The results of the present study revealed that 23% of diabetic patients have good glycemic control (<6% of HbA1c), 42% have moderate control (6 to 8% of HbA1c) and 35% have poor glycemic control (>8% of HbA1c). Figure 1 demonstrated the level of TGF-β1 in diabetic patients having good glycemic control and poor glycemic control. The level of TGF-β1 was increased in both groups of diabetic patients having good glycemic control and poor glycemic control revealing that glycemic effect also have an impact on the level of TGF-β1. Serum TGF-β1 level was significantly increased in patients with poor glycemic control with varible stages of renal function compared with those with good glycemic control with variable stages of renal fuction (Ibrahim, 2007). The results also revealed that there was significant positive correlation between HbA1c and TGF-β1 in diabetic patients. This may be due to hyperglycemia itself stimulates the production of TGF-β1 via activation of protein kinase C, showing TGF-β1 was related to the deteriorating kidney. In our study there was no significant correlation between TGF-β1 level and age, sex and duration of the disease. Previous studies also demonstrated the similar results that neither the age nor sex correlate significantly with the level of TGF-β1 in diabetic patients (Sharma *et al*., 1997). MDA was significantly increased in diabetic patients with nephropathy as compared to control group and diabetic patients without nephropathy. These results suggest that oxidative stress is involved in the development of nephropathy. Over expression of TGF-β1 in diabetes, promotes renal cell hypertrophy and ECM accumulation, leading to decline in renal function (mellasamy *et al*., 2008). In addition to this, MDA showed significant correlation with TGF-β1 in diabetic patients. FRAP assay is considered as a useful indicator of the system's ability to regulate the damage due to ROS and thus, a novel method of assessing total antioxidant capacity (Cao, 1998) as the individual antioxidant components may not fully reflect the protective efficiency of blood, probably because of interactions that occur *in vivo* among different antioxidant compounds. TAC was significantly reduced in all diabetic patients over that of control group. Consistent with our data, a number of studies demonstrated a reduction in total antioxidant status, in type II diabetes (Dordevic *et al*., 2008). Diminishing of the total antioxidant capacity, depletion of plasma antioxidants and inadequate metabolic control, may constitute the essential pathogenetic factor for vascular complications in diabetes . No significance was found in the level of TAC among the groups of the diabetic patients, however early study demonstrated that in patients with diabetic nephropathy with the development of the disease there is significant insufficiency of plasma antioxidant barrier (Blaszczak *et al*., 2005). MDA: TAC ratio may be considered as a new marker of oxidative stress rather than concentrating on increased MDA alone or decreased levels of individual antioxidants (suresh *et al*., 2010), and in our study there was increased MDA:TAC ratio in diabetic patients with nephropathy when compared to control group and diabetic patients without nephropathy stating that increased TGFb1 can lead to the increased production of ROS.

### Conclusion

Our results revealed that there were increased oxidative stress and TGF-β1 in diabetic nephropathy patients than in diabetic patients without nephropathy suggesting that TGF-β1, oxidative stress and poor glycemic control may associate in the development of renal disease in type 2 diabetic patients. Supplementation of antioxidants, strict metabolic control and new strategies against TGF-β1 may delay the onset of diabetic nephropathy in type II diabetes, which needs further investigation.

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