WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 10, 11-24 Research Article ISSN 2278 – 4357

TRANSFORMING GROWTH FACTOR-BETA 1 AN EARLY PREDICTOR OF DIABETIC NEPHROPATHY

Mehwish Subhan*[1], Shabana Usman Simjee[1,2] and Saiyid Ali Jaffar Naqvi[3]

[1]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

[2]Dr. Panjwani Centre for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

[3]National Institute of Kidney & Urological Diseases, University of Karachi, Pakistan.

Article Received on
29 July 2017,
Revised on 18 August 2017,
Accepted on 07 Sep. 2017
DOI: 10.20959/wjpps201710-10133

***Corresponding Author**
Dr. Mehwish Subhan
H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

ABSTRACT

Objectives: The common complication of diabetes is nephropathy which is a leading cause of renal failure in one third of patients undergoing dialysis. Transforming growth factor-beta 1 (TGF-β1) plays a crucial role in the progression of diabetic nephropathy. The present study aimed to evaluate if serum TGF-β1concentrations has any diagnostic role in predicting the incidence of diabetic nephropathy in the Pakistani patients before nephropathy starts. **Patients and Methods:** The study was performed on 140 subjects including normal, type 2 diabetes patients with and without proteinuria, and type 1 diabetes patients without proteinuria. Serum level of TGF-β1 was estimated by ELISA technique. **Results:** The mean serum level of TGF-β1estimated in the normal subjects was 507.28 ± 33.52 pg/mL. In comparison to normal, the TGF-β1 in type 2 diabetes with or without proteinuria and type 1 samples was significantly found to be elevated i.e., 1869.65 ± 238.59 pg/mL ($P < 0.002$), 1899.20 ± 211.03 pg/mL ($P < 0.001$), and 2866.91 ± 460.56 pg/mL ($P < 0.001$) respectively. Statistical analysis revealed no significant difference between type 2 diabetes with and without proteinuria, however, the type 1 diabetes samples reflect significant difference from type 2 samples with ($P < 0.03$) and without proteinuria ($P < 0.04$). **Conclusion:** Increased serum TGF-β1 in diabetes samples with or without proteinuria suggest that it can serve as a

predictive marker of diabetic nephropathy in diabetic patients before they suffer from chronic renal failure or diabetic nephropathy.

KEYWORDS: ACE inhibitors, diabetic nephropathy, diabetes mellitus, microalbuminuria, proteinuria, TGF-β1.

INTRODUCTION

Diabetes has become a global challenge because of its rising prevalence all over the world. About 5 to 10% people are diagnosed with type 1 diabetes which is due to destruction of beta cells of pancreas[1, 2] and it is commonly found in children. About 90-95% people are affected by type 2 diabetes. For the confirmation of diabetes WHO recommend to measure patient glycosylated hemoglobin i.e., HbA1c which according to the International Expert Committee (IEC) should not be ≥ 6.5% or else the patient will be classified as diabetic.[3] According to the WHO and American Diabetic Association (ADA) fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT) can conclude whether the patient is suffering from diabetes or not. If FPG is ≥ 7.0 mmol/L (126 mg/dL) and OGTT ≥ 11.1 mmol/L (200 mg/dL) then according to WHO and ADA standards these results shows that the patient having diabetes. According to the International Diabetic Federation (IDF), Pakistan stands on 7th number among those countries having highest degree of diabetes. It was estimated that in 2007 the diabetes population of Pakistan was 6.9 million and it will increase up to 11.5 million by the year of 2025.[4] Long term diabetes leads towards several other severe complications causing damage, dysfunction and failure of various organs, e.g., blood vessels, eyes, heart, kidneys, and nerves.[5] Complications of diabetes are divided into a) Macrovascular complications and b) Microvascular complications. As the diabetes progress it will lead towards nephropathy.

In diabetic nephropathy, there is a progressive increase in urine albumin excretion; along with an increase in the blood pressure that leads to decline in glomerular filtration and ultimately end-stage kidney failure.[6] As the nephropathy progresses, GFR start to decline and serum creatinine become double. Nephropathy is responsible for renal failure in about one third of patients who undergo dialysis and therefore it becomes leading cause of chronic renal failure and end stage renal disease (ESRD) throughout the world.[5,7,8] Diabetes patient with increasing blood pressure are more susceptible to develop microalbuminuria as compared to those patients that have blood pressure in normal range.[9] It has been reported that ~ 20-40%

of patients with diabetic history of 15-20 years develops proteinuria leading to renal failure.[10,11] Renal failure cannot be prevented by controlling hyperglycemia once the albuminuria exceeds > 300 mg/day.[6, 10, 11]

The incidence of ESRD in diabetes mellitus is estimated about 33.8% of the total ESRD cases at leading dialysis facility in Pakistan.[12] The nephropathy is one of the main outcomes of the progression of type 2 diabetes. This not only reduces the quality of life of patients, but also incurs heavy burdens to the health care systems, and increase diabetic mortality.[13, 14] Access to diabetic care is limited in Pakistan which is facing a rapid rise of type 2 diabetes population especially in urban population.[15, 16] The dialysis and transplantation facilities are expensive all over the world particularly in developing and under developing countries. In Pakistan, dialysis is very serious problem affecting the economy of country. It was reported that cost of the dialysis prescriptions per month is Rs. 29,852 (USD 609) for 2 heamodialysis (2 HD sessions/week) and Rs. 28,763 (USD 585) for 3 peritoneal dialysis (3 PD exchanges/day).[17] The total cost of dialysis per patient is approximately $3000 per year, which is eight times the average annual per capita income.

Screening of diabetic nephropathy is necessary for both type 1 and type 2 diabetes patient. It is recommended that if microalbuminuria is absent then screening should be repeated every year for both type 1 and type 2 diabetes patients. Various studies suggest that first screening for type 1 patient should start at 5 years of their diagnosis and for type 2 patients screening must be start at the time of diagnosis. According to the Gross et al., puberty is also an important factor for the microalbuminuria. For type 1 diabetes; microalbuminuria can be screened after onset of puberty or after 1 years of diabetes.[18]

Among the cytokines which are associated with inflammatory responses in type 2 diabetes, the transforming growth factor-β (TGF-β) has been identified as a central player in the diabetic nephropathy. Transforming growth factor beta (TGF-β) is a multifunctional cytokine isolated from platelets.[19] It is also present in brain, kidney, liver and heart. It controls cell proliferation, cell differentiation and also involved in paracrine signaling. It plays a crucial role in different diseases including diabetic nephropathy. Three isoforms of TGF-β has been reported i.e., TGF-β1, β2, and β3. Among these, TGF-β1 is the most potent cytokine that stimulates synthesis of extracellular matrix molecules. TGF-β1 also decreases matrix degradation by inhibiting protease and activating protease inhibitors.[20- 22] As glucose concentrations increases in the cell, it stimulates hypertrophy of mesangial cell and also

stimulates the production of matrix molecules such as fibronectin and collagen in endothelial, epithelial and interstitial fibroblastic cells. Hyperglycemia also increases renal production of vasoactive agents such as angiotensin-2, thromboxane and advanced glycated end (AGE) products. They are responsible for increase production of growth factors including TGF-β1 by kidney cells. Advanced glycated end (AGE) products are formed due to activation of protein kinase C (PKC) in response to certain pathways such as polyol pathway which is stimulated by hyperglycemia.[19, 22, 23] The TGF-β mRNA and protein level has been reported to increase in human during diabetes.[21] TGF-β starts its fibrogenic effect by acting as a transcription factor for various genes including βig-h3. βig-h3 is the TGF-β induced gene human clone 3 and is responsible for the regulation of matrix deposition and cell growth, and is over expressed in the patients of diabetes.

Keeping in mind the role of TGF-β1 in the diabetic nephropathy, we hypothesized that serum TGF-β1 concentrations may have a diagnostic role in predicting the incidence of diabetic nephropathy in the Pakistani population. If TGF-β1 is detected earlier then it will reduce the chances of kidney failure that require dialysis. Realizing the importance of TGF-β1 in initiation and progression of diabetic nephropathy which is responsible for nearly 60% of CKD patients in Pakistan we decided to measure TGF-β1 in Sindh Pakistan.

MATERIALS AND METHODS

Patients

The present study includes 140 subjects which are classified into groups shown in (Fig. 1). The exclusion and inclusion criteria adopted in this study are outlined in (Table: 01).

Table 01: Exclusion and inclusion criteria adopted in this study.

Inclusion Criteria	Exclusion Criteria
Diabetic Patients	Heart disease
Diabetic + CKD Patients	Neuropathy
	Retinopathy
	Chronic disease
	Smokers
	Pregnant women
	Surgery or diabetic induce disease

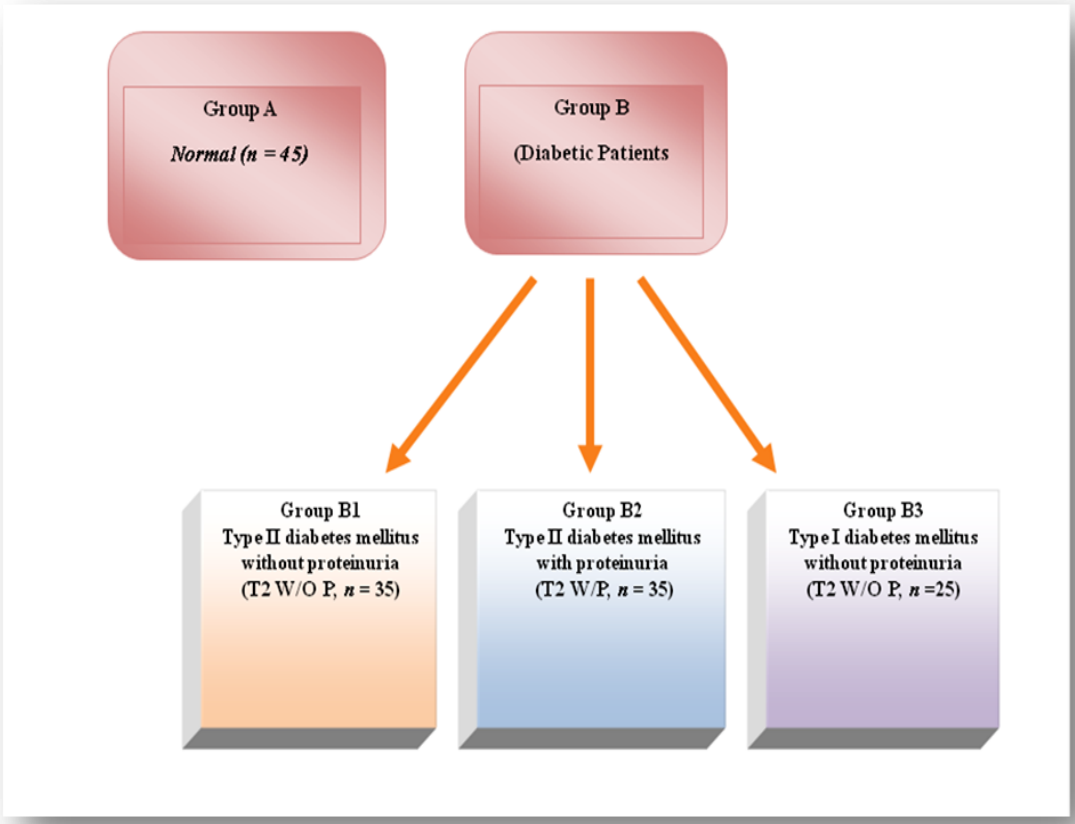

Figure 1: **Schematic diagram of groups included in this study.**

Sample Collection

In the present study 5cc blood samples were collected from those patients who attended OPD of Diabetic Association of Pakistan and National Institute of Kidney & Urological diseases (NIKUD), University of Karachi. Patient's history including age, sex, duration of diabetes and all clinical lab values were recorded. Blood samples from normal subjects were randomly collected from International Centre for Chemical and Biological Sciences, University of Karachi, Pakistan. In this analysis we excluded all those patients having any type of diabetic induced disease or the people with heart disease, neuropathy, retinopathy, or having any other chronic disease or surgery. Smokers and pregnant women were also excluded from our present study. Ethics committee approval was obtained. All procedures followed were in accordance with the standards of the responsible committee on human experimentation (Independent Ethics Committee, International Center for Chemical and Biological Sciences) and with the Helsinki Declaration of 1975 (revised 2008).

Sample Preparation for TGF-β1 Determination

The whole blood samples were centrifuged to separate serum. These serum samples were used to estimate TGF-β1 by using enzyme-linked immunosorbent assay (ELISA). The samples were run in triplet and standards in duplicate.

Assay Procedure

100 µL of diluted sample of activated human serum were added to each well and 100 µL of sample diluents buffer into control well (zero well). Plate was sealed with cover and incubated for 90 min at 37ºC. After 90 min wells were aspirated and 100 µL of biotinylated anti-human TGF-β1antibody working solution were added in each well and then plates were re-incubated for 1 hr at 37ºC. After incubation, plate was washed 3 times with 0.01M phosphate buffered saline (PBS) and then 100 µL Avidin-biotin-peroxidase complex (ABC) working solution were added into each well and plate was re-incubated at 37ºC for 30min. After 30 min of incubation plate was washed 5 times with 0.01M PBS and then 90 µL 3,3',5,5'-tetramethylbenzidine(TMB) color developing agent was added into each well and incubated at 37ºC in dark for 25-30 min. Finally 100 µL stop solution was added into each well and optical density was measured at 450 nm using (Sunrise™ ELISA reader, Tecan Group Ltd., Switzerland). The average zero (blank) was subtracted from average reading of each standard to plot standard curve. Average zero was also subtracted from each reading of control and test sample. These values were used to determine the concentration of TGF-β1.

The range TGF-β1 assay was 15.6pg/mL-1000pg/mL with sensitivity of < 1 pg/mL. The assay did not have any cross-reactivity with human interleukin (IL)-1α, -1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, tumor necrosis factor(TNF)-α, interferon(IFN)-α, -β, and –γ, and TGFβ2.

Biochemistry

In addition to age, history, hemoglobin, and blood pressure recordings, biochemical analysis including fasting glucose level, cholesterol, creatinine, serum iron, Na, K, Ca, Cl, P, HCO_3, total iron binding capacity (TIBC), alkaline phosphatase, serum glutamic-pyruvic transaminase (SGPT), albumin, total protein, urea, urinary protein, uric acid and urinary volume of type 2 diabetes patients were measured in the laboratory of National Institute of Kidney & Urological Diseases (NIKUD),University of Karachi.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 15) software. Throughout this study mean ± SEM were used to describe the data in figure. Analysis of variance (ANOVA) was used to determine the distribution of TGF-β1levels in various groups included in this study. The Bonferroni's post hoc test was used to determine group mean difference. Values equal to or below $p< 0.05$ were considered as significant.

RESULT

Mean serum level of TGF-β1

The mean ± SEM values of TGF-β1 measured in the serum samples of normal, diabetes patients with or without proteinuria are shown in (Table: 02). The statistical significance between groups is outlined in (Table: 03).

Table: 02. Mean serum level of TGF- β1 in diabetes and normal control.

Group A (Normal)	Group B1 (Type 2 without proteinuria)	Group B2 (Type 2 with proteinuria)	Group B3 (Type 1 without proteinuria)
507.28pg/mL ±33.52	1899.20 pg/mL ±211.03	1869.65pg/mL ±238.59	2866.91pg/mL ±460.56

Table: 03. The statistical significance between groups.

Group	Statistical Significance (*P* value)
Normal T2 WOP T2 WP T1 WOP	0.001 0.002 0.001
T2 WOP T2 WP T1 WOP	1.000 0.040
T2 WP T1 WOP	0.034

Where T1 and T2 stands for Type 1 and Type 2 diabetes. WP and WOP stand for with and without proteinuria respectively.

The mean serum level of TGF-β1estimated in the normal subjects was 507.28 pg/mL. In comparison to normal, the TGF-β1 in type 2 diabetes with or without proteinuria and type 1

diabetic samples was found to be elevated i.e., 1869.65 pg/mL, 1899.20 pg/mL, and 2866.91 pg/mL respectively (Fig. 2).

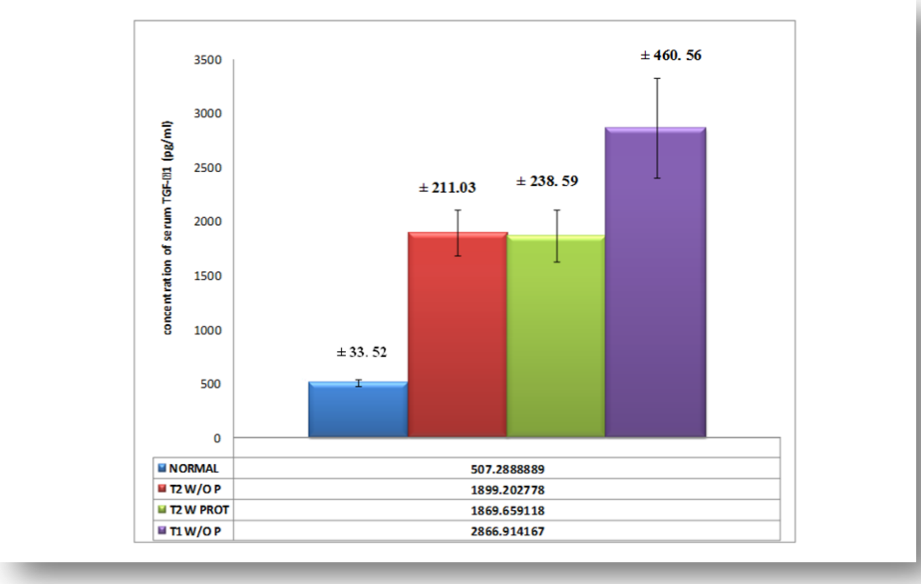

Figure 2: **Graphic representation of the serum level of TGF-β1 in diabetes (type 1 and type 2) and normal healthy control.**

Each bar represents the mean ± SEM. A significantly high level of Serum TGF-β1 was detected in diabetes sample type 2 without proteinuria ($P< 0.001$) and with proteinuria ($P < 0.002$) as compared to the normal. Likewise, the type 1 samples also demonstrated significantly increased levels of TGF-β1 ($P< 0.001$) as compared to normal. The serum TGF-β1 of type 1 diabetes samples was also significantly increased from type 2 diabetic samples with ($P< 0.03$) and without proteinuria ($P< 0.04$). The value presented over the bar represents the ± SEM of their means.

Analysis of variance (ANOVA) with bonferroni's post-hoc test revealed that all these three groups were significantly different from the normal control samples. When the diabetic groups were compared with each other, no significant difference was found in the type 2 diabetes samples with or without proteinuria however, the serum TGF-β1 of type 1 diabetes samples was significantly different from type 2 samples with ($P< 0.03$) and without proteinuria ($P< 0.04$).

Correlation of creatinine, urea, urinary protein with expression of TGF-β1 in kidney

The biochemical parameters measured in diabetes patients are shown in Table: 04.

Table 04: Biochemical parameters of normal and diabetes patients.

Parameters	Normal	T2 W/P	T2WOP
Age	36	57	55
B.P	120/80	146/85	128/77
History	-	17	13
Hb	12-17g/dl	9	12
Sugar	70-110 mg/dL	194.9	267
Cholesterol	< 200 mg/dL	169	185
Urea	10-50 mg/dL	99	33
Creatinine	0.5-1.5 mg/dL	4.8	1.1
SGPT	0-30 U/L	31	29
Uric acid	2.0-4.0 mg/dL	6	5
Total protein	5-7 gm/Dl	7.2	7.4
Urinary protein	150-250mg/24 hr	906	208
Albumin	3.4-4.7 g/dL	3.6	3.7
Urinary volume	400 to 2,000 mL	1646	1932
Alkaline phosphatase	45 to 150 IU/L	245.53	234
Iron	60-170 mcg/dL	52.35	67
Na	135 - 145 mmol/L	139	139
K	3.5 - 5 mmol/L	4	4
Ca	8.5 - 10.3 mg/dL	9	10
Cl	95 - 108 mmol/L	99	100
HCO3	22 to 26mEq/L	23	23.7
TIBC	240-450 mcg/dL	227.6	218.5
P	2.5 - 4.5 mg/dL	4.36	4

It was observed that glucose, creatinine, urea, uric acid, urinary protein, and alkaline phosphatase are elevated in diabetic patients with or without proteinuria. When the diabetes groups were compared with each other, it was found that type 2 diabetic patients with proteinuria showed significant difference in the levels of creatinine, urea, and urinary protein. The serum concentration of alkaline phosphatase enzyme was found to be elevated in the diabetes type 2 patients with proteinuria however this increase was not significant (Fig. 3).

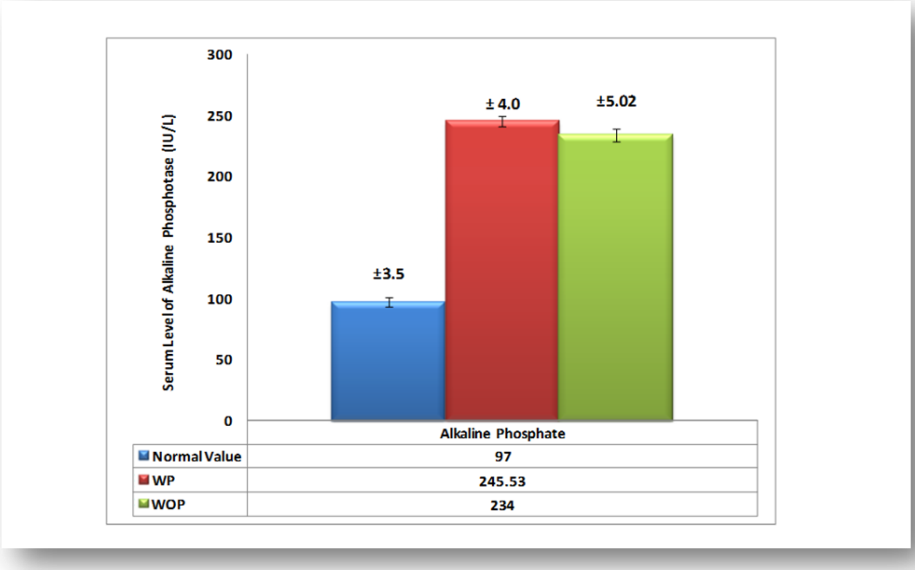

Figure 3: **Graphic representation of the serum level of alkaline phosphatase in diabetes (type 1 and type 2) and normal healthy control.**

Each bar represents the mean ±SEM. A significantly high level of serum phosphatase was detected in diabetes sample type 2 with and without proteinuria ($P< 0.001$) as compared to the normal. The value presented over the bar represents the ± SEM of their means.

Marked elevation of these factors indicates their contributing role in the progression of renal damage and also correlate with the over expression of TGF-β1 in kidney.

DISCUSSION

This study demonstrated high level of TGF-β1 in urban population of Sindh in diabetes. This is much higher than non-diabetic population. This is similar to study carried out by Vijay vishwanath *et.al* in south India. As in south India Vijay vishwanath found high level of TGF-β1. [6] We also found a significantly elevated level of TGF- β1 in type I diabetic samples as compared to type 2 (with and without proteinuria) and normal healthy control. As glucose concentration increases in body it stimulates formation of angiotensin-2, which results in increase production of growth factors including TGF- β1. Studies have reported increased production of TGF- β1 in the kidneys of diabetic patients which is then released in the systemic circulation. In contrast, the normal kidneys extract TGF- β1from the circulation.[24,28]

When we analyzed the results between the groups, it was found that serum TGF- β1 is non-significantly decrease in type 2 with proteinuria as compared to the type 2 without proteinuria. The reason behind this non-significant reduction seen in our study might be due to the use of angiotensin converting enzyme (ACE) inhibitors by the patients of type 2 with proteinuria for their hypertension. The intake of ACE inhibitors suppresses the development of proteinuria and renal fibrosis by inhibiting stimulation of vasoactive agents i.e. angiotensin 2 (Ang 2) which is responsible for the increased production of TGF- β1. Several studies suggest that ACE inhibitors protect kidneys by lowering serum TGF- β1.[29-31]

In addition to the TGF-β1, a marked increase in alkaline phosphatase level was also observed in both groups (type 2 with or without proteinuria) as compared to normal subject. Our observation is supported by the study reporting that hyperglycemic condition along with increasing TGF-β1 also simultaneously increases the osteoclastic activity which is responsible for bone resorption process and since alkaline phosphatase is by byproduct of osteoclastic activity therefore it is also raised. [32]

Because of increase prevalence of diabetes in 3rd world almost on 4th number under world map of diabetes, high economic cost of therapy under kidney fails (dialysis transplant). It is important that we should find out early markers of renal damage in diabetes. Our findings are same as vijay that TGF-β1 is early marker and early management of diabetes may slow down the renal damage. Our patients have shown that TGF-β1 is lower in those who are on ACE inhibitors or ARB. This finding can be taken as the result of therapy.

CONCLUSION

The current study estimated the serum TGF-β1 in diabetic samples (type 1 and type 2 with or without proteinuria) compared with normal healthy control in order to determine if it is also a predictive marker of diabetic nephropathy in diabetic patients before they suffers from chronic renal failure or diabetic nephropathy. We observed that serum TGF-β1 were significantly higher in diabetic samples (type 1 and type 2) as compared to the normal healthy controls. However, we were unable to see any significant difference between the patients with type 2 diabetes with proteinuria or without proteinuria. The reason was due to the fact that these patients were already on ACE inhibitor which alone is a factor to reduce the TGF-β1. However, as a part of our next step, we aim to target RAAS which is a major pathway involved in diabetic nephropathy and then correlate it with TGF-β1.

DISCLOSURE

The authors have no conflicts of interest.

ACKNOWLEDGEMENT

This work was supported by National Institute of Kidney and Urological Diseases, under the auspices of The Kidney Foundation (Pakistan).

REFERENCES

1. Hackett EA, Jackson SNJ. Diabetes mellitus, In: Clinical Pharmacy and Therapeutics*:* 5th *Edn*. Walker R., Whittlesea : Elsevier Health Sciences UK: 2011, 685-710.
2. American Diabetes Association. Classification and Diagnosis of Diabetes. Diabetes Care, 2015; 38: S8-S16.
3. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: A review of current trends. Oman Med J, 2012; 27(4): 269-273.
4. Mahar PS, Awan MZ, Manzar N, Memon MS. Prevalence of type-2 diabetes mellitus and diabetic retinopathy: The Gaddap study. JCPSP, 2010; 20(8): 528-532.
5. Dabla PK. Renal function in diabetic nephropathy. World J Diabetes, 2010; 1(2): 48–56.
6. Shaker YM, Soliman HA, Ezzat E, Hussein NS, Ashour E, Donia A, Eweida SM. Serum and urinary transforming growth factor beta 1 as biochemical markers in diabetic nephropathy patients. BJBAS, 2014; 3: 16-23.
7. Khaki A, Fathiazad F. Diabetic Nephropathy – Using Herbals in Diabetic Nephropathy Prevention and Treatment – The Role of Ginger (Zingiber officinale) and Onion (Allium cepa) in Diabetics Nephropathy. In: A Compendium of Essays on Alternative Therapy, Dr. Arup Bhattacharya (Ed.): 2012; 207-230. ISBN: 978-953-307-863-2.
8. Shafi S, Tabassum N, Ahmad F. Diabetic nephropathy and herbal medicine. Int J Phytopharmacol, 2012; 3(1): 10-17.
9. Sara G, Barit D and Cooper ME. Mechanisms of diabetic nephropathy role of hypertension. Hypertension, 2006; 48(4): 519-526.
10. Parchwani DN, Upadhyah AA. Diabetic nephropathy: Progression and pathophysiology. Int J Med Sci Public Health, 2012; 1(2): 59-70.
11. Ahmedani MY, Hydrie MZ, Iqbal A, Gul A, Mirza WB, Basit A. Prevalence of microalbuminuria in type 2 diabetic patients in Karachi: Pakistan: a multi-center study. J Pak Med Assoc, 2005; 55(9): 382-386.

12. Naqvi SAJ. Nephrology services in Pakistan. Nephrol Dial Transplant, 2000; 15: 769-771.
13. Wang W, Fu CW, Pan CY, Chen W, Zhan S, Luan R, et al. How do type 2 Diabetes Mellitus related chronic complications impact direct medical cost four major cites of urban China? Value Health, 2009; 12(6): 923-929.
14. Solli O, Stavem K, Kristiansen IS. Health related quality of life in diabetes associations of complications with EQ-ED Scores. Health Qual Life Outcomes, 2010; 8: 18.
15. Ali A, Iqbal F, Taj A Iqbal Z, Amin MJ, Iqbal QZ. Prevalence of microvascular complications in newly diagnosed patients with Type 2 diabetes. Pak J Med Sci, 2013; 29(4): 899–902.
16. Kazmi WH. A Greater than Expected Prevalence of Chronic Kidney Disease (CKD) in Pakistan. Annals Abbasi Shaheed Hospital & Karachi Medical & Dental College, 2013; 18(1).
17. Jha V. Current status of end-stage renal disease care in India and Pakistan. Kidney Int, Suppl, 2013; 3: 157–160; doi:10.1038/kisup.
18. Gross JL, De Azevedo MJ, Silveiro SP, Cananni LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: Diagnosis, prevention, and treatment. Diabetes Care, 2005; 28(1): 164-176.
19. Ahmed KY, El-Bazz WF, Mohamed Ha, Mohamed HA, and Elkeshky M. Transforming growth factor-β in diabetic nephropathy. Egypt J Intern Med, 2013; 25(1): 20-26.
20. Lan LY. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. Int. J. Biol. Sci, 2011; 7(7): 1056-1067.
21. Reeves WB, Andreoli TE. Transforming growth factor β contributes to progressive diabetic nephropathy. Proc Natl Acad Sci, 2000; 97(14): 7667–7669.
22. Goldfarb S, Ziyadeh FN. TGF-β: A crucial component of the pathogenesis of diabetic nephropathy. Trans Am Clin Climatol Assoc, 2001; 112: 27-33.
23. Kolset SO, Reinholt FP, Jenssen T. Diabetic nephropathy and extracellular matrix. J Histochem Cytochem, 2012; 60(12): 976-86.
24. Border WA, Noble NA. Evidence that TGF-β should be a therapeutic target in diabetic nephropathy. Kidney Int, 1998; 54: 1390-1391.
25. Gomes KB, Rodrigues KF, Fernandes AP. The Role of Transforming Growth Factor-Beta in Diabetic Nephropathy. Int. J Med Gen, 2014; 2014 (2014), Article ID 180270.

26. Lee HS. Pathogenic Role of TGF- β in Diabetic Nephropathy. J Diabetes Metab, 2013; S9.
27. Border WA, Noble NA. Workshop on Vascular Biology & Hypertension: From Molecules to Humans: Interactions of transforming growth factor-β and angiotensin 2 in renal fibrosis. Hypertension, 1998; 31: 181-188.
28. Ibrahim S, Rashed L. Estimation of transforming growth factor-β1 as a marker of renal injury in type 2 diabetes mellitus. Saudi Med J, 2007; 28(4): 519-523.
29. Loeffler I, Wolf G. Transforming growth factor-β and the progression of renal disease. Nephrol Dial Transplant, 2013; 29(suppl 1): i37-i45.
30. Albuquerque DA, Saxena V, Adams DA, Boivin GP, Brunner HI, Witte DP, Singh RR. An ACE inhibitor reduces Th2 cytokines and TGF-β1 and TGF-β2 isoforms in murine lupus nephritis. Kidney Int, 2004; 65: 846–859.
31. Zhu S, Liu Y, Wang L, Meng QH. Transforming growth factor-β1 is associated with kidney damage in patients with essential hypertension: renoprotective effect of ACE inhibitor and/or angiotensin 2 receptor blocker. Nephrol Dial Transplant, 2008; 23(9): 2841-2846.
32. Wu YY, Xiao E, Graves DT. Diabetes mellitus related bone metabolism and periodontal disease. Int J Oral Sci, 2015; doi: 10.1038/ijos.2015.2 Epub 2015 R, Whittlesea : Elsevier Health Sciences UK, 2011.