ORIGINAL RESEARCH **PERIOPERATIVE MEDICINE**

Association of TGF-β1 with cystatin-C in patients with diabetic nephropathy

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ABSTRACT

Background & Objective: Diabetes is considered a condition that is characterized by an increase in oxidative stress and inflammation. Transforming growth factor-beta (TGF-β) belongs to the TGF-β subfamily of the TGF-β superfamily. Numerous biological processes, such as cell growth, differentiation, adhesion, proliferation, tissue repair, morphogenesis, and apoptosis, are regulated by the TGF-β subfamily. Five TGF-β isoforms in this subfamily have been found in vertebrates; however, only TGF-β 1-3 has been demonstrated to be expressed in mammals.

Methodology: The current research comprised of a total of 130 individuals, all of whom were placed into one of three primary groups ; 50 (25 males and 25 females) diabetic patients without nephropathy; 50 (25 males and 25 females) diabetic patients with nephropathy as cases, and 30 (15 males and 15 females) persons in good health, who were of the same age as the patients being studied. In order to measure the concentrations of the parameters, the standard procedures and techniques were used.

Results: The mean value of serum TGF-β1was significantly higher in diabetic patients without nephropathy and diabetic patients with nephropathy (40.19 ± 3.56 ng/ml),(51.21 ± 5.20 ng/ml) respectively as compared to controls (24.80 ± 3.51 ng/ml) with a high significant difference ($P < 0.01$). The level of TGF β1 showed a positive correlation in the study population with fasting blood sugar ($r = 0.273$, $P = 0.006$), creatinine ($r = 0.546$, $P = 0.004$), and cystatin C ($r = 0.597$, $P = 0.005$). The main finding of the present study was that the mean value of serum TGF-β1 in diabetic patients without nephropathy was 40.19 ± 3.56, and for diabetic patients with nephropathy 51.21 ± 5.20, while for controls was 24.80 ± 3.51, a significant difference ($P < 0.05$). In addition, there was a statistically significant and positive link between the blood TGF 1 levels and the serum creatinine levels of the individuals who participated in the study ($r = 0.611$, $P = 0.003$). In addition, patients who had diabetes for a longer period of time (more than five years) had higher levels of TGF β1 than those who had just been diagnosed with the condition. This difference, however, was not statistically significant ($P > 0.05$), which may be due to the fact that the level of TGF 1 rises with increasing duration of the participant's cases.

Conclusion: There is no significant correlation between cystatin-C with fasting blood sugar. There was a statistically significant and positive link between the blood TGF-β1 levels and the serum creatinine and cystatin-C levels.

Key words: Type-2 Diabetes Mellitus; TGF-β1; Cystatin-C.

1. INTRODUCTION

The prevalence of diabetes mellitus type 2 (T2DM), which now exceeds 9% globally, is estimated to impact around 463 million people, and is one of the top 10 main causes of mortality.[1] This condition has been rising at an exponential rate. Therefore, one of the primary concerns of public health researchers and clinical health professionals[2] is the development of methods to postpone the onset of type 2 diabetes mellitus and early treatment techniques to halt its progression. It has been shown that inflammation has a significant role in the development and progression of T2DM, including an increase in the levels of inflammatory biomarkers; hence, the treatment of T2DM should focus on lowering inflammation.[3] Diabetes mellitus is a chronic metabolic illness that causes hyperglycemia. This hyperglycemia may be the consequence of a malfunction in insulin synthesis, insulin action, or both. Diabetes mellitus can raise the risk for the development of microvascular as well as macrovascular problems. An increase in oxidative stress and inflammation are two of the hallmarks of diabetes, which is recognized as a disorder in and of itself.

TGF-β is a member of the TGF-β subfamily, which is a part of the TGF-β superfamily.[4,5] A wide variety of biological processes are controlled by the TGF-β subfamily, which is engaged in their regulation, such as the development of cells and differentiation, adhesion, proliferation, tissue repair, morphogenesis, and apoptosis.[6,7] These are only some of the functions. Only TGF-β isoform 1-3 have been shown to be expressed in mammals,[8] despite the fact that there have been five different TGF-β isoforms discovered in vertebrates belonging to this subfamily. The TGF-β1 isoform that is prominent in the kidney is the one that causes responses to be triggered by many signaling pathways. These pathways include SMAD (an acronym from the fusion of *Caenorhabditis elegans Sma* genes and the *Drosophila Mad*, Mothers against decapentaplegic), protein mitogen-activated kinases, extracellular regulatory kinase, p38, and Jun kinase[9] were examples of proteins that play a role in signal transduction.

Through three mechanisms, renal fibrosis has been firmly linked to TGF-β1 activity in the setting of kidney disease:

(1) A pro-fibrotic phenotype is brought about in the kidneys as a result of an increase in the production of extracellular matrix (ECM) as well as epithelial-mesenchymal transition (EMT).

(2) Increased type I and IV collagen, fibronectin, and laminin synthesis.

(3) The matrix breakdown may be reduced by increasing the expression of matrix metallo-protease inhibitors.[10,11,12].

This study aimed to explore correlation between cystatin-C with fasting blood sugar, and a link between the blood TGF-β1 levels and the serum creatinine and cystatin-C levels.

2. METHODOLOGY

This case-control research was conducted using data obtained at the Faiha Specialised Diabetes, Endocrinology and Metabolism Centre (FDEMC) located in Basrah governorate between November 2022 to May 2023. The study included 100 patients (50 males and 50 females) with T2DM, and 30 controls apparently healthy subjects (15 males and 15 females).

Statistical analysis

The data were represented as means with the standard deviation. The statistical studies were conducted with SPSS, version 26, developed by IBM, United States. A significance level of $P < 0.05$ was deemed to be statistically significant.

3. RESULTS

There were 30 healthy controls to compare with the group containing known patients of T2DM, consisting of equal number of male and female patients (Table 1).

The data regarding BMI, FBS, HbA1c, creatinine, cystatin-C and TGF β1 are given in Table 1. BMI was insignificantly higher in the diabetics as compared to the healthy volunteers. The mean value of HbA1c% for diabetic patients was 8.83 ± 1.99. Concerning the serum TGF β1, the mean value of diabetic patients without nephropathy was 40.19 ± 3.56, and for diabetic patients with nephropathy 51.21 ± 5.20, while for controls was 24.80 ± 3.51. A statistically significant and positive association between the levels of blood TGF β1 and serum creatinine (Table 2).

4. DISCUSSION

Type 2 diabetes mellitus is the prevailing form of diabetes, characterized by a complex interplay of several factors and a persistent metabolic dysfunction. Its incidence has been progressively rising on a worldwide scale. Almost more than 95% worldwide of an individual with DM have T2DM.[5]

The objective of this research was to assess the use of TGF β1 as a biomarker for diabetic nephropathy in individuals with T2DM. This study linked the

Table 1: The research sample was distributed based on the biochemical characteristics:

Parameters		Controls (n = 30)	Cases (n = 100) DM	DN	P-value[*] (between groups)
Gender	Male	15 (11.5)	50 (38.5)		-
	Female	15 (11.5)	50 (38.5)		-
BMI (kg/m^2)		26.87 ± 1.92	29.77 ± 5.15		> 0.05[**]
FBS (mg/dl)		98.23 ± 11.42	211.10 ± 68.70	244 ± 92.27	< 0.01
HbA1c (%)		4.91 ± 0.58	8.78 ± 2.22	8.87 ± 1.76	< 0.01
Creatinine (mg/dl)		0.68 ± 0.11	0.81 ± 0.25	3.54 ± 2.44	< 0.01
Cystatin-C (ng/ml)		43.05 ± 8.92	77.02 ± 4.41	90.55 ± 8.06	< 0.01
TGF β1		24.8 ± 3.51	40.19 ± 3.56	51.21 ± 5.20	< 0.01

Data presented as mean ± SD or n (%); P < 0.05 considered as significant

Table 2: The use of receiver-operating characteristics (ROC) curve analysis in the diagnosis of Type 2 Diabetes Mellitus (T2DM).

Variables	Area under the ROC curve (AUC)	Best cut-off	Sensitivity (%)	Specificity (%)
Cystatin-C	0.970	82.35	88.0	86.0
Serum TGF-β1	0.972	44.45	92.0	94.0
Creatinine	0.998	1.6	94	88.0

association between serum TGF β1, creatinine, cystatin c, blood glucose, HbA1c, and anthropometric measures in patients with T2DM. The mean value of HbA1c for diabetic patients was was lower than the results of other study.[6] More than 61% of the patients in the present study had poor glycemic control and only 18%, and 21% of them achieved good and fair glycemic control respectively. Concerning the serum TGF β1, a statistically significant difference was observed (P < 0.05). A similar result was obtained by Shaker et al, who found that there is association between serum TGF β1 to T2DM.[7] Moreover, The present investigation revealed a statistically significant and positive association between the levels of blood TGF β1 and serum creatinine within the study cohort (r = 0.546, P = 0.004), (r = 0.611, P = 0.003) respectively, Similar results were revealed by Shaker et al.[7] The findings of the current investigation demonstrated that the average value for TGF β1 levels in females was higher but not significant when compared to male participants, as well as a higher significant difference in both males and females when compared with control (P < 0.05), Furthermore, it is worth noting that a statistically insignificant positive connection was seen between TGF β1 levels and gender (r = 0.043, P = 0.674) (Table 3).

The findings of this research indicate that individuals diagnosed with T2DM have notably elevated levels of cystatin C in comparison to the control group (83.79 ±

9.38 vs. 43.05 ± 8.92 ng/ ml). Similar results were revealed by Jeon YL et al.[8] They stated that the higher level of cystatin C is most likely due to that the level of cystatin C was strongly correlated with increment in the creatinine level and nephropathy. Jeon YL et al.[8] found significantly higher levels of cystatin C in diabetic patients than in controls. The observed increase in the parameter is hypothesized to be attributable to the occurrence of the tubular phase prior to the onset of glomerular symptoms. This observation implies a potential association between serum cystatin C levels and subclinical tubular dysfunction.

Patients with a duration of diabetes exceeding five years exhibited elevated levels of TGF β1 compared to individuals who had recently been diagnosed. Nevertheless, this disparity did not reach statistical significance (P > 0.05), potentially due to the observed trend of TGF β1 levels increasing with the progression of the participants' cases.[9]

Table 3: Correlations of serum TGF-β1 levels with biochemical and clinical characteristics.

Variables	r-value	P-value
Age (y)	0.179	0.264
Gender	0.043	0.674
BMI (kg/m^2)	0.202[*]	0.044
Duration of DM	0.045	0.654
FBG (mg/dL)	0.273[**]	0.006
HbA1c (%)	0.064	0.527
Cystatin-C	0.597[**]	0.005
Creatinine	0.546[**]	0.004

Although the present study revealed a non-significant difference in the level of TGF β1 with respect to diabetic treatment (P > 0.05), as well as the TGF β1 levels were higher in patients who used oral antihyperglycemic drugs (46.72 ± 7.18) and lower in patients who were kept on dietary control (40.55 ± 4.63). These findings were consistent with the results obtained by Shaker et al,[7] who demonstrated that the TGF β1 levels had significant association with serum glucose. They suggested that the alteration in TGF β1 levels might be associated with nephropathy. Regarding HbA1c the research investigation revealed that there existed no statistically significant link between the levels of HbA1c and TGF β1 within the examined sample (r = 0.064, P = 0.527). These findings were consistent with the outcomes obtained by Shaker et al.[7]

The study revealed that serum TGF-β1 correlates positively and significantly with creatinine and cystatin-C. This study also shows that there is no significant correlation between cystatin-C with FBS. These results are consistent with the findings of the research carried out by Shaker et al.[7]

The current study demonstrated, that there was a significant positive correlation between serum TGF-β1 with creatinine, and cystatin-C (r = 0.546, P = 0.004) and (r = 0.597, P = 0.005) respectively. An increased level of serum TGF-β1 in type 2 diabetic patients may be associated with nephropathy.

5. LIMITATIONS

We studied a moderate sample size due to strict inclusion criteria, which may have some bearing on the results of the study. A larger sample and inclusion of multiple centers may yield similar results, but these would be more credible.

6. CONCLUSION

 The findings of the present research show that the mean levels of serum TGF-β1 were substantially greater for diabetic individuals suffering from nephropathy than in controls. These results also revealed that serum levels of serum TGF-β1were significantly higher with poorly controlled diabetes patients.

7. Conflict of interest

The authors declare no competing interests.

8. Authors' contribution

AHJ : writing the manuscript

NSH: conduction of the study work and manuscript editing

NTY: conduction of the study work and manuscript editing

MMMA: Evaluation and sending the manuscript

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