Original Article

Evaluation Of The Relationship Between Tgf-B Expression And Clinical Symptoms In Patients With Diabetic Nephropathy

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Abstract

Background and Aims: Diabetic nephropathy (DN) is a common diabetic complication that results in chronic renal failure. It has been suggested that pro-inflammatory mediators have a significant role in the development and progression of this disease. As a result, the present study aimed to examine the association between transforming growth factor-β (TGF-β) expression and DN to define a prognostic biomarker that would accelerate DN diagnosis and treatments.

Methods: The study recruited 30 DN patients and collected demographic and clinical data on them. A real-time polymerase chain reaction was used to examine TGF-β expression in peripheral blood. For statistical analysis, the Shapiro-Wilk test, the student t-test, the Mann-Whitney U, and linear regression analysis were used.

Results and conclusion: TGF-β mRNA expression was found to be significantly linked to DN, renal impairment, and a consequent decrease in eGFR. TGF-β expression, on the other hand, had no relation to FBS or HbAlc. More research is needed to fully understand the link between hyperglycemia and TGF- gene expression, as well as the influence of this correlation on the pathogenesis of nephropathy.

Keywords: TGF-β, Diabetes mellitus, Diabetic nephropathy, PCR.

Introduction

Diabetes mellitus (DM) is a chronic metabolic syndrome characterized by high blood glucose, which can lead to serious complications (1). According to the World Health Organization (WHO), DM affects 422 million people in 2016 and results in 1.6 million deaths every year (2). Given the global prevalence of DM, the disease is expected to affect 693 million people by 2045 (3). Additionally, hyperglycemia can induce complications including cardiovascular disease, retinopathy, neuropathy, and nephropathy over time (4). In this regard, diabetic nephropathy (DN) is a common complication of diabetes, which can be caused by both type 1 and type 2 diabetes, although not all diabetics will develop it (5). DN is a glomerular microvascular complication that is characterized by renal hypertrophy, mesangial cell proliferation, matrix expansion, and diffuse and nodular glomerulosclerosis (4, 6). Over time, DN leads to chronic renal failure which, is characterized by a progressive decline in glomerular filtration rate (GFR), albuminuria, uremia, and elevated arterial blood pressure (7). Besides, DN is the primary cause of end-stage renal disease (ESRD) globally, with high morbidity and mortality (8).

There is evidence suggesting that pro-inflammatory cytokines have an important role in the development and progression of DN (9, 10). Among the cytokines associated with DN, transforming growth factor-β (TGF-β) plays an important role in the progression of glomerulosclerosis and interstitial fibrosis, as seen in ESRD (11, 12). TGF-β is a secreted polypeptide that affects cell proliferation, differentiation, adhesion, and migration (13). Furthermore, TGF-β promotes the synthesis of collagen, fibronectin, and proteoglycans while inhibiting matrix degradation (14). Therefore, it plays an important role in mammalian tissue development, homeostasis, and disease (13). Evidence suggests that TGF-β expression increases in patients with DN and rises as the disease progresses (15). In patients with DN, TGF-β overexpression by mesangial tubular cells or infiltrating renal cells has been associated with hyperglycemia, increased intraglomerular pressure, stretching of mesangial cells, activation of the renin-angiotensin system, reactive oxygen species (ROS), and advanced glycation end products (AGEs) (12, 15). These findings have led to the hypothesis that TGF-β inhibition could be an effective therapeutic approach for DN (16-18). However, the significance of TGF-β in the development of DN, and its relationship with hyperglycemia, remain controversial. Determining the precise relationship between TGF-β and DN can have a significant influence on the disease's prognosis, management, and treatment. Therefore, the present study aimed to assess the association between TGF-β expression and DN to develop a prognostic biomarker that would speed up DN diagnosis and treatment.

Materials and Methods

Ethical Considerations

This study was conducted in accordance with the seventh edition of the Helsinki Declaration on the Treatment of Humans (Helsinki Declaration) (19). The Mashhad University of Medical Sciences Ethics Committee authorized the research protocol (Approval no IR.IAU.MSHD.REC.1400.062). The goals of the study were described to the participants, and written informed consent was obtained.

Participants

This randomized, double-blinded, placebo-controlled, and active comparator study was conducted on 30 patients with DN who were admitted to the 17th Shahrivar and Alavi hospitals, Mashhad, Iran, between 2018 and 2019. In addition, an equal number of healthy subjects were included in the control group using simple random sampling. The inclusion criteria were: 1) diagnosis of DN based on a clinical and paraclinical examination, 2) age more than 20 years, 3) a history of nephropathy for at least one year, and 4) ability to give informed consent. Exclusion criteria were also included: 1) diagnosis of liver disease, 2) history of non-diabetic nephropathy, 3) malabsorption disorders, 4) fever for more than a week, 5) infection, 6) pregnancy, 7) consumption of vitamin D and mineral supplements. In addition, the demographic and clinicopathological features of patients, such as age, gender, weight, diabetes duration, and laboratory blood indices, were recorded on specific forms.

RNA extraction and complementary DNA (cDNA) Synthesis

The TGF-β mRNA expression in the blood samples of participants was measured using a real-time polymerase chain reaction (real-time PCR). In this regard, total RNA was extracted from participants' serum samples, and cDNA was synthesized. The TRIzol reagent (Invitrogen) was used to extract total RNA according to the manufacturer's instructions. Also, the Nanodrop ND-1000 (EPOC, USA) and gel electrophoresis (agarose 1%; Pars Tous, Iran) were utilized to quantify and qualify the RNA samples, respectively. Only RNA samples with a 260/280 ratio of 1.8 to 2.2 were used for cDNA synthesis. Finally, cDNA was synthesized using the cDNA Synthesis Kit (Pars Tous, Iran).

real-time PCR

The human TGF-β gene sequence was provided by the National Center for Biotechnology Information (NCBI) GenBank database. The specific primers were then designed using Gene Runner, and their specificity was evaluated using the NCBI Primer-Blast tool. In addition, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was measured as an internal control. The TGF-β primer sequences were sense: 5'- GGAAGGCATGGTTCCAAGTAGTC -3' and antisense: 5'- GTCATTGATGGCACCTAGTACGA -3'. Besides, the GAPDH primer sequences were sense: 5'-GGAAGGTGAAGGTCGGAGTCA-3' and antisense: 5'-GTCATTGATGGCAACAATATCCAT-3'. Power SYBR Green Master Mix and appropriate primers were used to perform real-time PCR on the cDNA. In a microtube with 50-200 ng/2 µl of template cDNA, 10 µl of SYBR Green PCR Master Mix (Amplicon, Denmark), 10 pmol/L of each primer, and 6 µl of RNase-free H_2O, the main reaction was carried out. Following the identification of the optimal temperature, the amplification procedure was performed in the Corbett thermal cycler (Corbett Research, Australia) for an initial denaturation step at 95°C for 5 min, 35 cycles, each cycle consisting of a denaturation step at 94°C for 30 sec, an annealing step at 57°C for 20 sec, and an elongation step at 72°C for 30 sec, and eventually a final elongation stage at 72°C for 5 min. Finally, the ΔΔCT method was used to calculate the fold change expression of TGF-β mRNA expression.

Statistical analysis

The statistical analysis was carried out using SPSS Statistics 25.0 (SPSS Inc., Chicago, Illinois, USA). The data normality was determined using the Shapiro-Wilk test. The Student's t-test was used to compare the differences in TGF-β expression between groups. Additionally, Kruskal-Wallis and Mann-Whitney tests were employed to compare groups with non-normal data. The correlation between variables' values was also determined using Pearson's correlation coefficient and Binary Logistic Regression. All data were presented as mean±standard deviation (SD), and $P \leq 0.05$ was considered statistically significant.

Results

This research was aimed to study the association between TGF-β and DN. In this regard, expression of the TGF-β mRNA and pathological features of 30 patients with DN and 30 healthy subjects were analyzed.

Demographic and clinical features of participants

A total of 30 patients with DN, including 11 males (36.7%) and 19 females (63.3%) participated in this study. Demographic information showed that the mean age of patients and healthy controls were 58.10±17.33 years (range 19 to 84) and 48.53±10.36 years (range 33 to 69), respectively. As a result of statistical comparison, patients and controls had significantly different mean ages ($P<0.05$). The analysis of the age distribution also reveals that 29.9% of patients were under the age of 50, while the remaining were over 50. Additionally, patients and healthy controls had a mean BMI of 28.43±2.16 (range 25.10 to 32.00) kg/m^2 and 28.12±3.74 (range 20.31 to 35.64) kg/m^2, respectively. However, the Student t-test revealed no significant difference in BMI between the two groups. The results showed that 70% of the patients were overweight with a BMI of 25-29.9 kg/m^2, and 30% of them were obese with a BMI of ≥30 kg/m^2. Therefore, none of the participants with DN were of normal BMI. Moreover, estimated glomerular filtration rate (eGFR) and blood parameters such as fasting blood sugar (FBS), hemoglobin A1c (HbA1c), insulin, urea, creatinine, and lipid profile were assessed in nephropathy patients, and the findings are described in Table 1. The findings revealed that 70% of patients had abnormal FBS levels and, all of them had abnormal HbA1c values. Patients had a significantly higher mean FBS level (128.33±17.92 mg/dl) than healthy controls (95.93±16.43 mg/dl) ($P < 0.001$). Additionally, the HbA1c values in patients decreased significantly from 7.14±0.29 to 5.02±0.57 in controls ($P < 0.001$). Furthermore, all patients had abnormal creatinine and GFR values (Tab. 1). There was a statistically significant difference between the patients and the controls, with a mean creatinine level of 1.34±0.07 and 1.05±0.2, respectively ($P < 0.001$). In addition, the mean eGFR decreased from 90 in the controls to 72 in the patients,

a statistically significant increase ($P < 0.001$). The healthy controls, on the other hand, had mean levels of cholesterol, triglycerides, LDL, and HDL of 177.60±50.27 mg/dl, 125.33±18.13 mg/dl, 101.77±33.77, and 43.37±14.48, respectively. When compared to controls, triglyceride, and LDL levels are shown to be considerably higher in patients.

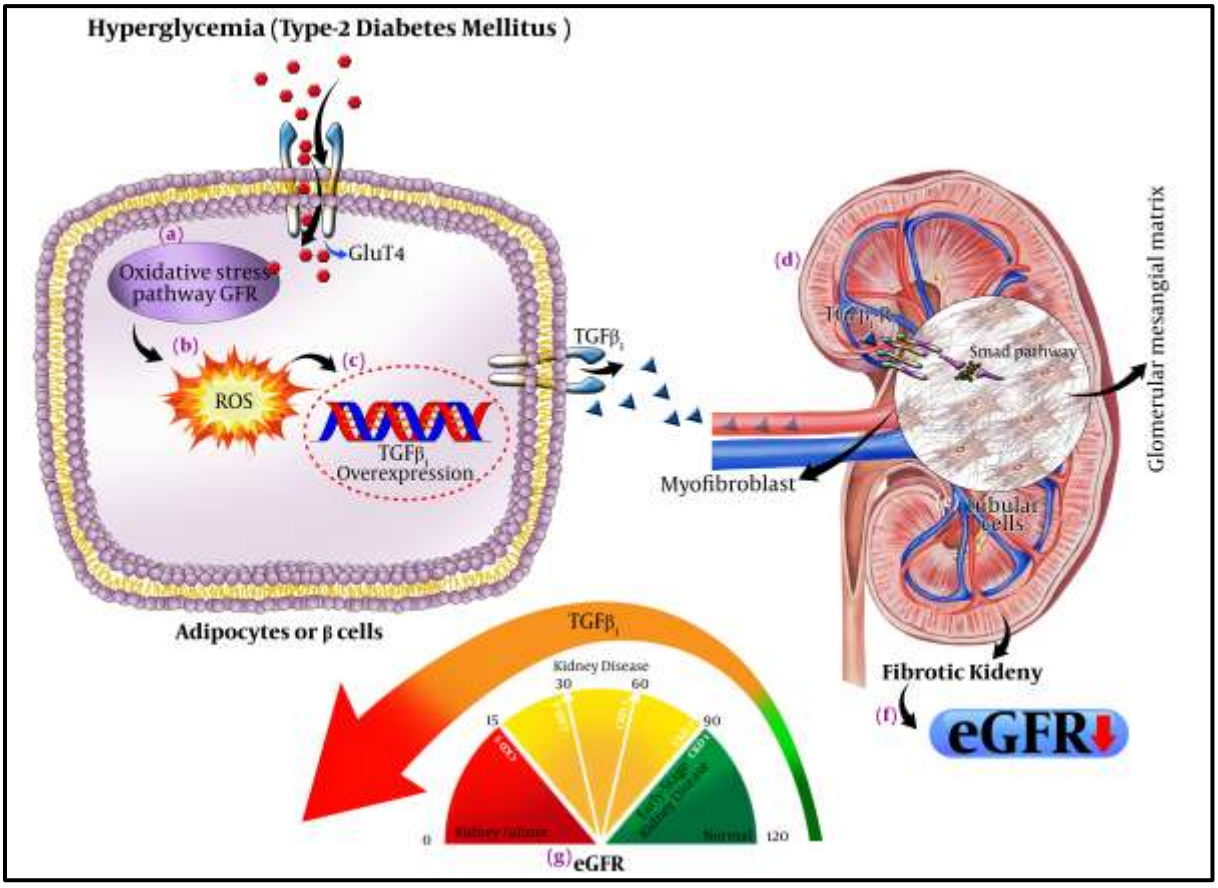


Figure 1: The regulatory role of TGF-β signal and eGFR in DN

In adipocytes or beta cells, the overproduction of mitochondrial ROS and the altered antioxidant system plays a crucial role in the pathogenesis and the development of CKD through the enhancement of oxidative stress (OS) in the kidney;

a) Glucose enters the cells through the GluT4 channel in hyperglycemia

b) The production of mitochondrial superoxide is increased because of the enhancement of intracellular glucose transport and the augmentation of intracellular glucose oxidation.

c) Dysfunction in cell components and organelles, especially mitochondria, results in the overproduction of mtROS, persistence inflammation, and overexpression of Transforming growth factor β1 (TGF β1), which is a key profibrotic growth factor. Notably, ROS act as a signal amplifier in diabetes.

d) Epithelial-mesenchymal transition (EMT) is induced by the activation of SMAD signaling pathway through the binding of TGF-β to its receptors (TGFβ-R).

e) Eventually, renal tubulointersitial fibrosis is developed which is involved in the progression of chronic kidney disease.

f) Lower level eGFR leads to the higher risk of chronic kidney disease (CKD) which can progress to kidney failure.

g) Hence, as the amount of TGF-β is rising, the amount of eGFR is falling, indicating a negative correlation between them, which leads to the CKD aggravation.

Table legends:

Tab. 1: Distribution of blood indices in patients with diabetic nephropathy (FBS: Fasting blood sugar, eGFR: estimated glomerular filtration rate, SD: standard deviation)

Tab. 2: Evaluation of the association between blood indices and glomerular filtration with the TGF-β mRNA expression in patients with diabetic nephropathy. ** $P \leq 0.01$

Tab. 3: The correlation coefficient of the studied variables with TGF gene expression in patients with nephropathy (BMI: Body mass index, FBS: Fasting blood sugar, eGFR: estimated glomerular filtration rate, SD: standard deviation). *** $P \leq 0.001$

Tab. 4: Evaluation of the relationship between TGF-BETA gene expression and the studied variables by analysis of variance (BMI: Body mass index, FBS: Fasting blood sugar, eGFR: estimated glomerular filtration rate, SD: standard deviation). ** $P \leq 0.01$

Figure legends:

Fig. 1: Frequency distribution of TGF-β gene expression in patients with diabetic nephropathy using real-time PCR

Fig. 2: Relationship between age and TGF-β expression in patients with nephropathy

Fig. 3: Relationship between gender and TGF-β expression in patients with nephropathy

Fig. 4: Relationship between BMI and TGF-β expression in patients with nephropathy

Fig. 5: Relationship between eGFR and TGF-β expression in patients with nephropathy

Fig. 6: Regression analysis of the relationship between TGF-β expression and A) FBS, B) HbAc1 and C) GFR. D) Three-dimensional graph of the simultaneous effect of TGF-β and HbA1C on eGFR score in patients with diabetic nephropathy

Table 1.

Variables	Incidence				Minimum	Maximum	Mean±SD
	Normal		Non-normal				
	n	%	n	%			
FBS	9	30.0%	21	70.0%	99.00	157.00	128.33±17.92
HbA1c (%)	0	0.0%	30	100.0%	6.70	7.60	7.14±0.29

Insulin	24	80.0%	6	20.0%	0.30	58.60	13.55±16.64
Triglyceride (mg/dl)	0	0.0%	30	100.0%	190.00	272.00	233.30±21.01
Cholesterol (mg/dl)	30	100.0%	0	0.0%	174.00	190.00	183.43±5.02
HDL	13	43.3%	17	56.7%	40.00	56.00	46.75±4.77
LDL	1	3.3%	29	96.7%	92.00	141.00	119.73±12.70
Urea (mg/dl)	30	100.0%	0	0.0%	27.00	42.00	35.20±4.66
Creatinine (mg/dl)	0	0.0%	30	100.0%	1.21	1.51	1.34±0.07
eGFR	0	0.0%	30	100.0%	68.00	78.40	72.89±3.19

Table 2.

Indices		Minimum	Maximum	Mean±SD	Test statics
FBS	Normal	0.1426	1.9319	0.683±0.585	U=-1.02 P-Value=0.308
	Non-normal	0.0579	4.1411	1.262±1.266	
HbA1c	Normal	-	-	-	-----
	Non-normal	0.0579	4.1411	1.088±1.128	
Insulin	Normal	0.1539	4.1411	1.282±1.171	U=-2.72 P-Value=0.006**
	Non-normal	0.0579	1.1329	0.312±0.405	
Triglyceride (mg/dl)	Normal	-	-	-	-----
	Non-normal	0.0579	4.1411	1.088±1.128	
Triglyceride (mg/dl)	Normal	0.0579	4.1411	1.088±1.128	-----
	Non-normal	-	-	-	
HDL	Normal	0.1539	3.1383	0.805±0.893	U=-1.00 P-Value=0.315
	Non-normal	0.0579	4.1411	1.305±1.262	
LDL	Normal	0.1539	0.1539	0.154	-----
	Non-normal	0.0579	4.1411	1.120±1.134	
Urea (mg/dl)	Normal	0.0579	4.1411	1.088±1.128	-----
	Non-normal	-	-	-	
Creatinine (mg/dl)	Normal	-	-	-	-----
	Non-normal	0.0579	4.1411	1.088±1.128	
eGFR	Normal	-	-	-	-----
	Non-normal	0.0579	4.1411	1.088±1.128	

Table 3.

Variables	Coefficient correlation	P-value
Age	0.273	0.144
BMI	0.331	0.074
FBS	0.334	0.071
HbA1c (%)	0.075	0.692
Insulin	-0.242	0.198
Triglyceride (mg/dl)	0.237	0.208
Cholesterol (mg/dl)	-0.005	0.980
HDL	-0.324	0.081
LDL	0.253	0.178
Urea (mg/dl)	0.142	0.453
Creatinine (mg/dl)	0.012	0.951
eGFR	-0.760	0.0001***

Table 4.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.080[a]	13	2.083	3.397	0.011
Intercept	3.189	1	3.189	5.201	0.037
Gender	0.008	1	0.008	0.014	0.909
Age	0.507	1	0.507	0.827	0.377
BMI	0.292	1	0.292	0.476	0.500
FBS	2.347	1	2.347	3.827	0.068
HbA1c	0.897	1	0.897	1.463	0.244
Insulin	0.017	1	0.017	0.028	0.868
Triglyceride	0.084	1	0.084	0.137	0.716
Cholesterol	0.079	1	0.079	0.129	0.724

HDL	0.633	1	0.633	1.032	0.325
LDL	0.712	1	0.712	1.162	0.297
Urea	0.183	1	0.183	0.298	0.592
Creatinine	0.095	1	0.095	0.156	0.699
eGFR	11.135	1	11.135	18.158	0.001**
Error	9.811	16	0.613		
Total	72.416	30			
Corrected Total	36.892	29			

Figure 1.

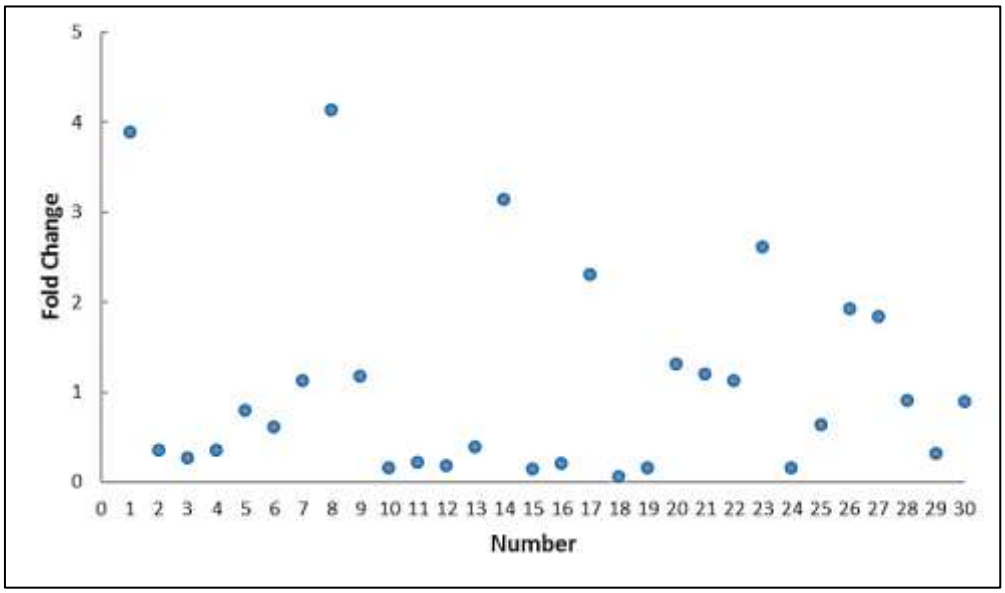
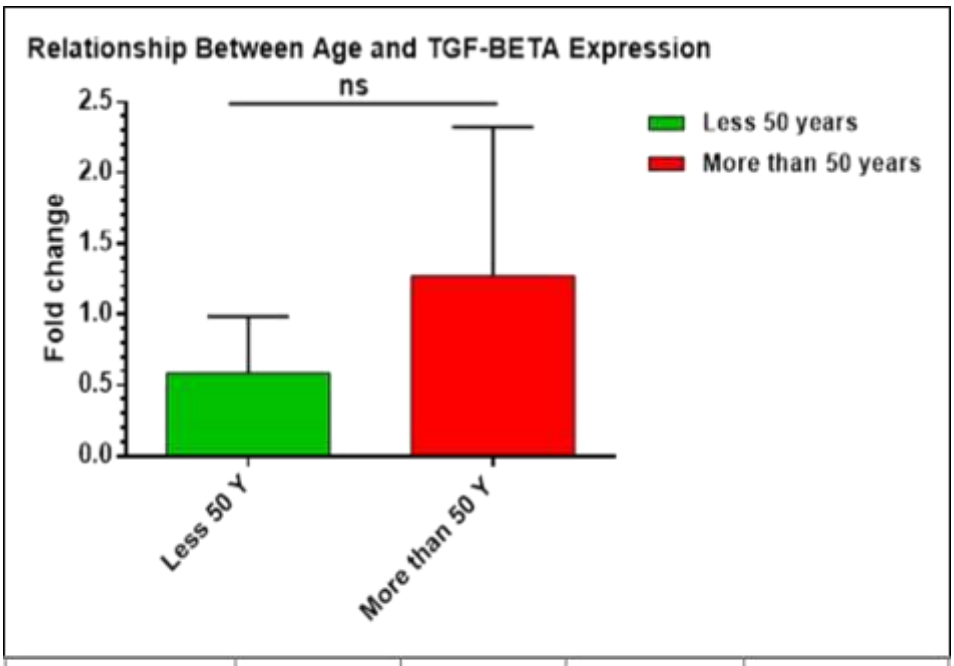
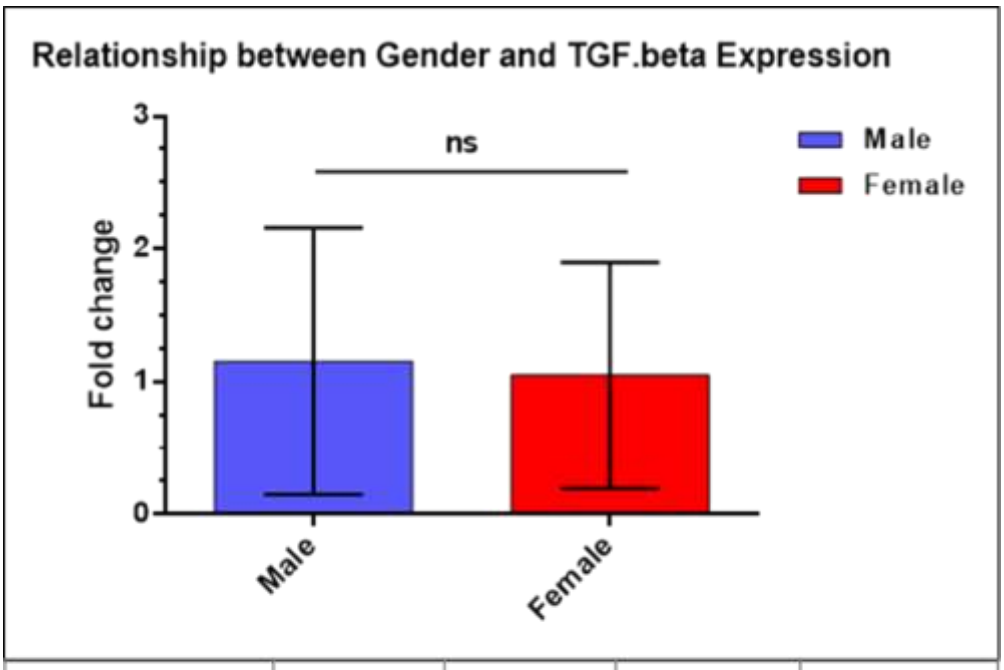


Figure 2.



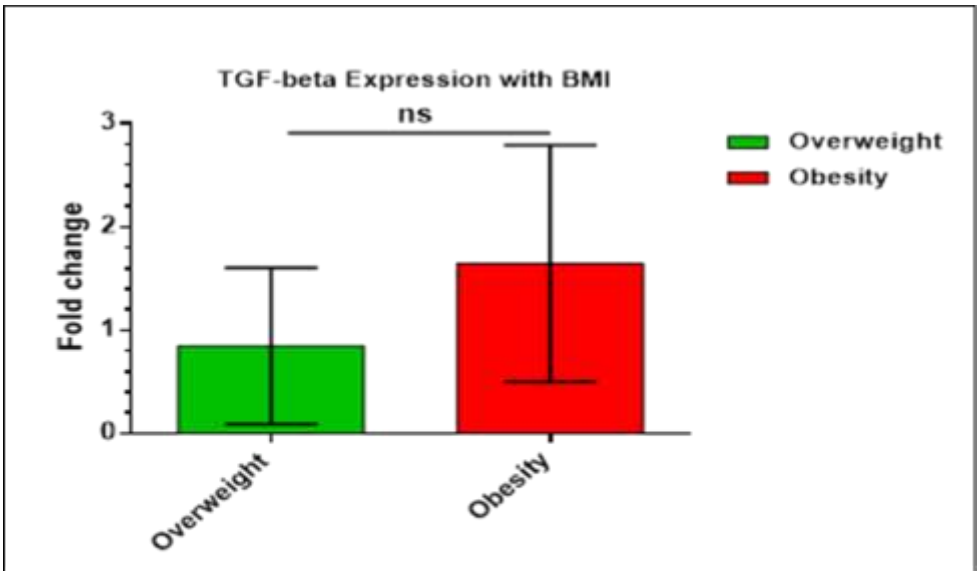
Age (years)		Minimum	Maximum	Mean±SD	Test statics
TGF-β	≤50	0.154	1.310	0.585±0.400	U=-0.94 P-Value=0.348
	≥50	0.058	4.141	1.271±1.254	

Figure 3.



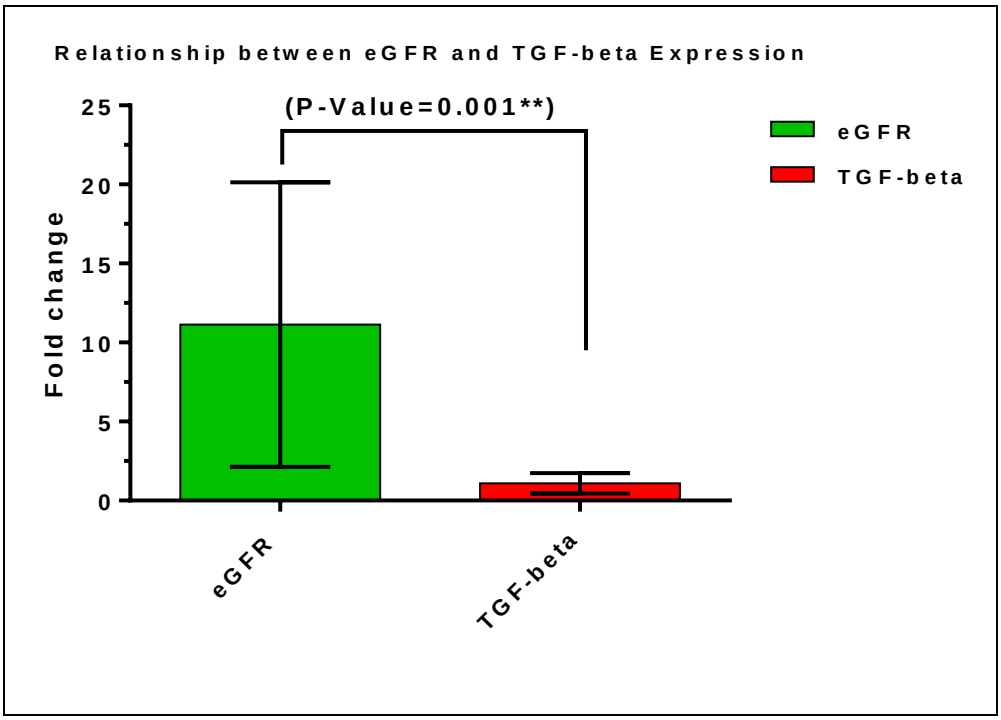
Gender		Minimum	Maximum	Mean±SD	Test statics
TGF-β	Male	0.154	4.141	1.155±1.364	U=-0.02 P-Value=0.983
	Female	0.058	3.891	1.050±1.006	

Figure 4.



BMI		Minimum	Maximum	Mean±SD	Test statics
TGF-β	Overweight	0.1426	2.6208	0.849±0.757	U=-0.99 P-Value=0.319
	Obesity	0.0579	4.1411	1.645±1.637	

Figure 5.



Association of TGF-β expression and clinicopathological features

The ΔΔCT method was used to calculate the fold change in the expression level of the TGF-β mRNA in participants. According to the results, the mean fold change of TGF-β expression in patients with DN was 1.088±1.128, with a range of 0.058 to 4.141 (Fig. 1). The effect of age on TGF-β expression was investigated by comparing TGF-β mRNA expression in patients aged ≤50 years to those over 50 (Fig. 2). The results show an increase in the mean expression level of TGF-β from 0.585±0.400 in patients aged ≤50 to 1.271±1.254 in those over 50. However, the Mann- Whitney test did not reveal any statistically significant differences between the two groups in terms of TGF-β expression. Furthermore, the Mann-Whitney test showed no statistically significant difference in the average of TGF-β expression levels between male (1.155±1.364) and female (1.049±1.006) patients (Fig. 3). In addition, obese patients had a higher mean expression level of TGF-β (1.645±1.637) than overweight individuals (0.849±1.757), albeit the Mann-Whitney test revealed that this difference was not statistically significant (Fig. 4). The assessment of the blood indices in patients also revealed that only insulin was significantly associated with TGF-β expression, with the mean TGF-β expression decreasing from (1.282±1.171) in patients with normal insulin levels to (0.312±0.405) in those with abnormal insulin levels (Tab. 2; $P<0.01$). TGF-β expression, on the other hand, had no significant association with other blood parameters such as FBS, HbA1c, insulin, urea, creatinine, and lipid profile (Tab. 2). To investigate the relationship between all variables and TGF-β gene expression, a linear model was used. The correlation coefficients of variables with TGF-β gene expression are shown in Table 3. The results revealed a significant inverse correlation between eGFR and TGF-β mRNA expression ($r= -0.760$, $P < 0.001$) (Fig. 5). We also employed analysis of variance to investigate the relationship between TGF-β expression levels and the patients' demographics, eGFR, and blood indices. As indicated in Table 4, analysis of variance shows that, except for eGFR, no other parameters had a significant association with TGF-β expression. Additionally, regression analysis revealed a direct correlation between TGF-β mRNA expression and FBS and HbA1c. However, the observed correlation was not statistically significant (Fig. 6A, B). Moreover, regression analysis confirmed that there was a significant regression equation in terms of eGFR and change in TGF-β expression ($r= -0.760$, $P < 0.0001$) (Fig 6C). The result showed that TGF-β expression was reduced by the increase in eGFR. Furthermore, it was found that the higher Hb.A1C level and lower TGF-β expression were associated with a higher eGFR in patients with DN (Fig. 6D).

Discussion

DN affects one-third of diabetic patients and is one of the most significant vascular complications of DM. This complication is characterized mostly by persistent albuminuria, elevated blood pressure, and a progressive decline in renal function (20). Furthermore, the GFR, which reflects renal function, gradually declines in these patients, causing them to enter ESRD (21). Currently, controlling the patient's blood pressure with medication and lifestyle changes is the most effective treatment for DN (22). However, due to the influence of blood pressure from a variety of factors, precise management of blood pressure is impossible. As a result, the present study aimed to see if TGF-β mRNA expression was associated with the development of DN to create a prognostic biomarker that would speed up DN diagnosis and treatment. In this regard, the expression level of TGF-β mRNA in 30 patients with DN was measured by the real-time PCR assay. Measuring mRNA expression can reveal critical information about protein levels and biological function.

Although the mean age of the patients and healthy controls in this study differed significantly, there were no significant differences in the parameters of gender and BMI. Our findings revealed a relationship between TGF-β expression and the probability of developing nephropathy in diabetic patients. In addition, the levels of FBS and HbA1c in patients were significantly higher than in controls. It seems that nephropathy may have a considerable impact on blood sugar management based on these findings. In confirmation of this assumption, many studies have found that diabetic patients with nephropathy have considerably higher FBS and HbA1c levels than diabetic patients without nephropathy. In contrast, some studies have suggested that poor glycemic control, as manifested by elevated FBS and HbA1c, may be the underlying cause of DN. Although TGF-β expression increases in all diabetic patients, Gewin (2020) stated in a review of the role of TGF-β in the development of DN, that despite the finding that TGF-β expression increases in all diabetic patients, it is still unclear how hyperglycemia causes this increase (17). As a result, more research is needed to determine the causal relationship between hyperglycemia and DN. In our study, the rate of renal creatine excretion in patients with DN was significantly higher than in the control group, whereas their eGFR was significantly lower. These findings support the impairment in renal function in DN patients. Given that regression analysis reveals a direct relationship between TGF-β gene expression and eGFR value, it is reasonable to conclude that greater TGF-β expression is strongly associated with lower eGFR in DN patients. However, because creatinine and eGFR levels are considered inclusion criteria for participants to enter the study, many studies do not report the values of these parameters in the results. Furthermore, an assessment of the patients' blood lipid profile revealed a statistically significant difference in blood triglyceride levels and LDL between DN patients and the controls. However, it is worth noting that the blood indices of diabetic patients in this study were somewhat closer to normal values as a result of getting anti-hyperglycemia medicines. Moreover, whereas the blood lipid profile analyses revealed a statistically significant difference in triglyceride and LDL levels between patients and controls, no clear association was found between TGF-β expression and these parameters. Hyperlipidemia has been implicated in some studies as a risk factor for the development of nephropathy. For example, Chen et al. (2020) demonstrated that treatment with free saturated fatty acid palmitate activates Smad2/3 and increases the expression of ECM proteins in human glomerular mesangial cells (23). The results of other studies indicate that the association between DN and changes in blood lipids in diabetic patients is not clear and requires further investigation. The TGF-β signaling pathway is shown to be significantly enhanced in glomeruli, and tubule cells in both humans and animal models with DN (24, 25). Hyperglycemia appears to enhance TGF-β transcription in a variety of kidney cell types, including proximal tubular cells, mesangial cells, and fibroblasts (24). Evidence suggests that AGEs, which are formed by irreversible glycosylation of proteins, may play a role in DN pathogenesis by activating the TGF-β signaling pathway in the kidneys of diabetic patients (25). It has been shown in vitro that AGEs activate TGF-β/Smad signaling in renal tubular cells and vascular cells, resulting in increased collagen production in the extracellular matrix and fibrogenesis (26, 27).

In support of our findings, John and Yadla (2019) demonstrated that TGF-β1, as a proinflammatory factor, plays a

significant role in the pathogenesis of DN and that measuring these cytokines could potentially distinguish diabetic from non-diabetic nephropathy (28). They also discovered that TGF-β1 played a significant role in the development of renal fibrosis by generating persistent alterations in the histology of renal tissue. As a result, it was suggested that TGF-β1 could be a viable biomarker in nephropathic diabetes prognosis. In this regard, several studies have shown that TGF-β promotes the production of the extracellular matrix while preventing its degradation (29, 30). Overproduction of TGF-β, on the other hand, can result in fibrosis due to a pathological accumulation of the extracellular matrix (29, 31). In another study, Rivarola et al. (1999) investigated examined TGF-β in urine samples from type 2 diabetic patients(32). They discovered that patients with nephropathy have a greater rate of urine TGF-β excretion than healthy controls. They also discovered a relationship between urine TGF-β excretion and proteinuria, which is one of the indicators of renal failure. In an animal model of glomerulonephritis, Border et al. (1990) found that administering a TGF-β inhibitor stops glomerular cells from producing more matrix proteins and inhibits ECM deposition (33). Furthermore, it was reported that transfection of the TGF-β gene into the rat kidney results in high TGF-β levels in the glomeruli and fast glomerulosclerosis development (34, 35).

Voelker et al. (2017), on the other hand, employed a monoclonal specific antibody against TGF-β1 to reduce renal failure in patients with DN using a chronic stable renin-angiotensin system inhibitor (36). Their findings demonstrated that TGF-β1–specific monoclonal antibody did not reduce the progression of DN when combined with renin-angiotensin system inhibitors. Although the expression of TGF-β increased with rising blood sugar in the current study, the correlation coefficient analysis revealed that there was no significant association between the expression of TGF-β with HbA1C and FBS. These results could be due to the small sample size or the patients' use of anti-hyperglycemic medicines, but more research is needed. Another point to consider is that some studies have indicated that a high glucose level can activate latent TGF-β through thrombospondin action, in addition to enhancing TGF-β gene expression (25). As a result, in addition to assessing the level of TGF-β mRNA, quantifying the amount of active protein is also essential. Moreover, due to reports on the influence of hyperglycemia on TGF-β receptor activation in renal mesenchymal cells, it is important to assess TGF-β receptor expression simultaneously in similar research.

Conclusion

In summary, TGF-β mRNA expression was directly correlated with DN, renal impairment, and a subsequent decrease in eGFR. However, there was no association between TGF-β expression and FBS or HbAlc. More research is needed to understand the relationship between hyperglycemia and TGF-β gene expression, as well as the impact of this relationship on the development of nephropathy.

Acknowledgment

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Conflict of interest

The authors declare that there is no conflict of interest.

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