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Transforming Growth Factor-beta 1 (TGF-β1) as a Master Regulator of Diabetic Nephropathy in Patients with type-ll Diabetes attending Aswan University Hospital

Aly Mahmoud Obaed[1*], Zain El-Abdeen Ahmed Sayed[2], Hala Mahmoud[3], Emad Farah Mohammed Kholef[4], Mohammed Mohiey El-Din Fouad[5], Abdallah Elebidi[6]

[1]Master's degree in internal medicine faculty of Medicine Ain Shams University, Email: aly.obaed@gmail.com
[2]Professor of Internal Medicine, Faculty of Medicine -Assiut University
[3]Assistant Professor of Internal Medicine, Faculty of Medicine -Aswan University
[4]Assistant Professor of Clinical Pathology, Faculty of Medicine -Aswan University
[5]Lecturer of Internal Medicine, Faculty of Medicine -Aswan University
[6]Professor of Biochemistry Aswan University, Email: abdallahelebidi@aswu.edu.eg
*Corresponding Author

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ABSTRACT

Objective: The objective is to study type ll diabetic patients at Aswan University Hospital and determine the function of converting growth factor-beta 1 (TGF-β1) toward the development of diabetic kidney disease (DKD).

Methods: We used a case-control design in this investigation. Our research includes 90 participants. The first group was the control group, which included healthy people randomly selected from the Aswan University Hospital blood bank.; second, there was the group of type 2 diabetics who did not have nephropathy; and third, there was the group of type 2 diabetics who did have nephropathy. The subjects for Groups 2 and 3 were sourced from the nephrology and diabetes clinics at Aswan University Hospital in Aswan, Egypt.

Results: According to these findings, TGF-β1 may serve as an early and highly specific marker of diabetic neuropathy in individuals who have diabetes of the type 2 variety. In the Type 2 diabetes. with nephritis group, the mean TGF-β1 level was 1424.3 ± 95.8 ng/ml, which was substantially higher than in the controls group (392.5 ± 14.9, $p<0.001$) and the people with type 2 diabetes with no nephropathy group (523.3 ± 22.5, $p<0.001$). Similarly, the mean level was much higher ($p=0.003$) in the group of type 2 diabetics without nephropathy compared to the control group.

Conclusion: Compared to adults without diabetes and healthy controls, those with diabetic neuropathy had considerably greater levels of TGF-β1. Moreover, T2DM cases without nephropathy group had significantly higher mean level than control. Furthermore, at the cutoff value of 435 ng/ml,; the TGF-β1 had better ability for prediction of T2DM with nephropathy as sensitivity 94%, specificity 87% PPV 88%, NPV 93.5% and overall, the test had 90.5% accuracy. Indicating that TGF-β1 has the potential to be a very sensitive and specific early indication of diabetic neuropathy in adults with type 2 diabetes.

Keywords: Diabetes mellitus, Diabetic Nephropathy, Transforming growth factorB1

INTRODUCTION

In Egypt nowadays; Diabetes mellitus (DM (is a conundrum and a growing issue. 15.2% of Egyptian people have diabetes, as estimated by the International Diabetes Federation (IDF)(1). Acute coronary syndrome, stroke, chronic renal failure, blindness, and amputation of the lower extremities are all most commonly brought on by diabetes mellitus (DM)(2).

Continuous kidney disease (CKD) and other microvascular complications are common among diabetics. The development of end-stage kidney disease (ESKD) is among the several potential consequences of chronic kidney disease (CKD). Therefore, therapeutic regimens are continuously developed to delay or prevent the progression of CKD in DM based on understood pathophysiologic mechanisms of CKD in DM(3) .

Mesangial enlargement, progressive glomerular scelrosis, extracellular protein deposition, and increased growth factor synthesis are all symptoms of persistent hyperglycemia, all of which decrease GFR(4).

To be acknowledged, TGF-β is known as the transforming growth factor an immunological mediator with anti-inflammatory properties. It functions by impeding or reversing the activation of macrophages through its ability to interfere with signaling pathways that are dependent on toll-like receptors(5). According to **Herder et al.,**

high levels of TGF-1 are related with T2DM and elevated IL-1Ra concentrations, which increase prior to the onset of T2DM. T2DM thus contains both pro- and anti-inflammatory mediators, and TGF-β1 may act on both sides(6).

People with diabetes who have type 2 diabetes commonly have elevated blood sugar levels, which is caused by cells infiltrating their kidneys or the tubules in the blood arteries overexpressing TGF- β. In addition to factors previously established to enhance TGF- β creation in the kidneys or in cultured mesangial or tubular cells, additional factors include raised intraglomerular pressure, extending of mesangial cells, activation of the renin and angiotensin system, reactive oxidant species (ROS), and advanced glycation end products (AGEs). There are two mechanisms that TGF- β uses to promote ECM production and signaling receptors: paracrine and autocrine. (5) (7).

METHODS

Invasive renal cells or mesangial tubular cells upregulating TGF-β causes hyperglycemia, which is often seen in people with type 2 diabetes. Some other elements that have been found to boost TGF-β production in kidneys or in cultured cells of the mesangial or tubular type include elevated intraglomerular pressure, expansion of the mesangial cells, activation of the renin-angiotensin system, reactive oxygen species (ROS), and advanced glycation end products (AGEs).

Inclusion criteria

1. Age between 30 to 70 years with different sex.
2. Type-lldiabetes mellitus (T2DM) individuals who have had diabetes for at least five years.
3. Type-lldiabetes mellitus (T2DM) with nephropathy.

Exclusion criteria

1. Individuals with essential hypertension.
2. Individuals who have experienced any chronic renal problems not related to DM.
3. people who have type 1 or secondary diabetes.
4. Exclusion criteria for the study included the presence of a urinary tract infection, systemic infection, aspirin use, and systemic steroid use.
5. Lack of informed consent.

Our research comprised Ninety participants, who were then separated into three groups:

1- First group (healthy controls): 30 people, ranging in age from 30 to 70, with normal blood pressure and glucose levels .

2- Group 2 (T2DM patients without nephropathy) (n=30).

3- The third group, which consists of people who are type 2 diabetic with nephropathy (n=30), includes individuals with diabetes mellitus, diabetic retinal degeneration, microalbuminuria, or overt proteinuria.

The participants in Groups 2 and 3 Participants were aged between 30 and 70 years and had a minimum diabetes duration of 5 years.

Each of the ninety participants underwent:

1- Complete history and physical examination: includeage , sex, underlying diseases, age of the onset of diabetes and diabetic kidney disease (DKD) , history of smoking and vital signs.

2 - Anthropometric measurements: (waist circumference - BMI).

3 –Investigations:

(A) Serum transforming growth factor-beta 1 (TGF-β1) by ELISA Kits

(B) Complete blood picture.

(C)Renal function tests and albumin-to-creatinine ratio.

(D) Serum electrolytes.

(E)Glycated hemoglobin (HbA1c) level.

(F)Fundus examination by direct ophthalmoscopy.

As per the criteria set out by the American Diabetes Association (ADA) for the year 2021, type 2 diabetes was officially diagnosed. As per the 2020 guidelines put out by the National Kidney Foundation (NKF) (8), diabetic nephropathy is defined as the presence of diabetic retinopathy in conjunction with two separate episodes of either overt albuminuria (ACR > 300 mg/g creatinine) or persistent microalbuminuria (ACR 30-299 mg/g creatinine) six weeks apart.

A direct ophthalmoscopy fundus examination was conducted to seek for symptoms of diabetic retinopathy at the ophthalmology clinic at Aswan University Hospital. The laboratory tests needed, such as measuring serum transforming growth factor-beta 1 (TGF-β1) using ELISA kits, were carried out at the Aswan University Hospital Laboratory Departments. The study was authorized by the Institutional Ethics Committee-Human Research (IEC-HR) of Aswan University Hospitals, and all subjects provided written informed permission prior to their participation.

Ethical consideration

1- Written consent was taken from each participant.
2- Confidentiality was secured as we give each participant a code.

Statistical analysis

Version 24 of IBM-SPSS was used in order to carry out the statistical analysis. In addition to being reported as frequencies, categorical variables were also displayed as percentages. For the purpose of analyzing the proportions of different groups, we used either the Chi-square test or the Monte Carlo exact testing, depending on the specifics of the situation. Any and all quantitative numbers, together with their respective standard deviations (SD), were presented to the audience. For the purpose of determining whether or not the data followed a normal distribution, the Shapiro-Wilk test was used. In order to determine whether or not the data followed a normal distribution, we carried out an analysis of variance (ANOVA) on constant variables that included a number of categories. When computing the post hoc test, Bonferroni adjustments were taken into account. In order to demonstrate the effectiveness of biomarkers in predicting and diagnosing diabetic nephropathy, a receiver operating characteristic curve (ROC) was used. The use of the area under the curve (AUC), the standard error (SE), and the confidence interval (CI) of 95% simplified the process of evaluating the effectiveness of the diagnostic procedure. When calculating validity metrics, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were among the metrics that were taken into consideration. When the p-value was less than 0.05, statistical significance was determined to have been achieved.

RESULTS

Regarding patient's age, controls were significantly ($p<0.001$) younger (38.5 ± 9.7 years) compared with T2DM cases with and without nephropathy (58.1 ± 9.3 and 53.3 ± 10.5 years). Contrarily, T2DM cases without nephropathy were insignificantly ($p=0.064$) younger than T2DM cases with nephropathy .Nonetheless, there was a sex match between the three groups ($p=0.935$).

In terms of the rate of comorbidity, all three groups were similar ($p=0.345$). Similarly, the amount of smoking past rate was similar among the three groups ($p=0.424$). On the other hand, T2DM cases without nephropathy had shorter duration of DM (11.3 ± 4.4 years) compared with those with nephropathy (16.4 ± 4.7 years) ($p<0.001$) .

Regarding the clinical examination results

Respecting the vital signs, the three groups were comparable ($p>0.05$).

However, there was a substantial difference in the mean anthropometric measurements across the groups (BMI and WC). ($p=0.005$ and <0.001).

For the BMI, the mean value was significantly lower in control (23.4 ± 3.1) compared with both T2DM without nephropathy group (26.1 ± 4.1, $p=0.003$) and T2DM with nephropathy group (25.8 ±3.1, $p=0.010$). Contrarily, T2DM cases without nephropathy were insignificantly ($p=0.656$) heavier than T2DM cases with nephropathy .

For the WC, the mean value was significantly higher in T2DM cases without nephropathy (95.6 ± 9.8 cm) compared with both control group (86.2 ± 5.7, $p<0.001$) and T2DM with nephropathy group (84.5 ± 4.8, $p<0.001$). Contrarily, control cases had insignificantly ($p=0.427$) higher mean WC than T2DM cases with nephropathy .

Regarding the laboratory investigations

Forty% (n=12) of the T2DM cases with nephropathy group had IDA compared with no cases in both control and T2DM cases without nephropathy groups. This was statistically significant ($p<0.001$) .

Moreover, ESR was significantly different between groups ($p<0.001$). The mean ESR at 1st hour was significantly higher in T2DM cases with nephropathy (15.6 ± 1.8 mm/hr) compared with both control group (11.7 ± 1.6, $p<0.001$) and T2DM without nephropathy group (11.8 ± 1.3, $p<0.001$). Contrarily, control cases had insignificantly ($p=0.796$) lower mean level than T2DM cases without nephropathy. Additionally, the mean ESR at 2nd hour was significantly higher in T2DM cases with nephropathy (29.8 ± 8.9 mm/hr) compared with both control group (23.1 ± 5.9, $p<0.001$) and T2DM without nephropathy group (23.5 ± 3.3, $p<0.001$). Contrarily, control cases had insignificantly ($p=0.916$) lower mean level than T2DM cases without nephropathy .

There was a significant difference between the groups with respect to the HbA1C level ($p<0.001$). When comparing pairwise, the control group's mean value was much lower (4.9 ± 0.2 mmol/L) compared with both T2DM without nephropathy group (9.9 ± 2.3, $p<0.001$) and T2DM with nephropathy group (10.6 ± 1.8, $p<0.001$). Contrarily, T2DM cases without nephropathy had insignificantly ($p=0.109$) lower mean HbA1c level than T2DM cases with nephropathy .

There was probably a significant difference in creatinine levels across the groups ($p<0.001$). T2DM patients with nephropathy had a substantially higher mean s. creatinine (2.42 ± 0.3 mg/dl) than both the control group

(1.07 ± 0.1, p<0.001) and the T2DM group without nephropathy (1.09 ± 0.1, p<0.001). On the other hand, the mean level of control cases was marginally (p=0.931) lower than that of T2DM subjects without nephropathy.
Similarly, urinary ACR level was significantly different between groups (p<0.001). The mean u. ACR was significantly higher in T2DM cases with nephropathy (773.5 ± 68.5 mm/mg cr.) compared with both control group (14.1 ± 3.5, p<0.001) and T2DM without nephropathy group (17.51 ± 5.6, p<0.001). Contrarily, control cases had insignificantly (p=0.987) lower mean level than T2DM cases without nephropathy .

Table 1: Comparison of Laboratory Investigations between studied cases

	Control (1) (n = 30)	**T2DM no NP (2) (n = 30)**	**T2DM with NP (3) (n = 30)**	**P-value**
CBC (IDA)	0 (0%)	0 (0%)	12 (40%)	**< 0.001***
ESR				
• 1st hour	11.70 ± 1.7	11.80 ± 1.3	15.60 ± 1.8	**< 0.001****
P-value***	1 vs 2 = 0.796	**2 vs 3 < 0.001**	**1 vs 3 < 0.001**	
• 2nd hour	23.07 ± 5.9	23.50 ± 3.3	29.77 ± 8.9	**< 0.001****
P-value***	1 vs 2 = 0.916	**2 vs 3 < 0.001**	**1 vs 3 < 0.001**	
HbA1C%	4.88 ± 0.2	9.90 ± 2.3	10.61 ± 1.9	**< 0.001****
• P-value***	**1 vs 2 < 0.001**	2 vs 3 = 0.109	**1 vs 3 < 0.001**	
S. Creatinine (mg/dl)	1.07 ± 0.1	1.09 ± 0.1	2.42 ± 0.3	**< 0.001****
• P-value***	1 vs 2 = 0.931	**2 vs 3 < 0.001**	**1 vs 3 < 0.001**	
U. ACR (mg/gm cr)	14.01 ± 3.5	17.15 ± 5.6	773.45 ± 68.5	**< 0.001****
• P-value***	1 vs 2 = 0.982	**2 vs 3 < 0.001**	**1 vs 3 < 0.001**	

Regarding the laboratory investigations (serum electrolytes)
For the Na level, there was significantly different level between groups (p<0.001). The mean Na level was significantly higher in T2DM cases with nephropathy (136.6 ± 7.9 mmol/L) compared with both control group (130.1 ± 1.7, p<0.001) and T2DM without nephropathy group (130.6 ± 2.9, p<0.001). Contrarily, control cases had insignificantly (p=0.659) lower mean level than T2DM cases without nephropathy .
Contrarily, there was insignificant difference in the mean K and Mg levels (p=0.126 and 0.632).
For the ionized Ca level, there was significantly different level between groups (p<0.001). The mean Na level was significantly higher in control group (1.15 ± 0.1 mmol/L) compared with both T2DM cases without nephropathy group (1.09 ± 0.1, p=0.019) and T2DM with nephropathy group (1.03 ± 0.1, p<0.001). Similarly, T2DM cases without nephropathy group had significantly (p=0.019) higher mean level than T2DM cases with nephropathy .

Regarding the laboratory investigations (liver function parameters)
For the s. albumin level, there was significantly different level between groups (p<0.001). The mean s. albumin level was significantly higher in control (4.23 ± 0.2 g/dl) compared with both T2DM cases without nephropathy group (4.01 ± 0.3, p=0.020) and T2DM with nephropathy group (3.84 ± 0.5, p<0.001). Contrarily, T2DM cases without nephropathy cases had insignificantly (p=0.060) higher mean level than T2DM cases with nephropathy .
Contrarily, there was insignificant difference in the mean INR (p=0.059), PC% (p=0.318) and t. bilirubin levels (p=0.601).
For the PT, there was a significantly different level between groups (p=0.003). The mean PT was significantly higher in control group (13.1 ± 1.2 second) compared with both T2DM cases without nephropathy group (12.13 ± 0.8, p=0.001) and T2DM with nephropathy group (12.4 ± 1.2, p=0.013). on the contrast, T2DM cases without nephropathy group had insignificantly (p=0.398) lower mean PT than T2DM cases with nephropathy .

Regarding the fundus examination and TGF-β1
In respect to fundus examination results, all control had normal findings. In T2DM cases without nephropathy groups, 20% (n=6) had mild NPDR. In T2DM cases with nephropathy groups, 43.3% (n=13) had mild NPDR, 23.3% (n=7) had moderate NPDR, 10% (n=3) had severe NPDR and 6.7% (n=2) had PDR. This difference was statistically significant (p<0.001) .
For the TGF-β1 level, there was significantly different level between groups (p<0.001). The mean TGF-β1 level was significantly higher in T2DM with nephropathy group (1424.3 ± 95.8 ng/ml) compared with both control group (392.5 ± 14.9, p<0.001) and T2DM without nephropathy group (523.3 ± 22.5, p<0.001). Similarly, T2DM cases without nephropathy group had significantly (p=0.003) higher mean level than control .

Table 2: Comparison of fundus examination and TGF-β1 between studied cases

	Control (1) (n = 30)	**T2DM no NP (2) (n = 30)**	**T2DM with NP (3) (n = 30)**	**P-value**
Fundus Examination				
• Normal	30 (100%)	24 (80%)	5 (16.7%)	
• Mild NPDR	0 (0%)	6 (20%)	13 (43.3%)	
• Moderate NPDR	0 (0%)	0 (0%)	7 (23.3%)	**< 0.001***
• Severe NPDR	0 (0%)	0 (0%)	3 (10%)	
• PDR	0 (0%)	0 (0%)	2 (6.7%)	
TGF-β1 (ng/ml)	392.47 ± 14.9	523.33 ± 22.5	1424.23 ± 95.8	**< 0.001****
•P-value***	**1 vs 2 = 0.003**	**2 vs 3 < 0.001**	**1 vs 3 < 0.001**	

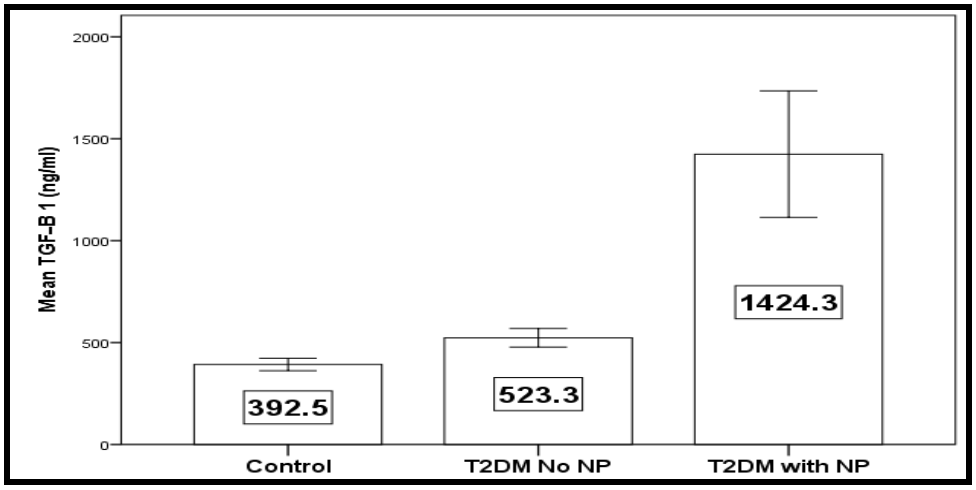

Fig. 1: Difference in the Mean TGF-β1 of the studied Groups

The validity of TGF-β1 for disease activity prediction was shown in Table 3 and Figures 2-3. Predictive power was good for T2DM without nephropathy (AUC = 0.815, p < 0.001; 95% CI: 0.71 - 0.92). Additionally, the following validity requirements were met at 435 ng/ml: TGF-β1 accurately detected 80% of positive patients as having T2DM without nephropathy, or 80% sensitivity. Additionally, 77% specificity means that 77% of controls were accurately classified as negative by the test. Furthermore, the test's Positive Predictive Value (PPV), or its capacity to identify individuals who are ill out of all positive instances, was 78%. Additionally, it had a 79% Negative Predictive Value (NPV), meaning that it could predict control out of all test positives. The test's overall accuracy was 78.5%.

Predictive power was high for T2DM with nephropathy (AUC = 0.968, p < 0.001; 95% CI: 0.93 - 1.00). Additionally, the following validity requirements were met at 435 ng/ml: TGF-β1 accurately detected 94% of positive patients as having T2DM with nephropathy, or 94% sensitivity. Additionally, the test properly identified 87% of controls as negative, or 87% specificity. Furthermore, the test's Positive Predictive Value (PPV), or its capacity to identify individuals who are ill out of all positive instances, was 88%. Additionally, it exhibited a Negative Predictive Value (NPV) of 93.5%, meaning that it could predict control out of all test negatives. The test's overall accuracy was 90.5%.

Table 3: Diagnostic criteria of TGF-β1 for Prediction of Disease

	TGF-β1	
Diagnostic criteria	**T2DM without NP**	**T2DM with NP**
• AUC	0.815	0.968
• 95% CI	0.710 - 0.920	0.931 - 1.000
• P-value***	< 0.001	< 0.001
• Cutoff Point	435 ng/ml	
• Accuracy	78.5%	90.5%
• Sensitivity%	80%	94%
• Specificity%	77%	87%
• PPV%	78%	88%
• NPV%	79%	93.5%

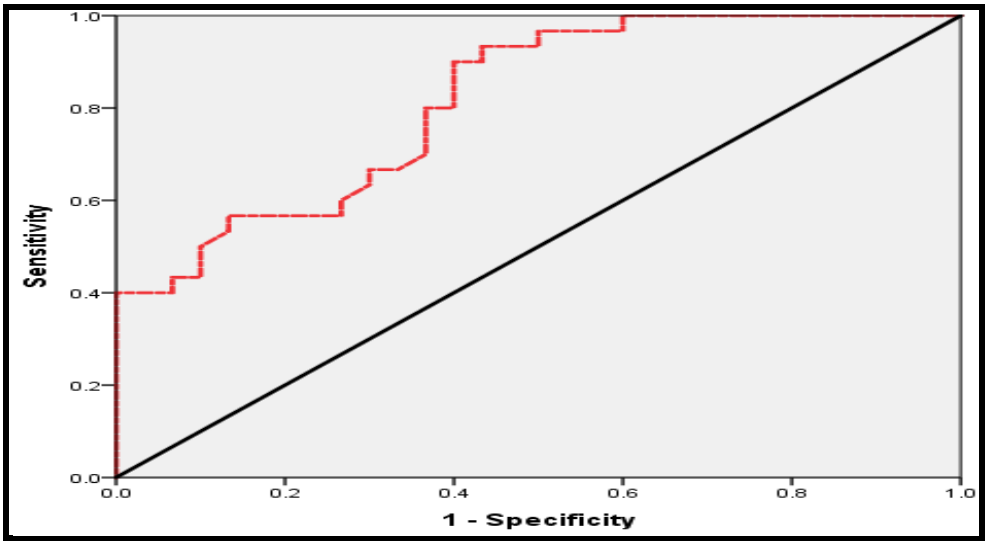

Fig. 2: ROC curve for TGF-β1 for Prediction of T2DM with no NP

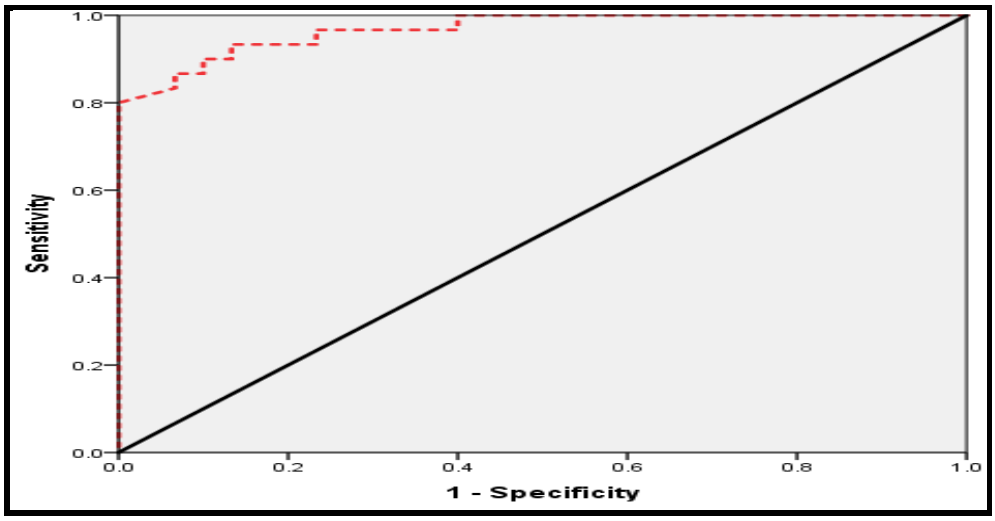

Fig. 3: ROC curve for TGF-β1 for Prediction of T2DM with NP

DISCUSSION

The objective of our work was to elucidate the role of transforming growth factor-beta 1 (TGF-β1) in the pathogenesis of diabetic kidney disease (DKD). Group 1 (Control), Group 2 (Type 2 diabetes. individuals without Nephropathy), and Group 3 (Type 2 diabetes. individuals who had Nephropathy) consisted of three equal groups (n=30), totaling 90 patients enrolled for the study.

Regarding patient'sage, there was statistically significant younger age among controls (38.5 ± 9.7 years) than T2DM cases with and without nephropathy (58.1 ± 9.3 and 53.3 ± 10.5 years). Moreover, T2DM cases without nephropathy were insignificantly (p=0.064) younger than T2DM cases with nephropathy

Our findings regarding the mean age of T2DM cases with nephropathy were in harmony with(9)(Abd-Elfattah et al., 2023) study in Egypt as the mean age of group with Type 2 DM patients with macroalbuminuria was 57.08 ± 9.42 years.

Our results, however, were marginally older than the mean age of 50.38 ± 14.41 years in the (10) sample (Hafez et al., 2019). According to Afifi (2008), the median age for ESKD in Egypt rose from 45.6 years in 1996 to 49.8 years in 2008. Improvements in healthcare are reflected in the rising mean age of ESRD patients.

However, in(12) (Kebede et al., 2021) study in Ethiopia the mean age was 53.2 ± 10.1years old .

We are recently near to developed countries as the mean age for DN in (Limkunakul et al., 2019) study in US as the mean age was 58-64 years old regarding to their subgroups(13).

In the current study, the three groups were matched for sex .With females predominance in all groups , the female predominance in DN cases were reported also in (El-Sherbini et al., 2013)study(7), However in (Hassan et al., 2022) study there was a male predominance in T2DM patients with microvascular complications as there was 11 (36.7%) females and 19 (63.3%) males(14).

Despite hormonal variables indicating a general safeguarding function for estrogens and progesterone, along with variations in genes and differences in significant risk factors, the underlying causes of the observed gender gaps in diabetic kidney disease (DKD) remain ambiguous (15). In diabetics women, whom seem to forfeit cardiovascular preventive benefits of estrogens prior to menopause, sex hormones are vital to the pathophysiology of diabetes and its consequences. In the past few years, much research has been conducted on the multiple benefits of estrogens outside the reproductive system, particularly their potential role in diabetic kidney disease (DKD). Estrogen receptors are specialized and distributed throughout the body, especially in cells called endothelial cells and the vascular system (15).

In the current research, T2DM patients without nephropathy had significantly greater WC than T2DM cases with nephropathy and in comparison to the control group, and their body mass index was much greater.

A greater body mass index was associated with diabetes-related microvascular complications compared to non-diabetic individuals, according to the study. Research that followed up on studies that looked at the impact of body mass index (BMI) at the time of diagnosis of diabetes mellitus (DM) and its subsequent complications in both men and women found that being overweight or obese is a major risk factor for type 2 diabetes and its consequences.

A greater BMI was also linked to microvascular complications in those with diabetes (Hassan et al., 2022). Consequently, Hu et al. (2012) found that being overweight or obese significantly increases the chance of developing type 2 diabetes and its complications in both sexes (18).

According to a study conducted by Gray et al. in 2015, individuals with a body mass index (BMI) of 30 or above were up to 168% more likely to have issues related to diabetes. There is an increased risk of cardiovascular disease, kidney disease, eye disease, and lower limb problems in obese males compared to obese women of the same body mass index (19).

Obesity in the abdomen region increases the risk of diabetic ketoacidosis (20)(21) more than obesity overall. Patients with type 2 diabetes who also have DKD are more likely to be abdominally obese, as measured by WC, and a new meta-analysis found that the features of abdominal obesity, such as continuous VFA and WC, are associated with an increased risk of DKD (22).

However, Man et al. (2018) found Among individuals who have diabetes type 2, there is no association between DKD and belly fat (23). in keeping with our findings, (Kanakamani et al., 2010) also found no association between WC and microalbuminuria in 670 patients seen at the endocrine outpatient clinic. (24).

The current study illustrated statistically significant **IDA (40%)** of the T2DM cases with nephropathy group had IDA compared with no cases in both control and T2DM cases without nephropathy groups.

In a cross-sectional research, El Minshawy and El Bassuoni (2010) discovered that 39% of Egyptian patients with type 2 diabetes and chronic renal disease also had anemia (25). Comparatively, a global study of 1205 patients with T2DM and CKD from 11 European countries found a prevalence of anemia that was 34.0% lower in those with CKD and T2DM (26) . Anemia was found to be 31.7% prevalent in a previous study conducted in Malaysia (27). In addition, an Omani study **(Alsalmani et al., 2023)**identified that 29.3% of individuals afflicted with both type 2 diabetes and chronic renal impairment (28).

identified that 29.3 percent of individuals afflicted with both diabetes type 2 and chronic renal impairment

Different population sample sizes, locations, lifestyles, racial and genetic compositions, and so on could all contribute to the discrepancies seen in prevalence rates of anemia between the aforementioned studies. Possible causes for the discrepancy include variations in the prevalence and severity of diabetes and CKD.

A high leukocyte count, low serum albumin levels, smoking, and an increased body mass index (BMI) are all risk factors, and diabetic nephropathyfor anemia in CKD. Also, the elderly and those with coexisting conditions like diabetes, cardiovascular disease, and hypertension are at a higher risk of developing anemia (30)(31).

In the current study; ESR was statistically significant higher in T2DM cases with nephropathy compared with both control group and T2DM without nephropathy group, in the same line **(Guo et al., 2020)** reported that in patients with T2DM, the ESR was independently correlated with the prevalence and severity of diabetic renal disease. ESR is therefore a useful biomarker for monitoring the progress of diabetic patients(32) .

HbA_1C as a predictor of glycemic control has a strong relation with proteinuria, levels of kidney failure; its filtration rate and hypoglycemia (33).In the present study, there was significant lowerlevel of HbA1C in control compared with both T2DM without nephropathy group and T2DM with nephropathy group. However no statistically significant difference between last 2 groups

In a related research (AHMED et al., 2019)(34), the DN patient group had a little higher HbA1c than the DN patient group, but the difference was not statistically significant. A p-value of 0.33 is used. Additionally, research done in Oman in 2012 by Alrawahi et al. (35) demonstrated this link.Baig et al. (2016)(36) found that in type 2 diabetics with inadequate glycemic control, elevated microalbuminuria levels were significantly associated with elevated HbA1c levels.

Compared to both the control group and the T2DM without nephropathy group, our research indicated that Creatinine and urine albumin creatinine ratio (ACR) levels were considerably higher in T2DM patients with nephropathy.

A greater level of creatinine and urine ACR was found in diabetic nephropathy patients compared to controls and diabetic DM patients without problems, which is in line with the findings of the research (Abd-Elfattah et al., 2023).

The research found that the group of individuals with microvascular issues had significantly higher blood creatinine levels (P < 00.001) compared to both the control group and the group of persons with diabetes who did not have any difficulties (Hassan et al., 2022) (15). Case group blood creatinine levels were much greater than control group levels (p 0.001), according to the research (Mohamed et al., 2021)(37). (Siddappa and Ramprasad, 2020) also found similar results, Relative to the control group, the case group had significantly higher blood creatinine levels (p < value.05) (38). Research conducted by Veluri and Mannangatti (2022) and Sueud et al. (2019) indicates that diabetic type 2 individuals with microalbuminuria had higher urine ACR levels compared to those with normoalbuminuria and healthy controls (p-value less than 0.0001).(39)(40).

Elemental salts including magnesium (Mg2+), potassium (K+), calcium (Ca2+), and sodium (Na+) may be found in serum. Enzyme activity, electrical gradients, and intermediate metabolism are all impacted by these electrolytes. Patients with type 2 diabetes have an imbalance in electrolytes because of osmotic fluid shifts caused by hyperglycemia or total-body deficiencies from osmotic diuresis (41).

When comparing T2DM patients with and without nephropathy, we discovered that those with nephropathy had significantly greater Na and lower Ca levels, indicating electrolyte disruption. Nonetheless, there was no discernible variation in the average K and Mg levels between the categories.

Different studies have shown different effects on electrolyte levels; for example, one looked at diabetics with nephropathy and found that According to Rao and Kuldeep (2022), the electrolyte levels in their blood were noticeably lower for phosphorus, magnesium, and sodium compared to the control group, while another found the opposite for calcium, potassium, and chloride (41).

The research conducted by Unachukwu et al. (2018) found that type 2 diabetes patients had a substantially higher blood K+ level (p< 0.05) than non-diabetic patients (ND), while there was no significant increase in Na+ level (p>0.05) (42).

(Lindner and Funk, 2013, Datchinamoorthi et al., 2016) illustrated thatwith hyperglycemia, elevated or normal plasma levels of sodium point to a clinically severe body water deficit. Hypo- and hypernatremia are connected to DM(43)(44), demonstrating the presence of processes related to hyperglycemia that can affect serum sodium in opposing directions(45).

In diabetic nephropathy, which occurs as a result of aberrant glucose metabolism, hyperglycemia, and alterations in the Renin-Angiotensin system, an osmotic force forces water to flow from the intracellular space to the extracellular region (46).

In respect to fundus examination results in our study, all control had normal findings. In T2DM cases without nephropathy groups, 20% (n=6) had mild NPDR. In T2DM cases with nephropathy groups, 43.3% (n=13) had mild NPDR, 23.3% (n=7) had moderate NPDR, 10% (n=3) had severe NPDR and 6.7% (n=2) had PDR. The statistical significance of this difference was high (p<0.001).

Figure 2: (a) Narrow-band NPDR color image. (b) In the early stages of FA, microaneurysms are associated with dot hyperfluorescence. (47).

Previous studies have shown a high association between diabetic retinopathy and diabetic kidney disease, which is consistent with our finding (48-50).

Risk factors for diabetic retinopathy (DR) and diabetic nephropathy (DN) include hypertension and poor glycemic control.moreover, Renal interstitial inflammation affected the likelihood of DR as well. Studying the pathogenesis of DN and DR has significantly advanced over the past few years, with an emphasis on oxidative

stress & inflammatory state. The development of diabetic vascular problems, such as DN and DR, is significantly influenced by oxidative stress(49).

Significant differences were seen between the groups in terms of the TGF-β1 level ($p<0.001$). The group with type 2 diabetes and nephropathy had a significantly higher mean TGF-β1 level (1424.3 ± 95.8 ng/ml) compared with both control group (392.5 ± 14.9, $p<0.001$) and T2DM without nephropathy group (523.3 ± 22.5, $p<0.001$). Similarly, T2DM cases without nephropathy group had significantly ($p=0.003$) higher mean level than control.

In line with Hassan et al. (2022),(14), the researchers set out to show how TGF-1 levels in the blood differed between those with type II diabetes and those who did not develop microvascular problems. Participants in groups I, II, and III with type 2 diabetes and microvascular problems, TGF-1 levels in the blood were measured using an enzyme-linked immunosorbent assay (ELISA) in both the experimental group and the healthy control group. The researchers found that groups III and I had significantly different serum TGF levels ($P=0.001$). The levels of TGF-1 in the blood were noticeably greater in the diabetic microvascular group (group III).

Our results are in line with those of a research by Zhou et al. (2018), who looked at the link between TGF-1 levels in the blood and microvascular problems in people with diabetes. They discovered that those with type 2 diabetes and microvascular problems had much higher blood TGF-1 levels. Moreover, they found that increased TGF-1 levels are associated with glomerulosclerosis, fibrosis, and ECM accumulation, and that TGF-1 could initiate the transcription of ECM proteins (51).

In order to establish the correlation between TGF-1 levels in the urine and serum of people with diabetes mellitus (DM) or diabetic nephropathy (DN), a meta-analysis was conducted by Qiao et al. (2017) using 26 studies that included 1968 cases and 2100 controls. They found that levels of TGF-1 in both urine and serum were significantly greater in those with T2DM and T2DN (52).

To determine whether blood TGF-1 levels are associated with the risk of diabetic nephropathy, Mou et al. (2016) reviewed nine publications including 264 patients and 227 healthy controls. Diabetes was associated with increased blood TGF-1 levels and an increased risk of renal involvement, according to their results (53).

Contrary to what was found in a previous research by Castro et al. (2014), which found a higher level of TGF-1 in type 2 diabetic patients with microvascular complications compared to both healthy persons and T2DM patients without these complications, our study found no such difference (54). An earlier research (Kim et al., 2011)(55) confirmed these findings.

By ROC curve; our study illustrated that at cutoff value 435 ng/ml the sensitivity of the TGF-β1 for prediction of T2DM without nephropathy activity was 80%, specificity 77%, PPV 78% and NPV 79% and 78.5% accuracy However, at the same cutoff value of 435 ng/ml, the TGF-β1had better ability for prediction of T2DM with nephropathy as sensitivity 94%, specificity 87% PPV 88%, NPV 93.5% and The test's overall accuracy was 90.5%.

Our results were supported by an observational research that included fifty type 1 diabetic individuals (Sawires et al., 2019). Microalbuminuria was used to classify patients into two categories, A and B. Consequently, TGF-1 is an early marker of DN in T1DM patients with 100% sensitivity and specificity (56).

This is the first research that we are aware of that demonstrates TGF-β1 as an early sign of diabetic neuropathy in adults with type 2 diabetes.

LIMITATION

Because we conducted this study in a single location, we were unable to generalize our findings.

RECOMMENDATION

We recommended to:Conduct future studies with higher sample size in multicenter

CONCLUSION

TGF-β1 was significantly higher in adult with diabetes with DN in comparison with adult without and healthy controls. Moreover, T2DM cases without nephropathy group had significantly higher mean level than control. Furthermore, at the cutoff value of 435 ng/ml,; the TGF-β1 had better ability for prediction of T2DM with nephropathy as sensitivity 94%, specificity 87% PPV 88%, NPV 93.5% and overall, the test had 90.5% accuracy. This suggests that TGF-β1 has the potential to be a very sensitive and specific early indication of DN in adults with type 2 diabetes.

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