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Serum transformation growth factor β1 levels in nephropathy diabetic patients attending specialist hospital and Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria

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Abstract

Introduction: Diabetic nephropathy is among the major causes of mortality and morbidity in type 1 & type 2 diabetes and is strongly associated with cardiovascular disease outcomes. Higher blood pressure and poor glycaemic control were reported to be the most common risk factors influencing the progress of diabetic nephropathy. This study was designed to determine the diagnostic and prognostic potential of the serum transformation growth factor β1 [TGF β1] in normoalbuminuric, microalbuminuric, macroalbuminuric and end-stage renal disease type 2 diabetic nephropathy patients.

Materials and Methods: The transformation growth factor β1 was measured using ELISA test kits and serum creatinine was assessed by the modified Jaffe method while estimated glomerular filtration rate [EGFR] in the study was evaluated by the MDRD equation (adjusted for four variables).

Results: The levels of serum transformation growth factor β1 and urine albumin/creatinine ratio were significantly increased ($p < 0.05$) across the diabetes nephropathy stages, while the estimated glomerular filtration rate was progressively decreased ($p < 0.05$). Serum creatinine was significantly increased ($p < 0.05$) with the progression of diabetic nephropathy.

Conclusion: Transformation growth factor β1 might have the potentials to be used as a diagnostic and prognostic marker for diabetic nephropathy.

Keywords: Diabetic nephropathy; Transformation growth factor β1; DN stage; Nigeria

1. Introduction

Diabetes nephropathy (DN) is the most common complication of diabetes mellitus, leading to end-stage renal disease, which has been identified as a medical disaster worldwide (1). DN is an advanced kidney disease, resulting from alterations in the glomerular capillary, the structure of the glomerular tubules and glomerular functions (2, 3). It is associated with elevated urea albumin clearance coupled with a rising blood pressure, which gives rise to disturbances in glucose homeostasis. The resultant effect here is the decreased glomerular filtration, which contributes majorly to

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end-stage kidney dysfunction (ESRD) (4, 5, 6). At the beginning of DN, microalbuminuria is present (≥ 30mg/day - <300mg/day). In the absence of specific interventions, microalbuminuria may progress to diabetic nephropathy, which initiates the progressive decline in renal functions (5). DN is found in 35% - 40% of patients affected with type 1 and 2 DM and remains the major risk factor of end-stage renal dysfunction in the developed world (2, 3, 7). DN is becoming more widespread worldwide due to increased incidences of obesity and types 2 DM (4). The major risk for developing DN in type 2 diabetes is based on ethnicity, ranging between 25% in the individual of European descent to around 50% in individual of other ethnic descent (e.g Africans, Afro-Caribbean, Asian-Indian, and Japanese). Diabetic subjects of Asian-Indian descent, or Afro- Caribbean descent develops type 2 diabetes more easily and more often at an early age. This is attributed to the increased risk of cardiovascular disease and nephropathy (8). The typical features of DN can be described by glomerulosclerosis, characterized by severe mesangial accumulation of extracellular matrix (ECM), and renal hypertrophy associated with albuminuria, an advanced glomerular capillary occlusion, and a gradual decline in glomerular filtration rate (GFR) (6). The apparent effect on the quality of life is significant and the treatment strategy is expensive, especially to some individual with ESRD. Persistent renal dysfunction is also attributed to early mortality in patients with diabetes (9).

Prolonged exposure to high blood glucose levels is associated with tubulointerstitial in overt diabetic nephropathy, described by thickening of the tubular basement membrane, tubular atrophy and interstitial fibrosis (7). High blood pressure, suboptimal control of glucose and albuminuria, which are recognized risk factors for DN could not give a detailed explanation of all the differences observed in the rates of developing nephropathy among various individuals (10). The prognostic importance of the minute quantity of albumin in urine that indicates the progress to kidney damage in patients with type 1 or 2 DM was established in the early 1980s and was referred to as the microalbuminuric stage or initial nephropathy (11). Albuminuria is widely recognised as a predictor for the early stages of diabetic nephropathy, even though it is limited by structural changes that might occur before the excretion of albumin in the urine (12). Hyperfiltration and hyperperfusion of the glomerulus are the early signs of DN, because of reduced resistance in the afferent and efferent arterioles of the glomerulus. The afferent arteriole is more effective in reducing resistance than the efferent arteriole. Nitric oxide (NO), proteinoids, vascular endothelial growth factor (VEGF) TGF-β1, and the rennin angiotensin system, specifically angiotensin II, are the various factors described, which are involved in this defective autoregulation (13).

Irregularities in TGF-β1 were known to be associated with several conditions, including autoimmune disorders, malignancies, and chronic renal conditions (14). TGF-β1, TGF-β2, and TGF-β3 are groups of cytokines with unique and potent anti-inflammatory and immune-regulatory activities (15). Each of these isoforms is coded by genes at different locations in many chromosomes: TGF-β1 are coded by genes at chromosome 19 (19q13.1), TGF-β2 genes are located at chromosome 1 (1q41) while TGF-β3 genes are located at chromosome 14 (14q24) (16). In humans, TGF- β1 is produced by platelets, macrophages, glioma cells, monocytes, lymphocytes, dendritic cells, fibroblasts, keratinocytes and epithelial cells. TGF- β3 is seen mostly in embryonic heart and lung tissue, and to a negligible extent in the liver, kidney and spleen (17). An *In vitro* study of the three isoforms indicated the similarities in their functions, while the in vivo procedure revealed that the TGF-β1 is strongly implicated in fibrosis (18). Virtually all the facilitators and intracellular signalling pathways associated with diabetic renal dysfunction have been recognised to facilitate the renal TGF- β activity as an intermediary step (19). Transforming growth factor-beta (TGF-β1) is a multitasking cytokine associated with the pathogenesis of various types of advanced renal conditions, such as diabetic nephropathy. TGF-β1 have been found to stimulate the transcription of numerous extracellular matrix (ECM) genes directly in renal cells, such as mesangial, tubular and endothelial cells (6). TGF-β1 also serves an essential role in facilitating the accumulation of ECM and fibrosis, which could lead to renal tissue dysfunctions (20). In its active form, TGF-β1 isoform is a homodimer comprising of two polypeptide chains with 112 amino acid residues each, linked by a disulphide bond and form a complex of 25 kDa molecular weight (17, 16).

This study was aimed to assess the diagnostic/prognostic potential of the serum transformation growth factor β1 in normoalbuminuric, microalbuminuric, macroalbuminuric and end-stage renal disease DN patients. We hypothesized that TGF β1 might have the potentials to be used as either a diagnosis or prognosis marker for diabetic nephropathy in type 2 DM patients presenting DN, in conjunction with either ACR or blood creatinine and EGFR.

2. Material and methods

2.1. Study area

This study was conducted in Sokoto Metropolis. Sokoto is in the extreme North- western region of Nigeria. According to the 2006 population census by National population commission, the population figures stand at 3,702, 676 million persons spread over 33,776.89 square kilometres of land. (PHC, 2006) The Population mainly consists of predominantly

Hausa-Fulani with a good number of other ethnic groups. Sokoto has a semi-arid climate and a vegetation that is largely Sudan Savannah with an annual rainfall of 500mm – 300mm and temperature ranges of 15 °C – 40 °C (UNFPA, 2013)

2.2. Ethical consideration

This study was approved by the ethical committee of the two renounce hospitals (Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital Sokoto) in Sokoto. All practical procedures were performed strictly in accordance with the two Hospitals Ethical Committee guidelines.

2.3. Inclusion criteria

Consenting diabetic subjects coming for routine diabetes follow-up with microalbuminuria, macro albuminuria or ESRD were consecutively recruited.

2.4. Exclusion criteria

Diabetic subjects without either microalbuminuria or macroalbuminuria were excluded from the study, so also patients who presented with febrile illness, urinary tract infection, or pregnancy were excluded.

2.5. Informed consent/ethical approval

Informed consent was obtained from the study subjects.

2.6. Research design

The research is a cross-sectional observational study, designed to assess the diagnostic and prognostic potential of the serum transformation growth factor β1 (TGF-β1) in normoalbuminuric, microalbuminuric, macroalbuminuric and end-stage renal disease DN patients in 224 diabetic patients attending Usmanu Danfodiyo University Teaching Hospital Sokoto and Specialist hospital Sokoto. The patients were enrolled on the study, after explaining the objectives of the research and consent obtained. The blood sample was collected from the patients for the assessment of TGF- β1, Blood Glucose, Urea and Creatinine. Urine samples were also obtained from the subjects and assessed for urine microalbuminuria and creatinine. From the selected subjects, about 5.0ml of venous blood specimen was collected and about 4.0ml of the blood was transferred into a plain vacutainer bottle. The sample was centrifuged at 4000xg for 5 minutes. The serum was harvested stored at -20°C and assayed in batches; for serum levels of TGF-β1, urea and creatinine. The remaining 1.0ml of the blood was dispensed into a fluoride oxalate vacutainer bottle, for plasma glucose levels estimation, done within one hour of the sample collection. A urine spot sample (10mL) was collected in a sterile container with no preservatives for the estimation of urine microalbumin and creatinine.

2.7. Statistical analysis

The values obtained was analysed by Invivostat and SAS Institute JMP Pro® Version 14.2.0 statistical software, 62 Bit for windows (2018). The results of serum TGF-β1, urea, creatinine, plasma glucose, urine microalbumin and creatinine were compared between normo-A, micro-A, macro-A and ESRF groups, with those of controls using a paired two-tailed student's t-test for samples that matched. While Multivariate analysis of variance (MANOVA) was used for comparisons of the mean values of the parameters in the various groups, a post-hoc analysis was carried out using Tukey HSD test. The relationship between TGF-β1 levels and Albumin/creatinine ratio and TGF-β1 and EGFR were analysed using a non-parametric test (Spearman's Rho) because the assumptions of Pearson`s moment correlation analysis (parametric) were not met e.g. non-linearity and normality of the distribution of data. A P-value of equal to or less than ($p \leq 0.05$) was considered statistically significant. The Modification of Diet in Renal Disease (MDRD) simplified equation adjusted for 4 variables was used to calculate the estimated GFR (EGFR) in ml/min per 1.73 m^2 (21).

3. Results

A total of 224 diabetic subjects comprising four groups of 56 each with an equal number of males and females (M= 28, F=28), aged between 19 – 78 years (Table 1) were used for this study. The majority of the subjects were Hausa/Fulani 175 (78.1%) and the least was the Igbo`s 4 (7.1%) (Table 1). Many of them were full-time housewives 72 (32.1%) followed by those engaging in business 55 (24.5%) while the remaining were students 10 (3.4%) (Table 1). Most of them attended Arabic school 114 (50.8%) and few with primary education 29 (12.9%) (Table 1).

Table 1 Demographic and socio-economic characteristics of the study subjects

Variable	N	NormoA	MicroA	MacroA	ESRD
Age (years)	224	51.4±11.9	54.7± 10.4	53.6± 9.4	59.1± 6.0
Ethnicity					
Hausa/Fulani	175(78.1%)	45 (80.3%)	43 (76.7%)	45 (80.3%)	42 (75%)
Yoruba	14(6.2%)	4 (7.1%)	3 (5.3%)	3 (5.3%)	4 (7.1%)
Igbo	4(7.1%)	0 (0%)	2 (3.5%)	1 (1.7%)	1 (1.7%)
Others	31(13.8%)	7 (12.5%)	8 (14.2%)	7 (12.5%)	9 (16%)
Gender					
Males	112 (100%)	28 (25%)	28 (25%)	28 (25%)	28 (25%)
Females	112	28 (25%)	28 (25%)	28 (25%)	28 (25%)
Occupation					
Civil servant	36 (12.9%)	9 (16%)	10 (17.8%)	6 (10.7%)	11 (19.6%)
Housewife	72 (32.1%)	19 (33.9%)	23 (41.0%)	12 (21.4%)	18 (32.1%)
Business	55 (24.5%)	13 (23.2%)	12 (21.4%)	18 (32.1%)	12 (21.4%)
Retired	22 (9.8%)	5 (8.9%)	5 (8.9%)	6 (10.7%)	6 (10.7%)
Student	10 (4.4%)	3 (5.3%)	1 (1.7%)	3 (5.3%)	3 (5.3%)
Farmer	12 (5.3%)	3 (5.3%)	2 (3.5%)	5 (8.9%)	2 (3.5%)
Unemployed	17 (7.5%)	4 (7.1%)	3 (5.3%)	6 (10.7%)	4 (7.1%)
Education					
Primary	29 (12.9%)	11 (19.6%)	3 (5.3%)	7 (12.5%)	8 (14.2%)
Secondary	40 (17.8%)	10 (17.8%)	12 (21.4%)	8 (14.2%)	10 (17.8%)
Tertiary	41 (18.3%)	8 (14.2%)	13 (23.2%)	11 (19.6%)	9 (16.0%)
Arabic school	114 (50.8%)	27 (48.2%)	28 (50%)	30 (53.5%)	29 (51.7%)

*Data are presented as mean ± SD (n = 56). Values are number of subjects with percentage in parenthesis, n=number of subjects, NormoA=normoalbuminuric, MicroA = microalbuminuric, MacroA = macroalbuminuric, ESRD = end stage renal disease. The table shows the ethnicity, Gender, occupation and educational qualification of the study subjects.

The result of blood pressure and BMI levels of the subjects indicated a significant ($p = 0.0091$) increase in systolic blood pressure with advancing DN stages. ESRD group has the highest mean value for systolic blood pressure (SBP) of 142 mmHg, followed by macro-A with a mean value of 136.5mm Hg and normal A has the least mean value 125.5 mmHg of the study subject. Also, no significant difference recorded in both the means of diastolic blood pressure and that of BMI ($p = 0.1498$) across all the DN stages (Table 2).

Table 2 Blood pressure and BMI levels of the study subjects

GROUP	N	SBP (mmHg)	DBP (mmHg)	BMIkg/m2
NormoA	56	125.5±16.8	81.5±9.9	26.5±4.9
MicroA	56	128.4±15.8	82.2±11.5	27.6±5.1
MacroA	56	136.5±14.5	85.5±9.1	27.7±2.9
ESRD	56	142.5±13.3	85.5±6.3	28.6±4.7
P-value		0.0001*	0.0901	0.1498

*Results expressed as Mean ± SD (n = 56), SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index: * = values that are statistically significant ($p < 0.05$). the table shows the mean and standard deviations of systolic and diastolic blood pressures as well as BMIs of the various study groups and the number of participants.

The result of blood glucose, serum urea and creatinine in the study subjects indicated no significant difference (p >0.9231) in the DN stages. Serum urea was significantly (p = 0.0001) raised in ESRD patients compared to normo-A, micro-A and macro-A. Serum creatinine was also significantly (p = 0.0001) increased with advances in stages of DN (Table 3). Urinary albumin/Creatinine ratio (ACR) was observed to show a significant difference (p = 0.0001) in increase with DN progression from normo A - ESRD. Estimated glomerular filtration rate (EGFR) was observed to show a statistically significant (p = 0.0001) decline with increase progression in DN from normo A - ESRD. The TGF-β1 levels show a significant increase (p = 0.0001) with an increase in the progression in DN stages from normo A – ESRD (Table 4).

Table 3 ACR, EGFR and TGF-β1 levels of the study subjects

Group	N	ACR (mg/gCr)	EGFR (ml/min/1.73m²)	TGF-β1 ng/ml
NormoA	56	21.5±6.4	102.5±18.7	21.3±6.7
MicroA	56	104.7±72.8	67.2±12.3	47.4±8.4
MacroA	56	357.3±43.1	39.0±7.7	71.9±9.9
ESRD	56	536.0±38.8	22.7±3.4	97.5±18.4
P-value		0.0001*	0.0001*	0.0001*

*Results are expressed as mean ± SD (n = 56). ACR- albumin/creatinine ratio, EGFR estimated glomerular filtration rate, TGF-β1, transformation growth factor beta1, *= values statistically significant (p < 0.05). the table shows number of subjects in each test group and the mean and Standard Deviation of ACR, EGFR and TGF-β1 levels in the respective groups.

Table 4 Blood glucose, serum urea and creatinine levels of the study subjects

Group	N	Glucose mmol/L	Urea mmol/L	Serum creatinine mg/dl
NormoA	56	8.2±2.9	5.6±1.7	0.9±0.1
MicroA	56	8.1±3.1	5.6±1.8	1.3±0.3
MacroA	56	7.8±3.1	5.7±2.2	2.0±0.4
ESRD	56	8.9±2.3	7.1±2.6[a]	3.1±0.4
P-value		0.9231	0.0001*	0.0001*

*Data are expressed as mean ± SD (n = 56), * = values that are statistically significant, the only group with significance. The table shows mean and standard deviation of Glucose, Urea, Serum creatinine in the different groups.

Spearman`s rank correlation between Albumin/creatinine ratio and TGF-β1 levels indicates a negative very weak relationship that was not significant, in both the normo-A (ρ = -0.1013, p = 0.4578) and Micro-A (ρ = -0.0266, p = 0.8454) groups and a weak positive relationship at the Macro-A (ρ = 0.0311, p = 0.8201) and ESRD (ρ = 0.2490, p = 0.0643) stages (Table 5). Spearman`s rank correlation between EGFR and TGF-β1 levels indicates a very weak positive relationship within all the DN stages, with Normo-A (ρ= 0.1321, P = 0.3319), Micro-A (ρ= 0.0184, p = 0.8932), Macro-A (ρ = 0.1679, p = 0.2160) and ESRD (ρ = 0.0063, p = 0.9633) even though the relationship was not statistically significant (Table 5).

Table 5 Spearman Rank correlation between Albumin/creatinine ratio by TGF-β1

Comparison between TGF-β1 vs ACR within groups		
Group	**Spearman ρ**	**P-value**
NormoA	-0.1013	0.4578
MicroA	-0.026	0.8454
MacroA	0.0311	0.8201
ESRD	0.2490	0.0643

*Results are presented as mean ± SD (n = 56). The significant difference was set at p ≤ 0.05. the table shows the spearman`s rho correlation between ACR and TGF--β1

Table 6 Spearman Rank correlation between EGFR by TGF-β1

Comparison between EGFR vs TGF-β1 within groups		
Group	**Spearman ρ**	**P-value**
normoA	0.1321	0.3319
microA	0.0184	0.8932
macroA	0.1679	0.2160
ESRD	0.0063	0.9633

*Values are presented as mean ± SD (n = 56). The significant difference was set at $p \leq 0.05$. the table shows the spearman`s rho correlation between EGFR and TGF--β1

4. Discussion

Diabetic nephropathy is one of the major causes of morbidity and mortality in diabetes mellitus, associated with CVDs, such as ischemic heart disease. Majority of patients with diabetes, particularly those living with type 2 DM associated with kidney disorder die from cardiovascular diseases even before they develop end-stage renal disease. This rate is about 15-times higher in diabetic nephropathy patients than those without nephropathy. The death rate among individuals with DN is about 20-40 times greater than those without nephropathy (22). Blood pressure, lack of glycaemic control and hypertension are the major contributing factors for diabetic nephropathy (8). Glomerular hyperfiltration and renal hypertrophy arises at the early stages of DM in diabetic nephropathy subjects and is linked with increased GFR. Although the appearance of microalbuminuria is a prime risk factor for progression to macroalbuminuria, as the presence of macroalbuminuria is established, there is a firm decrease in GFR and 50% of these patients reach ESRD within 7-10 years. The socio-demographic data obtained in this present study showed that the majority of the subjects were Hausa/Fulani, and a good percentage of the participants were housewives with the majority of them engaged in businesses. The age ranges from 51 -59 years across the groups. Most of them have an informal level of education (Arabic school), with a considerable percentage of the subjects with some level of western education (Table 1).

This study evaluated TGF β1 levels in the serum, ACR and EGFR in normo-A, micro-A, macro-A and ESRD in type 2 DN patients. The study explored and ascertained the usefulness of TGF β1 as an indicator of early kidney contribution to DN which in turn can aid in the diagnosis or prognosis of DN. The findings of an increased urinary albumin/creatinine ratio (uACR) across the various DN stages (Table 2) concurred with the report by Babazono et al*.* (2009) in a study that compared baseline ACR levels between normo-A, micro-A, macro-A groups with the level of change in EGFR at a follow-up period of 5–13 years. Previous research has shown that elevated levels of ACR, even at a normal range of less than 30 mg/g or an ACR greater than or equals to10 mg/g in women and ≥ 5 mg/g in men is characterized by a higher rate of decline in EGFR in diabetic patients (23) for both sexes (Table 4). When the mean ACR was compared between the sexes, a significant rise was observed, with men having a higher ACR than women (Table 2), this disagreed with the findings by Carter et al. (2012), in a study on the effect of urine creatinine on the relationship between ACR and CVD events. Although recent research has reported a higher spot ACR levels in women (24). This difference might be the result of the difference in ethnicity among the study subjects and possibly geographical location. Existing studies have associated ACR with risk of death rate linearly on the log-to-log scale without threshold effects (25). In another study reported that subjects with baseline ACR ranging between 150 mg/gCr - 300 mg/gCr (micro-A) had a higher rate of macroalbuminuria and elevated risk of nephropathy progression than patients in lower ACR category normo-A, irrespective of the cause of microalbuminuria or macroalbuminuria. Urinary albumin/creatinine ratio (uACR) even at the early stages of microalbuminuria is a great indicator of possible death arising from heart diseases, especially in aged subjects with type 2 diabetes (26, 27). Proteinuria that are stiffly increasing are mostly linked with a rapid decline in renal functions rates, regardless of baseline GFR (28).

A statistically significant difference was observed in increased systolic blood pressure (SBP) values in DN stages in this study (Table 3). This finding agreed with the study of Yang et al. (2009) that reported a SBP of 126, 145, and 157 from normo-A, micro-A and macro-A diabetes patients, respectively while measuring NGAL levels in DN patient. Previous research reported a rising systolic blood pressure across DN stages, (29). In a similar but more recent study reported in another study that raised systolic blood pressure and glucose are the most common risk factors in the development of DN (30. 9), When comparing the mean SBP between sexes, there is a significant difference with the females having a higher SBP compared to men. This difference might be due to the sedentary lifestyle of the females, who are mostly full-time housewives. A sedentary lifestyle is a predisposing factor for obesity, while obesity is an initiating risk factor for

diabetic kidney diseases, including DN (26). Elevated blood pressure (especially SBP) has an extremely harmful effect on the kidneys, the heart and the retina of the eyes (8). Furthermore, Alicic et al. (2017) reported that each 10-mmHg rise in the mean systolic BP correlates with a 15% rise in the hazard ratio for the progression of both micro - and macroalbuminuria and impaired renal function. The blood creatinine level can also double in concentration. Blood pressures are known to rise progressively around the macroalbuminuric stages in type 1 and type 2 diabetic patients respectively. Stabilizing renal functions at this stage can be difficult to attain (8). In general, baseline systolic BP 140mmHg in some individuals with type 2 DM has been linked with a higher risk of ESRD and death (9). The results of Table 3 indicate no significant difference in BMI at the various DN stages, even though the BMI falls within the overweight categories. This study agreed with a study by Nauta et al. (2011), who investigated a non-statistically significant difference in BMI amongst their study groups. Additionally, current research reported that a moderately elevated BMI (25-29.9) is attributed to a heightened risk of developing complications of DM, including nephropathy (31). An elevated BMI is an independent forecaster of vital renal changes in patients with type 2 diabetes (32, 33). Existing evidence has indicated that higher BMI is a risk factor for the progress to diabetes, leading to DN (34).

Fasting blood glucose was observed to be raised across the various groups. Even though a non-significant difference was noticed between the various DN stages and between sexes (Table 3). This might be due to their diabetic state and poor glycaemic control in the subjects. Moreover, the high blood glucose level is a well-known risk factor for the initiation and development of diabetic kidney diseases including nephropathy (26). This coincides with a study by Huang et al. (2017) that recorded raised blood glucose between normo-A, micro-A and macro-A groups, and concluded that raised blood glucose is significantly influenced by the development of DN. In DN patients, elevated expression of TGF- β genes is caused by hyperglycaemia (35), TGF- β proteins and their receptors through the polyol pathway activation and activation of hexosamine pathway. This raises the level of advanced glycation end products (AGE) and enhances oxidative stress. Hyperglycaemia also activates the TGF- β pathway via the activation of glucose transporters (36). The activation of PKC by *de novo* formation of diacylglycerol and the ensuing oxidative stress occurs because of raised glucose levels. The increase in the synthesis of TGF- β1 genes expression and elevated production of extracellular matrix protein synthesis during hyperglycaemia is mediated through the PKC pathway (36, 28). Hypercalcemia causes glucose to bind at first reversibly and later irreversibly to proteins in the kidneys and other circulating proteins forming advanced glycated end products (AGEs), which can form complex cross-links over years and contribute to renal damage occurs, due to prolong hyperglycaemia, AGEs (37). The findings of the increased serum urea levels observed in the ESRD patients only (Table 3) concurred with results of the study by Abbas et al. (2017) on subjects with type 2 diabetic nephropathy and healthy controls which reported raised serum urea levels in diabetic nephropathy subjects, then in healthy control. This might be due to a reduction in renal functions which usually occurs in patients with a longer duration of DM due to reduced filtering capacity of the kidney. This leads to the deposition of waste products, and eventually a raised serum creatinine and urea levels (37). The duration and severity of diabetes significantly correlate with serum urea levels, and poorly controlled blood-sugar levels cause a rise in serum urea levels thereby raising the possibilities of developing diabetic nephropathy by the patient (38). Blood urea levels are reported to increase when there is damage to the kidney or renal failure. Raised blood urea levels in patients with diabetes may be an indication of a pre-renal problem (39). Both serum urea and creatinine levels are markedly raised in cases of kidney dysfunction or urinary flow damage (40).

The findings for increased serum creatinine levels observed with advancement in DN stages (Table 3) agreed with reports of a study by Yang et al. (2009), on serum levels of NGAL in type 2 DN patients, that reported steadily increased serum creatinine levels of 0.8, 1.0 and 2.5 mg/dl for normo-A, micro-A and macro-A respectively. This result also agreed with a study by Sur A (2016) that reported a value of (0.9 and 1.10 mg/dl) for normo-A and macroalbuminuric groups respectively. The males having a higher mean value than females. Creatinine is known to be more in males than females which could be due to the presence of high muscle mass in males compared to females as reported in earlier studies (39). Serum creatinine levels have been reported to rise with the increase in DN stages and ACR levels in numerous studies (41). The findings of increased urine microalbumin levels in this present study agreed with the report by Mattix et al. (2002), which reported no statistically significant difference between men and women while examining the distribution of urine albumin and creatinine concentrations by one ACR value and sex-specific cut points. In type 2 DM patients with hypertension, a decline in renal function may occur even when albumin excretion is still in the macroalbuminuric range (42). Normally, GFR begins to drop around the macroalbuminuric stage, although the GFR can still be within the normal ranges (8). Without intervention, microalbuminuria progresses to proteinuria within the period range of roughly 10–15 years. Although abnormal values are usually not noticed until a higher level of proteinuria is attained, while the filtration capabilities of the glomerulus may already be plummeting all the time (43, 8). It is a fact that Diabetic nephropathy is a familiar and potentially life-threatening complication of both type 1 and type 2 diabetes. Significant reduction in the disease progression can be achieved through early detection of microalbuminuria and subsequent measures taken to protect against further progression (28).

The results showing a significant increase in serum level of TGF β-1 as recorded with advance DN stages across the groups (Table 4) agreed with results from a study by Qiao et al. (2017), which assessed the changes attributed to TGF-β1 in type 2 DM and diabetic nephropathy. Previous studies have reported significantly increased serum TGF-β1 levels in diabetic subjects with albuminuria (44). A more recent study has also reported similar findings in another research that compared the TGF-β1 levels among non-diabetic, diabetic without nephropathy and Type 2 DM with nephropathy, and concluded that raised serum TGF-β1 levels in diabetic individuals were dependent on the glycaemic control and some level of kidney dysfunction (45). Earlier research also reported similar findings of elevated levels of TGF β-1 and concluded that the raised levels of TGF β-1 in individuals with type 2 diabetes could be an indicator of renal and endothelial damage tendencies in such patients (46). Pathologically, TGF β1 serves a vital role in the promotion of glomerulosclerosis, tubular fibrosis, and decrease infiltration rate of the glomerulus, TGF β1 also increase the albumin level, water level, electrolytes levels, and the level of glucose excreted via the urine of diabetic patients (44). The elevated number of TGF-β1 receptors expressed is reported to be necessary for fibrosis and remodelling of tissues in many organs during the progression of the disease, which includes glomerular fibrosis in the kidney (47). Several findings have shown that TGF- β1 assists in the progress of albuminuria in diabetics (44). Additionally, TGF-β, growth factor derived from platelets and vascular endothelial tissues are raised in DN patients (37). When the healing process has been completed, under ideal conditions, TGF-β1 secretion is checkmated by feedback mechanisms. However, if the secretion is not turned off by the feedback mechanism, accumulation of extracellular matrix components (ECM) ensues which results in the occurrence of tissue fibrosis (48). Recent studies have shown that most of the molecular facilitators and intracellular signalling pathways connected with the development of diabetic nephropathy are linked with transforming growth factor-beta 1 (TGF- β1) at certain stages (49).

The findings of declining EGFR between the DN stages (Table 4) agreed with the discoveries of Huang, et al. (2017) that reported a significant difference in EGFR between the three DN groups (normo-A, micro-A and macro-A) in their study. Previous research has also reported a significant difference ($p < 0.0001$), in a multivariable survival analysis, where they compared EGFR and albuminuria and reported that EGFR and albuminuria were independently and firmly associated with developing ESRD (50). In another study by Leoncini et al. (2010) which is a collective meta-analysis of the general population cohorts. They established that lower ACR measurements and higher EGFR levels between 75 - 105 mL/min/1·73 m^2 are not related to mortality and the risk of mortality increases as EGFRs declines. Impairment of the glomerular filtration rate is the final common pathway of kidney dysfunction. The progression of ESRD to CVDs occurs because of impaired GFR. The impairment takes place even with appropriate medical management (51). In patients with continuous clinical albuminuria, the GFR declines gradually from one stage to another; with the rate of decline fluctuating depending on how effective the promoters of development of DN, such as hypertension and level of albumin in urine are controlled. Deaths due to CVD and infections are extremely prevalent and compete with the development of ESRD (26). The findings of advanced age with the advancement in diabetic nephropathy stages among the study participants coincides with the similar result presented by Chae et al. (2012), earlier research reported a significant difference in both age and HbA_1C, among diabetic subjects, which is associated with advancement in DN stages (51). This supports the suggestion that the incidence of diabetic nephropathy increases with disease progression and poor glycaemic control. Huang et al. (2017) concluded that age and raised blood glucose were the major risk factor for DN. Recent studies have indicated that older age and sex (M) are known risk factors in the progression to DN (8). A correlation analysis was performed by Spearman's Rho (non-parametric) analysis to determine the relationship between TGF-β1, ACR and EGFR. A negative relationship was observed between TGF-β1 and ACR within normo-A and micro-A groups and a positive relationship between macro-A and ESRD groups, the strength of the relationship was very weak. A positive relationship was recorded between TGF-β1 and EGFR within the DN stages, even though it was also a very weak relationship.

5. Conclusion

Our finding suggested that the serum levels of TGF-β1 in DN patients were observed to increase with the increase in classes of albuminuria (normo-A, micro-A, macro-A, ESRD, blood creatinine levels and Urea. TGF-β1 was found to have a negative inverse relationship with EGFR, (as TGF-β1 increases with albumin classification, EGFR decreases). Hyperglycaemia and increased blood pressure were demonstrated to play a crucial role in the progress of the disease. Differences were also seen in urine creatinine and urine albumin levels with classes of albuminuria. As such, TGF-β1 might be utilized in the laboratories for diagnosis/prognosis of DN patients, couple with either ACR or blood creatinine or EGFR. In the future, the significance of this study will go a long way in stemming the rate at which diabetic patients develops nephropathy, hence reducing the needs for dialysis and costly transplants.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

This study was approved by the ethical committee of the two renounce hospitals (Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital Sokoto) in Sokoto.

Statement of informed consent

Informed consent was obtained from the study subjects.

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