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The Role of Transferrin and Laminin Biomarkers in the Diagnosis of Diabetic Nephropathy in Type II Diabetic Patients

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Authors' contributions

This work was carried out in collaboration among all authors. Author MEAEF designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors LAR and SMMN' managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background/Aim: Diabetic nephropathy is one of the most important microvascular complications associated with type II diabetic patients. It occurs in 20-40% patients with diabetes mellitus, and microalbuminuria is still considered as the first sign of diabetic nephropathy. Low sensitivity and specificity of microalbuminuria leads to more sensitive biomarkers that may be used to detect diabetic nephropathy at an earlier stage with higher accuracy. This study was carried out to detect the validity of using serum Transferrin and Laminin as a diagnostic biomarkers for diabetic nephropathy in type II diabetic patients.
Methods: Egyptian patients (n=96) included 72 type 2 diabetic patients who were classified into three groups: group 1 - normoalbuminuric patients (uACR up to 30 mg/g), group 2 - microalbuminuric patients (uACR from 30 – 300 mg/g), group 3 - macroalbuminuric patients (uACR from >300 mg/g) and 24 healthy control were surveyed in a cross-sectional study over a period of 6 months at biochemistry department, KASR ALAINY Hospital of Cairo University. Patients were

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subjected to measurement of Albumin creatinine ratio, eGFR, Serum creatinine, glycosylated hemoglobin (HbA1c) and lipid profile. The serum concentrations of transferrin and lamnin were measured using a highly sensitive one-step sandwich enzyme immunoassay kit.

Results: Serum laminin was significantly higher in macroalbuminuric patients than in the microalbuminuric and in microalbuminuric patients than in the normoalbuminuric and healthy control subject. By comparing these goups according to serum laminin concentration we found statistically significant positive correlation (p value <0.001, r= 0.670), serum transferrin was significantly lower in macroalbuminuric patients than in the microalbuminuric and in microalbuminuric patients than in the normoalbuminuric and healthy control subject. By comparing these goups according to serum transferrin concentration we found statistically significant inverse correlation (p value <0.001, r= -0.579). There was no correlation between level of serum transferrin /laminin and glycoregulation, and statistically significant positive correlation was found between serum laminin and duration of diabetes and statistically significant inverse correlation was found between serum transferrin and duration of diabetes.

Conclusions: The results from this study provide the evidence that serum laminin and transferrin could be used as a diagnostic markers of diabetic nephropathy.

Keywords: Diabetes mellitus; albuminuria; biomarker; transferrin; laminin.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disease whose incidence and prevalence show a steady increase. An increasing number of diabetic patients; mostly with type2 diabetes (90%) is associated with enhanced rate of diabetic complications, including diabetic kidney disease [1]. Diabetes is considered as the leading cause of chronic kidney disease (CKD) and end stage renal disease (ESRD). Costs of care for patients with diabetic kidney disease (DKD) are extremely high, especially after they enter ESRD, and it is necessary to establish the diagnosis of diabetic nephropathy as soon as possible [2,3]. Diabetic nephropathy (DN) is a serious and progressive complication related to diabetes. It can increase the risk and progression of end-stage renal disease. Diabetic nephropathy is clinically defined as a rise in urinary albumin excretion (albuminuria), generally measured as an albumin-to-creatinine ratio (A1 [<30 mg/day; ACR, <30 mg/g (<3 mg/mmol)], A2 [30–300 mg/day; ACR, 30–300 mg/g (3–30 mg/mmol)], and A3 [>300 mg/day; ACR, >300 mg/g (>30 mg/mmol)], decreased glomerular filtration rate (GFR) and elevated blood pressure [4]. Albuminuria have some limitations to detect early stages of DN such as it can be elevated in some cases such as exercise, acute illness, heart failure and there are some diabetic patients develop DN with normal albuminuria [5] so, new biomarkers are required [6]. Although Urinary Albumin excretion rate remains an essential tool for risk stratification and monitoring disease progression a number of factors have called into question its sensitivity and specificity. The presence of Microalbuminuria (MA) was originally thought to be predictive of future overt DN in 80% of patients. However more recent evidence suggests that only around 30% of microalbuminuric patients progress to overt nephropathy after 10 years of follow up [7]. It has also been shown that advanced structural alterations in the glomerular basement membrane may already have occurred by the time MA becomes clinically evident [8].

In addition, there is evidence that a significant proportion of patients with MA can revert to normoalbuminuria [9] and the concept of nonalbuminuricDN is well-documented, reflecting the fact that patients with diabetes can demonstrate a reduction in glomerular filtration rate without progressing from normo-to Microalbuminuria' [10] Taken together, these results suggest that MA is perhaps more a diagnostic marker than a tool to predict DN. Therefore, there is a need to identify and investigate alternative biomarkers for the earlier prediction of DN and these are subject to this review. Transferrin is a plasma protein with a slightly greater molecular weight (76.5 kDa) than albumin [11]. It is also less ionic than glycosylated albumin and thus less easily repelled by glomerular basement membrane polyanion [12]. Previous studies showed that low serum transferrin concentration was associated with liver transplantation and/or death [13]. But the relationship between transferrin and ESRD was unclear. Laminin is a major non-collagenous glycoprotein component of glomerular basement membrane (GBM) and mesangium and consists of three different polypeptide chains designated

α, β and γ. Laminin was first isolated from EHS mouse tumor and subsequently found to be a cross-shaped disulfide-bonded heterotrimer composed of a 400 kD α1 chain along with β1 and γ1 chains of approximately 200 kD. Eleven distinct laminin chains have been described to date (five α, three β and three γ chains). These chains can combine into at least 15 different laminin isoforms, Lm-111 to Lm-523 [14,15,16]. Laminins are the most abundant glycoproteins of the basement membrane extracellular matrix (ECM) and can be found in almost all tissues of the body. They play essential roles in the establishment of tissue architecture and stability, and provide cells with a structural scaffold. As such, laminins are involved in a variety of biological processes ranging from tissue survival, angiogenesis [17], and neural development [18], to skin re-epithelialization and wound healing [19,20,21], and even cancer metastasis [22,23].

Laminins have been shown to regulate core cellular activities, such as adhesion, apoptosis, proliferation, migration, and differentiation. Serum Transferrin and Laminin can be used as potential biomarkers for diabetic nephropathy and may be raised earlier than albuminuria. Additionally, there is considerable variation in the distribution of laminin isoforms in the developing kidney.

2. OBJECTIVES

The aim of this study was to determine the validity of using Serum Transferrin and Laminin as a diagnostic biomarkers for diabetic nephropathy in Egyptian patients.

3. PATIENTS AND METHODS

3.1 Study Design

This is a cross-sectional study aiming to predict the validity of using Serum Transferrin and Laminin as a diagnostic biomarkers for diabetic nephropathy.

3.2 Study Setting

This study was conducted over a period of 6 months at biochemistry department, KASR ALAINY Hospital of Cairo University.

3.3 Target Population

Patient having type II diabetes presented to diabetes clinic as well as inpatient ward of nephrology department of the KASR ALAINY University Hospital.

Sample size was calculated using the (G power software). As regarding the primary outcome (albuminuria) we found that 24 participants per group were appropriate sample size for the study with total sample size 96 participants (4 groups) The power is 80% and α error probability =0.05.

The magnitude of the effect to be detected was estimated as the mean and standard deviation of the variable of interest and obtained from the scientific literature of Idowu et al. [31].

F tests ANOVA: Fixed effects, omnibus, one-way.
Analysis: A priori: Compute required sample size.

Input:	Effect size f	=0.345
	α err prob	=0.05
	Power (1-β err prob)	=0.8
	Number of groups	=4
Output:	Noncentrality parameter λ = 11.426400	
	Critical F	=2.703594
	Numerator df	=3
	Denominator df	=92
	Total sample size	=96
	Actual power	=0.801913

Study population sample divided into four groups:

A) Consisted of 24 Non diabetic and without nephropathy healthy control.
B) Consisted of 24 Diabetic patients having normoalbuminuria.
C) Consisted of 24 Diabetic patients having microalbuminuria.
D) Consisted of 24 Diabetic patients with macroalbuminuria.

That is based on albumin /creatinine ratio where:

Normoalbuminuria is (less than 30 mg albumin / gm creatinine)
Microalbuminuria is (30 – 300 mg albumin / gm creatinine)
Macroalbuminuria is (more than 300 mg albumin / gm creatinine) according to [24].

3.4 Inclusion Criteria of the Patients

Patients included according to the following criteria:

All males and females with T2DM and associated DN (based on registration records) lacked

absolute contraindications, especially T2DM patients without diabetic retinopathy, or with obvious glomerular hematuria, or with sudden onset overt proteinuria.

3.5 Exclusion Criteria

-Patients having autoimmune diseases causing secondary diabetes (e.g. SLE)
-Patients with other chronic disease such as chronic liver disease.
-Patients suffering from Coexisting non-diabetic renal diseases (NDRD) such as congenital kidney diseases, renal artery stenosis hydro-nephritis and IgA nephropathy or systemic diseases, especially anti-neutrophil cytoplasmic antibody (ANCA) that are associated vasculitis, anti-glomerular basement membrane (GBM) disease and lupus nephritis In sum, we enrolled 72 patients who had DN as the only glomerular disease.

3.6 Clinical Examination

The selected patients were studied in detail with history and physical examination, including ultrasonography of the kidney. Age, gender, duration of diabetes mellitus, weight, height and blood pressure were noted too. Blood pressure measurement was after resting for 5 min in sitting position using mercury sphygmomanometer. Body mass index (BMI) was calculated according to formula based on the height and weight measurements of the patients by the following equation according to [25]:

$$BMI = (Weight/kg) / (Height/m^2)$$

3.7 Laboratory Investigations

-Including sampling blood samples for measurement of glycolated hemoglobin (HbA1C), serum creatinine, lipid profile, Concentration of serum Transferrin and laminin and Spot urine sample from each patient to measure urinary albumin/creatinine ratio (ACR).

-Calculation of estimated glomerular filtration rate (eGFR) by using the MDRD equation [26] =
$175 \times [\text{plasma creatinine (mg/dl)}]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if female}]$

-All of the above laboratory investigations except serum Transferrin and laminin levels were done by using UV 2300 Spectrophotometer.

Serum Transferrin (ng/ml) and laminin concentrations (pg/ml) were measured by using an Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available standard kits provided by Chongqing Biospes Co., LTD [27].

-All of the above laboratory investigations were performed at biochemistry department, KASR ALAINY Hospital of Cairo University. Blood samples were taken after overnight fasting, at least 8 hours, Urine was centrifuged (1000xg, 20 min), then Serum and urine samples were frozen until the time of assay. Minimum and detectable dose for serum transferrin was 1.5 ng/mL and for serum laminin was 156 pg/mL.

4. STATISTICAL DESIGN

Statistical analysis was performed with IBM® (IBM Corporation, NY, USA.)Statistical Package for the Social Science SPSS®(SPSS, Inc., an IBM Company.) Statistics Version 25. Categorical data were presented as frequencies and percentages and were analyzed using chi square test. Numerical data were presented as mean, standard deviation (SD). Data were explored for normality by checking the data distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. Parametric data were analyzed using independent t-test for comparisons between two groups and one-way ANOVA followed by Tukey post hoc test for multiple group comparisons. Categorical data were presented as frequencies and percentages and were analyzed using chi square test. Correlations between quantitative variables were done by Pearson's correlation. The significance level was set at probability $P \leq 0.05$ within all tests.

The Pearson correlation coefficient (r) was used to measure the strength and direction of a linear relationship between the 2-biomarkers and the studied patient's parameters. The value of r is always between +1 and −1. The closer the correlation to 1, the stronger will be the relationship. A correlation of 0.0 indicates the absence of a relationship.

Sensitivity, specificity, and area under the curve (AUC) were calculated as measures of diagnostic accuracy. Receiver operating characteristic (ROC) curve was used to calculate the area under the curve (AUC). A perfect test has an area under the ROC curve of 1.0.

5. RESULTS

A total of 96 subjects were enrolled in this study; the mean age for all study groups was from 36.21±10.24years to 59.38±10.5 years with statistical significant differences (p-value <0.001).

The gender distribution was 24 (26%) female and 72(74%) male with statistically significant decrease in the incidence of diabetic nephropathy in female patients compared to males (p-value <0.001).

Our study included 72 type 2 diabetic patients, The duration of type II diabetes mellitus ranged from 3.08±2.28 to 12.88±5.19 years in all diabetic patients in this study in group C&D compared to group B (p value 0.01, <0.001). And in group D compared to C (P value <0.001).

The mean values of body mass index (BMI) showed no significant difference between the three groups (p value >0.05). hypertension(HTN) is most significantly occurring and showed higher values in group D while group B is the least incidence of hypertension due to progression of the disease with (p-value <0.001).

In Table 3; The mean values of glycated hemoglobin (HbA1C) showed no significant difference between the three groups (p value >0.05), Serum creatinine (SCr) showed no significant difference between group B&C (p value =0.9) but there is Significant increase in serum creatinine in group D compared to group C, B (P value <0.001) and also (eGFR) showed no significant difference between group B&C (p value =0.5) but there is Significant decrease in group D compared to group C, B (P value <0.001, 0.003).

Table 1. Baseline characteristics of the study population

Study groups	Healthy Control group(A)	Diabetes without nephropathy group(B)	Diabetes with microalbuminuria group(C)	Diabetes with macroalbuminuria group(D)	P-value
Variables	**Mean± SD**	**Mean± SD**	**Mean± SD**	**Mean± SD**	
Age (years)	36.21±10.24	44.46±10.54	52.13±10.64	59.38±10.5	<0.001
Duration of DM (years)		3.08±2.28	6.81±5.3	12.88±5.19	0.01
duration of HTN		6.25±3.28	10.23±8.61	10.05±7.23	>0.05
BMI ($kg/m^{2)}$		30.27±6.56	31.16±5.15	29.29±4.68	>0.05

Statistical significant difference when P-value<0.05
BMI = Body Mass Index, DM = Diabetes mellitus, HTN = Hypertension

Table 2. Baseline characteristics of the study population

Study groups	group(B)	group(C)	group(D)	P-value
Variables	**%**	**%**	**%**	
Gender				<0.001
Male	32.4	26.8	28.2	
Female	4	20	16	
TTT of DM				0.004
Insulin	12.5	41.7	58.3	
incidence of HTN				<0.001
HTN (Yes)	33.3	54.2	83.3	
TTT of HTN	29.2	37.5	54.2	0.009
incidence of dyslipidemia				0.3
Dyslipidemia(yes)	45.8	33.3	54.2	
US finding				0.02
Normal	83.3	79.2	62.5	
fundus examination				<0.001
Normal	100	83.3	41.7	

Statistical significant difference when P-value<0.05
US finding=Ultrasonography of kidney, TTT of HTN=treatment of hypertension

Table 3. Showing laboratory investigations of the study population

Study groups	group(B)	group(C)	group(D)	P-value
Variables	Mean± SD	Mean ± SD	Mean± SD	
HBA1C%	8.29±1.89	7.45±1.28	7.81±2.03	>0.05
SCr (mg/dl)	1.1±0.27	1.14±0.37	1.62±0.62	<0.001 gp D compared to gp C, B
ACR (mg/g)	11.04±2.42	109.33±61.87	459.08±227.16	<0.001 gp D compared to gp C, B
eGFR (ml/min)	79.86±30.38	72±27.11	47.41±15.87	<0.001 gp D compared to gp C

Statistical significant difference when P-value<0.05
SCr= serum creatinine, eGFR=estimated glomerular filtration rate, HBA1C=glycated hemoglobin

ACR show significant increase in group C&D compared to group B (p value 0.03, <0.001) and in group D compared to C (P value <0.001).

In Fig. 1; Transferrin showed higher values in group A while group D had the least Transferrin concentration. The mean Transferrin concentration for all study groups was from (2.39±0.98) ng/ml to (14.69±5.22) ng/ml, the mean Transferrin concentration for group B (7.38±2.2) ng/ml & group C (4.65±1.87) ng/ml with statistical significant differences (p-value <0.001). There was statistically significant decrease in the transferrin in all diabetic nephropathy patients compared to normal controls (p value <0.001) and statistically significant decrease in transferrin of group C&D compared to group B (p value=0.012, <0.001).

In Fig. 2; Laminin showed higher values in group D while group A had the least Laminin concentration. The mean Laminin concentration for all study groups was from (85.64±17.84) pg/ml to (380.48±186.51) pg/ml, the mean Laminin concentration for group B (114.93±32.46) pg/ml & group C (196.22±68.52) pg/ml with statistical significant differences (p-value <0.001). There was statistically significant increase in the laminin in group C&D diabetic nephropathy patient compared to normal controls and group B (p value = 0.002, <0.001,0.03, <0.001).

In Table 4 There is a significant positive correlation between Laminin and Age (p value 0.008, r= 0.309), duration of DM (p value <0.001, r= 0.558), UACR (p value <0.001, r= 0.670), SCr (p value <0.001, r= 0.505) and a significant inverse correlation between Laminin and GFR (p value =0.001, r= -0.379). There is a significant inverse correlation between transferrin and Age (p value 0.021, r= -0.271), duration of DM (p value <0.001, r= -0.507), UACR (p value <0.001, r= -0.579), SCr (p value <0.001, r= 0.505) and a significant positive correlation between transferrin and GFR (p value =0.003, r= 0.342).

There was no significant correlation between transferrin/Laminin and BMI, Duration of HTN and HBA1C.

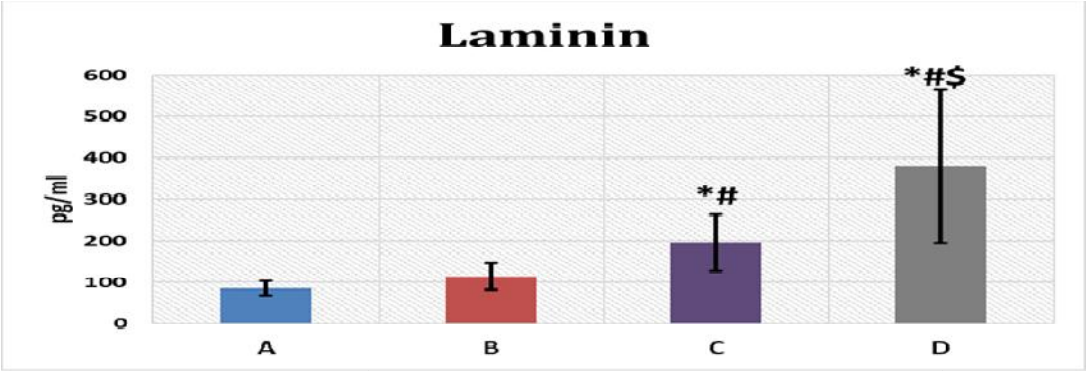

Fig. 1. Laminin among studied groups

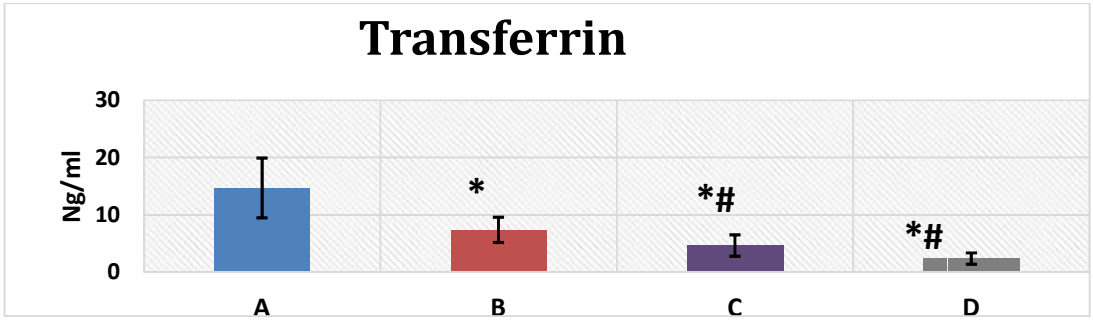

Fig. 2. Transferrin among studied groups

Table 4. Showing Correlation between Laminin and Transferrin and other study variables

Variables	Laminin		Transferrin	
	r	P value	r	P value
Age(years)	0.309**	0.008	-0.271*	0.021
Duration of DM (years)	0.558**	*<0.001	-0.507**	<0.001
Duration of HTN (years)	0.056**	0.729	-0.082*	0.611
BMI (kg/m2)	-0.042	0.725	0.040	0.741
UACR	0.670**	*<0.001	-0.579*	*<0.001
HBA1C%	-0.071**	0.555	0.075*	0.529
SCr (mg/dl)	0.505**	*<0.001	-0.339**	0.004
eGFR (ml/min)	-0.379**	0.001	0.342**	0.003

Pearson correlation coefficient test (r)

Statistical significant difference when P-value<0.05

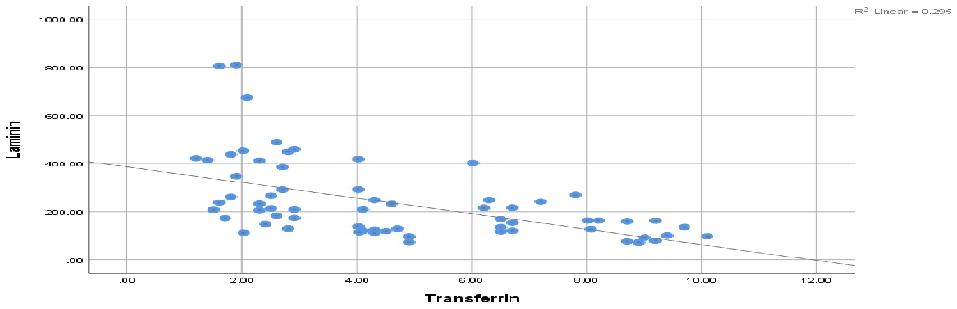

Fig. 3. Correlation between laminin and transferrin

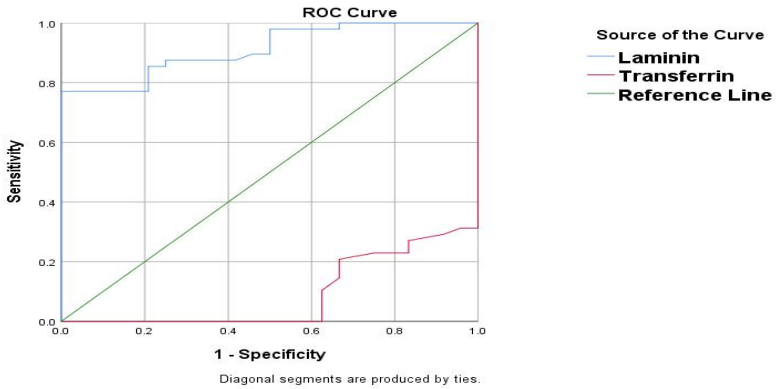

Fig. 4. The ROC curve to identify the accuracy of Laminin and Transferrin as a diagnostic markers of diabetic nephropathy

As demonstrated in Fig. 4: receiver operating characteristics (ROC) curve analysis was used to assess the clinical diagnostic accuracy of Laminin and transferrin levels as a diagnostic markers of diabetic nephropathy in the studied population, the result of Laminin ROC curve analysis showed p value <0.001 so; the Laminin diagnosed as statistically diagnostic with 85% sensitivity (true positive cases)and 80% specificity (true negative cases) and accuracy 82.5% , Lower Bound of Asymptotic 95% Confidence Interval = 0.850 and the Upper Bound = 0.975. AUC = 0.913 at a cutoff point level ≥ 135.7; the result of transferrin ROC curve analysis showed p value <0.001 so; the transferrin diagnosed as statistically diagnostic with 20% sensitivity (true positive cases) and 34% specificity (true negative cases) and accuracy 27% , Lower Bound of Asymptotic 95% Confidence Interval = 0.026 and the Upper Bound = 0.157. AUC = 0.092 at a cutoff point level ≤ 5.4.

6. DISCUSSION

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria; microalbuminuria then macroalbuminuria, a persistent decline in the glomerular filtration rate and elevated arterial blood pressure. Diabetic nephropathy is the leading cause of chronic renal failure. Due to the presence of some limitation for estimated glomerular filtration rate and albuminuria, the need for new biomarkers which can able to detect the disease in its early stages is required. Transferrin may be one of these biomarkers and also Laminin. In the current study there was no statistically significant difference in HBA1C among study groups. This finding agreed with Wu et al. [28] also, Zhao et al. [29] who not found statistical significant difference in HBA1C among study groups.

On the contrary, Zeng et al. [30] study which consisted of 146 type II diabetic patients divided into 42 subjects with Diabetic kidney disease (DKD) and 104 non-DKD in which there was statistically significant difference in HBA1C among study groups.

Idowu et al. [31] found that there was statistical significant difference in ACR among study groups (a study of 50 type II diabetic patients and 30 non-diabetic subjects as a control group), in this respect, the present study represented statistical significant difference between ACR among study groups.

In agreement with Zhao et al. [29] who suggested that there was statistical significant difference in ACR among study groups.

In the present study, serum creatinine was measured for GFR estimation by using MDRD equation which used in staging and monitoring the early stages of diabetic nephropathy and chronic kidney disease (CKD) stages. Concerning to that, statistically significant increase in serum creatinine and decrease in GFR in macroalbumiuric group compared to micoalminuric and normoalumiureic groups was found in this study but not found statistical significant difference in serum creatinine between micoalminuric and normoalumiureic groups that was in agreement with Wu et al. [28] study who found statistical significant difference in serum creatinine among study groups.

In the current study there was statistically significant difference in the incidence of Hypertension among study groups. This finding agreed with Zhou et al. [32] study which consisted of 250 older adults were enrolled during the study period, including 124, 82, and 44 with normal albuminuria, microalbuminuria, and macroalbuminuria, respectively. They found that an elevated blood pressure was independent risk factor for albuminuria.

In the current study there was no statistically significant difference in the BMI among study groups (p value >0.05) and there was statistically significant difference in the incidence of diabetic nephropathy in male patients compared to females among study groups (p value <0.001).

In the current study there was statistically significant difference in the concentration of serum transferrin among study groups as a result it decreased significantly early in normoalbuminuric patients (group B DN) which may possess more sensitive marker for DN.

Previous studies showed that low serum transferrin concentration was associated with liver transplantation and/or death Frazer et al. [33]. But the relationship between transferrin and ESRD was unclear.

Zhao et al. [29] demonstrated that serum transferrin was an indicator for predicting ESRD in patients with T2DM and biopsy-proven DN During a mean follow-up of 30.9 months, 66 (59.5%) patients progressed to ESRD. Compared with patients without ESRD, ESRD patients had higher levels of baseline creatinine and proteinuria, lower baseline eGFR, and lower concentrations of serum albumin, hemoglobin, serum iron and transferrin. Independent of other clinical features and pathological findings. This study is the first to reveal the associations between iron status and renal outcomes in patients with T2DM.

This finding agreed with the current study although the levels of transferrin were significantly different between groups, it presented low sensitivity and specificity as a diagnostic value in diabetic nephropathy.

In the current study there was statistically significant difference in the concentration of serum Laminin among study groups, as a result serum Laminin increased significantly early in group B DN which may possess more sensitive marker for DN. This finding agreed with Uwaezuoke et al. [34], who demonstrated that several studies have shown that urinary laminin excretion is higher in patients with diabetes mellitus in comparison to their healthy controls, even prior to the onset of microalbuminuria. Banu et al. [35], Haiyashi et al. [36], Miyake et al. [37] To buttress the discriminatory role of this biomarker in diabetic and nondiabetic kidney disease, it has been reported that patients with T2DM who show evidence of nephropathy present with significantly higher laminin/albumin ratio compared to patients with nondiabetic nephropathy Banu et al. [35].

Pietschmann et al. [38], In 97 patients with type I diabetes mellitus, 155 patients with type II diabetes mellitus, and two matched control groups, serum concentrations of laminin P1, a non-collagenous component of basement membranes, were determined by radioimmunoassay to see whether laminin P1 might be a valuable indicator of microangiopathic complications in diabetics. Independent of the type of diabetes, serum laminin concentrations in patients without nephropathy or with early renal damage as assessed by microalbuminuria were comparable with those of the control subjects. Patients with macroproteinuria or with renal insufficiency had significantly increased serum laminin P1 concentrations. Diabetic retinopathy was not found to influence serum laminin P1 concentrations. These data indicate that serum laminin P1 concentrations are increased in advanced diabetic nephropathy.

Haiyashi et al. [36] Serum and urinary concentrations of laminin were measured by enzyme-linked immunosorbent assay (ELISA) in diabetic patients and compared with normal control subjects. In diabetic patients with proteinuria or with renal insufficiency, urinary concentrations of laminin were significantly higher in diabetes, even in the absence of nephropathy, than in normal controls ($p < 0.05$).

Serum concentrations of laminin ($p < 0.025$) were significantly higher in diabetic patients than normal controls and the difference between patients with and without retinopathy was not significant.

The first obvious increase in serum Laminin concentration was in group B DN before the elevation of albuminuria (the trade marker which elevated in group C DN) with high specificity and sensitivity so, serum Laminin can be used as an earlier diagnostic biomarker for DN than albuminuria.

Along the same lines, serum Transferrin concentration was lower in DN group rather than the control group. The first obvious decrease in serum Transferrin concentration was in group B DN before the elevation of albuminuria with low specificity and sensitivity so, serum Transferrin can be used as an earlier diagnostic biomarker for DN than albuminuria.

This research is an extension of Mohy Eldin et al. [6] and Abd Elfattah et al. [39, 40,41].

7. CONCLUSION

It is concluded from this study that serum Laminin had the best diagnostic value and needs to be further investigated with longitudinal prospective studies to evaluate the predictive power of this marker for diabetic nephropathy before any structural damage occurs. Serum transferrin had less discriminative diagnostic value.

The current study is limited by being a Cross-sectional study design which only provides the basis for associations and not causality and relatively low number of patients. Another limitation of the current study was non

adjustment for hypertension and hyperlipidaemia as confounding factors, however, such adjustment is difficult since diabetic nephropathy would predispose for hypertension and hyperlipidaemia. On the other hand, this study draws its strength from assessing the diagnostic values of a 2 various biomarkers in different stages of diabetic nephropathy.

ETHICAL CONSIDERATIONS AND CONSENT

Study was approved by the Ethical Committee of the faculty of medicine Suez Canal University and written informed consent was taken from all the patients (protocol approved by date 26/03/2019).

RECOMMENDATION

More studies with larger sample size are needed to confirm the observed associations.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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