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Assessment of SH3YL1 protein as a marker for diabetic nephropathy in type 2 diabetes mellitus

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Abstract:

The goal of this study was to explore the association between Src homology 3 domain of SH3 domain containing Ysc84-like 1 (SH3YL1) protein and diabetic nephropathy (DN) in type 2 diabetes mellitus (T2DM). The study included 90 participants, 60 patients with T2DM all recruited from Outpatients Clinic and Internal Medicine Inpatient Department, Beni-Suef University Hospital; and 30 apparently healthy controls. Patients were sub-divided into group I: 20 patients with no albuminuria, group II: 20 patients with microalbuminuria and group III: 20 patients with macroalbuminuria. All participants were subjected to full history taking, routine laboratory investigations and serum SH3YL1 analysis using enzyme-linked immunosorbent (ELISA) assay. Serum SH3YL1 was significantly higher in T2DM patients (5.69 ± 1.6 ng/ml) versus controls (3.76 ±0.80 ng/ml) ($p<0.001$).There was statistical significant difference with $p <0.05$ between cases of group I, and each of group II, and group III as regards SH3YL1 level with lowest mean among group I. There was statistical significant positive correlation between SH3YL1 level and each of fasting blood glucose, 2hours postprandial, glycated hemoglobin, urea, creatinine, albuminuria, total cholesterol and triglycerides. SH3YL1 showed sensitivity of (88.3%) and specificity of (53.3%) at cut off value (3.85 ng/mL) among T2DM patients versus controls ($p<0.001$). Moreover, sensitivity of (70%, 90%, and 90%) and specificity of (56.7%, 70%, and 86.7%) at cutoff (4 ng/mL, 4.45 ng/mL, and 4.65 ng/mL) was found among groups I, II and III versus control group ($p= 0.01$, <0.001 and <0.001), respectively. In conclusion, SH3YL1 serum level revealed statistically significant increase among T2DM patients and showed statistical difference between the studied no albuminuria, microalbuminuria and macroalbuminuria groups. Our study suggests SH3YL1 as a promising diagnostic and prognostic marker among DN patients.

Keywords: SH3YL1, biomarker, diabetic nephropathy, type 2 diabetes mellitus.

1. Introduction:

Diabetes mellitus (DM) is a disease characterized by hyperglycemia which is caused by deficiency in insulin secretion and/or its biological action, prolonged diabetes results in chronic damage and dysfunction of various organs, mainly eye, kidney, heart, blood vessels, and nerve [1]**.** Diabetes mellitus is divided into 2 types, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), which can develop into diabetic nephropathy (DN) in about 30% of T1DM and 20% of T2DM [2]**.**

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria, progressive decline in renal function, presence of a typical pattern of glomerular disease; and is considered the single commonest cause of end-stage renal disease (ESRD) in many populations [3]**.** Diabetic nephropathy is typically associated with arterial hypertension and increased cardiovascular morbidity and mortality; outcomes for people with T2DM who develop DN are significantly worse than those who do not; yet despite the large and increasing number of people affected by devastating consequences, DN is also an area that has seen significant therapeutic advances which would reduce the medical and economic burden associated with it [4]**.**

In Kidney Disease Improving Global Outcomes (KDIGO) 2021, albuminuria was described according to urinary albumin-to-creatinine ratio (ACR) : values of 3 to 30 mg/mmol or 30 to 300 mg/g (corresponding to microalbuminuria) referred to as moderately increased (A2); and ACR values of >30 mg/mmol (>300 mg/g), corresponding to macroalbuminuria, referred to as severely increased (A3) [5].

Sufficient evidence indicates that oxidative stress (OS) is a very important factor in the development of T2DM, caused primarily by uncontrolled hyperglycemia [6]. The generation of advanced glycation end-products (AGE), reactive oxygen species (ROS), activation of transforming growth factor β and enzymes that digest the extracellular matrix (ECM) have the capability to cause structural and histological damage in the kidneys through the expression of cyclin-dependent kinases; leading to glomerular sclerosis and interstitial tubular damage through the abnormal formation of ECM [7].

The increase of ECM leads to renal fibrosis primarily generated by the mesangial cells accumulation, which favors ECM deposition, glomerular and tubular membranes thickening, dysfunction of the podocytes, and encouraging apoptosis; where all of these events are results of changes in the redox system that lead to proteinuria, glomerulosclerosis, and interstitial tubular fibrosis [8].

Guidelines from the American Diabetes Association (ADA) and KDIGO group

recommend that all people with diabetes should have renal function, serum creatinine based estimated glomerular filtration rate (eGFR) calculation and albuminuria measured at diagnosis and annually [9]. Given the increased prevalence of DN, early detection of DN is essential in order to provide appropriate treatment to prevent ESRD; where most diagnoses are based on a clinically recognized definition of DN, which requires the detection of albuminuria or decreased glomerular filtration rate (GFR) [10]. Albuminuria is not a sufficiently accurate predictor of DN risk given some limitations where not all diabetics with microalbuminuria will end up with ESRD, and 30% of them may actually have normo albuminuria [11]. Biomarkers of glomerular injury, tubular injury, inflammation and oxidative stress precede albuminuria in some patients and thus may be useful for the early prediction of DN [12].

The Src family of protein tyrosine kinases contains three conserved domains, Src homology 1 (SH1), Src homology 2 (SH2) and Src homology 3 (SH3) domains [13]. Src homology 3 (SH3) domain is a small protein domain composed of around 60 amino acid residues; initially, the SH3 domain was described as a conserved region in cytoplasmic tyrosine kinases [14]. Src homology 3 (SH3) domain-containing YSC84-like 1 (SH3YL1) Protien is a protein having Src homologydomain3 (SH3) in the carboxyl terminal region and SYLF (YSC84) in the amino terminal region [15]. The SH3YL1 was first reported in skin keratinocytes as a regulator of the hair cycle, it was also detected in various tissues such as kidneys, stomach, small intestine, and colon, this suggests a possible role of SH3YL1 in development of gastrointestinal and renal diseases where SH3YL1 protein was found to be over expressed in renal tissue compared to non-renal tissue [16]. SH3YL1 protein has been found in many protein families involved in signal transduction and is thought to mediate specific protein protein interactions in signal transduction pathways [17]. SH3YL1 protein has a unique lipid-binding domain which strongly binds to phosphorylated phosphoinositides so has a role in endosomal sorting of epidermal growth factor (EGF) [18].
Reactive oxygen species are formed upon incomplete reduction of oxygen and have been viewed as harmful biomolecules leading to oxidative stress and damage to DNA, proteins, lipids and carbohydrate; however ROS are important second messenger in various cell signalling [19]. Reduced nicotinamide adenine dinucleotide phosphate (NADPH), its related enzymes NADPH oxidase (Nox), dual oxidase (Duox) and their six homologs (Nox1, Nox2, Nox3, Nox4, Nox5, Duox1, and Duox2) have been identified to be present in various types of cells or tissues and to generate ROS in a regulated manner, producing reactive oxygen in various cells and tissues in response to growth factors, cytokines and calcium signals;

where NADPH oxidase 4 (Nox4) was originally identified as a NADPH oxidase homolog which is highly expressed in the kidney [20]. SH3YL1 binds and stimulate the activity of Nox4; and is considered as a regulator for Nox4 through interaction with COOH-terminal region of Nox4, where SH3 domain of SH3YL1 interacts with proline rich region of the human neutrophil cytochrome b light chain and this makes a stable complex with Nox4 which is formed by renal tubular epithelial cells in response to stimulation by lipopolysaccharides (LPS); SH3YL1-dependent Nox4 activation and H2O2 generation are essential in lipopolysaccharides-induced pro-inflammatory cytokine production; where SH3YL1-Nox4-H2O2 cascade plays an important role in LPS-induced kidney injury and in macrophage and neutrophil infiltration, leading to kidney injury and inflammation [21]. The aim of this study was to explore the association between SH3YL1 and DN in T2DM.

2. Patients and Methods:

This was a randomized study performed in Beni-Suef University Hospital within six months involving 90 participants, 60 patients with T2DM and 30 apparently healthy controls who were provided with complete information about the study. Written informed consent was obtained from all included participants.

Data confidentiality was preserved according to the Revised Helsinki Declaration of Bioethics 2008 [22].

2.1 Inclusion criteria:

1. Both males and females above 20 years old.
2. Type 2 diabetic patients diagnosed according to American Diabetic Association criteria2019 [9] as followings:

• Fasting blood glucose (FBG) ≥126 mg/dL (7.0 mmol/L).

• 2hours postprandial (2hpp) ≥200 mg/dL (11.1mmol/L).

• Glycated hemoglobin (HbA1c) ≥6.5% (48 mmol/l).

• Random blood glucose (RBG) ≥200 mg/dL (11.1 mmol/L).

- Patients were further stratified based on urine albumin-creatinine ratio (UACR) into group I: 20 patients with no albuminuria (<30 mg/g), group II: 20 patients with microalbuminuria (30 to 300 mg/g) and group III: 20 patients with macroalbuminuria (>300 mg/g).

2.2 Exclusion criteria:

1. Patients Less than 20 years old.
2. Patients with malignancy.
3. Patients with diabetic macrovascular complications.
4. Patients with other endocrine diseases which affect glucose metabolism and lipid metabolism.
5. People with chronic hepatitis, pregnancy, and history of drug abuse.
6. The presence of hematuria, renal insufficiency of unexplained origin, urinary tract infection, history of rapidly progressive renal failure, polycystic kidney disease and glomerulonephritis.

2.3 Methods:

1. Careful history taking with special stress on diabetic manifestations, complications and treatment and family history.
2. Measurement of Body mass index (BMI), systolic and diastolic blood pressure.
3. Estimation of GFR according to the Modification of Diet in Renal Disease (MDRD) equation based on creatinine and patient characteristics.
4. Clinical examination.
5. Routine laboratory investigations were analyzed on an automated chemistry analyzer (Bechman CX5 automated chemistry analyzer, Ireland) by its own commercial kits, according to manufacturer's instructions; including:

-Fasting blood glucose (FBG) and 2hours postprandial (2hpp).

-Glycated hemoglobin (HbA1c).

-Kidney function tests (creatinine, urea).

-Lipid profile including: total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides.

-Albumin/creatinine level in urine test.

6. Special test:

-Quantitative detection of serum SH3YL1 was assayed by enzyme linked immunosorbent assay (ELISA) sandwich principle using Human SH3YL1 ELISA Kit supplied by Sunredbio (SRB) Technology co.,Ltd,Shanghai, China (Cat no 201-12-5643) according to manufacturer's instructions.

7. Radiological investigation:

-Abdominal ultrasound.

2.4 Statistical methods:

Data were analyzed using Statistical Package of Social Science (SPSS) software version 22 in windows 7 (SPSS Inc., Chicago, IL, USA). Numerical data were expressed as mean and standard deviation (SD). Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using either student t-test or Mann-Whitney test (non-parametric t-test). Correlation between numerical variables was done using Pearson product-moment. Receiver operating characteristic (ROC) curve was used for sensitivity, specificity and prediction of cut off values. A P-value < 0.05 was considered significant.

3. Results :

Sixty T2DM patients and 30 apparently healthy controls were included in this study. Patients were sub-divided into group I: 20 patients with no albuminuria, group II: 20 patients with microalbuminuria and group III: 20 patients with macroalbuminuria.

Patients and controls were age and sex matched (p=0.21 and 0.92, respectively). Statistically significant higher mean of BMI was found among cases versus controls (p <0.001) (Table 1).

Table (1): Comparisons of demographic characters in different study groups

Variables	**Patients** (N=60)	**Controls** (N=30)	**P-value**	**Sig.**
	Mean± SD	**Mean± SD**		
Age (years)	58.3±11.1	55.6±6.4	0.21	NS
BMI (kg/m^2)	32.1±3.2	24.1±4.6	**<0.001**	**HS**
Sex	**Frequency (%)**	**Frequency (%)**		
Male	31(51.7%)	16(53.3%)	0.92	NS
Female	29(48.3%)	14(46.7%)		

P-value < 0.01 is highly significant (HS)

P-value > 0.05 is not significant (NS)

There was statistically significant difference with p <0.05 as regard FBG, HbA1C, creatinine, eGFR, urea, creatinine, albuminuria and LDL-C between studied groups; with highest mean among G III. On the other hand, no statistical significance was found as regards other parameters (Table 2).

Table (2): Comparison of routine laboratory investigations in different subgroups of cases

Variables	**G I** (N=20)	**G II** (N=20)	**G III** (N=20)	**P-value**	**Sig.**
	Mean± SD	**Mean± SD**	**Mean± SD**		
Glucose profile					
FBG (mg/dL)	198.9±44.2	231.8±60.3	237.1±39.3	0.1 [a] 0.9 [b] **0.04 [c]**	NS NS **S**
2hpp (mg/dL)	252.3±53.5	269.7±67	291.6±45.6	0.09	NS
HbA1c (%)	8.1±0.65	8.5±0.86	8.8±0.76	0.2 [a]	NS NS

				0.9 b **0.02 c**	S
Kidney function tests					
Urea (mg/dL)	63.4±49.5	65.5±33.8	119.8±73.2	0.9 a **0.008** b **0.005**c	NS **HS** HS
Creatinine (mg/dL)	1.4±1.1	1.6±1.2	3.8±2.9	0.9 a **0.002** b,c	NS **S**
eGFR (mL/min/1.73m2)	65.6±33.1	60.6±32.5	38.2±40.1	0.9 a 0.1 b **0.05** c	NS NS **S**
Urine albumin-creatinine ratio (mg/g)	18.4±7.8	171.1±83.9	2187.9±1617.3	0.9 a **0.001** b,c	NS **HS**
Lipid profile					
Cholesterol(mg/dL)	196.6±50.9	183.7±48.8	160.1±73.2	0.1	NS
TG(mg/dL)	128.5±33.9	114.6±38.9	139.2±34.5	0.1	NS
LDL-C (mg/dL)	101.1±34.5	109.6±35.4	133.5±43.2	0.9 a 0.1 b **0.02** c	NS NS **S**
HDL-C (mg/dL)	40.7±13.5	41.8±14.4	36.9±16.6	0.6	NS

a:significance between GI, and G II

b:significance between GII, and G III

c:significance between GI, and G III

P-value < 0.05 is significant (S)

P-value < 0.01 is highly significant (HS)

P-value > 0.05 is not significant (NS)

Serum level of SH3YL1 was significantly higher in patients with T2DM (5.69 ± 1.6 ng/ml) when compared with healthy control subjects (3.76 ±0.80 ng/ml) (p<0.001).

There was a statistically significant difference in serum SH3YL1 level between patients of G I, and each of G II, and G III (p=0.02 and 0.001, respectively) with lowest mean among G I and highest mean in G III (Table 3).

Table (3): Comparison of serum SH3YL1 level in different patients subgroups

Variables	SH3YL1 protein (ng/mL)	P-value	Sig.
	Mean ± SD		
GI	4.6 ± 1.3	**0.02** [a]	S
G II	5.9 ± 1.5	0.5 [b]	NS
G III	6.5 ± 1.6	**0.001** [c]	**S**

a:significance between GI, and G II

b:significance between GII, and G III

c:significance between GI, and G III

P-value < 0.05 is significant (S)

P-value > 0.05 is not significant (NS)

Among cases there was statistical significant positive correlation with p <0.05 between SH3YL1 protein level and each of systolic blood pressure, FBG, 2hpp, HbA1c, urea, creatinine, albuminuria, TC, and TG. On the other hand, there was no statistical significant correlation with p >0.05 between SH3YL1 protein and other studied variables (Table 4).

Table (4): Correlation between serum SH3YL1 with different studied variables among cases

Variables	SH3YL1 protein		
	R	P-value	Sig.
Age	0.04	0.7	NS
BMI	-0.03	0.8	NS
Blood pressure			
Systolic	0.46	**0.001**	**HS**
Diastolic	0.19	0.1	NS

Glucose profile			
FBG	**0.37**	**0.003**	**HS**
2hpp	**0.45**	**<0.001**	**HS**
HbA1c	**0.65**	**<0.001**	**HS**
Kidney function tests			
Urea	**0.37**	**0.004**	**HS**
Creatinine	**0.38**	**0.003**	**HS**
eGFR	-0.24	0.07	NS
Urine albumin-creatinine ratio	**0.29**	**0.02**	**S**
Lipid profile			
Cholesterol	**0.40**	**0.002**	**HS**
TG	**0.38**	**0.003**	**HS**
LDL-C	0.22	0.09	NS
HDL-C	-0.20	0.1	NS

P-value < 0.05 is significant (S)

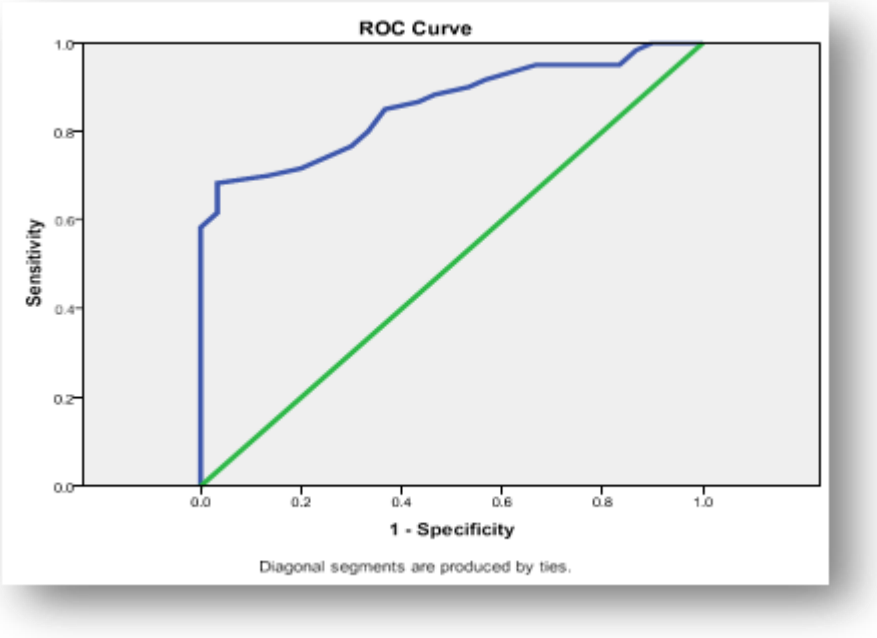
P-value < 0.01 is highly significant (HS)

P-value > 0.05 is not significant (NS)

Using ROC curve, SH3YL1 protein showed a sensitivity of (88.3%) and a specificity of (53.3%) at cut off value (3.85 ng/mL) in differentiation between T2DM patients and controls (p<0.001) (Table 5) (Figure 1). Moreover, sensitivity of (70%, 90%, and 90%) and specificity of (56.7%, 70%, and 86.7%) at cutoff (4 ng/mL, 4.45 ng/mL, and 4.65 ng/mL) was found among groups I, II and III versus control group (p= 0.01, <0.001 and <0.001), respectively (Table 5) (Figures 2, 3 and 4) respectively.

Table (5): Sensitivity and specificity of serum SH3YL1 in the studied groups

Variable	Sensitivity	Specificity	Cut off point (ng/mL)	P-value
Case versus control	88.3%	53.3%	3.85	**<0.001**
G I versus control	70%	56.7%	4	**0.01**
GII versus control	90%	70%	4.45	**<0.001**
G III versus control	90%	86.7%	4.65	**<0.001**



Figure(1): Receiver operating characteristic (ROC) curve of serum SH3YL1 in T2DM patients

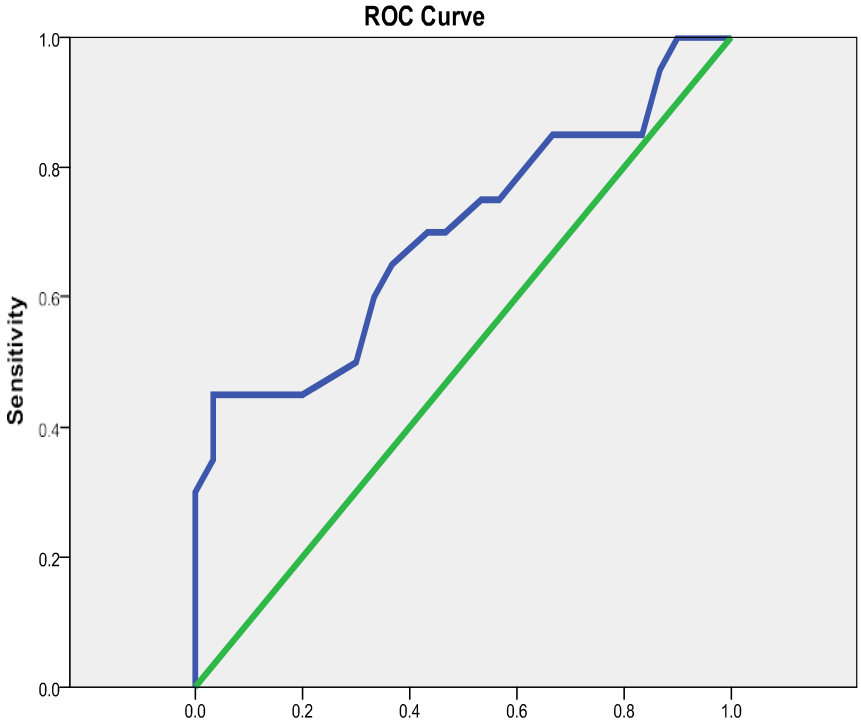


Figure (2): Receiver operating characteristic (ROC) curve for serum SH3YL1 in no albuminuria group

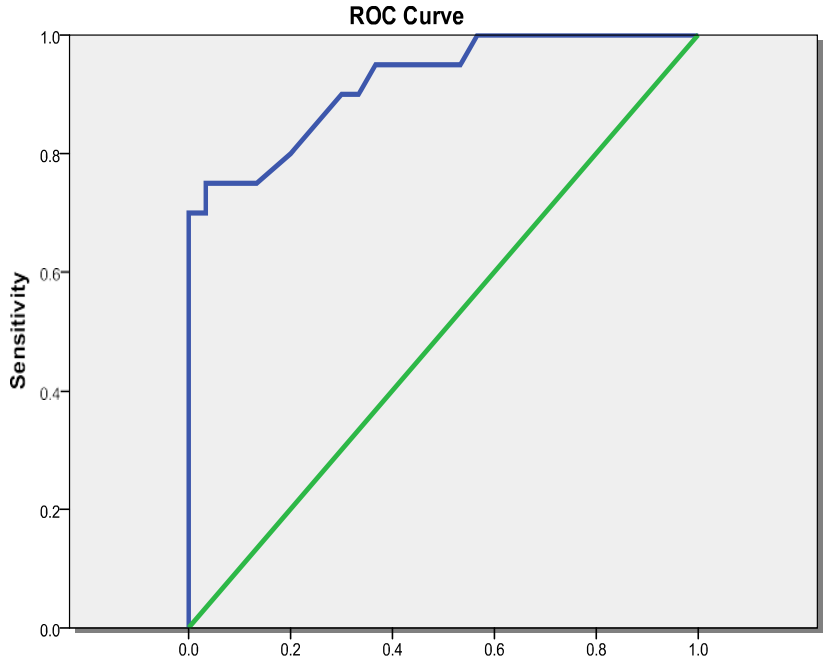


Figure (3): Receiver operating characteristic (ROC) curve of serum SH3YL1 in microalbuminuria group

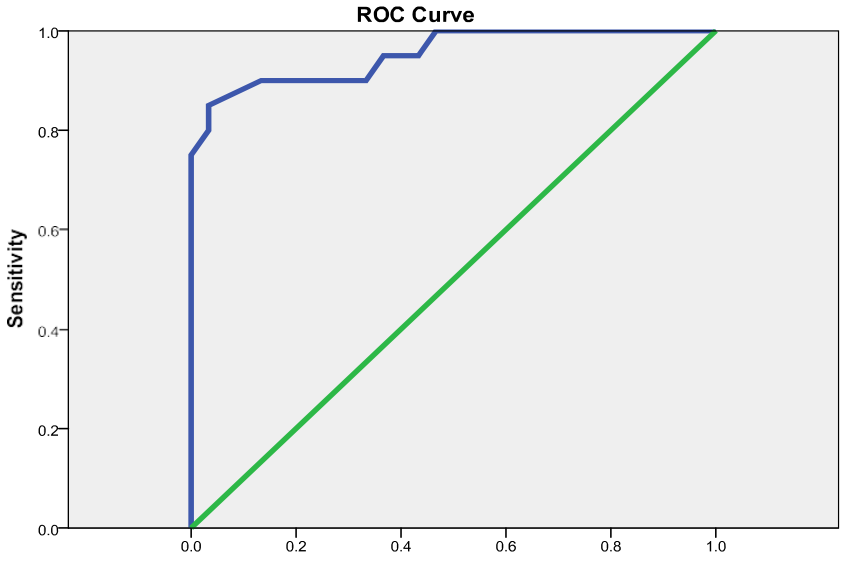


Figure (4): Receiver operating characteristic (ROC) curve of serum SH3YL1 in macroalbuminuria group

4. Discussion:

Prolonged diabetes can cause a variety of micro and macrovascular complications, including some serious long term complications, such as cardiovascular disease, retinopathy, neuropathy, and as well as chronic renal failure,

which is also known as diabetic nephropathy [23].

Diabetic nephropathy is an important factor affecting the prognosis of patients resulting in disability and is considered the main cause of adult hemodialysis in developing countries [24].

Studies have stated that microalbuminuria may develop in non-diabetic patients with progressive chronic kidney disease; so microalbuminuria is not specific for patients with DN alone and also, not all diabetic patients with microalbuminuria progress to ESRD where the correlation between both albuminuria and eGFR has been perceived to be obviously weak and urinary albumin is deficient of both specificity and sensitivity to establish initial stages of diabetic nephropathy; and therefore sensitive and specific biomarkers that can early predict susceptibility to diabetic nephropathy is needed [25].

Based on this knowledge, this study was designed to assess the level of SH3YL1 protein in a group of T2DM patients to emphasize its possible role as a predictor marker for DN in T2DM. This study included 90 participants, 60 patients with T2DM and 30 healthy controls. Our patients were subdivided according to their urinary albumin-creatinine ratio into three groups, GI patients with no albuminuria and G II patients with microalbuminuria and G III patients with macroalbuminuria. In our results, SH3YL1 level showed significantly elevated level in T2DM patients (5.69 ± 1.6 ng/ml) compared to the normal controls (3.76 ± 0.80 ng/ml) ($p < 0.001$). There was a statistically significant difference in serum SH3YL1 level among cases of G I, and each of G II ($p = 0.02$), and G III ($p = 0.001$) with lowest mean among GI (no albuminuric) (4.6 ± 1.3 ng/ml) and highest mean in G III (macroalbuminuric) (6.5 ± 1.6 ng/ml). In our study there was a significant positive correlation with $p < 0.05$ between SH3YL1 serum level and each of systolic blood pressure, FBS, 2hpp, HbA1c, urea, creatinine, albuminuria, TC, and TG. We found a sensitivity of (88.3%) and a specificity of (53.3%) at cut off value (3.85 ng/mL) in differentiation between T2DM patients and controls ($p < 0.001$). Moreover in differentiation of the three diabetic groups G I, G II and G III at cutoff (4 ng/mL, 4.45ng/mL, and 4.65ng/mL), the sensitivity was (70%, 90%, and 90%) and specificity was (56.7%, 70%, and 86.7%) with ($p = 0.01$, <0.001 and <0.001), respectively versus control group.

Our results were in agreement with the study conducted by Choi et al., 2021 [26] in South Korea on a total of 171 patients with type 2 diabetes and 65 healthy control subjects who found that SH3YL1 level was significantly elevated in patients with diabetes compared to the normal controls and was positively correlated with systolic blood pressure, postprandial glucose concentration and albuminuria and not significant with the eGFR. However the authors found no significant relationship with other risk factors, including HbA1c, fasting plasma glucose

concentration, serum creatinine concentration, or lipid profile compared to our study which has a high significance with them. The authors reported no significant difference in SH3YL1 concentration between no albuminuric and microalbuminuric groups but SH3YL1 level was significantly higher in the overt proteinuria group among patients with diabetes.

The physiological and pathological events underlying SH3YL1 protein relation with DN was investigated by Yoo et al., 2020 [21] in korea in a study conducted to investigate the interaction of Nox4 and SH3YL1 and its effect on the enzymatic function of Nox4 who reported that stimulation of human embryonic kidney cells expressing Nox 4 cells, overexpressing SH3YL1, with EGF resulted in increasing reactive oxygen species (ROS) generation compared to cells of control vector, demonstrating a positive role for SH3YL1 in ROS generation by Nox4 which plays a critical role in glucose-induced oxidative stress and hypertrophy in the kidney concluding SH3YL1 to take part in cellular injury by Nox4 in kidney.

Choi et al., 2021 [26] reported that in an in vitro study, expression of SH3YL1 gene was up regulated in kidneys with diabetes; where in accordance with gene expression, the level of SH3YL1 protein expression was progressively increased in kidneys with diabetes which exhibited markedly increased immunoreactivity in both glomeruli and tubulo-interstitium compared with control kidneys. In addition, SH3YL1 immunoreactivity was primarily increased in podocytes and the proximal tubular epithelium. The authors found that high glucose, angiotensin II, and free fatty acids, are all major mediators for tissue injury in diabetes which cause increased SH3YL1 synthesis and this explanation also potentiates the idea of the presence of relationship between the SH3YL1, high glucose level of T2DM and DN. There is a strong belief that novel DN markers among T2DM patients offer new promising diagnostic, prognostic and therapeutic opportunities [27].

5. Conclusion and Recommendations:

Our study suggests an association between SH3YL1 and T2DM with differentiation between the three studied no albuminuria, microalbuminuria and macroalbuminuria groups but small sample size and interactions with other implicated factors affecting kidney functions such as smoking, drugs and others were not considered in this study, were limitations in our study. Further research and intense work in larger groups of patients is recommended for evaluation of SH3YL1 as promising diagnostic and prognostic marker among DN patients.

Declaration of Conflicting Interests

The auther(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study was approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University (approval number FMBSUREC/06072021/Gomaa).

Informed consent

A signed consent form was obtained from each study participant.

6. References:

1. Nasri, H., & Rafieian-Kopaei, M. (2015). Diabetes mellitus and renal failure: Prevention and management. Journal of research in medical sciences 20(11), 1112.
2. Collins, A. J., Foley, R. N., Herzog, C., Chavers, B., Gilbertson, D., Ishani, A., & Agodoa, L. (2011). US renal data system 2010 annual data report. American Journal of Kidney Diseases, 57(1), A8.
3. Byrne, C., Caskey, F., Castledine, C., Dawnay, A., Ford, D., Fraser, S., & Williams, A. J. (2018). UK Renal Registry. Nephron, 139.
4. Andrésdóttir, G., Jensen, M. L., Carstensen, B., Parving, H. H., Hovind, P., Hansen, T. W., & Rossing, P. (2015). Improved prognosis of diabetic nephropathy in type 1 diabetes. Kidney international, 87(2), 417-426.
5. Hodel, N. C., Hamad, A., Reither, K., Kasella, I. M., Abdulla, S., Schoetzau, A., & Mayr, M. (2021). Comparison of two different semiquantitative urinary dipstick tests with albumin-to-creatinine ratio for screening and classification of albuminuria according to KDIGO. A diagnostic test study. Diagnostics, 11(1), 81.
6. Oates, P. J. (2002). Polyol pathway and diabetic peripheral neuropathy. International review of neurobiology, 50, 325-392.
7. Forbes, J. M., Cooper, M. E., Oldfield, M. D., & Thomas, M. C. (2003). Role of advanced glycation end products in diabetic nephropathy. Journal of the American Society of Nephrology, 14(suppl 3), S254-S258.
8. Manda, G., Checherita, A. I., Comanescu, M. V., & Hinescu, M. E. (2015). Redox signaling in diabetic nephropathy: hypertrophy versus death choices in mesangial cells and podocytes. Mediators of Inflammation,2015:604208.doi:10.1155/2015/604208.
9. American Diabetes Association. (2019). Microvascular complications and foot care: standards of medical care in diabetes. Diabetes Care, 42(Supplement_1), S124-S138.
10. Jerums, G., Panagiotopoulos, S., Premaratne, E., & MacIsaac, R. J. (2009). Integrating albuminuria and GFR in the assessment of diabetic nephropathy. Nature Reviews Nephrology, 5(7), 397-

406.

11. An, J. H., Cho, Y. M., Yu, H. G., Jang, H. C., Park, K. S., Kim, S. Y., & Lee, H. K. (2009). The clinical characteristics of normoalbuminuric renal insufficiency in Korean type 2 diabetic patients: a possible early stage renal complication. Journal of Korean medical science, 24(Suppl 1), S75-S81.
12. Gluhovschi, C., Gluhovschi, G., Petrica, L., Timar, R., Velciov, S., Ionita, I., & Timar, B. (2016). Urinary biomarkers in the assessment of early diabetic nephropathy. Journal of diabetes research, 2016:4626125. doi:10.1155/2016/4626125.
13. Pawson, T., Gish, G. D., & Nash, P. (2001). SH2 domains, interaction modules and cellular wiring. Trends in cell biology, 11(12), 504-511.
14. Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R., & Soltoff, S. (1991). Oncogenes and signal transduction. Cell, 64(2), 281-302.
15. Shrestha, P., Yun, J. H., & Lee, W. T. (2010). Expression, Purification and NMR studies of SH3YL1 SH3 domain. Journal of the Korean Magnetic Resonance Society, 14(2), 105-116.
16. Aoki, N., Ito, K., & Ito, M. (2000). A novel mouse gene, SH3YL1, is expressed in the anagen hair follicle. Journal of investigative dermatology, 114(5), 1050-1056.
17. Mayer, B. J., & Baltimore, D. (1993). Signalling through SH2 and SH3 domains. Trends in cell biology, 3(1), 8-13.
18. Hasegawa, J., Tokuda, E., Tenno, T., Tsujita, K., Sawai, H., Hiroaki, H. & Itoh, T. (2011). SH3YL1 regulates dorsal ruffle formation by a novel phosphoinositide-binding domain. Journal of Cell Biology, 193(5), 901-916.
19. Auten, R. L., & Davis, J. M. (2009). Oxygen toxicity and reactive oxygen species: the devil is in the details. Pediatric research, 66(2), 121-127.
20. Lyle, A. N., Deshpande, N. N., Taniyama, Y., Seidel-Rogol, B., Pounkova, L., Du, P., & Griendling, K. K. (2009). Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells. Circulation research, 105(3), 249-259.
21. Yoo, J. Y., Cha, D. R., Kim, B., An, E. J., Lee, S. R., Cha, J. J., & Bae, Y. S. (2020). LPS-induced acute kidney injury is mediated by Nox4-SH3YL1. Cell Reports, 33(3), 108245.
22. World Medical Association (2008) Declaration of Helsinki: ethical principles for medical research involving human subjects. The 59th WMA General Assembly, Seoul, South Korea.
23. Filla, L. A., & Edwards, J. L. (2016). Metabolomics in diabetic complications. Molecular BioSystems, 12(4), 1090-1105.
24. Conserva, F., Gesualdo, L., & Papale, M. (2016). A systems biology overview on human diabetic nephropathy: from genetic

susceptibility to post-transcriptional and post-translational modifications. Journal of diabetes research, 2016:7934504. doi:10.1155/2016/7934504.

25. Green, J. B., Bethel, M. A., Armstrong, P. W., Buse, J. B., Engel, S. S., Garg, J., & Holman, R. R. (2015). Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. New England Journal of Medicine, 373(3), 232-242.
26. Choi, G. S., Min, H. S., Cha, J. J., Lee, J. E., Ghee, J. Y., Yoo, J. A., & Cha, D. R. (2021). SH3YL1 protein as a novel biomarker for diabetic nephropathy in type 2 diabetes mellitus. Nutrition, Metabolism and Cardiovascular Diseases, 31(2), 498-505.
27. Fiseha, T. (2015). Urinary biomarkers for early diabetic nephropathy in type 2 diabetic patients. Biomarker research, 3(1), 1-7.