

Role of Supar and Some Other Biochemical Parameters in Diabetic Nephropathy

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Abstract

Diabetes mellitus (DM) is a disorder that occurs due to metabolic problems and is most frequent globally. Diabetic nephropathy is a common complication of diabetes and the leading cause of chronic kidney disease. Soluble urokinase plasminogen activator receptor (suPAR) is the circulating form of a glycosyl-phosphatidylinositol-anchored three-domain membrane. Protein Urokinase-type plasminogen activator is a trypsin-like serine protease that plays a vital role in the extracellular conversion of inactive plasminogen into catalytically active plasmin. Methods: Blood samples were taken from 90 patients and 30 healthy people, the suPAR levels were determined by using Elisa kits, while protein urine was by urine dipstick. Result: the levels of suPAR in the studied groups there was a highly significant increase of ($p < 0.001$) in the three patients' groups compared with a control group. Also, there was a highly significant increase of ($p < 0.001$) in the diabetic nephropathy and nephropathy groups compared with the diabetes mellitus group and a highly significant increase of ($p < 0.001$) in the diabetic nephropathy group compared with the nephropathy group. The criteria diagnosis validity of suPAR level in groups: The optimal cut-off value for suPAR in all groups of patients compared with control estimated from ROC curves, according to these results, the test is positive if test \leq threshold value (criterion values) for diabetes group, while $>$ threshold value for nephropathy and diabetes nephropathy groups. These results indicated that: the variable is closely related to diabetic nephropathy more than it is to diabetes.

Keywords: Health, suPAR; Diabetic

1. Introduction

According to the International Diabetes Federation, diabetes has become a global health burden, affecting 425 million people worldwide. This number is expected to rise to 630 million by 2045 [1]. Diabetes mellitus (DM) is a disorder that occurs due to metabolic problems and is most frequent globally [2], [3]. Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. Diabetes can be classified into two main categories: Type 1 diabetes (T1DM), and Type 2 diabetes (T2DM).

Diabetic nephropathy (DN) is a common complication of diabetes and the leading cause of chronic kidney disease (CKD). Diabetic nephropathy is characterized by an increased urinary albumin excretion in the absence of other renal diseases [4]. About 40% of diabetic individuals acquire DN, which is defined by a rise in albuminuria and blood pressure and a decline in kidney function that leads to end-stage renal disease (ESRD) or premature mortality [5]. Kidney disease is more common in T1DM; this is mainly because, in T2DM, cardiovascular deaths are more common than deaths from renal failure. Microalbuminuria (MA) (urinary albumin excretion 30–299 mg/24 hr or 30–299 mg albumin/g creatinine) in patients with diabetes is an independent predictor of overt nephropathy, renal function decline, and cardiovascular disease. Given the increased prevalence of diabetes and the resulting increased prevalence of DN, as well as the limitations of MA in detecting early tissue changes in the kidney, early detection of renal damage in diabetic patients is

critical to provide timely therapy that slows or even prevents progression to ESRD [6]. The development of macroalbuminuria often accompanies the decline of glomerular filtration rate (GFR) and may progress to ESRD [7].

Soluble urokinase plasminogen activator receptor (suPAR) is the circulating form of a glycosyl-phosphatidylinositol-anchored three-domain membrane. Protein Urokinase-type plasminogen activator (uPA) is a trypsin-like serine protease that plays a vital role in the extracellular conversion of inactive plasminogen into catalytically active plasmin. uPA is synthesized by endothelial and tumor cells as a single-chain glycoprotein without catalytic activity. When it is converted to a two-chain protein by plasmin, an active serine protease center develops, which activates plasminogen. Thus, uPA (55 kDa) results in the amplification of fibrinolysis [8].
Aim: To specifically understand the role of suPAR in diabetic nephropathy

2. Material and Methods

Blood samples were taken from 90 patients and 30 healthy people from medical city/Gazzi al-Hariri hospital/ center of kidney disease and transplantation from (November 2021 to February 2022) the participants' range (18–65) years of age were divided into four groups study: A Control group of healthy individuals, Diabetic without nephropathy, Nephropathy, and DN.

The suPAR levels were determined in the blood serum of patients and control groups using Elisa kits supplied by Sun long Bio-Technology Laboratory company. The principle of the ELISA kit uses

Sandwich-ELISA as the method. Determination of Glucose done by kit from AGAPE-Switzerland company. Estimation of glomerular filtration rate (GFR) was calculated for each patient and controlled by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

$$GFR = 141 * \min (Scr/\kappa, 1) \alpha * \max (Scr/\kappa, 1) - 1.209 * 0.993Age * 1.018 [if female] * 1.159 [if black]$$

Quantitative assessment of proteinuria

Proteinuria is an indicator of renal damage that is often detected earlier than any apparent decline in GFR. Proteinuria is frequently measured using the urine protein-to-creatinine ratio (PCR). Urine dipstick primary detects albumin and is highly specific.

3. Statistical Analysis

Data were analyzed by MedCalc statistical analysis software. Normality of distribution was determined by Student’s t-test. The probability $P < 0.001$ =highly significant, $P < 0.05$ = significant, $P > 0.05$ = non-significant.

4. Result and Discussion

The results represent the statistical values of the clinical variables measured in the serum of four groups: A Control group of healthy individuals, a diabetic without nephropathy, Nephropathy, and DN. Each group was divided into several categories depending on: Age divided into (A1=35-50, A2=50-65 year), gender (Male, Female).

urine albumin and urine glucose in studied groups The percentage of urine albumin and urine glucose in the studied groups was illustrated in table (1).

Parameters	urine Albumin			urine glucose		
	+	++	>+++	+	++	>+++
Control (n=30)	0%	0%	0%	0%	0%	0%
DM (n=30)	0%	0%	0%	50%	15%	25%
Nephropathy (n=30)	10%	50%	40%	0%	0%	0%
DN (n=30)	40%	30%	30%	70%	15%	15%

suPAR pg/ml	Mean ± SD										
	Control		Diabetic		Nephropathy		DN				
	11.5 ± 1.99		34.1 ± 2.61		132 ± 14.3		220 ± 22.8				
	P value										
G1/G2		G1/G3		G1/G4		G2/G3		G2/G4		G3/G4	
P< 0.001		P< 0.001		P< 0.001		P< 0.001		P< 0.001		P< 0.001	

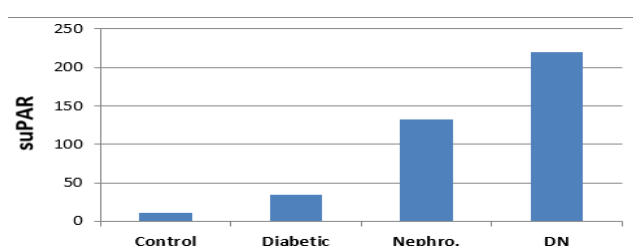


Figure (1): Serum Level of suPAR in studied groups

Table (4) showed that there was no significant

Previous studies agreed with the present study’s findings. Studies have pointed out that increased urine protein excretion is related to prolonged DN, Yang et al. (2021), and Lim et al. (2017).

B. levels of eGFR in studied groups

The Percentage of eGFR in studied groups were illustrated in table (2).

Groups	eGFR (ml/ min/1.73 m ²)		
	≥ 125	90-30	<30
Control (n=30)	100%	0%	0%
DM (n=30)	20%	80%	0%
Nephropathy (n=30)	0%	100%	0%
DN (n=30)	0%	70%	30%

The result of Noor et al. study (2020) agreed with the present study results concerning eGFR. They reported that the patient group with Nephropathy and DN had lower levels of eGFR compared with the diabetic and control groups. As the GFR indicates the amount of blood filtered per minute by the glomeruli. These rates of occurrence can be explained according to the functions of the kidney, it is decreased due to injury or disease, thus filtration rate decreases and waste products begin to accumulate in the blood clinically. Progressive renal decline (eGFR) is associated with the presence of specific pro-inflammatory cytokines[9].

Serum Level of suPAR

The (mean ± SD) suPAR levels in serum of DN, Nephropathy, Diabetic, and control groups were illustrated in tables (3) and figure (1). Results showed that the levels of suPAR in the studied groups there was a highly significant increase of ($p < 0.001$) in the (DN, DM and, nephropathy) groups compared with control group. Also, there were a highly significant increase of ($p < 0.001$) in the (DN and Nephropathy) groups compared with DM group and a highly significant increase of ($p < 0.001$) in DN group compared with Nephropathy group.

difference between female and male in all groups.

Groups	Mean ± SD		P value
	Male (n=12)	Female (n=18)	
Control (n=30)	33.9 ± 4.01	34.1 ± 3.09	$P > 0.05$
DM (n=30)	31.46 ± 3.787	33 ± 12.7	$P > 0.05$
Nephropathy (n=30)	135 ± 12.02	129.39 ± 13.03	$P > 0.05$
DN (n=30)	218.83 ± 18.3	220.19 ± 19.20	$P > 0.05$

Table (5) showed that: there were no significant between A1 and A2 in all groups.

Table (5): Serum Level of suPAR according to Age

Groups	Mean ± SD		P value
	A1 35-50 (n=13)	A2 50-65(n=17)	
Control (n=30)	35.1 ± 4.6	34.33 ± 3.61	P>0.05
DM (n=30)	12.415 ± 4.744	9.914 ± 4.161	P>0.05
Nephropathy (n=30)	130.8 ± 12.74	134 ± 13.9	P>0.05
DN (n=30)	228 ± 22.8	209 ± 19.8	P>0.05

Table (6) and figures (2), (3), (4) shows the criteria of diagnosis validity (sensitivity, specificity, criterion and Accuracy tests) of suPAR level in groups.

The optimal cut-off value for suPAR in all groups patients compared with control estimated from ROC curves, according to these results, test is positive if test ≤ threshold value (criterion values) for diabetes group, while > threshold value for nephropathy and diabetes nephropathy groups. These results indicated that: the variable is closely related to the syndrome more than it is to diabetes.

Table (6): Predictive values of serum suPAR level

Groups	Sensitivity	Specificity	Criterion	AUC
Control/Diabetes	36%	60%	≤ 25.38	0.513
Control/nephropathy	100%	100%	>44	1.000
Control/DN	100%	100%	>41	1.000

Sensitivity = positive in disease, expressed as percent.
Specificity = negative in healthy, or absence of particular disease, expressed as percent.

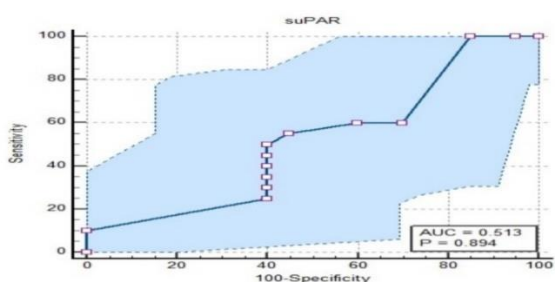


Figure (2): ROC curve for diabetic group

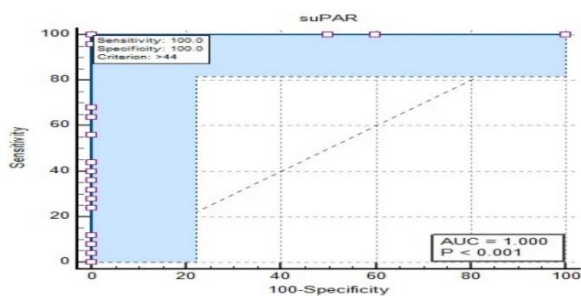


Figure (3): ROC curve for nephropathy group

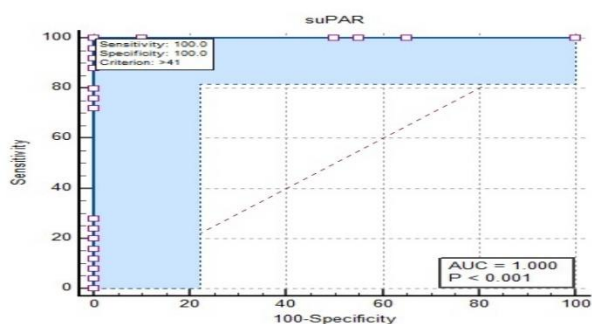


Figure (4): ROC curve for diabetic nephropathy group

Urokinase-type plasminogen activator receptor (uPAR) and its soluble form (suPAR) are new injury biomarkers that have been recently suggested to play a vital role in renal diseases[10]. In healthy tissues, the expression of the membrane-bound uPAR is limited [11].

However, uPAR is strongly expressed by many different cell types during physiologic and pathologic tissue remodelling. For example, it is expressed in osteoclasts during bone resorption and in macrophages, vascular smooth muscle cells and endothelial cells during the formation of the atherosclerotic plaque[12].

Previous studies agreed with the present study's findings, Zhou et al. (2019) study the uPAR expression in Diabetic Kidney Disease (DKD) patients was significantly increased compared to that in healthy controls and was widely colocalized with the podocyte marker, serum suPAR levels gradually increased as DKD progressed to the advanced stage. This increase was more significant in DKD patients with macroproteinuria and decreased renal function, also No significant differences were found regarding sample size, sex, age or smoking status among all groups[10].

Also, Gabriela et al. (2021) study agreed with the present study, increased level of serum suPAR in patients with DKD, the level of suPAR was positively associated with diabetes duration, presence of diabetic retinopathy, serum creatinine level, 24 h proteinuria, ACR, DN class[13]. While, Wlazel et al. (2020) study reported suPAR level is higher in the population aged ≥ 74 years than on one composed of middle-age individuals, and it increases with advancing age[14].

Also Mehta et al. (2020) reported women have higher circulating levels of suPAR, and elevated suPAR is associated with cardiovascular risk and elevated sex-specific plasma suPAR levels are equally predictive of risk of adverse events in both sexes. The independent association of sex with suPAR and the impact of sex on its association with cardiovascular risk are unknown[15].

5. Conclusions

suPAR was raised in case podocytes damage in DN patients. Circulating suPAR gradually increased with DN progression and was associated with proteinuria and eGFR levels.

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