suPAR is a Bad Omen for Chronic Kidney Disease Progression

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Abstract

The rationale for considering serum soluble- urokinase plasminogen activator receptor (s-uPAR) concentration as a potential biomarker for incipient CKD was primarily based on reports that increased levels of this receptor will serve as a circulating permeability variable that may be impaired at the start of FSGS and DN, a crucial explanation for CKD. So in early stages(1-3) of CKD, suPAR was evaluated. The aims of present paper are to investigate whether patients with CKD have distinct circulating suPAR, that could lead to a potential development of noninvasive diagnostic biomarkers of the disease. And finally using healthy subjects as a control group to determine specificity and sensitivity . 135 subjects were incorporated in this study, 30 were healthy control, with mean age 48.7±9.7years, and they were 14 males and 16 females. 105CKD patient , with mean age 50.1±10.0years, they were 54 males and 51 females. Blood samples were collected. Serum after centrifugation was isolated for estimation of suPAR by Enzyme linked immune sorbent assay , creatinine, and urea by enzymatic method . Early morning urine sample was collected to be used for determination albumin creatinine ratio in patient . suPAR level was higher in CKD patients than in healthy control, 6.179±2.221 versus 2.303±0.475, respectively; the difference was highly significant ($P < 0.001$) .To study the potential role of suPAR in diagnosis of patients with CKD, ROC showed that the cutoff value of suPAR was > 3.5 ng / ml fold shift with sensitivity and specificity; the AUC was 0.826 (95 % confidence interval: 0.690-0.920) and therefore 82.6 % precision and significance level of ($P < 0.001$). In conclusion, The suPAR is significantly more effective and also more comprehensive to CKD clinical assessment and can be supported with creatinine blood analysis.

***Key words**: CKD , soluble- urokinase plasminogen activator receptor, creatinine, albumin creatinine ratio*

Introduction

Chronic kidney disease (CKD) is increasingly recognized worldwide as a major concern for public health. It is a leading cause of mortality and morbidity and a major economic burden on health care systems [(1)]. It is difficult to recognize the early stages of CKD since people affected are typically asymptomatic [(2)]. It often stays undiagnosed until kidney function failure is serious [(3)]. CKD is distinguished by a progressive deterioration of kidney function. The most common risk factors of CKD are hypertension, diabetes, and conditions that less commonly induce CKD include glomerulonephritis[(4)]. In medical practice, a strategy of checking kidney disorder is restricted to measure urinary protein secretion and estimate the glomerular filtration rate (eGFR). Proteinuria and reduction in eGFR is enormously insensitive to early infection and has restrained advantage in screening for CKD[(5)]. Therefore, more sensitive biomarkers are wanted to discover at-threat sufferers in advance in the disorder process. So in early stages of CKD , suPAR novel marker was evaluated. SuPAR is the uPAR receptor's soluble form and can be determined in serum/ plasma.. SuPAR is considered a biomarker of inflammation and is high in both acute and chronic diseases. Increased levels of suPAR were related to poor effects in numerous patient populations[(6)]. Further, suPAR has been involved in the kidney disease pathogenesis, particularly diabetic nephropathy and focal segmental glomerulosclerosis, via intervention with migration of podocyte and apoptosis [(6,7)] despite the fact that those findings are nonetheless beneath research, they recommend a likely increase suPAR function in kidney disorder. We examined the hypothesis that levels of serum suPAR are related to early stages (1-3) of CKD.

Material and Method

The study was conducted on 105 Iraqi CKD patients, 52 females and 49 males, The age mean of 55.6 ± SE 1.13randomly selected from those attending the to Baghdad Teaching Hospital /Medical City form between Nov. 2018 to Mar. 2019. A well-structured questionnaire was filled for every subject and patient after full clinical exanimation by their consultant physicians. Patients define by using both GFR and urinary ACR. For the purpose of comparisons, 30 Iraqi control subjects comparable to CKD patients in respect to age (35-59 year) and gender (16 females and 14 males), were included in the study. Blood and urine were collected at the same visit from each subject. Before clinical examination, a permission was taken from the about the following clinical examination and biochemical tests performance.BMI,PB,eGFR. GFR was calculated using the modification of Diet in Renal Disease (MDRD),and ACR estimated from dividing the value of urine microalbumin in (mg/L) to urine Creatinine (mg/dl). Serum after centrifugation was isolated for estimation of suPAR by Enzyme linked immune sorbent assay , creatinine, and urea by enzymatic method .

Result and Discussion

The mean (± SD) values ,rang, and distribution of studied groups (number and %) for the groups , age (in years), gender, body mass index, SBP, DBP ,stages of diseases and duration of the disease in years of the studied groups with their significanc are shown in Table 1 . Primary diagnosis of CKD has been categorised as presented by the UK Renal Registry (UK Renal Registry, 2018) by the main diseases which cause CKD. As shown in table 1 below, The CKD groups had similar etiology of CKD, includes 90 CKD patients; of whom 35 (33.3%) type 2 diabetic patients, 35(33.3%) hypertension patients and 35 (33.3%) GN patients. The mean age for CKD group was (50. 1±10.0) which was comparable to that of healthy control mean (48.7±9.7) years which was statically non-significant (P >0.05), the highest proportion of the CKD patients were found to be in the age group of more than 60 years. For older patients with diabetic , raise BMI , higher systolic and diastolic blood pressure (BP), and higher levels of hemoglobin A1c, the prevalence of CKD is higher.54 of CKD patients were males while the rest were female compared to16 females and 14 males in the healthy controls which statically was not significant (P <0.05), highest proportion of the CKD patients 51.5%were male . The findings matched previous study [8] . The development of CKD may differ depending on sex. Male patients have a significantly higher prevalence of CKD and occurrence of ESRD than female patients [9]. ACR incidence and decreases in eGFR may be higher for men with diabetes than for women with diabetes[10]. Chang et at (2016) concluded that maintaining blood pressure at normal levels may prevent the development of ESRD in both male and female patients[11] .Regarding BMI, the table shows that mean BMI for CKD group was (27.21±3.65) which was comparable to that of healthy control mean (27.02±2.91) years which was statically non-significant (P >0.05). high proportion of patients (41.9%) were overweighed .Patients with CKD groups clearly showed that BMI had no significant difference in comparison with controls, and no significant differences were observed in BMI between the (normal, overweight and obese) ranges as in table 1.

These results are consistent with Tian-Jong Chang etal 2018 The incidence of hypertension and DM in overweight and obese patients with CKD (all stages of CKD) was significantly higher. This clinical correlation may not reflect cause and effect; because obesity as a whole is correlated with multiple adverse sequelae of metabolic syndrome, It is consistent with CKD as well as with comorbidities like DM and hypertension[12]. Obvious variation in means of systolic blood pressure between CKD and controls(127.6±20.1)and (116.3± 7.7)with range (99.9-180) and (100-130) receptivity. Patients subjects showed a significant elevation in diastolic BP Hypertension affects ~30% of the general adult population and up to 90% of those with CKD[13].

Table 1: The mean (± SD) values and Distribution (number and %) for some epidemiological

Epidemiological variables		CKD		Healthy controls		P value
		No	%	No	%	
Groups	Diabetes	35	33.3	-	-	
	Glomerulonephritis	35	33.3	-	-	
	Hypertension	35	33.3	-	-	
Age (years)	<40y	14	13.3	3	10.0	0.572
	40---44	12	11.4	11	36.7	
	45---49	18	17.1	3	10.0	
	50---54	25	23.8	4	13.3	
	55---59	9	8.6	4	13.3	
	=>60y	27	25.7	5	16.7	
	Mean±SD (Range)	50.1±10.0 (35-69)		48.7±9.7 (35-66)		
Gender	Male	54	51.4	14	46.7	0.645
	Female	51	48.6	16	53.3	
BMI (Kg/m2)	Normal (18.5-24.9)	31	29.5	6	20.0	0.051
	Overweight (25-29.9)	44	41.9	20	66.7	
	Obese (=>30)	30	28.6	4	13.3	
	Mean±SD (Range)	27.21±3.65 (20.55-33.50)		27.02±2.91 (20.98-33.5)		
SBP (mmHg)	Mean±SD (Range)	127.6±20.1 (99.9-180)		116.3±7.7 (100-130)		0.003*
DBP (mmHg)	Mean±SD (Range)	84.5±15.1 (60-118)		75.7±6.3 (60-80)		0.002*
Stage	Stage I	11	10.5	-	-	
	Stage II	37	35.2	-	-	
	Stage IIIa	33	31.4	-	-	
	Stage IIIb	24	22.9	-	-	
Duration (years)	1---4	16	15.2	-	-	
	5---9	32	30.5	-	-	
	10---14	21	20.0	-	-	
	=>15y	36	34.3	-	-	
	Mean±SD (Range)	7.4±4.9 (1-16)				
*The Pearson Chi-square test is significant at the 0.05 level of significance for two means						
* Students-t-test test is significant at the 0.05 level of significance for two means						

Concerning the stages and duration of CKD, table 1 shows that mean ±SD was7.4±4.9 with range from 1 to16, highest proportions of CKD cases were mainly in stage 3 and duration more than 15years . Elaine et al (2018) noted that the majority of patients spend a considerable amount of time in stage 3a of CKD, the presence of specific risk factors such as proteinuria diabetes, and uncontrolled systolic BP, significantly Reduces the time invested at this stage. Aggressive management of such risk factors in stage 3a of CKD could be correlated with significant absolute gain (in years), and identifying patients at risk of progression at this early stage of CKD can encourage more concentrated efforts to prevent progression of CKD (For example, by avoiding non-steroidal anti-inflammatory drugs or iodine contrast), even though it's early and mild [14].

Consider table 2 and a histogram 1 derived from suPAR concentration values representing various early stages of the CKD group. Within this distribution a suPAR levels of 5.570 is associated with the 50th percentile. In other words, 50% of the measured suPAR values are less than 5.570 as shown that the median is a strong estimate of the central direction while 50% of the controls fall below the level of 2.530 of suPAR. Moreover the percentile means below the level of 11.210 in CKD are 99% of sample fall while 99% of the controls fall below the level of 2.855 of suPAR. Serum suPAR level ranged between (1.210-2.855) and (3.450-12.340) ng/ml in healthy controls and CKD cases respectively, statistically significant differences were found between mean serum suPAR level of CKD patients and healthy controls. Biomarkers are still scarce for risk assessment in early stages of CKD patients. Recently, suPAR has been correlated with deteriorating renal function, the

risk of ESRD progression. Besides its function in activating αV β3 in podocytes, suPAR can mediate renal damage in several molecular pathways. Blocking β3 integrin with the use of a monoclonal antibody was protective in animal models of diabetic kidney disease. Therefore, suPAR can associate with several other molecules to cause podocyte dysfunction and mediate CKD progression under a wide range of conditions[15] .

Table 2: Assessment mean ± SD of suPARfor CKD and healthy groups

suPAR (ng/ml)	CKD	Healthy controls
Mean±SD	6.179±2.221	2.303±0.475
Standard Error of Mean	0.217	0.087
Range	3.450-12.340	1.210-2.855
Percentile 05th	3.585	1.210
25th	4.520	2.110
50th (Median)	5.570	2.530
75th	7.800	2.660
95th	10.500	2.810
99th	11.210	2.855
P value (Compared to healthy control)	0.0001*	

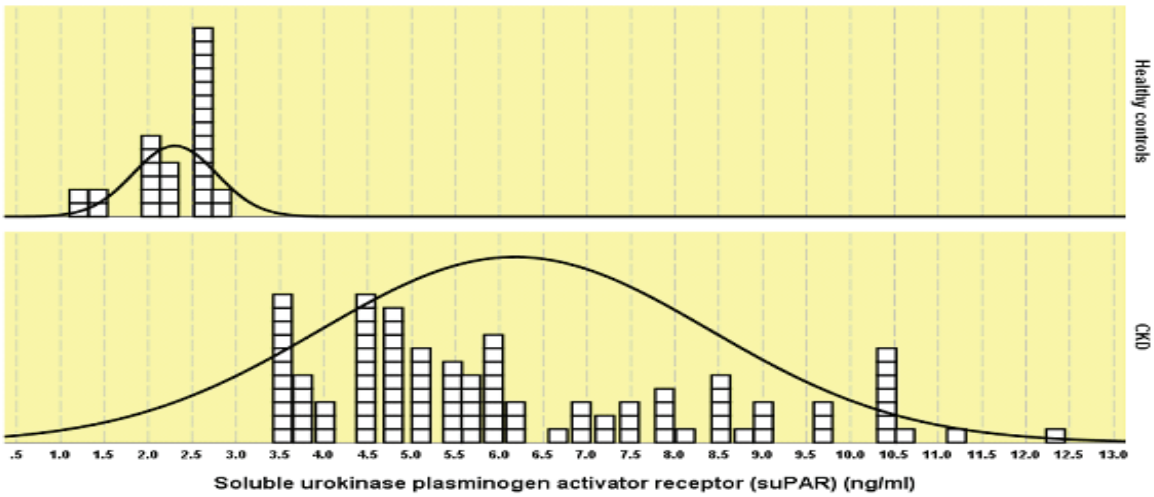

Figure 1.: Distribution of suPAR in the CKD and control groups

Table 3 shows that the mean suPAR was higher in patients with age from (55-59) years, in males compared to females. Result was agreement with recently study. Schulz et al (2017) who stated that higher baseline levels of suPAR are linked with increased incidence of CKD and hospitalization as a result of impaired kidney function in the middle-aged cohort[16]. Regarding the anthropometric measurements, mean was found to be higher in those with overweight and centrally obese. Existing evidence suggests that suPAR in the obese patients may be correlated with macrophage accumulation in adipose tissue and is strongly influenced by adiposity [17] Also in the present stuty the mean suPAR level was higher in obese individuals than the overweight and lean individuals.

Table 3 shows the mean (± SD) value of serum suPAR level by epidemiological variables in CKD and healthy control groups.

Variable		**suPAR (ng/ml)**			
	CKD		**Healthy controls**		
	No	**Mean±SD**	**No**	**Mean±SD**	
Age (years)	<40y	14	5.417±1.555	3	1.885±0.394
	40---44	25	4.984±1.044	11	2.296±0.562
	45---49	18	5.442±1.598	3	2.695±0.020
	50---54	12	7.136±2.296	4	2.639±0.026
	55---59	9	8.238±2.075	4	2.389±0.206
	=>60y	27	7.061±2.743	5	1.998±0.504
	P value		0.0001*		0.113
Gender	Male	54	6.657±2.397	14	2.275±0.557
	Female	51	5.673±1.913	16	2.328±0.408
	P value		0.023*		0.766
BMI (Kg/m2)	Normal (18.5-24.9)	31	4.681±1.289	6	2.136±0.709
	Overweight (25-29.9)	44	6.787±2.066	20	2.338±0.436
	Obese (=>30)	30	6.836±2.496	4	2.380±0.259
	P value		0.0001*		0.636
Stage	Stage I(GFR ≥90) ACR= 10.1	11	4.980±1.873	-	
	Stage II (GFR= 60-89 ACR= 32.98	37	5.808±1.513	-	
	Stage IIIa (GFR= 45-59) ACR= 294.2	33	6.225±2.558	-	
	Stage IIIb(GFR= 30-44) ACR=593.5	24	7.239±2.450	-	
	P value		0.019*		
Duration (years)	1---4	36	5.132±1.323	-	
	5---9	32	6.698±2.438	-	
	10---14	21	6.657±2.476	-	
	=>15y	16	6.870±2.369	-	
	P value		0.005*		

*ANOVA test is significant at the 0.05 level of significance for difference of more than two means

*The Pearson Chi-square test is significant at the 0.05 level of significance for two means

Cancello *et al* found that the total amount of suPAR protein was significantly higher in obese individuals, compared to lean controls. suPAR was significantly more expressed in white adipose tissue of obese individuals, compared to lean controls[17].

The mean suPAR concentrations were determined for all the different stages after classifying patients according to the proposed KDIGO classification of CKD. As shown in table 3 the suPAR concentrations increased significantly with increased albuminuria. ($p< 0.005$). Interestingly, a total of 11 of the patients had no signs of kidney damage (i.e., eGFR >90 ml/min/1.73m2 and albuminuria <30 mg/g creatinine) together with suPAR levels > 3.5 ng/ml

In this research, the consequence was an inverse relation of the suPAR level with the eGFR. Previous research has shown an inverse correlation between suPAR and eGFR; [18] consequently, the present observation is aligned with the Wei et al results, in their analysis of two large populations, found inverse relationship between eGFR and suPAR[19]. Because the major suPAR fragment's molecular weight is 22kDa, which should be sufficiently low to cross through the glomerular filtration barrier, the correlation of suPAR levels with kidney function inversely seems reasonable. While a kinetic analysis will be needed to verify this hypothesis, suPAR is likely to accumulate in patients with renal disease[20].

ROC analysis allowed the estimation of the utility of suPAR as the indicator of the patient's progression. A suPAR concentration equivalent to 3.5 ng / mL has been identified as the best fit cutoff value with a sensitivity of 93 % but a precision of 91.5 % ($p=0.001$).That means, the test value higher than 3.5 ng/ml represents the abnormal case (CKD), whereas the value is less than 3.5 ng/ml consider healthy condition as shown in Figure2. ROC analysis further demonstrated that suPAR was a sensitive indicator for the stage of CKD, suggesting that serum suPAR could be a valuable biomarker for early diagnosis of CKD:

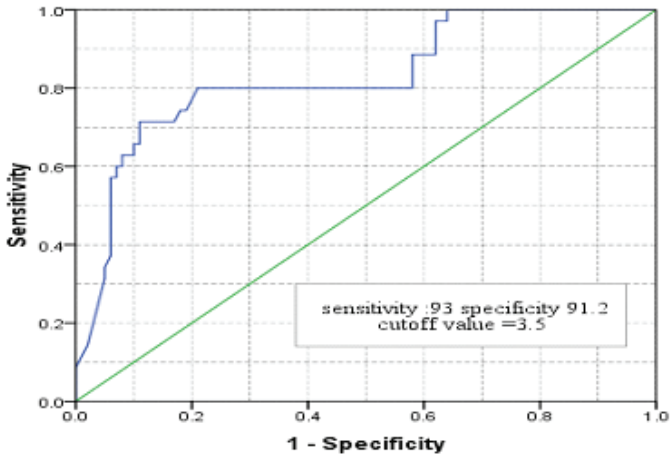

Figure 2: ROC curve showing the trade-off between sensitivity (rate of true positive) and rate of false positive (1-specificity) for fasting serum suPAR concentration when used to predict cases with CKD differentiating them from healthy controls.

The comparative ROC analysis is presented in Fig 3 , we took the area under the curve as a measure of assay efficiency, i.e., 0.89($P <0.001$), for the suPAR assay and 0.68($P=0.032$) for S.Cr assay in the case when the two assays used to predict cases with CKD differentiating them from healthy control, by this measure, the suPAR assays was clearly more valid than S.Cr, the difference statistically significant between them correspondingly when the two assays used to predict CKD progression , from the area, the suPAR assays was valid and efficient and the suPAR area seems to be slightly bigger than S.Cr.

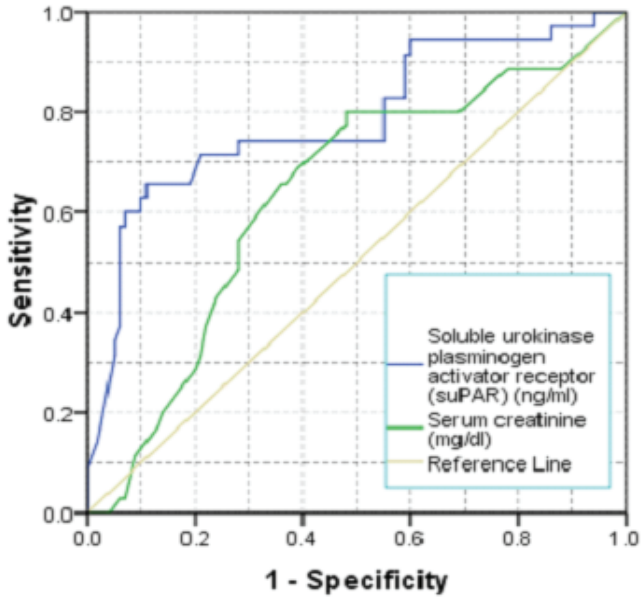

Figure 3:ROC curve showing the trade-off between sensitivity (rate of true positive) and rate of false positive (1-specificity) for fasting serum(suPAR, and SCr) concentration when used to predict cases with CKD differentiating them from healthy controls.

Conclusion

The suPAR is significantly more effective and also more comprehensive to CKD clinical assessment and can be supported with creatinine blood analysis.

Conflict of Interest: Nill

Source of Funding- Self

Ethical Clearance – This study was conducted with the consent of the volunteers and without mentioning the names with the complete privacy of volunteers

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