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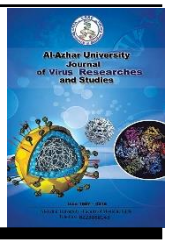

Relation of Serum Soluble Suppression of Tumorigenicity 2 with Cardiovascular Complications in Chronic Kidney Disease Patients

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Abstract

Chronic kidney disease (CKD) is defined as a reduced glomerular filtration rate, increased urinary albumin excretion, or both, and is an increasing public health issue. Prevalence is estimated to be 8–16% worldwide. The aim is to study the correlation between the level of soluble Suppression of Tumorigenicity 2 (sST2) in patients with chronic kidney disease and cardiovascular (CV) complication in these patients. This study was carried out on 89 patients having CKD aged above 18 years, of both sexes. Patients were collected from Al Zahraa University Hospital Internal Medicine Department in the period from January 2021 to June 2021. They were divided into: Group one (30 CKD patients) having cardiovascular complications, Group two (59 CKD patients) without cardiovascular complications. Their analyses were done at Al Zahraa University Hospital, Clinical Pathology Department. The present study showed that Serum sST2 was significantly increased in CKD patients with cardiovascular complications represented by decreased LVEF%. Also, this biomarker is independent of eGFR, age, sex, creatinine, BUN, uric acid, HbA1C, cholesterol, triglycerides, LDL and HDL. The present study proved that sST2 is a promising new biomarker to detect cardiac affection in chronic kidney disease patients and this biomarker is independent of eGFR and age.

Keywords: Serum Soluble Suppression, Tumorigenicity, Cardiovascular Complications, Chronic Kidney Disease

1. Introduction

Chronic kidney disease (CKD) is a type of kidney disease in which there is gradual loss of kidney function over period of time (months or years) and affects about 11.5% of the overall population with increasing age-dependent prevalence of up to 47% in persons older than 70 years. Apart from old age, CKD is associated with diabetes mellitus and hypertension. Due to an increase of these precipitating and often causative diseases, the prevalence of CKD is expected to rise even further in the future Mirna et al. [1].

The majority of patients with CKD are at risk of accelerated cardiovascular disease and death. CKD complications such life and metabolic acidosis and secondary hyperparathyroidism affect cardiovascular

health and quality of life and require diagnosis and treatment***. Because*** of shared risk factors and the fact that CKD constitutes an independent risk factor itself, CKD and cardiovascular disease (CVD) often occur concomitantly. Also, heart failure is the most common complication of CKD Plawecki et al. [2].

Hence, biomarkers established in the evaluation of patients with CVD are increasingly used in patients with decreased renal function. Unfortunately, some of the most common biomarkers in this field, such as troponin or brain natriuretic peptide (BNP), are chronically elevated in patients with CKD, which may in part be due to impaired renal clearance Mirna et al. [1].

Therefore, their clinical applicability in patients with CKD is limited and hence, novel biomarkers are warranted to improve diagnosis and risk stratification in these disease entities. In the following study, serum concentrations of a novel cardiovascular biomarker, the soluble suppression of tumorigenicity 2 was investigated in patients with various stages of CKD is Mirna et al. [1]. The protein ST is an alternative name to protein interleukin-1 receptor-like 1 (IL-1RL1). It is a member of the interleukin (IL)-1 receptor family with ST2-ligand (ST2L) and soluble (sST2) is oforms Pascual-Figal [3].

sST2 is the circulating form of the cellular receptor ST2L, expressed by cardiomyocytes and vascular endothelial cells together with its ligand interleukin-33(IL-33) after cardiovascular injury. Binding between IL-33 and ST2L is likely to inhibit myocardial hypertrophy and fibrosis and mitigate adverse cardiac remodeling. sST2 competes with ST2L for IL-33 binding, thus likely blunting the cardiovascular protective effects exerted by the IL-33/ST2L interaction Aimo et al. [4]. The level of sST2 does not appear to be significantly affected by age, sex, BMI, etiology of heart failure, atrial fibrillation and anemia. Unlike almost any cardiac biomarker in use, sST2 does not appear to be significantly affected by renal function. The fact that sST2 has the lowest intra-individual variation and smallest relative change value compared to other biomarkers makes it suitable for accurate serial measurements Maisel [5]. The aim is to study the correlation between levels of sST2 in patients with chronic kidney disease and cardiovascular complication in these patients.

2. Patients and Methods

In this cross-sectional study 89 patients having chronic kidney disease aged above 18 years, of both sexes. Patients were collected from Al Zahraa University Hospital Internal Medicine Department in the period from January 2021 to June 2021. They were divided into: Group one (30 CKD patients) having cardiovascular complications, Group two (59 CKD patients) without cardiovascular complications. Their analyses were done at Al Zahraa University Hospital, Clinical Pathology Department.

2.1 Inclusion Criteria

Adult CKD patients were diagnosed and categorized according to the National Kidney Foundation Disease Outcomes Quality Initiative (NKF-K/DOQI) clinical practice guidelines.

2.2 Exclusion Criteria

- Patients with GFR > 60 ml /min
- Age below 18 years
- History of kidney transplantation.

Informed consents were obtained from all subjects and the study protocol was approved by the Research Ethics Committee of Faculty of Medicine for Girls Al-Azhar University.

2.3. All the participants were subjected to the following

- Careful medical history and clinical examination.
- Radiological investigation: Echocardiography.

2.4 Measurement of serum sST2 level

The concentration of sST2 in the serum was analyzed according to the manufacturer's instructions by ELISA technique; with a complete set of ELISA reader model SLT Spectra 216687, Human sST2 ELISA Kit (Catalog No: E-EL-H6082), Size: 96T. supplied by Ela science Technology Laboratory. China. Detection Range: 0.31-20ng/ml.

2.5 Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 24. Quantitative data were expressed as mean± standard deviation (SD) (for normal distributed data) and Median (IQR) (for abnormal distributed data). Qualitative data were expressed as frequency and percentage. P-value < 0.05 was considered significant, P-value < 0.001 was considered as highly significant, P-value > 0.05 was considered insignificant.

3 .Results

In this study 89 CKD patients of both sexes with CV complications and without CV complication are compared to each other in relation to serum levels of sST2. Table.1 shows the description of age and sex in all studied patients. As regard age, the mean age of all studied patients was 62.7±9 years with minimum age of 44 years and maximum age of 82 years. As regard sex, there were 47 males (52.8%) and 42 females (47.2%) in the studied patients. Table.2 shows no statistically significant difference (p-value > 0.05) between studied patients with cardiovascular complications and patients without cardiovascular complications as regard age and sex. Table.3 shows highly statistically significant difference (p-value < 0.001) between studied patients with cardiovascular complications and patients without cardiovascular complications as regard systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Table (1): Description of age and sex in all studied patients.

		Studied patients. (N = 89)	
Age(years)	**Mean ±SD**	62.7± 9	
	Min – Max	44 – 82	
Sex	**Male**	47	52.8%
	Female	42	47.2%

Table (2): Relation between cardiovascular complications and demographic data.

		CV complications				Stat. test	P-value
		Yes (N = 30)		No (N = 59)			
Age(years)	**Mean**	63.7		62.2		T = 0.73	0.467 NS
	±SD	9.2		8.9			
Sex	**Male**	14	46.7%	33	55.9%	X^2 = 0.68	0.408 NS
	Female	16	53.3%	26	44.1%		

T: independent sample T test. NS: p-value > 0.05 is considered non-significant. X^2: Chi-square test.

Table (3): Relation between cardiovascular complications and blood pressure.

		CV complications		Stat. test	P-value
		Yes (N = 30)	No (N = 59)		
SBP (mmHg)	Mean	152.9	128.1	T = 11.8	<0.001HS
	±SD	14.4	5.3		
DBP (mmHg)	Mean	93.2	86.8	T = 4.08	<0.001HS
	±SD	5.5	7.5		

Table.4 shows no statistically significant difference (p-value > 0.05) between studied patients with cardiovascular complications and patients without cardiovascular complications as regard Creatinine, BUN, Uric acid, eGFR, Cholesterol, Triglyceride, LDL and HDL; highly statistically significant difference (p-value < 0.001) between studied patients with cardiovascular complications and patients without cardiovascular complications as regard sST2. Table.5 shows highly statistically significant difference **(p-value < 0.001)** between studied patients with cardiovascular complications and patients without cardiovascular complications as regard left ventricular ejection fraction % (LVEF%).

Table (4): Relation between cardiovascular complications and studied laboratory data.

		CV complications		Stat. test	P-value
		Yes (N = 30)	No (N = 59)		
Creatinine (mg/dl)	Median	8.2	8	MW = 869	0.890 NS
	IQR	6.8 - 9.8	6.8 - 10.2		
BUN (mg/dl)	Median	62.5	56	MW = 713.5	0.136 NS
	IQR	50 - 81.5	51 – 70		
Uric acid(mg/dl)	Median	6.35	5.8	MW = 749.5	0.239 NS
	IQR	5.5 - 7.05	5.2 - 6.8		
HbA1C (%)	Median	6.65	6.6	MW = 835	0.667 NS
	IQR	6 - 7.5	5.7 - 7.9		
eGFR (ml/ min/1.73 m2)	Median	7	7	MW = 754	0.251 NS
	IQR	5 - 9	6 - 9		
Cholesterol(mg/dl)	Mean	180.8	184.9	T = 0.33	0.735 NS
	±SD	44.4	57.5		
Triglyceride(mg/dl)	Median	130.5	122	MW = 833.5	0.655 NS
	IQR	94.8 - 176.3	91 - 159		
LDL (mg/dl)	Median	103	101.5	MW = 861.5	0.838 NS
	IQR	74 – 149	83 – 124.5		
HDL (mg/dl)	Mean	45.6	47.8	T = 0.81	0.417 NS
	±SD	9.9	15.6		
sST2(ng/ml)	Median	26.8	3.11	MW = 0.0	< 0.001 HS
	IQR	24.6 - 28.3	0.93 - 4.5		

T: independent sample T test. NS: p-value > 0.05 is considered non-significant.
MW: Mann Whitney U test. NS: p-value > 0.05 is considered non-significant.

Table (5): Relation between cardiovascular complications and LVEF%.

		CV complications		Stat. test	P-value
		Yes (N = 30)	No (N = 59)		
LVEF (%)	Median	34	71	MW = 0.0	<0.001HS
	IQR	32 - 35.3	67 – 72		

Table (6): Correlation study between sST2 and other studied data in all studied groups.

Variables	With CV Complications (n = 30)		Without CV Complications (n = 59)	
	r	p-value	R	p-value
sST2 vs age	-0.103	0.587 NS	-0.057	0.67 NS
sST2 vs Creatinine	0.059	0.758 NS	0.011	0.936 NS
sST2 vs BUN	0.145	0.445 NS	0.153	0.246 NS
sST2 vs Uric acid	-0.183	0.334 NS	-0.003	0.983 NS
sST2 vs HbA1C	-0.019	0.921 NS	-0.092	0.487 NS
sST2 vs eGFR	-0.032	0.868 NS	-0.046	0.731 NS
sST2 vs Cholesterol	0.297	0.111 NS	0.109	0.409 NS
sST2 vs Triglyceride	0.001	0.996 NS	-0.023	0.863 NS
sST2 vs LDL	0.28	0.124 NS	0.09	0.497 NS
sST2 vs HDL	0.311	0.095 NS	0.134	0.311 NS
sST2 vs LVEF	**-0.825**	**< 0.001 HS**	**-0.707**	**< 0.001 HS**

(r): Pearson correlation coefficient. HS: p-value < 0.001 is considered highly significant. NS: p-value > 0.05 is considered non-significant.

In patients with CV complications there were highly statistically significant (p-value < 0.001) Negative correlation (r = -0.825) between sST2 and LVEF%, not statistically significant (p-value > 0.05) correlation between sST2 and other studied data. In patients without CV complications there were highly statistically significant (p-value < 0.001) Negative correlation (r = - 0.707) between sST2 and LVEF%, not statistically significant (p-value > 0.05) correlation between sST2 and other studied data. Using roc curve, it was shown that serum sST2 can be used to discriminate between patients with CV complications and patients without CV complications at a cutoff level of > 13.23, with 100% sensitivity,100% specificity, 100% PPV and 100% NPV **(AUC = 1.0& p-value < 0.001)**. Table.8 shows highly statistically significant (p-value < 0.001) correlation between sST2 and blood pressure of studied patients. It was higher in hypertensive patients (26.4 ± 2.4) than normal patients (4.5 ± 6.2), not statistically significant (p-value > 0.05) correlation between sST2 and HbA1C in studied patients. It was 9.93 ± 11.4 in normal patients, 11.2 ± 11.3 in pre-diabetic patients and 10.7 ± 11.4 in diabetic patients.

Table (7): Diagnostic performance of serum sST2 in discrimination of patients with CV complications and patients without CV complications.

Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
> 13.23	1.0	100 %	100 %	100 %	100 %	<0.001

PPV: positive predictive value. AUC: Area under curve. NPV: negative predictive value.

Table (8): Correlation between sST2 and (HbA1C & blood pressure).

		N		sST2	Test	P-value
HbA1C	**Normal**	19	21.3%	9.93± 11.4	KW = 1.37	0.503 NS
	Pre-diabetes	21	23.6%	11.2 ± 11.3		
	Diabetes	49	55.1%	10.7± 11.4		
Blood pressure	**Normal**	64	71.9%	4.5± 6.2	**MW = 42**	**< 0.001 HS**
	Hypertensive	25	28.1%	26.4± 2.4		

4. Discussion

Chronic Kidney disease is a great health problem, being one of the most leading causes of death worldwide. Individuals with CKD are more liable to premature death rather than progressing to end stage renal disease (ESRD). This increased risk of death is mainly attributed to developing cardio -vascular disease as heart fatigue, myocardial infarction or sudden cardiac arrest and stroke. However, biomarkers of prognosis of life-threatening effects in CKD are not yet satisfactory as they may be chronically elevated with CKD. Therefore, we aimed to study serum sST2 as a biomarker for CVD in CKD patients. sST2 is known to be a biomarker of CVD in CKD patients independent of renal function, age and dialysis process, unlike other cardiac markers Guo et al., [6]. This cross-sectional study was carried out on 89 patients with chronic kidney disease of both sexes aged above 18 years. Their age range was 44 – 82 years with a mean of 62.7±9. The patients were 47 males (52.8%) and 42 females (47.2%).

On grouping patients according to the presence or absence of cardiovascular complications, there was no significant difference ($p>0.05$) between the two groups as regards to age or sex.

This was in accordance with Shajahan et al. [7] who made a meta- analysis study and concluded that the appropriate risk awareness of cardiovascular complications is necessary for both sexes.

Regarding renal function, all our patients were stage 5 CKD (eGFR<15) undergoing hemodialysis. Comparing both groups of patients with CV complications and those without CV complications, there was no statistically significant difference between them as regards to creatinine, BUN, Uric acid, and eGFR.

We observed that 55% of our patients were diabetic (HbA_{1c}>6.5%), 23.5% were prediabetic (HbA_{1c} 5.7 -6.4 %) and 21.5 % were nondiabetic (HbA_{1c}<5.7%). Hernandez et al. [8] observed a strong and linear cross-sectional association between HbA_{1c} concentration and CKD and CVD. HbA_{1c} undergoes intracellular glycoxidation and peroxidation to form advanced glycated end products which are implicated in activation and progression of atherosclerosis. Changes in glycemia may lead to activation of some growth factors contributing to the development of intraglomerular hypertension and renal injury. Both of these mechanisms explain the relationship between HbA_{1c} and microvascular and macrovascular complications even in a person with prediabetic state.

Li et al. [9] conducted a study on 3641 patients that investigated the prognostic value of the sST2 in patients with established coronary artery disease (CAD) and its predictive value in CAD patients with and without T2DM. They found that sST2 was increased in patients with diabetes versus those without T2DM, yet still sST2 was significantly associated with cardiovascular event (CVE) in patients with and without T2DM.

Concentrations of sST2 increased under myocardial overload and were found to be related to adverse left ventricular (LV) remodeling and cardiovascular outcomes Huang et al., [10]. We observed that 28.1% of our patients were hypertensive (systolic blood pressure >140 mmHg, or diastolic blood pressure >90 mmHg), there was significant difference (p-value < 0.001) correlation between sST2 and blood pressure of studied patients and was higher in hypertensive patients. Similarly, Huang et al., [10] mentioned that concentrations of sST2 increased under myocardial overload and were found to be related to adverse LV remodeling and cardiovascular outcomes. They conducted study on 186 patients that found the relationship between sST2 and cardiovascular outcomes in patients with ST-segment elevation myocardial infarction (STEMI) and reported that sST2 is correlated to both LV ejection fraction

(P = 0.002) and blood pressure (P = 0.009). In opposition to our results Plawecki et al. [2] had a study on 218 CKD patients and they found no correlation between sST2 and both systolic blood pressure (p = 0.209) and diastolic blood pressure (p = 0.167). The prevalence of CV complications in patients with CKD is very high especially in the presence of risk factors such as diabetes, hypertension and dyslipidemia. Patients with CV complications had higher triglycerides, elevated LDL-C and lower HDL-C than those patients without CV complications, but statistically insignificant. Some clinical evidence suggests that vascular effects of HDL may vary in different conditions and that progressive kidney dysfunction causes dramatic changes in the composition and quality of HDL and triglycerides – rich lipoproteins leading to atheromatous changes Zewinger et al., [11]. Similar to our results, Parikh et al. [12] conducted a study on age and sex matched patients for the prevalence of CVD in CKD patients. They reported that CKD patients having CV complications had low levels of HDL-C, high triglycerides level, and elevated LDL-C. ST2 is expressed by cardiac myocytes in two forms: soluble subtype (sST2) and the membrane bound (ST2L). Its ligand IL-33 which is released from cardiac cells to protect myocardium under pressure overload. sST2 acting as a decoy receptor neutralizes the protective effect of IL-33 when bound to it. It is speculated that IL-33/ST axis reduces risk of plaque rupture by inhibiting the immune response in atherosclerosis, an effect that can be antagonized by ST2 Liu et al., [13].

When sST2 was assessed in included patients, there was a significant increase in sST2 levels in patients with CV complications versus those without CV complications (p<0.001). Moreover, there was a statistically significant difference in LVEF% (p<0.001) between the two studied groups. This present study showed no correlation between sST2 and age, sex, renal function and HbA_{1c}. However, sST2 correlated significantly with blood pressure and LVEF%.

Circulating sST2 levels are increased in response to inflammatory diseases and heart diseases Mueller et al., [14]. Inflammation is said to play an important role in the pathogenesis of different cardio-vascular diseases, higher concentrations of sST2 are associated with the progression of diseases. Since sST2 is a soluble decoy receptor of IL-33, it attenuates protective effects of IL-33/ST2L signaling pathway Sunita, [15].

These protective effects are in the form of preventing myocardial maladaptive hypertrophy, reducing cardiac dysfunction and fibrosis and cardiac myocyte apoptosis. As ventricular hypertrophy is almost found in most kinds of heart failure, then sST2 by its action on IL-33/ST2L has a powerful value as a biomarker of heart failure, moreover it has a role in myocardial fibrosis and remodeling, so it is used as a powerful prognostic biomarker in both acute and chronic heart failure Ding et al., [16]. Mirna et al. [1] had results going hand in hand to our results. They studied 5 cardiovascular biomarkers sST2, growth differentiation factor 15 (GDF-15), heart-type fatty acid-binding protein (H-FABP), insulin-like growth factor-binding protein 2 (IGF-BP2) and soluble urokinase plasminogen activator receptor in 219 CKD patients. They concluded that sST2 diagnostic performance is least affected by renal function and suggested its potential viability in management of patients with CVD and concomitant CKD. Similarly, Plawecki et al. [2] conducted a retrospective study on 218 CKD patients. In agreement with our results, they found no correlation between sST2 and both age and eGFR, but it was negatively correlated with LVEF (p=0.04). Mancianti et al. [17] enrolled 40 HD patients in a prospective, observational cohort study. After 12 months 10 of the patients developed CVE predicted by increased sST2 levels (p<0.0001) and was correlated with LVEF (p=0.01).

In opposition to our results, Allam et al. [18] had a study on 561 patients with CKD (eGFR>15 and <90 ml/min/1.73m^2) reported that sST2 significantly correlated with eGFR($p<0.05$).

Recent research and meta-analysis had provided more understanding of the role and value of sST2 in prediction and prognosis of CVD in CKD patients. Current findings of our study suggested that sST2 being not correlated to age, sex, renal function and hemodialysis process is a powerful biomarker in predicting CVD patients and concomitant CKD. This exciting area of research deserves further study to find its implication on therapy and reducing the overall mortality of these patients. **Limitations of the study**: First: the cross-sectional design of the study and the absence of matched healthy control did not allow us to clarify the association of sST2 and CKD and CVD. Second: there is no consensus among investigators on the criteria for the diagnosis of CVD in CKD. Third: we did not follow up patients to know the outcome and detect value of sST2 in survivors and non survivors.

5. Conclusion

- Serum sST2 was significantly increased in CKD patients with cardiovascular events represented by hypertension and decreased LVEF%.
- This biomarker was independent of age, sex, renal function and HbA1c.

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