

Salivary Cystatin C as a Biochemical Marker for Chronic Renal Failure

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ABSTRACT

A case control study was conducted on 49 subjects 29 of them with chronic renal disease and 20 subjects as control with age range between (45-75) years were investigated to determine the correlation of salivary and serum cystatin, urea and creatinine to assess their significance in the diagnoses of patients with chronic renal disease. The results indicated that the level of salivary cystatin was significantly ($P \leq 0.05$) elevated in chronic renal disease (292.517 ± 37.19 ng/ml) as compared to control (144.45 ± 22.963 ng/ml). The level of serum cystatin was significantly ($P \leq 0.05$) elevated in chronic renal disease (498.414 ± 59.091 ng/ml) as compared to control (391.60 ± 49.488 ng/ml). The level of serum creatinine was significantly ($P \leq 0.05$) elevated in chronic renal disease (4.369 ± 0.799 mg/dl) as compared to control (1.007 ± 0.180 mg/dl). The level of urea was significantly ($P \leq 0.05$) elevated in chronic renal disease (56.138 ± 5.908 mg/dl) as compared to control (31.80 ± 5.176 mg/dl). From all the above results, we can conclude that salivary cystatin can be considered as a better biochemical marker for renal function compared to serum cystatin in chronic renal disease.

Keywords: biochemical marker, Salivary Cystatin, Chronic renal disease, Creatinine, Blood urea

INTRODUCTION

Cystatin C is a low molecular weight of (13.3 kDa) basic protein, it is an endogenous marker of the function of kidney [1, 2]. It is a member of the super family of inhibitors for cysteine proteinase, produced by all nucleated cells [2]. Because it has low molecular weight, it is filtered freely by the glomeruli and almost reabsorbed completely and catabolized in the tubules, without suffering tubular secretion, that make it more sensitive and accurate than creatinine [3]. Cystatin C is found in many biological fluids, such as plasma, serum, tears, synovial fluid, amniotic fluid, bile, urine, cerebrospinal fluid (with high concentration), milk (with low concentration), and also in saliva [4].

Saliva is a filtrated from the blood where various molecules pass through paracellular routes (extracellular ultrafiltration) or transcellular (passive intracellular diffusion and active transport) into saliva. As a result, saliva may represent equivalent to serum, thereby reflecting the physiological body state [5]. Thus, It has been proposed, to be a good source, for diagnostic purposes, Several chronic diseases, cardiovascular diseases, cancer, renal diseases (especially Chronic Kidney Disease-CKD) can be diagnosed by saliva [6, 7].

Chronic Kidney Disease is a reduction in renal function progressively [8]. It is a condition where the renal tissues lose their normal function, especially regulatory and excretory functions [9]. CKD is progressing towards becoming a major problem for health [10]. Renal diseases contribute a common component to mortality and morbidity [8]. It has been considered a problem that needs an early detection, evaluation, and preventive precaution to delay the progression and to prevent unwanted effects [9].

For glomerular filtration Assessment, cystatin C concentration can be considered an excellent correlate of the glomerular filtration level that is not influenced significantly by other effects (diet, liver function, infections, myopathies, malignancies, body fat content). An indicator for filtration of glomeruli used is creatinine, in practice is only estimate roughly the glomerular filtration because it reflects changes with low specificity and sensitivity. Its value depends on muscular mass and thus it depends on tubular secretion sex and age. It has been demonstrated that cystatin C level increases even with creatinine clearance decrease below $1.57 \text{ ml}\cdot\text{s}^{-1}$ when the creatinine level has not yet changed [11].

No data in the literature were available about the salivary cystatin C in patients with CKD, thus that this study aimed to evaluate the salivary cystatin C as biochemical markers in CKD patients with varying stages of disease.

MATERIAL AND METHODS

Study Design

A case control study was conducted on (49) persons attended to Samarra General Hospital between the periods from the first of October 2017 to the end of January 2018, their ages ranged between 38- 75 years.

Study subjects classified according to clinical and laboratory findings into the following groups:

A. Group I (C): (20) subjects who were apparently healthy as a control group.

B. Group II (P): (29) subjects who were diagnosed as patients with chronic renal disease.

Sample Collection

Blood and saliva had been collected from subjects attended to Samarra General Hospital after an overnight fasting in plain tube in the absence of any anticoagulants, and serum had been harvested by allowing the sample to clot within 30 minutes then centrifugation for 10 minutes at 3000 rpm, the sera supernatant of blood and saliva were aliquoted and stored at -20 until assayed.

Exclusions Criteria

Patients with acute or chronic illnesses apart from patients with chronic renal disease have been excluded from the study. Smokers are also excluded from this study.

Methods

Serum and salivary Cystatin, urea and creatinine were determined by using ELISA Kit for Cystatin and enzymatic colorimetric methods for urea, provided from Elabscience (China), Biolabo (France) and Randox (U.K) respectively.

Statistical Analysis

Data was translated into codes using a specially designed coding sheet, and then converted to a computerized database. An expert statistical advice was sought and statistical analyses (correlation and T-test) were done using SPSS (Statistical Package for Social Science) version 22. P value less than 0.05 level of significance was considered significant.

RESULTS

During this study period, a total of 49 subjects with age range between (45-75) years were investigated to determine the correlation of some salivary and blood biomarkers to assess their significance in the diagnoses of patients with chronic renal disease.

Comparative Study

Salivary and serum cystatin

The level of salivary cystatin was significantly ($P \leq 0.05$) elevated in chronic renal disease ($292.517 \pm 37.19 \text{ ng/ml}$) as compared with control group ($144.45 \pm 22.963 \text{ ng/ml}$), while the level of serum cystatin was significantly ($P \leq 0.05$) elevated in chronic renal disease ($498.414 \pm 59.091 \text{ ng/ml}$) as compared with control group ($391.60 \pm 49.488 \text{ ng/ml}$) as shown in the [Table 1](#).

Table 1. Mean \pm SD of salivary and serum cystatin between control and chronic renal disease

Parameters	Mean \pm SD	
	Salivary cystatin (ng/ml)	Serum cystatin (ng/ml)
Control (n=20)	144.45 \pm 22.963	391.60 \pm 49.488
Patients (n=29)	292.517 \pm 37.19	498.414 \pm 59.091
P- value	0.0001	0.0001

Table 2. Mean \pm SD of serum creatinine and blood urea between control and chronic renal disease

Parameters	Mean \pm SD	
	Serum creatinine (mg/dl)	Blood urea (mg/dl)
Control (n=20)	1.007 \pm 0.180	31.80 \pm 5.176
Patients (n=29)	4.369 \pm 0.799	56.138 \pm 5.908
P- value	0.0001	0.0001

Table 3. Correlation between serum cystatin, salivary cystatin, serum creatinine and blood urea within control group

		Blood urea	Serum creatinine	Serum cystatin	Salivary cystatin
Blood urea	Pearson Correlation	1	.238	.568**	.491*
	Sig. (2-tailed)		.313	.009	.028
	N	20	20	20	20
Serum creatinine	Pearson Correlation	.238	1	.451*	.595**
	Sig. (2-tailed)	.313		.046	.006
	N	20	20	20	20
Serum cystatin	Pearson Correlation	.568**	.451*	1	.701**
	Sig. (2-tailed)	.009	.046		.001
	N	20	20	20	20
salivary cystatin	Pearson Correlation	.491*	.595**	.701**	1
	Sig. (2-tailed)	.028	.006	.001	
	N	20	20	20	20

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Serum creatinine and Blood urea

The level of serum creatinine was significantly ($P \leq 0.05$) elevated in chronic renal disease (4.369 \pm 0.799 mg/dl) as compared with control group (1.007 \pm 0.180 mg/dl), while the level of blood urea was significantly ($P \leq 0.05$) elevated in chronic renal disease (56.138 \pm 5.908 mg/dl) as compared with control group (31.80 \pm 5.176 mg/dl) as shown in **Table 2**.

Correlation Study

Correlation within control group (C)

A correlation study of serum cystatin, salivary cystatin, serum creatinine and blood urea show a significant positive correlation between all these parameters except between serum creatinine and blood urea which was non-significant within the control group as shown in **Table 3**, **Figures 1, 2, 3, 4, 5** and **6**.

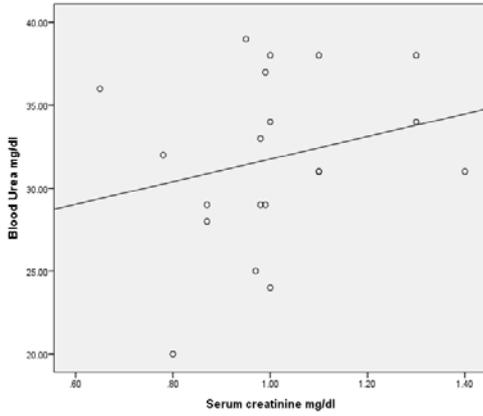


Figure 1. Correlation between blood urea and serum creatinine within control group

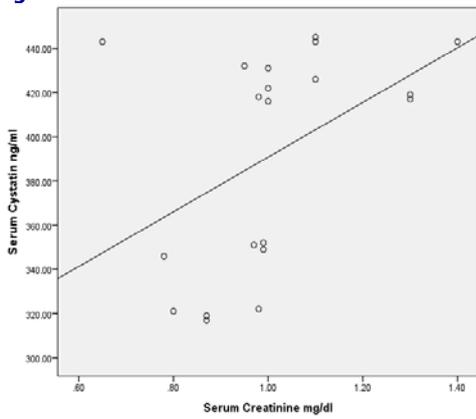


Figure 2. Correlation between serum cystatin and serum creatinine within control group

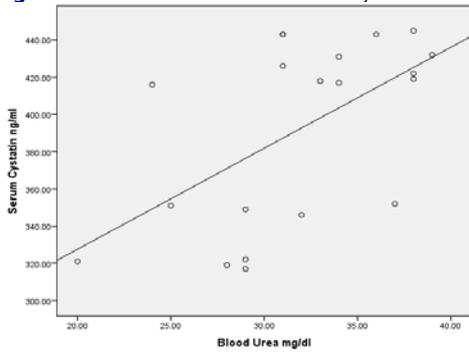


Figure 3. Correlation between serum cystatin and blood urea within control group

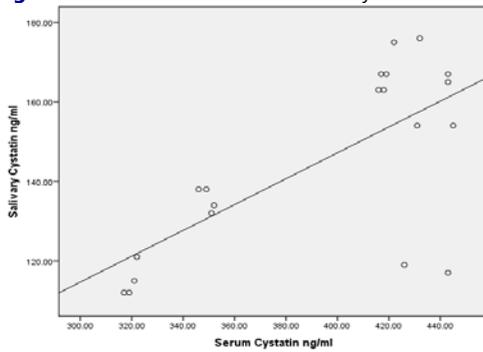


Figure 4. Correlation between salivary cystatin and serum cystatin within control group

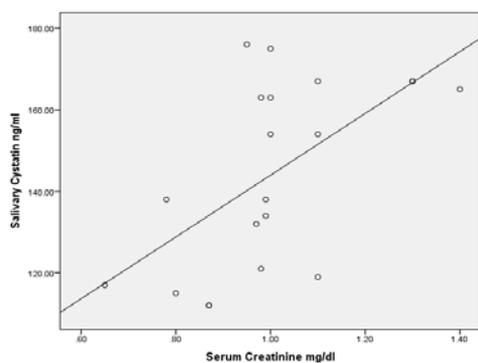


Figure 5. Correlation between salivary cystatin and serum creatinine within control group

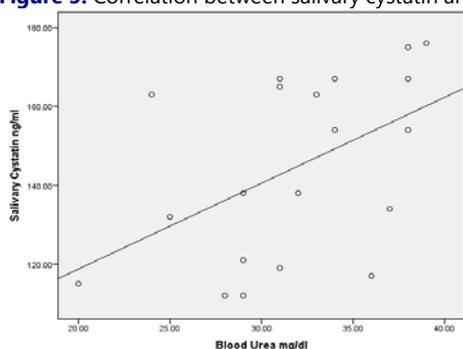


Figure 6. Correlation between salivary cystatin and blood urea within control group

Table 4. Correlation between serum cystatin, salivary cystatin, serum creatinine and blood urea within chronic renal disease group

		Blood urea	Serum creatinine	Serum cystatin	Salivary cystatin
Blood urea	Pearson Correlation	1	.166	.574**	.381*
	Sig. (2-tailed)		.390	.001	.042
	N	29	29	29	29
Serum creatinine	Pearson Correlation	.166	1	.427*	.465*
	Sig. (2-tailed)	.390		.021	.011
	N	29	29	29	29
Serum cystatin	Pearson Correlation	.574**	.427*	1	.679**
	Sig. (2-tailed)	.001	.021		.0001
	N	29	29	29	29
Salivary cystatin	Pearson Correlation	.381*	.465*	.679**	1
	Sig. (2-tailed)	.042	.011	.0001	
	N	29	29	29	29

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Correlation within chronic renal disease group

Correlation between serum cystatin, salivary cystatin, serum creatinine and blood urea

A correlation study of serum cystatin, salivary cystatin, serum creatinine and blood urea show a significant positive correlation between all these parameters within chronic renal disease group except between blood urea and serum creatinine, which was non-significant as shown in [Table 4](#) and [Figures 7, 8, 9, 10, 11](#) and [12](#).

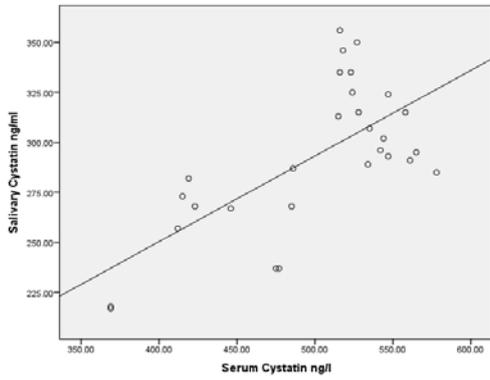


Figure 7. Correlation between salivary cystatin and serum cystatin within chronic renal disease group

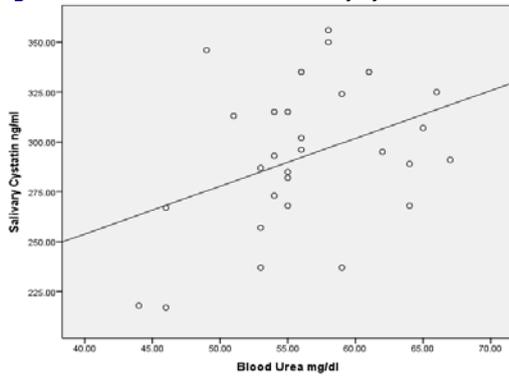


Figure 8. Correlation between salivary cystatin and blood urea within chronic renal disease group

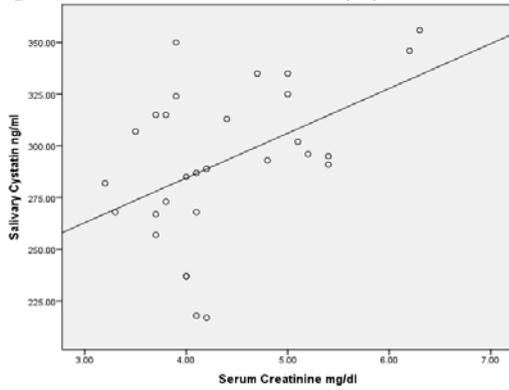


Figure 9. Correlation between salivary cystatin and serum creatinine within chronic renal disease group

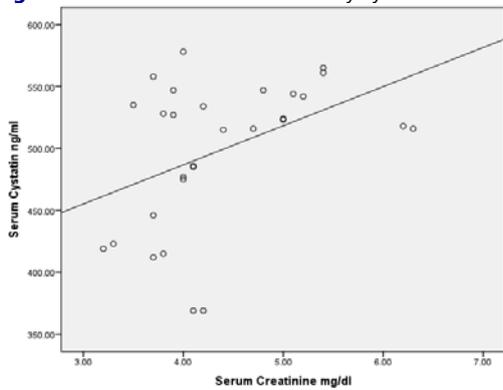


Figure 10. Correlation between serum cystatin and serum creatinine within chronic renal disease

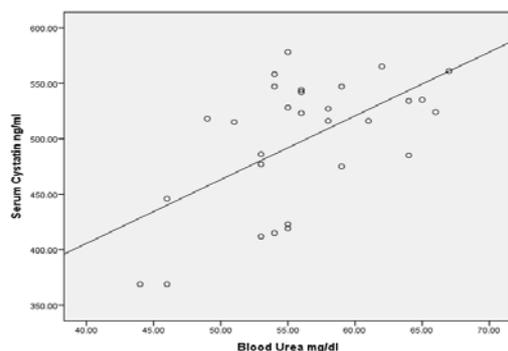


Figure 11. Correlation between serum cystatin and blood urea within chronic renal disease group

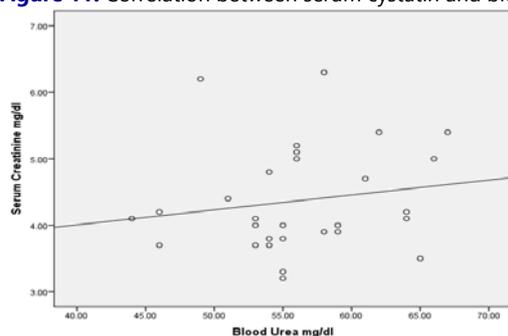


Figure 12. Correlation between serum creatinine and blood urea within chronic renal disease group

DISCUSSION

In chronic renal disease, many metabolic changes develop which may necessitate frequent biochemical blood analysis. Many studies concluded that the levels of Creatinine and urea in serum and saliva are closely related. The concentration of saliva Creatinine and urea can reflect to kidney damage, monitor the renal function of chronic renal disease patients, and help in the diagnosis of different stages of renal disease. It is a simple, noninvasive, and quick method [11-14]. Serum cystatin proved to be a better biochemical marker for renal function as compared to serum creatinine in chronic renal disease [15-18].

Our study shows that salivary cystatin positively correlate with serum cystatin, serum creatinine and blood urea in both control and patients group which give a conclusion that salivary cystatin could be used as diagnostic biomarker of chronic kidney disease which go with other studies in patients with chronic renal disease that showed the analysis of salivary creatinine and urea (a known biochemical markers of chronic renal disease) in patients with chronic renal disease reflects their levels in blood giving saliva analysis many advantages as an alternative to blood using [19].

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