ORIGINAL RESEARCH ARTICLE

Efficacy of Salivary Creatinine and Urea and their Association with Serum Creatinine and Urea Levels in Severe Chronic Kidney Disease Patients

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Abstract

An increase in the incidence of chronic kidney disease (CKD) is seen worldwide due to increased burden of noncommunicable diseases and ageing. There is accumulation of several waste products in these patients due to kidney impairment, leading to various metabolic complications. Thus, for survival they have to undergo repeated hemodialysis. Therefore, the levels of urea and creatinine have to be continuously monitored in blood. Saliva as a sample for estimation of these parameters is emerging nowadays. The present study is an attempt to check the efficacy of urea and creatinine levels in saliva for assessment of CKD patients in comparison to their levels in the blood. The findings indicated urea and creatinine levels were differed significantly between CKD patients and controls in blood as well as in saliva. We also reported the significant positive correlation of salivary creatinine and urea with serum creatinine and urea, respectively. By means of receiver-operating characteristic (ROC) curve analysis, we found the cut-off value of 0.8 mg/dL and 40 mg/dL for salivary creatinine and blood urea, respectively, at highest sensitivity and specificity. So, the conclusion can be made that, salivary creatinine and urea can be used as an alternative to serum creatinine and urea values in calculating eGFR and staging of CKD. Also, collection of saliva, a noninvasive method, could be an alternative to blood, an invasive method, for diagnosis and monitoring patients with CKD.

Keywords: Chronic kidney disease, Receiver-operating characteristic curve analysis, Salivary creatinine, Salivary urea.

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Introduction

Chronic kidney disease causes abnormal functioning of the kidney, which is caused due to the number of pathophysiologic processes with continual decrease in glomerular filtration rate (GFR).[1] Chronic kidney disease is a clinical syndrome due to irreversible kidney dysfunction leading to excretory, metabolic, and synthetic failure culminating into the accumulation of nonprotein nitrogenous substances and presenting with various clinical manifestations.[2,3]

The prevalence of CKD in India as reported by two studies was 0.79% and 0.9%. The number of end-stage renal stage (ESRD) patients is expected to rise every year from 1.65 million to 2.2 million in India considering the current Indian population of 1.23 billion.[4–6] Due to impairment in the functioning of the kidney in CKD patients, there is accumulation of the waste end products of various metabolisms seen, along with the involvement of different organs. These include the increase in the levels of serum creatinine and urea, along with other hematological parameters, electrolytes, and endocrine markers as well.[7]

The biological fluid containing a number of constituents secreted by salivary glands, saliva, shown to have a vital role in systemic as well as oral health. Compared to the collection of blood which is an invasive method, the collection of saliva is preferable for analysis of biochemical markers, as it is easy to collect, inexpensive, and the sample can be collected frequently. The method is also cost-effective as compared to blood collection, so can be used in large study population screenings.[8]

The contents of the salivary secretions are known to be affected in various systemic diseases, and CKD is one of them. The routine parameters which are assessed in the blood of CKD patients can also be estimated in the saliva of these patients, with its advantages for the estimation of salivary creatinine and urea. The invasive blood collection method in patients is often related to nervousness and distress. During the hemodialysis process and due to regular blood collection for investigations, there is some blood loss noted in CKD patients. Apart from this, there is an increased risk of blood-borne diseases to the physicians and other healthcare workers. Therefore, the noninvasive method which requires minimal skills, i.e., collection of saliva can be the better option, which provides comparative evaluation of disease condition with minimal risk to the healthcare professionals and patients as well.[7,9,10] So, we planned the present study, the association of levels of urea and creatinine in blood with salivary

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urea and creatinine, to assess the efficacy and find the cut-off values of salivary urea and creatinine in severe CKD patients.

Material and Methods

The present case–control study was performed in the Department of Biochemistry and Department of Medicine, Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Pune. The CKD patients were recruited as per KDIGO guidelines. A total of 50 diagnosed cases of CKD patients at stages 4 and 5, classified using KDIGO guidelines, were included after informed consent. A similar number of age and gender-matched healthy individuals with no major illness in the recent past were selected as controls. The study was approved by Institutional Ethics Committee, and informed consent was taken from participants.

Sample Collection

Sample collection was done at any time of the day. The blood and saliva samples of CKD patients were collected prior to hemodialysis under aseptic conditions. In total, 2 mL of blood was collected in plain vacutainers from the antecubital vein, and 2 mL of saliva was collected by spitting method in a graduated sterile container. No eating or drinking was allowed prior to sample collection, the mouth was thoroughly rinsed with distilled water. In a comfortable position, the patients were restricted to swallowing and movements of the mouth and asked to pool the saliva on the floor of the mouth, after every 60 seconds, the saliva was collected by spitting till 2 mL of sample is collected.

Methods

The serum from the blood sample and supernatant from the saliva were separated by centrifugation at 3000 rpm. Levels of urea (enzymatic kinetic method) and creatinine (Jaffe's method) in serum and saliva were estimated by standard commercially available kits (Randox Laboratories Ltd., United Kingdom).

Statistical Analysis

The quantitative variables were presented as mean ± SD. Categorical and nominal data were expressed in percentage. The unpaired Student's *t*-test was used for analyzing quantitative data. Categorical data were analyzed by using the Chi-square test. The correlation between the salivary and blood markers was tested by Pearson's correlation coefficient. The efficacy of salivary urea and creatinine in CKD patients was assessed by means of ROC curve analysis. The significance threshold of *p*-value was set at <0.05. All analysis was carried out by using SPSS software version 21.

Results

The study included a total of 50 diagnosed cases of stages 4 and 5 CKD patients. A similar number of age and gender-matched healthy controls were also taken. Out of 50 cases and 50 controls, 37 and 35 were males and 13 and 15 were females, respectively, with no statistical ($p = 0.82$) significant difference in gender among the two groups. There was no significant ($p = 0.44$) difference found in the ages of CKD patients (48.0 ± 13.87) and healthy controls (46.86 ± 12.86).

Among 50 cases of CKD, 31 (62%) were with diabetes, 30 (60%) were with hypertension, 4 (8%) and 3 (6%) with coronary artery disease and obstructive uropathy, respectively, and 1 (2%) each had atrial fibrillation, stroke, gout, peripheral neuropathy, polycystic kidney disease, and systemic lupus erythematosus (Table 1).

Table 1: Distribution of comorbidities among CKD cases

Comorbidities	*N*	*%*
Diabetes mellitus	31	62
Hypertension	30	60
Coronary artery disease	4	8.0
Obstructive uropathy	3	6.0
Atrial fibrillation	1	2.0
Stroke	1	2.0
Gout	1	2.0
Peripheral neuropathy	1	2.0
Polycystic kidney disease	1	2.0
Systemic lupus erythematosus	1	2.0

Table 2: Mean urea and creatinine values (mean ± SD) in saliva and serum samples among CKD patients and controls

Parameters	*CKD patients (N = 50)*	*Controls (N = 50)*	*p-value*
Salivary creatinine	0.88 ± 0.71	0.10 ± 0.03	<0.01
Salivary urea	115.72 ± 58.87	24.06 ± 7.95	<0.01
Serum creatinine	4.34 ± 2.29	0.81 ± 0.21	<0.01
Serum urea	119.84 ± 55.02	28.94 ± 6.94	<0.01

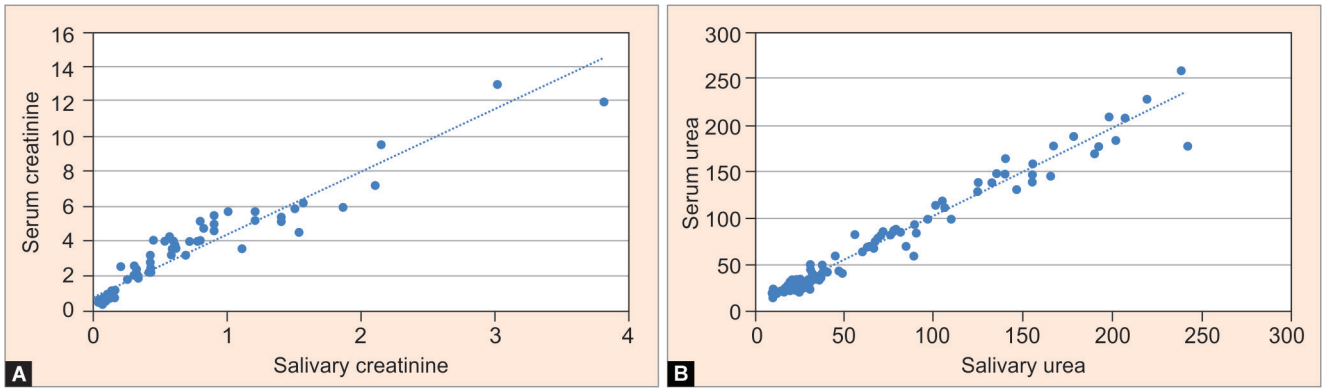
Table 3: Correlation of salivary and serum creatinine and urea values

Pearson corelation	*r-value*	*p-value*
Salivary creatinine vs serum creatinine	0.95	<0.01
Salivary urea vs serum urea	0.98	<0.01

The mean level of salivary creatinine in patients and controls were 0.88 ± 0.71 and 0.10 ± 0.03, salivary urea levels were 115.72 ± 58.87 and 24.06 ± 7.95, serum creatinine levels were 4.34 ± 2.29 and 0.81 ± 0.21, and serum urea levels were 119.84 ± 55.02 and 28.94 ± 6.94, respectively. There was a significant ($p < 0.01$) increase in the levels of salivary creatinine and urea, and serum creatinine and urea were observed in CKD patients when compared to controls. The results are shown in Table 2.

The association of salivary creatinine to serum creatinine and salivary urea to serum urea were analyzed by Pearson's correlation coefficient. A significant ($p < 0.01$) positive correlation was observed between salivary creatinine and serum creatinine, also there was an association between salivary urea and serum urea levels in patients with CKD. The results of correlation of salivary to serum creatinine and urea are depicted in Table 3, and Figure 1 shows the scattered diagram.

To assess the diagnostic potential of salivary creatinine and urea, ROC analysis was performed. The areas under the ROC curve (AUC) obtained were 1.000 and 0.98 for salivary creatinine and urea, respectively. Different cut-off values of salivary creatinine and urea with their respective sensitivity and specificity were calculated, and cut-off values of 0.2 mg/dL for creatinine (100% sensitivity and 100% specificity) and 40 mg/dL (92% sensitivity and 98% specificity) for urea were determined with highest sensitivity and specificity. The area under the ROC curve with standard error and 95% confidence interval for salivary creatinine and urea are shown in Table 4, and respective cut-off values with their highest sensitivity and specificity in Table 5 with receiver operating characteristics curve in Figure 2.



Figs 1A and B: Scattered diagram showing the association of (A) salivary creatinine to serum creatinine and (B) salivary urea to serum urea

Table 4: Area under the ROC curves of the salivary creatinine and urea levels with the corresponding 95% confidence interval

Test result variable(s)	*AUC ± SE*	*95% confidence interval*	*p-value*
Salivary creatinine	1.00 ± 0.00	1.00–1.00	<0.01
Salivary urea	0.98 ± 0.01	0.96–1.00	<0.01

Table 5: The cut-off values of salivary creatinine and urea levels with the corresponding highest sensitivity and specificity in CKD patients

	Cut-off value	*Sensitivity*	*Specificity*
Salivary creatinine	0.2	100.0	100.0
Salivary urea	40	92.0	98.0

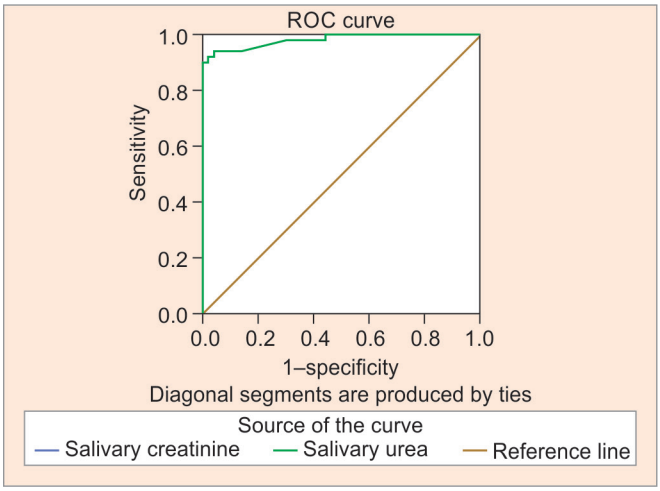


Fig. 2: The ROC curves of salivary creatinine and urea in CKD patients

Discussion

Creatinine and urea, excreted primarily by the kidneys, are the metabolic waste end products. The creatinine produced in the body is totally excreted without any reabsorption; therefore, estimation of creatinine in blood is used as an index of kidney function.[11] The invasive blood collection method in patients is often related with nervousness, distress, and needs skill. During the hemodialysis process and due to regular blood collection for lab investigations, there is some blood loss noted in CKD patients.[12]

The noninvasive method which required minimal skills, collection of saliva has the advantage over blood, which provides comparative evaluation of disease conditions irrespective of the age of the patients with minimal risk to the healthcare professionals and patients as well, along with its cost-effectiveness.[13–15] The present study thus aimed to estimate and compare serum and salivary creatinine and urea values in CKD patients and age and gender-matched healthy controls. We also aimed to correlate serum and salivary creatinine and urea values in CKD patients.

Our findings are also similar to those reported by the National Kidney Foundations K/DOQI study conducted by Fivush et al.[16] regarding the male predominance in the cases of CKD. In a study by Arvind and Manokaran,[17] 28.09% of patients of CKD fall into 51–60 years age group with males accounting 70% and females 30%.

Diabetes and hypertension as comorbidities were present in 62% and 60% cases of CKD patients, respectively. Other major comorbidities seen in CKD cases were of coronary artery disease (8%) and obstructive uropathy (6%). This trend is similar to that reported by Dash and Agarwal in the study conducted at the All India Institute of Medical Sciences.[18]

Lysaght has also demonstrated similar trends in American populations.[19] In the study conducted by Xue et al., the number of patients with diabetic nephropathy was almost 50% of the study group.[20] The etiological data are also concurrent with the data from other developing countries like Egypt and Bolivia.[21,22]

The present study reported that the mean salivary and serum creatinine values were significantly higher among cases as compared to controls. Also, mean salivary and serum urea values were significantly higher among cases as compared to controls. The positive association of salivary creatinine and urea with serum creatinine urea was noted.

Similar results were reported by Bader et al.,[23] Yajamanam et al.,[24] and Lasisi and Fasanmade[25] with significantly increased values of salivary creatinine and urea in CKD patients than the healthy individuals with significant positive correlation of salivary urea and creatinine to serum urea and creatinine, respectively. Renda[8] and Pham[26] in two independent studies observed significantly higher levels of salivary creatinine and urea and creatinine in CKD patients irrespective of disease stage when compared to controls, with a positive association between serum and salivary creatinine. Bagalad et al.,[15] Chand et al.,[27] and Arvind and Manokaran[17] also observed a significant positive association between blood and salivary urea and creatinine levels.

Chronic kidney disease is characterized by increase in the levels of urea and creatinine in blood, which are retained due to abnormality in kidney functioning. This rise in urea and creatinine is

known to increase their level in saliva too, because of the diffusion of urea and creatinine against concentration gradient, which causes dry mouth, uremic breath, tongue coating, and other oral complications of CKD. Normally, creatinine being low lipid soluble and large molecule across the cells and tight junctions of the salivary glands, it is not diffused in healthy individuals, in contrast, there is increase in the level of creatinine in CKD patients creating concentration gradient and making the diffusion of it easier from serum to saliva.[8]

To assess the diagnostic potential of salivary creatinine and urea, ROC curve analysis was performed. The area under ROC curve (AUC) achieved in the present study was 1.000 for salivary creatinine and 0.98 for salivary urea, with cut-off values of 0.2 mg/dL and 40 mg/dL, respectively, at higher sensitivity and specificity. This can be explained as the people with salivary creatinine and urea values above 0.2 mg/dL and 40 mg/dL are more likely to have increased risk of CKD in the future, and therefore, the appropriate management can be given to the patients.

In the present study, the obtained areas under the ROC curves for salivary urea and creatinine are suggestive of these markers, can be used as additional diagnostic parameters in CKD patients. Similar findings related to AUC of 0.967 and 0.897 for salivary creatinine were obtained by Venkatapathy et al.[9] and Yuncheng et al.[28] Venkatapathy et al.[9] determined a cut-off value of 0.2 mg/dL at promising sensitivity and specificity. Yajamanam et al.[24] calculated the cut-off values of salivary urea to be >6 mmol/L and creatinine to be >14.6 µmol/L and concluded salivary creatinine can be used to calculate eGFR in identifying CKD patients.

Conclusion

The present study provides a positive association between serum and salivary creatinine and urea levels, indicating the rise in the levels of creatinine and urea in serum, the values will increase in saliva and vice versa. Therefore, it is suggestive that the levels of salivary creatinine and urea can be used as an alternative to serum creatinine and urea values in calculating eGFR and staging of CKD. Also, the collection of saliva, a noninvasive method, could be an alternative to blood, an invasive method, for diagnosis and monitoring patients with CKD.

Limitations

Only severe stages (stages 4 and 5) of CKD patients were considered in the present study, testing among other stages was not considered, which in the future can be done. As this is a small sample study, the kits used were not validated for salivary samples, the same will be done with a large sample size in the future.

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