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Biochemical Evaluation of the Effectiveness of Salivary Urea and Creatinine Levels and Their Relationship with Serum Urea and Creatinine Levels in Patients with Chronic Kidney Disease

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ABSTRACT

Background: Individuals with chronic kidney disease (CKD) often experience blood loss as a result of treatments like hemodialysis and routine blood samples. The submandibular, sublingual and parotid salivary glands produce saliva, and this biological fluid is crucial to both dental and overall health. Saliva collection is easy, does not need much expertise, and has several benefits over serum. Regardless of age, regular saliva collection is safe and effective. In Pakistan there is a less attention of physicians, surgeon, and dentists on the importance of saliva in diagnostic field. **Objective:** This study has two main objectives, first is to determine the efficacy of urea and creatinine in saliva and the second was to find out any association with creatinine and urea levels in serum amongst CKD. **Study Design:** This is cross sectional study. **Settings:** This study was conducted at Department of Biochemistry Central Park Medical College, Lahore Pakistan. **Duration:** February 2022 to July 2022. **Methods:** Total 50 saliva and serum sample were taken in this study form the patients with stage four and five of CKD. The urea and creatinine were estimated in saliva and serum samples, collected from CKD patients. The statistical analysis was done by using SPSS version 20. **Results:** Out of sixty patients with stages 4 and 5 CKD, total 44 male and 16 female were enrolled in the study. Total age range 16 to 55 years with the mean of 24.67 ± 4.14 years. However, the BMI range 16 to 33 Kg/m^2 with 21.7 ±3.8 Kg/m^2 were calculated. The ROC analysis confirmed that the overall area under the curve was 0.967 (95% CI: = 0.937-0.997, standard error = 0.015) of urea analysis. **Conclusion:** The levels of urea and creatinine in the saliva of people with severe CKD were shown to be considerably higher and strongly correlate with blood levels. ROC analysis shows that salivary creatinine and urea have good specificity and sensitivity. The present study found that the levels of creatinine and urea in the blood are positively correlated with those in the saliva. Collecting a patient's saliva instead of blood for CKD diagnosis and monitoring is a less painful option.

Keywords: *Saliva, Serum/blood, CKD, Creatinine, Urea.*

INTRODUCTION

The kidneys are the body's primary excretory organs and play a crucial role in maintaining internal stability.[1] Nephrons are the structural and functional building blocks of the kidney. As nephrons gradually and chronically deteriorate, kidney function is reduced, a condition known as chronic renal failure. Permanent kidney damage is the hallmark of the chronic illness known as end-stage renal failure. Blood urea nitrogen (BUN) and nitrogen found outside of proteins both rise as a consequence.[2] At a condition of impaired renal function, the body may try to eliminate waste products via the mouth through saliva. Saliva secretion and its chemical make-up are known to be influenced by a wide variety of systemic disorders. Since it can be collected quickly and

painlessly, saliva has been gaining popularity as a diagnostic tool.[3] Chronic kidney disease (CKD) influences an rising number of cases throughout the world.[4] The buildup of metabolic waste products and the involvement of many organs are hallmarks of chronic kidney disease. Elevated levels of biochemical parameters including blood creatinine, blood urea and others like electrolytes, hematologic, skeletal, and endocrine problems, are common indicators of these alterations.[5,6] Salivary secretion has been shown to fluctuate significantly and detectably in response to a number of systemic disorders. Salivary secretion content may be altered by a number of systemic disorders, including chronic kidney disease. Patients who were suffering from CKD might have their creatinine and urea levels (parameters typically evaluated in blood samples) indicated by their saliva. There are a number of benefits of saliva newly accepted as a diagnostic tool for many parameters and in many studies it is used as to analyze creatinine and urea in the saliva of CKD patients.[6,7]

Patients often feel anxious and distressed when they need to have blood drawn for diagnostic purposes. In addition, individuals with chronic renal disease often experience blood loss as a result of treatments like hemodialysis and routine blood sample.[8] Also, those who work with CKD patients are at increased risk for contracting blood-borne illnesses. So, medical practitioners and patients alike would benefit from a low-risk, non-invasive diagnostic tool that can reliably assess patients' illness states.[9] The submandibular, sublingual and parotid salivary glands produce saliva, and this biological fluid is crucial to both dental and overall health. Collection is easy, does not need much expertise, and has several benefits over serum. Regardless of age, regular saliva collection is safe and effective. Screening huge populations can be done efficiently and affordably.[10,11] Salivary parameters may be influenced by environmental, dietary, and genetic variables. In Pakistan there is a less attention of physicians, surgeon, and dentists on the importance of saliva in diagnostic field.[12] This study has two main objectives, first is to determine the efficacy of urea and creatinine in saliva and the second was to find out any association with creatinine and urea levels in serum amongst CKD.

METHODS

This cross-sectional study was conducted at Department of Biochemistry Central Park Medical College, Lahore from February 2022 to July 2022. The demographic information was used to take from filled questionnaire for this investigation. The demographic factors used to create this survey were age, gender, height & weight for BMI, level of education, income, marital status, and ethnicity. Saliva and blood samples were used to evaluate biochemical parameters in CKD patients.

Patients in stages 4 and 5 of chronic kidney disease who consented to blood and saliva collection were enrolled. Patients were disqualified from the trial if they had preexisting conditions such as neuropathy, diabetes, heart attack, heart failure, or trauma.

The study's 60 participants were selected using the World Health Organization's sample size calculator, which assumed a diabetes prevalence of 23.3%[13] with a 95% confidence interval and a margin of error of 5%.

According to the spitting collection method of Ahmed *et al*. we collect the saliva sample.[14] Saliva samples were collected between ten and twelve hours of the day to decrease the effects of diurnal variation on both quality and quantity. Study participants were instructed to fast for one hour prior to treatment. For five minutes, researchers watched test subjects spit into a receptacle. The length of time required to collect saliva was measured with a stopwatch. By dividing the total amount of saliva collected by 5, we were able to calculate the flow rate (ml/min). After collecting the saliva samples, the researchers took them to analyze in more depth. Urea was measured using an enzymatic kinetic methodology, and creatinine was measured using the Jaffe's method; both techniques were performed according to the manufacturer's protocol provided by Randox Laboratories Ltd. UK.

The saliva and blood supernatant were separated and centrifuged at 3000 rpm for 10minutes. The amounts of urea and creatinine in the blood and saliva, respectively, were measured that employ the enzyme kinetic methodology and the Jaffe's method, respectively.

IBM-SPSS version 20.0 was used to analyze the data for this investigation. Demographic parameters were the subject of a descriptive study. The assay's sensitivity and specificity were evaluated using ROC analysis by comparing saliva to serum. Pearson's correlation was employed for the investigation of the link between saliva and serum. The data were considered to be statistically significant if the p-value was less than 0.05.

RESULTS

Out of sixty patients with stages 4 and 5 CKD, total 44 male and 16 female were enrolled in the study. Total age range 16 to 55 years with the mean of 24.67 ± 4.14 years. However, the BMI range 16 to 33 Kg/m^2 with 21.7 ±3.8 Kg/m^2 were calculated. In the demographic details of the patients were also included their marital status, socio-economic status, and ethnicity as shown in Table I. In this total 86.7 % individuals were married, mostly were belong from very low-income status.

Table 1: Demographic detail of the patients

Grouping		N	%
Gender	Male	45	74.3%
	Female	15	25.7%
Marital Status	Married	53	87.7%
	Unmarried	7	12.3%
Socioeconomic Status	Very Low-Income Status	32	53.3%
	Low Income status	6	10%
	Middle Income Status	7	28.3%
	High Income Status	5	8.3%
Ethnicity	Punjabi	19	31.7%
	Sindhi	2	3.3%
	Urdu Speaking	9	15%
	Baloch	13	21.7%
	Pathan	17	28.3%

ROC analysis (Fig. 1 and 2) was analyzed to compare the accuracy of urea and creatinine in serum and saliva at diagnostic level. The overall area under the curve was 0.967 (95% CI: 0.937-0.997, standard error = 0.015) of urea analysis. However, the overall area under the curve was 0.902 (95% CI: 0.850-0.954, standard error = 0.027) of creatinine analysis. Salivary urea levels were obtained, and a sensitivity and specificity cutoff were developed. The cut off value of urea was 72mg/dl and creatinine was 1.10mg/dl.

Figure 1: The ROC curve analysis urea in serum and saliva

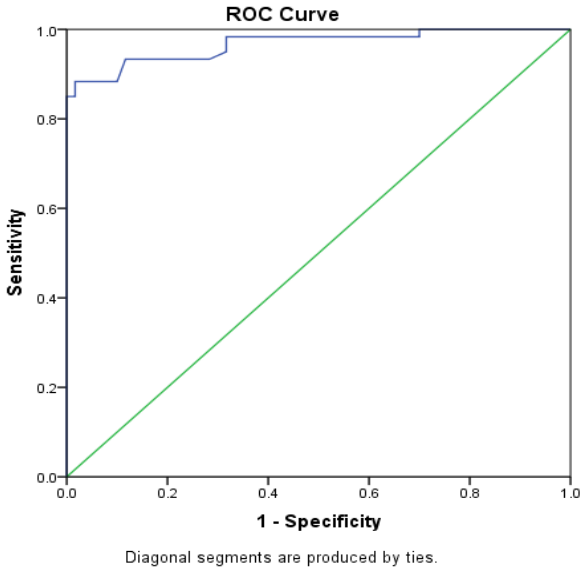
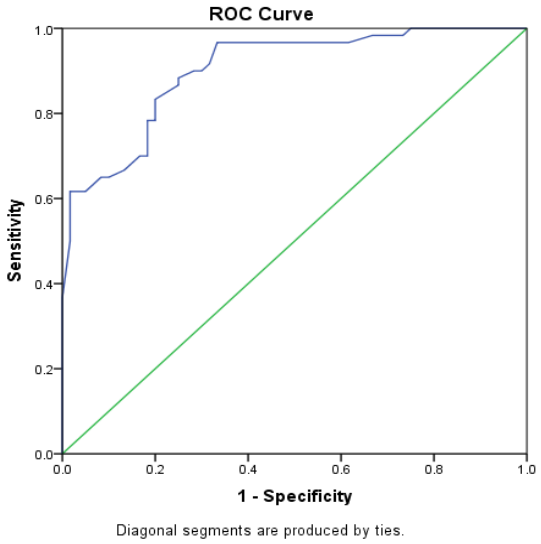


Figure 2: The ROC curve analysis creatinine in serum and saliva



In table 2 the Pearson's correlation coefficient was used to examine the relationship between salivary urea and serum urea, as well as serum creatinine and salivary creatinine. There was a correlation among salivary urea and blood urea levels in CKD patients, and a positive correlation among salivary creatinine & serum creatinine (p 0.05).

Table 2: Correlation of saliva and serum parameters

Correlation	Person's Test	
	r2	P-value
Saliva urea x serum urea	0.61	0.042*
Saliva creatinine x serum creatinine	0.82	0.032*

DISCUSSION

The kidneys are responsible for excreting metabolic remaining products urea and creatinine. Since the body's creatinine is completely eliminated without being reabsorbed, measuring creatinine levels in the blood is a good indicator of how well the kidneys are working.[15] Patient anxiety, anguish, and technical expertise are common during invasive blood collection. Some blood loss is seen in CKD patients during the dialysis procedure and owing to routine blood collection for lab studies.[16] Saliva collection is advantageous over blood collection because it is noninvasive, requires less technical skills, and benefits patients of all ages by allowing for cost-effective, risk-free examination of disease states via comparison.[11] This study has two main objectives, first is to determine the efficacy of urea and creatinine in saliva and the second was to find out any association with creatinine and urea levels in serum amongst CKD. Eight

percent of CKD patients additionally had coronary artery disease, while six percent of CKD cases had obstructive uropathy. The findings is similar with Vinters *et al*.[17] MR *et al* has shown that the same patterns hold true for the Nepal people.[18] The creatinine and urea in saliva were shown to have a positive correlation with creatinine and urea levels in serum. Salivary urea and creatinine were positively correlated with blood urea & creatinine in CKD patients when compared with healthy controls, as shown by Alexandra *et al*,[19] C Amadi *et al*,[20] & Medine *et al*.[21] Salivary creatinine plus urea and creatinine levels were significantly higher in individuals with CKD compared to controls in two studies.[22,23] Chronic renal disease is depicted by an elevated levels of urea and creatinine, both of which are retained in the body. An increase in urea and creatinine in the blood is known to cause dry mouth, uremic breath, and tongue coating. Because it is a big, poorly lipid-soluble molecule, creatinine cannot pass through the cells and tight connections of the salivary glands. In CKD patients, however, an elevated creatinine level generates a concentration gradient, allowing the amino acid to diffuse from the serum into the saliva.[22]

ROC curve analysis was used to evaluate the diagnostic accuracy of salivary creatinine and urea. At a lower threshold of 1.1 mg/dL for salivary creatinine and a higher threshold of 72 mg/dL for salivary urea, the current research produced an area under the ROC curve (AUC) of 0.902 and 0.967, respectively. This is because individuals whose salivary creatinine or urea levels are more than 1.1 mg/dL or 72 mg/dL, respectively, have a higher risk of developing chronic kidney disease in the future and may be managed accordingly. Salivary urea and creatinine were shown to have high areas under their respective ROC curves, indicating their potential utility as additional diagnostic measures in patients with CKD.[24] AUC values for salivary creatinine reported by R Venkatapathy *et al*[3] and Y Xia *et al*[25] were 0.967 and 0.897, respectively. With a good balance between sensitivity and specificity, Venkatapathy *et al*. settled on a cutoff value of 0.2 mg/dL. Salivary creatinine may be utilized to compute glomerular filtration rate in identifying CKD patients.[3]

Patients with CKD have been shown to have increased amounts of both creatinine and urea in their saliva, and these results have been validated by favorable correlations with their blood levels. CKD patients may have dry mouth, mouth odor, uremic breath, and tongue coating due to the increased amounts of creatinine and urea in their saliva.[26] The typical uremic fetor, an ammoniacal stink from the mouth, is caused by the excessive quantity of urea in the saliva and its resulting breakdown to ammonia in uremic patients.[27] This study's significant correlation between serum & salivary creatinine may be explained by the concentration gradient that promotes greater diffusion of urea and creatinine from blood into saliva in individuals with chronic kidney disease (CKD).[28]

The small sample size also accounts for the absence of gender and age-specific cut-off values in this investigation. Salivary analysis of urea and creatinine has to be compared to existing standard procedures before it can be utilized as a diagnostic tool to replace the usage of blood. The reliability of the new test will be determined by how well it classifies test subjects into diseased and healthy categories. The reliability of a diagnostic test may be measured in two ways: sensitivity and specificity. Therefore, ROC analysis is used to evaluate an alternate diagnostic tool as saliva to the gold standard i.e. blood.

CONCLUSION

Creatinine and urea levels in saliva were shown to be significantly higher in individuals with advanced CKD, correlating positively with blood levels. Salivary creatinine and urea have high levels of sensitivity and specificity, according to ROC analysis. According to the results of the current investigation, there is a positive correlation between the concentrations of creatinine and urea in the blood and in the saliva. Saliva is easy to collect without pricking technique and alternative to blood collection for diagnosis and monitoring of CKD patients. Hence, this study concluded that the salivary creatinine and urea levels has diagnostic potential for CKD, however more research is needed to encompass people in the early diagnosis.

LIMITATIONS

Because just one public hospital participated in the study, the findings cannot be applied generally.

SUGGESTIONS / RECOMMENDATIONS

The study suggested that frequent and ongoing health education programs be run for such issues to enhance awareness of self-care in various settings.

CONFLICT OF INTEREST / DISCLOSURE

None.

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