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Original Article

Biochemistry Section

Efficacy of Salivary Urea and Creatinine Compared to Serum Levels in Chronic Kidney Disease Patients: A Cross-sectional Study

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ABSTRACT

Introduction: Chronic Kidney Disease (CKD) has become an impending health concern due to the massive rise in the number of patients with diabetes mellitus and hypertension. Monitoring CKD patients typically requires regular invasive testing, and a simple diagnostic test that does not involve venipuncture would greatly benefit patients and healthcare professionals. Extensive research is being conducted to explore the use of saliva as a Non invasive tool for evaluation of systemic diseases like CKD. However, most of these studies have focused on End Stage Renal Disease (ESRD) patients.

Aim: To investigate the correlation between salivary urea and creatinine levels and their serum counterparts in CKD patients and healthy controls. Additionally, the study aimed to assess the efficacy of salivary urea and creatinine compared to serum urea and creatinine in predicting CKD.

Materials and Methods: The present cross-sectional study was conducted between January 2021 and July 2022 in the Department of Biochemistry, in collaboration with the Department of Medicine, at Heritage Institute of Medical Sciences in Varanasi, Uttar Pradesh, India. The study included a total of 60 participants: 30 CKD patients (stage 1-3) and 30 age-matched healthy controls. Serum and salivary urea were analysed using the Urease-Glutamate Dehydrogenase (GLDH) method, and creatinine was measured using the Modified Jaffe's method on the Dirui-300B autoanalyser. Data were statistically analysed using Pearson's correlation coefficient. The sensitivity, specificity, and Area Under the Curve (AUC) of salivary urea and creatinine were evaluated in comparison to their serum counterparts.

Results: The participants consisted of 30 CKD patients with a mean age of 54.8±8.8 years and 30 age-matched healthy controls with a mean age of 52.42±8.4 years. A significant difference in salivary urea and creatinine levels was observed between the control and CKD groups. There was a strong and significant correlation (p-value <0.01) between salivary creatinine and serum creatinine in both the control group (r-value=0.76) and the CKD group (r-value=0.82). Additionally, a strong and significant correlation (p-value <0.01) was found between salivary urea and serum urea in the CKD group (r-value=0.63). However, the correlation between salivary and serum urea was not significant in the control group, with an r-value of 0.58 and a p-value of 0.24. Both salivary urea and creatinine demonstrated high sensitivity (90% and 89%, respectively), specificity (80% and 80%, respectively), and AUC (0.78 and 0.86, respectively) compared to their serum counterparts, validating their practical clinical utility.

Conclusion: The concentration of urea and creatinine in saliva can reflect kidney damage and help monitor kidney function in CKD patients. Standardising the protocol for evaluation of salivary urea and creatinine and establishing a reference range will make it useful for screening for CKD.

Keywords: Non invasive biomarkers, Salivary analytes, Salivary biomarkers

INTRODUCTION

The CKD has emerged as a significant health issue in recent years, with a pooled prevalence of more than 10%, as stated by the World Health Organisation. This is primarily due to the significant increase in the number of patients suffering from diabetes mellitus and hypertension [1]. Consequently, CKD poses a serious threat and burden to health services in both rural and urban areas of the country. In addition to being a crucial risk factor for heart diseases and stroke, CKD can eventually progress to kidney failure [2].

A systematic review estimated the prevalence of CKD to range from 2% to 41% in the African subcontinent, and globally it ranges from 11.7% to 15.1%. The exact burden of CKD/ESRD in India cannot be accurately measured due to the absence of a renal registry. However, the number of deaths due to CKD increased to 1.18 million in 2016 from 0.59 million in 1990 [3].

Glomerular Filtration Rate (GFR) is estimated for the diagnosis and staging of CKD. GFR is measured using markers such as urea, creatinine (most commonly used), inulin, and cystatin C. In routine clinical practice, serum creatinine is used to estimate GFR using prediction equations, with the 'Modification of Diet in Renal Disease' (MDRD) study equation being the most commonly used [4].

Routine monitoring of CKD patients can ensure improved prognosis and delay clinical complications. However, this requires regular sample collection through venipuncture, which causes anxiety and discomfort to the patient, further discouraging them. Therefore, there is a need for the development of a painless, non invasive procedure that would make regular monitoring more acceptable to patients. Such a technique, free from venipuncture, would greatly ease the experience for patients as well as healthcare professionals.

Salivomics is a rapidly developing diagnostic field, and extensive research is being conducted to establish saliva as a tool for evaluating systemic diseases such as diabetes mellitus, CKD, and rheumatoid arthritis [5]. Recent studies have focused on the application of salivary urea and creatinine in diagnosing CKD, but these studies have primarily concentrated on patients with End Stage Renal Disease (ESRD) [6,7]. In order for saliva to be practically useful in

relation to CKD, it is important to investigate whether salivary levels of urea and creatinine reflect their respective serum levels in the early stages of the disease. Therefore, it is crucial to understand the relationship between the serum and salivary levels of these analytes.

The present study aimed to examine the correlation between salivary urea, creatinine, and their serum counterparts. The study will help determine whether salivary levels of urea and creatinine accurately reflect their respective serum levels. Additionally, the sensitivity, specificity, and Area Under the Curve (AUC) will be compared for serum analytes (urea and creatinine) and their salivary counterparts in CKD patients and healthy control participants. This comparison will help establish the practical utility of estimation of these salivary analytes.

MATERIALS AND METHODS

The present cross-sectional study was conducted between January 2021 and July 2022 in the Department of Biochemistry, in collaboration with the Department of Medicine, at Heritage Institute of Medical Sciences in Varanasi, Uttar Pradesh, India Ethical approval was obtained from the institute's Ethical Committee (HIMS/IEC/023), and written informed consent was obtained from all participants prior to the study.

Inclusion criteria: The study included a total of 60 participants aged between 18 and 70 years, consisting of 30 CKD patients (stage 1-3) and 30 age-matched healthy controls.

Exclusion criteria: Patients who were advised dialysis (having ESRD), diagnosed with acute kidney injury, hepatorenal syndrome, diseases of the salivary gland, periodontitis, or had a habit of chewing pan masala, betel nut, or tobacco were excluded from the study.

Study Procedure

Chronic kidney disease diagnosis and staging: The National Kidney Foundation Kidney Disease Outcome Quality Initiative (NFK KDOQI) defines the stages of CKD as follows: Stage 1 {Glomerular Filtration Rate (GFR) ≥90 mL/min/1.73 m^2} and Stage 2 (GFR 60-89 mL/min/1.73 m^2) are characterised by the presence of other markers of kidney damage, such as proteinuria, imaging abnormalities, or functional/histological abnormalities. Stage 3 CKD is defined as a GFR <60 mL/min/1.73 m^2. Stage 4 CKD is designated by a GFR <30 mL/min/1.73 m^2, and Stage 5 is defined by a GFR <15 mL/min/1.73 m^2 [8].

Data collection:

- Two milliliters of venous blood were collected in plain (serum) red top vacutainers, regardless of the prandial status, for the estimation of urea and creatinine.
- Saliva: Saliva was collected through the process of passive drooling. The mouth was rinsed twice with distilled water prior to collection. It is important to note that stimulated saliva may have altered pH, water content, protein content, and electrolyte levels [9,10]. The collected saliva samples were then transported to the laboratory in an icebox and analysed as soon as possible.

Serum and salivary urea were estimated using the Urease-GLDH method. The biological reference range for serum urea is 20-40 mg/dL, and for serum creatinine, it is 0.6-1.4 mg/dL [8]. The normal cut-off range for salivary parameters has not yet been standardised. Both serum and salivary creatinine were estimated using the Modified Jaffe's method on the Dirui-300B autoanalyser [8].

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Packages for Social Sciences (SPSS) version 16.0. Descriptive statistics were analysed, and Pearson's correlation coefficient was calculated and compared between the groups. A p-value less than 0.05 was considered significant. The sensitivity, specificity, and AUC of salivary urea and creatinine were assessed in comparison to their serum counterparts to determine the clinical utility of these salivary analytes.

RESULTS

The study included a total of 60 participants aged between 18 and 70 years. The participants consisted of 30 CKD patients with a mean age of 54.8±8.8 years and 30 age matched controls with a mean age of 52.42±8.4 years. There was no significant difference observed in age and gender distribution between the CKD and control groups [Table/Fig-1].

Parameters	Control group (n=30)	CKD group (n=30)	p-value
Age (Mean±SD) in years	52.42±8.4	54.8±8.8	0.345
Gender			
Male	17	16	0.46
Female	13	14	
Co-morbidity			
Hypertension	0	19	
Type 2 diabetes mellitus	0	07	

[Table/Fig-1]: Demographic characteristics of the control and CKD group.

The mean values of serum and salivary urea were 16.28±3.23 mg/dL and 9.26±2.18 mg/dL in the control group, and 58.62±18.06 mg/dL and 23.07±14.27 mg/dL in the CKD group, respectively. Similarly, the mean values of serum and salivary creatinine were 0.71±0.16 mg/dL and 0.17±0.15 mg/dL in the control group, and 4.52±2.16 mg/dL and 0.66±0.63 mg/dL in the CKD group, respectively [Table/Fig-2].

A positive correlation was found between the serum and salivary concentrations of both urea and creatinine. A significant positive correlation was observed between salivary creatinine and its serum counterpart in the control group (r-value=0.76, p-value <0.01) [Table/Fig-3]. The correlation between salivary and serum urea was found to be insignificant in the control group, with an r-value of 0.58 and a p-value of 0.24 [Table/Fig-3]. In the CKD group, a significant positive correlation was observed between salivary creatinine and its serum counterpart (r-value=0.82, p-value <0.01) [Table/Fig-3,4]. Additionally, a positive significant correlation was found between salivary urea and its serum counterpart in the CKD group (r-value=0.63, p-value <0.01) [Table/Fig-3,5].

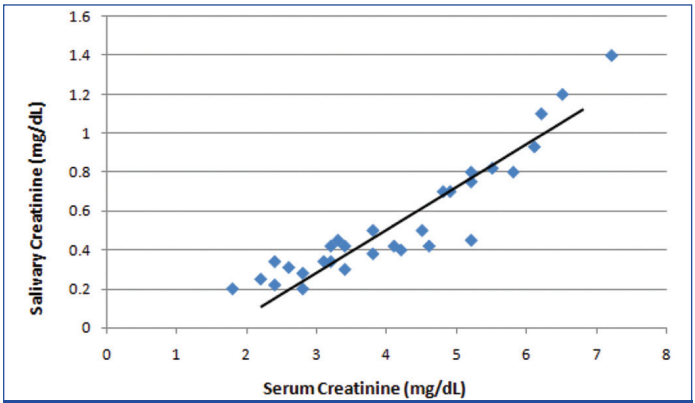
The sensitivity, specificity, and AUC of salivary urea were found to be 90, 80, and 0.78, respectively, and for salivary creatinine, they were 89, 80, and 0.86, respectively. These values were comparable to their respective serum concentrations [Table/Fig-6].

Group	Serum urea (mg/dL)	Salivary urea (mg/dL)	p-value[a]	Serum creatinine (mg/dL)	Salivary creatinine (mg/dL)	p-value[a]
Control group (Mean±SD)	16.28±3.23	9.26±2.18	<0.01*	0.71±0.16	0.17±0.15	<0.01*
CKD group (Mean±SD)	58.62±18.06	23.07±14.27	<0.01*	4.52±2.16	0.66±0.63	<0.01*
p-value[b]	<0.01*	<0.01*		<0.01*	<0.01*	

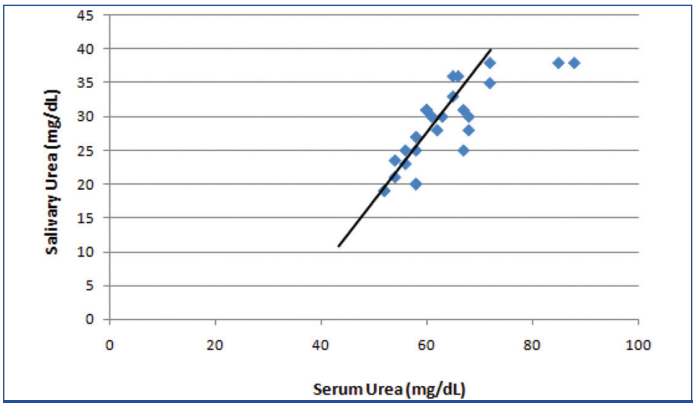
[Table/Fig-2]: Comparison of serum and salivary urea and creatinine between the control and CKD group.
*Statistically highly significant; p-value[a]: Comparison of serum and salivary parameter within the same group; p-value[b]: Comparison of serum/salivary parameter between the control and CKD group

Parameters	Pearson's correlation coefficient and significance	
	Control group	CKD group
Serum urea and salivary urea	0.58, p-value=0.24	0.63, p-value=0.048*
Serum creatinine and salivary creatinine	0.76, p-value=0.004*	0.82, p-value=0.002*

[Table/Fig-3]: Correlation of salivary urea and creatinine with their serum counter parts.
*Statistically highly significant



[Table/Fig-4]: Correlation between serum and salivary creatinine in the CKD group.



[Table/Fig-5]: Correlation between serum and salivary urea in the CKD group.

Parameters	Sensitivity	Specificity	AUC	Standard error	95% CI
Serum creatinine	90	90	0.93	0.033	0.841-0.986
Salivary creatinine	89	80	0.86	0.068	0.761-0.942
Serum urea	95	80	0.91	0.092	0.801-0.968
Salivary urea	90	80	0.78	0.041	0.662-0.881

[Table/Fig-6]: Sensitivity, specificity and Area Under the Curve (AUC) of serum and salivary urea and creatinine in the Control and CKD group.
Note: Since no test/parameter can be considered 100% efficient, therefore true value of serum parameters is compared with their salivary counterparts

DISCUSSION

In the present study, authors measured both serum and salivary levels of urea and creatinine in CKD patients and healthy controls. A systematic review has shown that approximately two-thirds of kidney failure patients die due to the unavailability or failure to access dialysis facilities in a timely manner. Type 2 diabetes mellitus and hypertension have been identified as the most common causes of chronic kidney failure in India. Additionally, around 16% of cases are attributed to an unknown aetiology. India is now considered the "diabetic capital" of the world, and the burden of CKD on the country's healthcare system is substantial [11].

As a result, there has been a shift in the approach to CKD management, with a focus on more aggressive primary and secondary prevention, particularly in developing nations like India. Currently, the diagnosis of CKD relies on information obtained from blood and urine analysis, as well as radiological and physical examinations of the patient [12]. Saliva, being a biological fluid secreted in large quantities by the body, has the potential to reflect the metabolic status of the body. Many metabolites and biomarkers can pass through the salivary gland basement membrane and therefore serve as indicators of health [13].

Authors concluded that there was a significant difference in the salivary levels of urea and creatinine between the control group and the CKD group. Similar results have been obtained by other researchers as well. Bader RS et al., also compared serum and salivary levels of urea and creatinine in CKD patients and controls and obtained similar results [14]. In their study, Lasisi TJ et al., concluded that patients with CKD showed elevated levels of salivary creatinine and urea compared to healthy individuals [15]. These results are consistent with present study findings. Venkatapthy R et al., studied the levels of creatinine in serum and saliva in control and CKD patients. They concluded that there is a progressive increase in salivary creatinine levels with the progression of CKD stages. This finding is similar to present study, as we also observed a significant difference between serum and salivary levels of creatinine in CKD patients [16].

In addition, when authors examined the correlation between serum urea and creatinine and their salivary counterparts, a significant correlation was observed. However, the correlation was found to be weaker for urea compared to creatinine. Furthermore, when comparing the two participant groups, the correlation was stronger in the CKD group compared to the control group. Lasisi TJ et al., concluded in their study that salivary levels of creatinine and urea are positively correlated with their plasma levels [15]. Ladgotra A et al., concluded that each chemistry parameter needs to be studied exclusively in healthy individuals as well as different disease states to determine the clinical utility of salivary parameters [17]. The study conducted by Briet M et al., suggests that the negative correlation between serum and salivary concentrations could be attributed to the presence of salivary gland diseases, which may affect the movement of creatinine from serum to saliva through the salivary glands [18]. Additionally, Xia Y et al., stated that the serum and salivary concentrations of urea, creatinine, and uric acid positively correlate in both healthy individuals and CKD patients [19].

Salivary urea concentration can be influenced by non renal factors such as protein intake, hydration status, metabolic status (e.g., prolonged starvation), and the presence of liver or gastrointestinal diseases. The use of steroids has also been shown to affect salivary urea concentrations. However, this is not the case with creatinine, as its salivary concentration remains relatively unchanged, similar to its serum counterpart [20].

In the present study, authors found comparable specificity and sensitivity of salivary urea and creatinine compared to their serum counterparts. This suggests that measuring these parameters in saliva could potentially have a practical clinical utility. Similar results were also reported by Lloyd JE et al., who concluded that salivary creatinine could serve as a potential screening tool for renal disease, which aligns with our study findings [21]. Temilola DO et al., conducted a study on CKD patients and stated that salivary creatinine assays can be used alongside serum creatinine to monitor CKD patients, which is in line with the aim of the present study as well [22].

Limitation(s)

There are certain limiting factors that need to be considered in present study. These include the oral retention of food, undiagnosed parotitis, and periodontitis, as they can potentially alter the results. Additionally, the intake of certain medications can cause hypo or hypersalivation, and therefore their impact on salivary parameters should be examined.

CONCLUSION(S)

Statistical analysis reveals that the diagnostic utility of salivary urea and creatinine is comparable to their serum counterparts.

The concentration of urea and creatinine in saliva can therefore serve as a reflection of renal damage, aiding in the monitoring of kidney function in CKD patients. Standardisation of assay kits for the estimation of salivary urea and creatinine, as well as the establishment of a reference range, will enhance the clinical relevance of these parameters. Regular monitoring of these salivary analytes, in conjunction with serum measurements, can make the monitoring of CKD less burdensome for patients.

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