

ORIGINAL ARTICLE



Validation of the salivary urea and creatinine tests as screening methods of chronic kidney disease in Vietnamese patients

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ABSTRACT

Objectives: The aims of this case control study were to correlate the serum and salivary urea as well as creatinine levels; and to evaluate salivary urea and creatinine as noninvasive alternatives to serum for creatinine estimation in chronic kidney disease (CKD) patients.

Methods: Blood and saliva samples were collected from 112 CKD patients and 108 subjects without CKD for quantitative analysis of urea and creatinine. Spearman's correlation coefficients between salivary and serum urea as well as creatinine were obtained. Receiver operating characteristic analysis was done to assess the diagnostic tests of salivary urea and creatinine. Cut-off values were determined based on the best trade-off between the sensitivity and specificity for both salivary urea and creatinine.

Results: Salivary urea and creatinine concentrations were significantly higher in CKD patients than those in control subjects; and increased by the stages of the severity of the disease. The positive correlation was significantly found between salivary and serum creatinine ($r = 0.90$) and between salivary and serum urea ($r = 0.73$). Area under the curve for salivary urea was 0.76 and a cut-off value of 14.25 mmol/L gave a sensitivity of 82.9% and specificity of 57.8%. Area under the curve for salivary creatinine was 0.92 and a cut-off value of 0.24 mg/dL gave a sensitivity of 86.5% and specificity of 87.2%.

Conclusions: Both salivary urea and creatinine have a high capacity for serum creatinine estimation. Salivary urea and creatinine tests can be used as low-cost, easily accessible and noninvasive tools for screening, diagnosing, monitoring treatment outcomes and ascertaining prognosis of chronic kidney disease.

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Introduction

Chronic kidney disease (CKD) is the result of progressive and chronic deterioration of nephrons, usually irreversible decline of the glomerular filtration rate (GFR), leading to an increase of serum creatinine and blood urea nitrogen levels. Glomerular filtration rate is clinically measured by creatinine clearance (CrCl) that is an indirect value for evaluating the amount of functioning renal tissue [1]. Chronic kidney disease requires repeated serum analysis to diagnose the disease status and stages, monitor treatment outcomes and evaluate prognosis. Patients involved in the CKD management are at greater risk of blood borne diseases such as Hepatitis B and C [2,3]. Individuals in haemodialysis stage frequently related to blood loss because of blood sampling. In addition, collection of blood samples for serum analysis is an invasive procedure that can cause anxiety, discomfort and increase the risk of infectious diseases to the patients, especially in end stage renal failure [4]. For health care professionals, saliva based diagnostics is therefore more accessible, more accurate, less expensive, and presents less risk to the patient than using blood tests, which are more likely to result in exposure to HIV or hepatitis. Hence, non-invasive, simple, economic diagnostic tests that provide reliable evaluation of the

disease with minimal risks would be of worth to both the health professionals and the patients [5].

Saliva is a multiconstituent biologic fluid secreted by the major and numerous minor salivary glands located throughout the oral mucosa. Saliva containing high amount of disease biomarkers are being explored as a means of monitoring general health or early diagnosis of disease. The systemic diseases can cause the changes of saliva and in contrast, the changes of saliva can be a predictive sign of a systemic disease [6]. In other cases, systemic disorders induce salivary changes without any significant local effects. Many such changes are of high diagnostic interest because they can be rather specific to the causing conditions and can be used for screening and early diagnosis of several local and systemic disorders [7]. Many researchers suggest that saliva would be an easily accessible body fluid, non-invasive diagnostic means for variety of clinical settings. Saliva sampling is an appropriate method which can be repeated more frequently without discomfort to the patient. It is also easy to collect data, to store, to transport and considered as the most cost-effective surgical procedure for the screening of a disease in large populations [8–10].

It has been well documented about the effects of CKD to the oral health status in many researches. Over the past two decades, many cross-sectional studies as well as clinical trials have been carried out regarding the salivary changes and clarified manifestations of oral symptoms (both sclerenchyma and parenchyma) under the CKD influence [11–13]. Urea was found in saliva since 1951 that is a product of amino acid and protein catabolism [14]. It easily diffuses throughout biological membranes, with homogeneous distribution throughout the body's water. The normal salivary urea level is 12–70 mg/dL and resolution above this level does not significantly change during the day [15]. Salivary urea level is independent of saliva volume and detection of salivary urea can be done at any time during the day. There is concomitant increase urea level in saliva when it increases in the blood due to diffusion of nitrogenous waste into saliva [16]. Creatinine is a waste product of muscle metabolism that is primarily excreted by kidney. The normal range of serum creatinine is 0.6–1.5 mg/dL and salivary creatinine is 0.05–0.2 mg/dL. Creatinine is filtered at the glomerulus and excreted without reabsorption in the renal tubules and so its level in the blood is used as a value to evaluate renal function [17]. Saliva exhibits biochemical indices of renal function and many studies have well shown association between salivary and serum creatinine levels. Salivary urea and creatinine procedures can dramatically reduce anxiety and discomfort for patients. In both clinical and public health settings, they can be used as non-invasive, inexpensive and easily accessible diagnostic methods for screening patients in early stages of CKD especially in developing countries like Vietnam where health care professionals are limited.

This study was performed with the aims to compare the salivary urea and creatinine levels between CKD patients and non-CKD subjects, to examine the relationship between the urea and creatinine levels in serum and saliva; and to determine the diagnostic ability of salivary urea and creatinine tests as alternatives to serum creatinine for the estimation of Vietnamese patients with CKD.

Materials and methods

This cross-sectional and diagnostic test validation study was carried out in the Department of Nephrology and Endocrinology and the Department of General Internal Medicine under Gia Dinh People Hospital, Ho Chi Minh City, Vietnam on 111 patients diagnosed with CKD (CrCl <60 mL/min/1.73 m² skin) and 109 patients without CKD (CrCl of >90 mL/min/1.73 m² skin) according the standard of KDIGO [18,19]. The patients gave written informed consent to participate in the study and ethical approval of the study was obtained from University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam.

The blood samples and unstimulated saliva samples were obtained from each participant between 9:00 am and 11:00 am before lunch and at least two hours after breakfast to minimize the effect of diurnal variation. In patients undergoing haemodialysis, the sample was collected prior to dialysis. Two millilitres of blood was drawn from an antecubital

vein with minimal trauma for quantitative analysis of the concentration of serum urea and creatinine. The patients were instructed to refrain from eating, chewing, drinking and smoking at least 90 minutes before saliva collection; and thoroughly rinse mouth with deionized water prior to the collection and to void the mouth of saliva. They were asked to sit in a comfortable upright position with eyes open, head tilted slightly forward and lips closed. They were also instructed to avoid swallowing and oral movements during collection; to pool the saliva on the floor of the mouth and spit every 30 seconds into a sterile plastic cup. Salivary sample was preserved in an icebox and then immediately transported to the laboratory for quantitative analysis of the concentration value of salivary urea and creatinine. Salivary sample was taken about 10 µL, centrifuged at 4000 rpm and the concentration of those substances were determined using Beckman Coulter Chemistry Analyzers 5820 (2012).

Statistical analysis

Spearman's correlation coefficients were calculated to explore the association between urea and creatinine concentrations in saliva as well as those in serum. All analyses were conducted using SPSS 20.0 software (SPSS, Chicago, IL). A *p* value of <.05 was considered statistically significant. Linear regression equations were derived to estimate the serum level of creatinine as well as urea from its level in saliva. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic potential of salivary creatinine or urea compared to serum creatinine to find whether salivary creatinine levels or salivary urea levels can distinguish the diseased subjects (CKD) from nondiseased (non-CKD). The overall performance was assessed by the total area under the curve and the cut-off values were determined based on the best trade-off between the sensitivity and specificity.

Results

Characteristics of the subjects

Of the 210 patients enrolled in the study, there were 98 men (44.5%) and 122 women (55.5%). No difference in gender was found between the two groups (*p* > .05). The average age of the study subjects was 49.8 ± 15.7 years. All the patients in the CKD group (mean age: 51.3 ± 12.9 years) were older than the non-CKD group (mean age: 48.2 ± 18.0 years) but there was no significant difference in the groups by age (*p* = .140). Based on the GFR estimated using Cockcroft Gault formula, the number of CKD patients was 27, 41 and 43 in stage 3, 4, 5 of the disease, respectively.

In the saliva of CKD patients, urea and creatinine concentrations were 23.60 mmol/L and 0.40 mg/dL, respectively; higher than those in non-CKD patients (13.89 mmol/L and 0.14 mg/dL, respectively; *p* < .001). In the CKD group, the concentration of urea was lowest in stage 3 (15.85 mmol/L), increased in stage 4 (25.51 mmol/L) and was highest in patients with stage 5 (26.65 mmol/L) (*p* < .001). Accordingly, the concentrations of creatinine in the saliva was lowest in

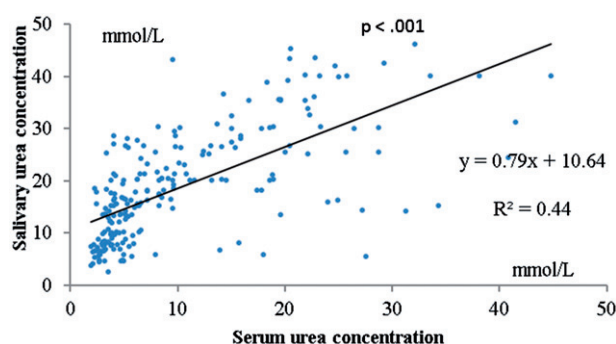


Figure 1. Correlation between salivary and serum urea in total sample.

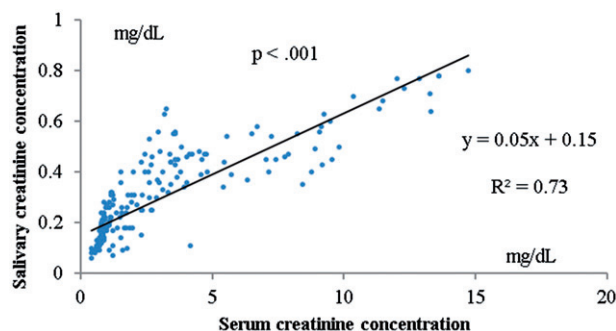


Figure 2. Correlation between salivary and serum creatinine in total sample.

patients with stage 3 (0.25 mg/dL), increased in patients with stage 4 (0.40 mg/dL), and lowest highest in patients with stage 5 (0.51 mg/dL) ($p < .001$).

Correlation between urea and creatinine in saliva with its serum

In total sample, the correlation between the urea or creatinine concentration in serum with its concentration in saliva is shown in Figures 1 and 2. The tight positive correlation was significantly found between serum and salivary urea concentrations ($r = 0.73$; $p < .001$) and between serum and salivary creatinine concentrations ($r = 0.90$, $p < .001$).

Assessing the diagnostic potential of salivary urea and creatinine

To evaluate the diagnostic potential of salivary urea compared to serum creatinine, that is to separate the groups into CKD group and controls, ROC analysis was performed (Figure 3). Total area under the curve was 0.76 ($p < .001$). Sensitivity and specificity for different values of salivary urea were established and cut-off value of 14.25 mmol/L was determined as this gave a best trade-off with sensitivity of 82.9% and specificity of 57.8%. That is, people with salivary urea values above 14.25 mmol/L are more likely to suffer from CKD. The other cut-off values with the sensitivity and specificity are presented in Table 1.

Similarly, to evaluate the diagnostic potential of salivary creatinine compared to serum creatinine, that is to separate the groups into CKD group and controls, ROC analysis was performed (Figure 4). Total area under the curve was 0.92

($p < .001$). Sensitivity and specificity for different values of salivary creatinine were established and a cut-off value of 0.24 mg/dL was determined as this gave a best trade-off with sensitivity of 86.5% and specificity of 87.2%. That is, people with salivary creatinine values above 0.24 mg/dL are more likely to suffer from CKD. The other cut-off values with the sensitivity and specificity are presented in Table 2.

Discussion

Our study examined the values of urea and creatinine measured in saliva, as useful methods to detect CKD. The strong positive correlations between salivary urea and creatinine concentrations and its serum allowed us to conclude that the salivary urea and creatinine tests can be used as noninvasive diagnostic tools with great utility to distinguish people with from those without CKD. This study also establishes the best salivary urea concentration cut-off value of 14.25 mmol/L and the best salivary creatinine concentration cut-off value of 0.24 mg/dL for detecting subjects with CrCl under 60 mL/min per 1.73 m², these values permit screening of CKD.

Findings in this study showed that the serum urea and creatinine levels were significantly higher in CKD patients compared to non-CKD subjects. These results are consistent with previous studies by Yajamanam et al. [9] and Lasisi et al. [16]. Davidovich et al. also reported that salivary creatinine and urea levels were higher than in the control group with a 2.5-fold increase in the dialysis group [20]. Our results indicated the levels of salivary urea and creatinine also increased by the severity of CKD condition. Tomás et al. also concluded the salivary urea concentrations increased significantly with the severity of the CKD condition [13]. A significant negative correlation was found between the GFR and the salivary urea concentration in a study by Davidovich et al. [20].

In our study, there was linear correlation between the salivary and serum urea as well as creatinine levels. This result is consistent with previous studies such as the research by Ahmed et al. [21], Lasisi et al. [16], Zúniga et al. [10], Venkatapathy et al. [22] and Yajamanam et al. [9]. Blood or serum components can get into saliva by either passive diffusion, active transport or by ultrafiltration of extracellular fluids induced by hydrostatic pressure through tight junctions between acinar cells [23]. Under normal circumstances, metabolic degradation of products such as urea, creatinine is excreted by the kidneys into the urine. In diseased kidneys, these compounds accumulate in the systemic circulation and get into saliva, either directly or are excreted by salivary glands [23]. In CKD patients, the highly increasing urea and creatinine levels in serum lead the disparity of the gradient concentration between serum and saliva thus increasing diffusion ability to saliva from serum [24].

In this study, there is strong correlation between serum and salivary creatinine level with the Spearman coefficient of 0.9. This correlation is lower than result of Yajamanam et al. ($r = 0.96$) [9], but higher than results of Bader et al. ($r = 0.58$) [25], Lasisi et al. ($r = 0.69$) [16], Venkatapathy et al. ($r = 0.73$) [22]. Although the results were different, they showed there was rather strong correlation between salivary

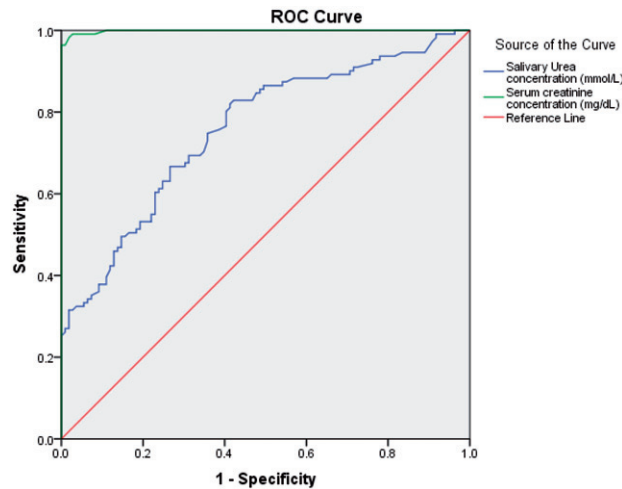


Figure 3. ROC curve for salivary urea levels. Total area under the curve is 0.76.

Table 1. Sensitivity and specificity analyses of salivary urea for different cut-off values considering serum creatinine as the gold standard.

Cut-off value (mmol/L)	Sensitivity (%)	Specificity (%)
14.25	82.9	57.8
8.25	89.2	31.2
24.40	45.9	87.2

Bold data indicate the cut-off value for salivary urea based on the best trade-off between sensitivity and specificity.

and serum creatinine. A positive correlation was observed between salivary and serum creatinine. However, salivary creatinine levels were lower when compared to serum in both patient and control group [9,22,25]. Creatinine enters saliva from blood by ultrafiltration. However, due to relative nonpolar nature of creatinine resulting in incomplete filtration, salivary levels are only 10–15% of serum levels [9]. Besides, creatinine is a large molecule (molecular radius of 3.2 Å) with high molecular weight (113 Da) and low lipid solubility. Due to these physical properties, creatinine molecule is unable to diffuse easily across the cells and the tight intercellular junction of the salivary gland under normal conditions. But in the diseased state, possibly there is an alteration in the permeability of the salivary gland cells and the increased serum creatinine levels in CKD patients create a concentration gradient that facilitates increased diffusion of creatinine from serum to saliva [22].

Similar to creatinine, urea enters by the process of passive diffusion from blood into saliva through acini of salivary glands [9]. Our findings show there is a strong positive correlation between blood and salivary urea levels. This result is consistent with previous studies [9,10,16,21]. Lasisi et al. carried out a case control study, involving 50 patients with late stage CKD and 49 healthy individuals as control in Nigeria. In Lasisi's study, there was positive correlation between blood and salivary creatinine as well as urea levels [16]. The results in this study and previous studies about the positive correlation between the serum and salivary urea and creatinine concentrations, suggested that analysis of salivary creatinine and urea in CKD patients reflects their levels in blood, and that the salivary concentration of urea and creatinine could be useful in screening, diagnosis, monitoring the CKD.

Many authors suggested to use saliva as a noninvasive alternative to serum for screening, diagnosing and monitoring the efficacy of CKD treatment [13,16,22]. While this is a new aspect, promising to bring many benefits for the CKD patients are taken blood sampling regularly. Salivary tests are non-invasive methods that the patient can self-sample with the instruction of medical staffs, have low cost and can be repeated easily. Besides, salivary tests have the potential to dramatically reduce anxiety and discomfort associated with blood sampling procedures in patients and this increases their willingness to have frequent health inspections in order to greatly increase the opportunity to early detection of disease. Sampling saliva instead of blood is suitable for all age groups and also reduces the infectious risks to both patients and medical staffs. However, salivary composition is under the influence of the method of collection, hydration status, body posture, lighting, circadian rhythm, seasonal variations, gland size, smoking, stimulants and medications. The positive correlations between saliva and serum urea as well as creatinine in this study could be explained by the increased concentration of serum creatinine and urea in patients with CKD which creates an increased concentration gradient which in turn increases the diffusion of urea and creatinine from serum into saliva leading to xerostomia, halitosis or uremic breath.

Before a salivary diagnostic test can replace a more conventional one, the diagnostic value of a new salivary test has to be compared with accepted diagnostic methods. The accuracy of this new test depends on how well it separates the group being tested into those with and without the disease. Sensitivity and specificity are the basic measures of the accuracy of a diagnostic test [22]. So, to ascertain the diagnostic potential of saliva as an alternative, ROC analysis was performed. Accuracy is measured by the area under the ROC curve (area under curve) and golden standard is the serum creatinine concentration (area under curve with serum creatinine approximately equal 1). Our findings show total area under the curve for salivary creatinine and urea was 0.92 and 0.76, respectively. These findings suggest that analysis of salivary creatinine and urea in patients with CKD reflects their levels in blood. Therefore, salivary urea and creatine tests

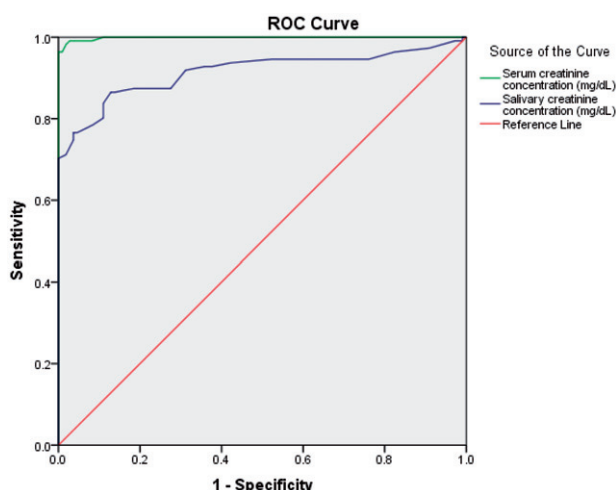


Figure 4. ROC curve for salivary creatinine levels. Total area under the curve is 0.92.

Table 2. Sensitivity and specificity analyses of salivary creatinine for different cut-off values considering serum creatinine as the gold standard.

Cut-off value (mg/dL)	Sensitivity (%)	Specificity (%)
0.23	87.4	81.7
0.24	86.5	87.2
0.30	76.6	96.3

Bold data indicate the cut-off value for salivary creatinine based on the best trade-off between sensitivity and specificity.

could be the best alternatives for the blood based diagnostic tools for CKD. Our results are in agreement with those reported by Yajamanam et al. [9] and Lasisi et al. [16].

In this study, ROC curve test analysis showed that the best cut off points for salivary urea and creatine were 14.25 mmol/L and 0.24 mg/dL, respectively, because they had the highest upper left extreme points of the curve. The ROC analysis of salivary creatinine and urea in our study showed a good accuracy with good sensitivity and specificity. With salivary creatinine, a cut-off value of 0.24 mg/dL gave a sensitivity of 86.5% and specificity of 87.2%. With salivary urea, a cut-off value of 14.25 mmol/L gave a sensitivity of 82.9% and specificity of 57.8%. It implies that individuals with salivary creatinine above 0.24mg/dL or salivary urea above 14.25 mmol/L are more likely to suffer from CKD and should be subjected for further medical evaluation for appropriate management. In the study of Venkatapathy et al. [22], with salivary creatinine, a cut-off value of 0.20 mg/dL gave sensitivity of 97.1% and specificity of 86.5%. Yajamanam et al. [9] reported a cut-off value of $> 14.6 \mu\text{mol/L}$ for salivary creatinine (100% sensitivity and 98.3% specificity) and $> 6 \text{ mmol/L}$ for salivary urea (96.7% sensitivity and 90% specificity) be useful to identify patients with CKD. In study of Lasisi et al., cut-off value for salivary creatinine and urea were 0.55 mg/dL and 27.50 mg/dL, respectively which gave sensitivity and specificity of 94.0% and 85.0% for creatinine; as well as 86.0% and 93.0% for urea [16]. Different cut-off values in the researches may be due to differences in the characteristics of study subjects as well as various CKD stages selected in each study. However, in agreement with most of the previous authors, our study also suggested that the saliva can be used

as an alternative diagnostic medium for estimating serum creatinine in CKD patients.

The present study had some limitations. A cross-sectional design and convenience sample in the hospital were used, thus the sample might not be representative of the general population. For further population based researches, using a representative sample would be needed to confirm the current findings. However, this study provided the salivary urea and creatinine values in which best estimate for the serum creatinine to diagnose CKD was provided. Our findings supported using salivary urea and creatinine samples are promising procedures for screening, diagnosis, monitor treatment outcomes and ascertain prognosis of CKD especially in public settings, where access to clinical diagnostic facilities and testings, and the number of available personnel as well, are limited.

Conclusions

The sensitivity, specificity and AUC of saliva urea and creatinine are comparable to serum creatinine. Salivary urea and creatinine tests can be used as simple, low-cost, easily accessible and noninvasive tools for screening, diagnosing, monitoring treatment outcomes and ascertaining prognosis of CKD.

Disclosure statement

The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

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