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CASE STUDY

CLINICAL SIGNIFICANCE OF SALIVARY UREAS AS AN ALTERNATIVE DIAGNOSTIC AID IN CHRONIC KIDNEY DISEASE PATIENTS

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ABSTRACT

Context: For most of us be young or old, the fear of needle prick for blood tests was more painful than actually getting it done. Suppose that had to be done very often, the level of anxiety and misery it could cause to the patients was no doubt immense. How would it be if the same parameters could be tested in a non-invasive way? Hence a study to see if it is possible to use easily accessible saliva as an alternative to serum was conducted.

Aim: To compare and correlate the serum and salivary urea levels in Chronic Kidney Disease (CKD) patients and to validate the role of salivary urea (SaU) as an alternative to Serum urea in these patients.

Study Design: Case-control study

Methods: Blood and saliva samples from 105 chronic kidney disease patients and 37 healthy volunteers were collected and centrifuged. Urea concentration was estimated in the serum and salivary supernatant on the same day using automatic analyser.

Statistical Analysis: The serum and salivary urea levels between controls and cases were compared using t test. Correlation between serum and salivary urea was done using Pearson correlation coefficient. The diagnostic performance of salivary Urea was done by Receiver operating characteristic (ROC) analysis. Cut off values were established for salivary Urea.

Results: Serum and salivary Urea levels were significantly higher in chronic kidney disease patients than healthy volunteers.

Conclusion: Saliva is a promising non-invasive alternative to serum for urea estimation.

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INTRODUCTION

One devastating medical, social, and economic problem frequently necessitating serum analysis is Chronic Kidney Disease (CKD). It is progressive decline in renal function over a period of months or years, the incidence of which has almost doubled in the last 15 years. The adverse outcomes of CKD such as kidney failure, cardiovascular disease, and premature death can be prevented or delayed if earlier stages of chronic kidney disease can be detected through laboratory testing [1].

Urea is an end product of protein metabolism and is excreted primarily through the kidneys. Serum urea is frequently monitored to assess the physiologic state, check morbidity initiation and progression and also to check post treatment

outcome in CKD patients. With impairment of renal function, its concentration in blood raises, resulting in a state of intoxication called uremia and is responsible for the various clinical manifestations noted in practically all body organs and systems [2].

Blood sampling is invasive and is associated with many risks. Like blood, saliva is a complex fluid secreted by salivary glands. With the emergence of more sophisticated techniques coupled with easy and non-invasive method of collection, saliva as a diagnostic tool has evolved to monitor essential features of overall health[3]. The greatest challenge of salivary diagnostics is to establish its usefulness in terms of sensitivity and specificity against the already established conventional methods. Urea is a normal constituent of saliva [4].The diagnostic value of salivary urea instead of serum urea in clinical practice is still unclear. With this background this study was undertaken to assess the potential role of Salivary urea (SaU) as an alternative to Serum urea in CKD patients.

Aims & Objectives

To compare and correlate the serum and salivary urea levels in CKD patients and to validate the role of salivary urea (SaU) as an alternative to Serum urea in these patients.

MATERIALS AND METHODS

Institutes' ethical committee clearance for the study was obtained. The case group comprised of 105 known cases of CKD who were under medical management with or without dialysis. The CKD patients were found to be in Stage 4 and stage 5 based on the Glomerular filtrate rate (GFR) obtained using Cockcroft Gault formula. The control group comprised of 37 healthy individuals, age & gender matched, with absence of any chronic illness in the recent past. Patients suffering from any systemic disease or under any medication that could affect GFR and/or salivary characteristics were excluded from the study.

The purpose of the study was explained to all the participants and a written informed consent was obtained. Under aseptic conditions 2 ml of blood from ante-cubital vein was drawn. Participants rinsed their mouth with distilled or deionised water and were refrained from eating and drinking one hour prior to saliva collection. Two ml of unstimulated whole saliva was obtained by spitting method under restful conditions [5]. Sample collection was done in the forenoon to avoid diurnal variation. In dialysis patients samples were obtained before the procedure.

Serum and salivary supernatant were obtained by centrifugation of samples collected at 3000rpm for 10 minutes. Then Urea levels were estimated using automatic analyser (EM360 chemistry analyser with ISE module) using urea estimation kit (Autospan, Span diagnostics limited) by Modified Berthelot method. The estimated Serum and salivary urea levels were subjected to descriptive, comparative, correlation, Regression and ROC analysis using SPSSv17.0.

Statistical Analysis

Study Population

Among the 37 healthy volunteers (control) 21 were males and 16 were females. The age range of healthy volunteers was 19 to 68 years with a mean age of 44.5 years [Standard deviation (SD) 14.97]. Among the 105 CKD patients (cases) 84 were males and 21 were females. Their age range was 19.0 to 70 years with a mean age of 47.5 years [SD 15.2]. The estimated GFR showed the patients to be in stage 4 (n=38) and stage 5(n=67) CKD. This is because patients referred to nephrology department were majority in late stages of CKD. Out of the 67 stage 5 patients 22 were on haemodialysis and 10 on peritoneal dialysis.

Descriptive statistics

The mean serum urea in the control group was 20.19 mg/dl [SD 3.38] and values ranged between 15.0 mg/dl and 28.0 mg/dl. The mean salivary urea in the control group was 30.38 mg/dl [SD 15.48] with range between 13.0 mg/dl and 92.0 mg/dl. The mean serum urea level in the case group was 90.18 mg/dl [SD 40.72]. The values ranged between 24.0 mg/dl and 305 mg/dl. The mean salivary urea level in the case group was 109.32mg/dl [SD 50.50]. The values ranged between 28.0 mg/dl and 352 mg/dl.

Comparative Analysis

The serum and salivary values were compared within the two groups using paired t test and found that salivary urea was significantly higher than the serum levels in both the groups ($p<0.01$) [Table 1]. The salivary values were compared between the two groups using Student t test. The result showed that like serum, salivary levels were also significantly higher in CKD patients compared to healthy individuals ($p<0.01$) [Table 2].

Correlation analysis

A Correlation analysis was performed using Pearson correlation coefficient. A statistically significant $r= 0.3$ ($p<0.05$) was obtained between the serum and salivary urea levels in controls. In case group a highly significant correlation $r=0.8$ ($p<0.01$) was obtained [Table 3].

Regression analysis

Using a linear regression analysis regression equations were derived to estimate the serum urea levels (y) from the salivary values (x) [Table 3]. The regression equation $y = 18.028 + 0.071x$ was obtained in controls ($p<0.05$) [Figure 1a] and $y = 19.68 + 0.646x$ was obtained in CKD patients ($p<0.01$) [Figure 1b].

ROC analysis

The diagnostic potential of saliva was ascertained by a ROC analysis [Figure 2]. The total area under the curve for serum urea was 0.999 ($p<0.01$) and for salivary urea it was found to be 0.981 ($p<0.01$) [Table 4]. Using Youden Index an optimal cut off value for salivary urea was found to be 56mg/dl with sensitivity 94.29%% and specificity 97.30%.

RESULTS

Table 1 Intra group comparison of serum and salivary urea levels in group 1 (control) and group 2 (cases) using paired t test

Group	Urea (mg/dl)	Mean	N	SD	Mean difference	t-value	p-value
Group 1 (Controls)	Serum	20.19	37	3.38	-10.19	-4.21	0.00**
	Saliva	30.38	37	15.48			
Group 2 (Cases)	Serum	90.18	105	40.72	-19.14	-6.47	0.00**
	Saliva	**109.32**	**105**	**50.50**			

** denotes statistically highly significant

Table 2 Comparison of serum and salivary urea levels between group 1 (control) and group 2 (cases) using Student's t test

	Group	N	Mean	SD	SE Mean	t-value	p-value
Serum (mg/dl)	1 (control)	37	20.19	3.38	0.556	17.443	<0.001**
	2 (cases)	105	90.18	40.72	3.974		
Saliva (mg/dl)	1 (control)	37	30.38	15.48	2.544	14.234	<0.001**
	2 (cases)	105	109.32	50.50	4.928		

** denotes statistically highly significant

Table 3 Table showing Correlation and Linear regression analysis between serum and salivary urea for group 1(controls) and group 2 (cases)

Group	Correlation (r)	Constant (b)	Regression Coefficient (a)	Regression equation Y = ax + b	P value
Group 1 (Controls)	0.3*	18.028	0.071	Y=18.028 + 0.071x	<0.05
Group 2 (Cases)	0.8**	19.68	0.646	Y=19.68 + 0.646 x	<0.001

* Correlation significant at 0.05 level (2-tailed)
**Correlation significant at 0.01 level (2-tailed)

Table 4 Table showing area under the ROC curve

Urea (mg/dl)	Area	Std. Error	Asymptotic 95% Confidence Interval		Asymptotic Sig.
			Lower Bound	Upper Bound	
Serum	0.999	0.002	.995	1.000	<0.001**
Saliva	0.981	0.011	.958	1.000	<0.001**

** denotes statistically highly significant

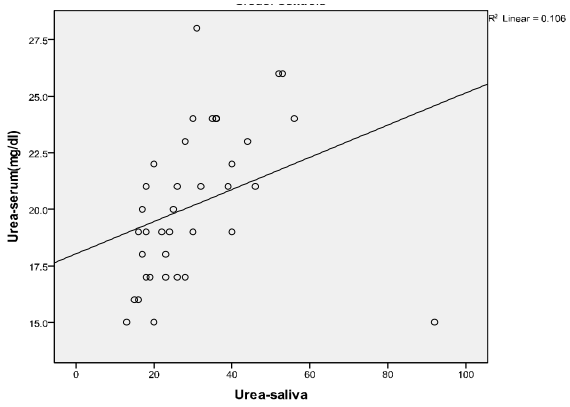


Figure 1(a) Scatter diagram showing linear correlation between salivary serum and urea levels among group 1 (controls) n=37

Linear regression equation

Y (Serum urea) = 18.028 + (0.071) x (Salivary urea) [Sig = 0.049, R^2-=0.106]

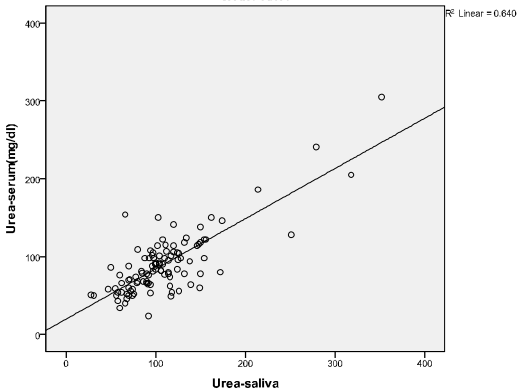


Figure 1(b) Scatter diagram showing linear correlation between salivary and serum urea (mg/dl) levels among group 2 (cases) n=105

Linear regression equation

Y (Serum urea) = 19.68 + (0.646) x (Salivary urea) [Sig = <0.001, R^2-=0.640]

Group	N
Positive(cases)	105
Negative (control)	37

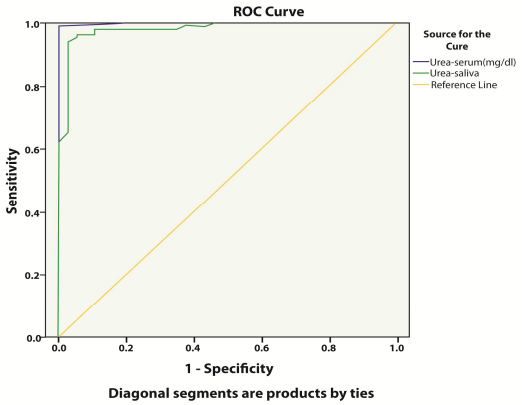


Figure 2 Receiver Operating Curve for serum and salivary urea levels (n= 142)

DISCUSSION

Urea is 60 Da water soluble organic compound. It is the major nitrogen containing metabolic product of protein catabolism in humans [2]. It is produced in the liver and more than 90% of the urea is excreted through the kidneys. Thus in CKD there is significant elevation of serum urea levels. The normal serum urea concentration is 15 - 40 mg/dl. The normal salivary urea level is 12-70 mg/dl [4]. The results of our study were also within this range. In CKD patients the serum levels were significantly elevated compared to the controls [Table 2]. This is due to the fact that kidneys are unable to clear the body of excess urea and hence the higher serum urea level.

It was also observed that the salivary urea levels of the cases were significantly higher than the controls [Table 2]. This may be because of increased serum urea which creates an increased concentration gradient which in turn increases the diffusion of urea from serum to saliva, hence accounting for the increased salivary urea levels observed in cases. Similar results were obtained by Ivanoski *et al* [6], Xia *et al* [7], and Davidovich *et al* [8].The comparative analysis showed a significantly high mean salivary urea levels compared to mean serum levels in both the case and controls [Table 1]. Similar observation was also made by Obry *et al* [9]. Correlation analysis was performed to know if changes in serum urea are accompanied by changes in salivary urea [Table 3]. The controls showed a significant ($p<0.05$) but low positive correlation ($r_= 0.3$) [Figure 1 a] unlike Xia *et al* (r=0.9) [7] who obtained a high positive correlation in controls. In CKD patients a significant ($p<0.01$) high positive correlation ($r_= 0.8$) [Figure 1b] was obtained similar to Xia *et al*[7].

As a next step, a regression analysis was performed to find the functional relationship between serum and salivary urea, considering saliva as an independent variable and serum urea as a dependent variable. Regression equations were drawn for both the groups to estimate the serum urea (y) from the values of salivary urea (x) [Table 3].

The diagnostic performance of salivary urea as an alternative to serum urea was assessed by ROC analysis [Figure 2]. That is how well salivary urea can identify the group being tested into those with and without CKD. The total area under the curve obtained was 0.999 for serum urea and 0.981 for salivary urea [Table 4]. The higher area under the curve obtained for salivary urea suggests that salivary urea is good diagnostic alternative to discriminate CKD patients from healthy individuals. A cut of value of 56 mg/dl provided the best trade-off between sensitivity and specificity. That is people with salivary urea values more than 56 mg/dl are more likely to suffer from uremia and should be appropriately investigated for early diagnosis and intervention of CKD.

The higher salivary urea levels obtained compared to serum urea [Table 1] suggest that in addition to passive diffusion several other mechanisms may play a role in increasing the salivary urea concentration against the concentration gradient. Hence a discussion on various potential mechanisms and possibilities are included here. One possible mechanism is the active transport which is a carrier mediated transport through membrane against a concentration gradient and requires metabolic energy. It shows high degree of specificity and here structure, conformation, size and charge are important in determining penetration [10]. Dix *et al* [11] in their study have proven the localization of UT-B urea transporters in the ductal system of the bovine parotid salivary gland, thus suggesting a possibility of ductal secretion of urea which contributes to higher salivary urea concentration than serum.

Sodium dependent secondary active transporters for urea have been established in IMCD of kidneys [12]. It is possible that such secondary active transporters are also present in salivary glands which help to transfer more urea from serum to saliva. However this is only a hypothesis and warrants further study.

It is found that there is an up regulation of urea transporters in pathologic conditions[12] and it is hypothesized that there is an increase in urea transporters in salivary glands also under pathologic condition like CKD and this may account for the increased salivary urea levels observed in CKD patients compared to controls. Another possible mechanism is ultrafiltration, a paracellular pathway in which small polar molecules enter tight junction between cells. The molecular weight cut off for ultrafiltration is 100-200 [13]. The molecular weight of urea is only 60 Daltons hence it is possible that urea can reach saliva by ultrafiltration [14].

Ivanoski *et al*[6] found a significantly higher values of salivary urea in diabetics, suggested that the increased concentration of urea in saliva was due to increased permeability of the acinar cells in the parotid gland in diabetics that allowed enhanced ultrafiltration of blood. It is possible that in CKD patients also (diabetes being a leading cause) there is an altered permeability of salivary gland and increased ultrafiltration occurring which contributes to the higher concentration of urea in CKD patients. Urea also reaches oral cavity through gingival crevicular fluid (GCF). Higher concentration of urea in GCF compared to plasma can be due to the fact that passage of water is slower than that of very diffusible urea or that water is reabsorbed during passage through the gingival tissue [15]. Urea from GCF can also contribute for increased urea concentration in whole saliva than serum. Martin *et al* [16] in their study have shown that the salivary glands are permeable to urea with sympathetic and parasympathetic stimulation. Substances with low solubility in lipids and with a molecular radius up to 3.4A° could permeate through the parotid gland on parasympathetic stimulation. They also found that epinephrine/ nor epinephrine/ electrical stimulation of the cervical sympathetic trunk increases the free diffusion of molecules (with molecular radius up to 5.7 A° and molecular weight 1450). It also increases the permeability of the ductal system permitting molecules up to 25A° diameter to penetrate. About two-thirds of the diffusional area for urea has been shown to be in the duct system. Urea being a small molecule must be able to appear in saliva in increased concentration with sympathetic or parasympathetic stimulation. The increased salivary urea also suggests a possibility that salivary glands may serves as an attempted alternative route of excretion (this may be significantly less when compared to kidney) when there is gross destruction of the nephrons which normally does this function [6]. Thus it is hypothesized that in CKD, with failure of primary excretory organ the kidneys, body is attempting to excrete metabolic waste urea through what all available routes like saliva which are otherwise insignificant in nature. The other possible routes in addition to saliva are sweat, tears etc. Further studies are warranted comparing the levels of urea in saliva, sweat, urine, and tears to arrive at a conclusion as whether the concentration of urea is increased in all other possible routes other than urine thus serving as an excretory route.

The potential to assess the serum levels, along with the non-invasive nature of salivary diagnostics, will be a motivating factor for all age groups to undergo frequent health checkup and thus help in early diagnosis and intervention for the patients. Also it is to be noted that a repeat sample if required can be easily obtained without causing much anxiety to the patients. The laboratory personnel will also be spared of the risk of accidental needle prick injuries.

CONCLUSION

Thus in this study, a positive correlation was obtained between serum and salivary urea levels in CKD patients and controls. The large area under the curve obtained in ROC analysis further suggest that saliva can be used as an alternative to serum urea in CKD patients.

Our study concludes that salivary urea correlates well with serum urea in chronic renal failure patients and is a promising body fluid to be used as an alternative to serum. To bring it in to a clinical reality further studies are warranted checking its reliability. These studies will undoubtedly remain a major focus of investigation in the future.

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