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TO EVALUATE THE EFFECTIVENESS OF SALIVARY UREA & CREATININE LEVELS AS A DIAGNOSTIC MARKER FOR CHRONIC KIDNEY DISEASE

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ABSTRACT:

Background: Chronic kidney disease (CKD) is defined as kidney failure with the organ functions reduced or lost completely and the kidneys are incapable of filtering excess fluids. CKD leads to many metabolic changes in blood that often warrants frequent biochemical analysis. Estimation in blood is an invasive and worrisome procedure. It would be quite easier if a noninvasive alternative to blood analysis is identified. Saliva can be collected noninvasively, repeatedly, and without the help of healthcare personnel.

Objective: This study analyses saliva as a medium to measure urea and creatinine levels in Chronic kidney disease(CKD) patients.The main objective of this study was to compare serum and salivary urea and creatinine levels in CKD patients and healthy controls, and to decide whether salivary creatinine and urea levels can be used to diagnose or monitor CKD in as efficiently as serum creatinine and urea levels.

Material & method: This study included 60 Chronic kidney disease(CKD) patients & 60 age matched healthy controls from Varun Arjun Medical college & Rohilkhand Hospital,Banthra, Shahjahanpur(U.P). Urea estimation in blood was carried out using system pack kit of transasia company using urease GLDH method. Creatinine levels was measured using enzymatic reaction colorimetric method done on ERBA-EM200 Transasia fully autoanalyzer. Unstimulated saliva along with blood sample were collected. Estimation of salivary urea & creatinine was done using the same method as discussed above.

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Result: In CKD patients , the mean salivary creatinine level was 1.51±0.45 mg/dL and the mean salivary urea level was 67.33±21.85 mg/dL, versus 0.21±0.1mg/dL and 16.4±6.7 mg/dL respectively, in the control group. Serum urea and creatinine, and salivary urea & creatinine were significantly higher in the CKD patients than in the controls ($p<.05$). There was a positive correlation seen between serum and salivary creatinine and serum & salivary urea also.

Conclusion: Based on the results of our study, we propose that salivary urea and creatinine has a strong potential to be used as a marker in diagnosis and monitoring of chronic kidney disease patients.

Keywords: Salivary, urea, creatinine

INTRODUCTION:

Chronic kidney disease is defined as increased levels of blood creatinine and serum urea, along with hematological, electrolyte, endocrine and skeletal disorders which leads to accumulation of metabolic waste products and thereby causing multiorgan involvement**(1)(2)**.The use of blood sample in order to determine the levels is still the gold standard method. Saliva due to its availability and non-invasive method of collection, it is gaining wide momentum in current times to be considered as a diagnostic tool for diagnosing chronic kidney disease**(3)(4)**.

Salivary levels can indicate any changes in urea and creatinine levels which are the usually done through blood sample collection in chronic kidney disease patients. Thus saliva may play an important role as a diagnostic tool in diagnosing patients with chronic renal disease. Chronic kidney disease occurs when the function of the kidneys is impaired towards 5--10% of the original capacity, defined by a reduction in glomerular filtration rate and decreased creatinine clearance rate**(5)**. Saliva is a dilute fluid, over 99% being made up of water and the rest are dissolved solids i.e. organic and inorganic constituents**(6)**.

In our country number of CKD patients are increasing rapidly as reflected by rising elderly populations and rising number of patients with hypertension and diabetes. Biochemical markers has a significant role in making proper diagnosis and in assessing risk and modulating treatment to improve clinical outcome. Blood sample collected for biochemical estimation is an invasive procedure and the patient has to repeatedly give sample making it very cumbersome for the patient as compared to saliva which is secreted by salivary gland and is easy to collect and non-invasive also. The main objective of our study is to measure urea and creatinine level both in saliva & serum and to evaluate the effectiveness of saliva as a diagnostic method for diagnosing chronic kidney disease.

MATERIAL & METHOD:

This cross-sectional study was carried out in 60 patients of chronic kidney disease who were undergoing treatment in the hemodialysis unit of Varun Arjun Medical College & Rohilkhand Hospital. Sample collection of saliva & blood was carried out by Biochemistry department of our hospital. Total of 60 chronic kidney disease patients

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aged > 18 years were selected over a period from March 2022 to November 2022 who were undergoing hemodialysis at our institution, similar age matched healthy controls were also selected.

Inclusion criteria:

(i) Chronic kidney disease patients undergoing hemodialysis
(ii) Age > 18 years

Exclusion criteria:

(i) Any systemic disease(Diabetes mellitus, any endocrine disorder)
(ii) Taking any drug which could affect saliva quality
(iii) Alcoholics & smokers

Sample collection:

On the basis of inclusion & exclusion criteria patients were selected who were undergoing hemodialysis. 3mL of blood sample was collected and immediately transferred to plain vial and was allowed to clot for 20 minutes and later centrifuged at 5000 rpm for 10 minutes. Serum sample obtained was used for estimating urea & creatinine levels. For salivary sample early morning unstimulated saliva was collected in a sterile tube by spitting method. Both the samples collected were immediately sent to biochemistry laboratory and was analyzed on the same day.

For urea & creatinine estimation both in saliva and serum, system pack kits were used provided by Transasia all tests were done on EM200 fully autoanalyser. Serum urea was estimated by Urease glutamate dehydrogenase(GLDH) method while creatinine estimation was done by enzymatic kit method. All test values were validated by controls provided by the manufacturer as well.

Statistical analysis:

All test values were expressed as Mean $\pm$ SD. Student's t-test was used for statistical analysis and p value <0.05 were considered significant. Correlation between salivary & serum creatinine as well as urea was done by pearson's correlation test.

RESULT:

The study population consists a total of 120 individuals among which 60 patients were suffering from CKD and 60 were healthy controls. One group consists of 60 CKD patients. There were 34 males and 26 females. The mean age of this group (CKD) was 34.3 years with a standard deviation of 10.1. The other group comprised 60 healthy volunteers as controls. There were 29 males and 31 females. The mean age of the controls was 32.4 years with a standard deviation of 12.2(Table I). In CKD patients the serum urea level shows mean of 155.57 mg/dL (SD 55.26) and salivary urea level with a mean of 67.33mg/dL (SD 21.85). Both serum urea and salivary urea shows positive correlation($r = 0.79$).(Table II). In CKD patients the serum creatinine level shows mean of 11.76 mg/dL (SD 3.63) and salivary creatinine level with a mean of 1.51 mg/dL (SD 0.45).(Table III).

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The mean salivary and serum creatinine & urea concentration were found to be significantly higher in CKD patients compared to controls & is statistically significant. If we want to consider salivary urea and creatinine as a reliable substitute to serum urea & creatinine respectively, then a strong correlation needs to be established between these two parameters.

To assess any correlation between salivary and serum creatinine and urea values we performed pearsons correlation analysis of CKD group correlating values of creatinine in serum & saliva and we found a significant positive correlation , r = 0.55. (Figure I,II).On correlating urea level in serum and saliva in CKD patients, we here also found a significant positive correlation, r = 0.79. (Figure III,IV). By seeing these findings we can say that as urea & creatinine increase or decrease in blood, there is corresponding increase or decrease in salivary urea & creatinine respectively.

TABLE 1: Age & gender wise distribution of CKD patients & Controls

	Chronic kidney disease	**Controls**
N(no. of individuals)	60	60
Age	34.3± 10.1	32.4± 12.2
Male	34	29
Female	26	31

TABLE 2: Comparision of Salivary urea & Serum urea levels between CKD patients and Controls.

Test	**No.of participants**	**Chronic kidney disease (Mean± SD)**	**Controls (Mean±SD)**	**p value**	**r value**
Salivary urea	60	67.33 ± 21.85	16.4 ± 6.7	<0.001	0.79
Serum urea	60	155.57 ± 55.26	29.1 ± 6.4	<0.001	

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TABLE 3: Comparision of Serum creatinine & Salivary creatinine levels between CKD patients and Controls.

Test	No.of participants	Chronic kidney disease (Mean± SD)	Controls (Mean±SD)	p value	r value
Salivary creatinine	60	1.51 ± 0.45	0.21 ± 0.1	<0.001	0.55
Serum creatinine	60	11.76 ± 3.63	0.71 ± 0.4	<0.001	

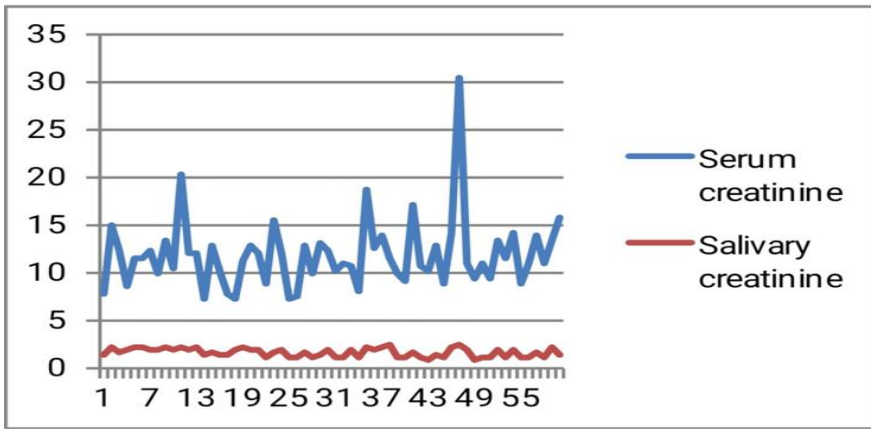

Figure. 1 Correlation between salivary & serum creatinine in CKD patients

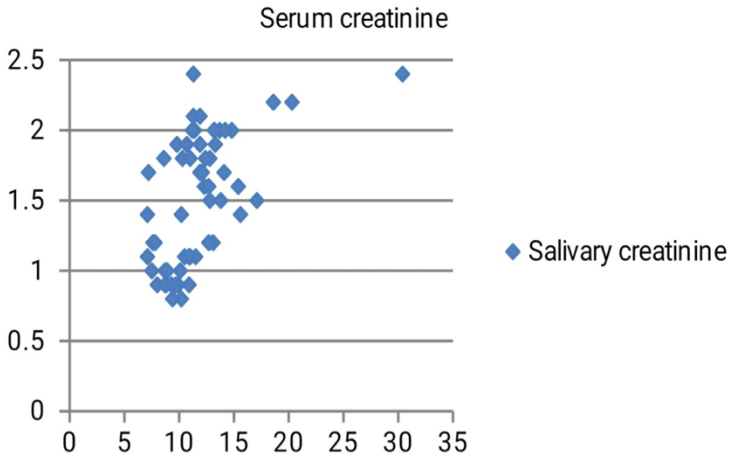

Fig. 2 Scatter plot between serum and salivary creatinine level of CKD patients

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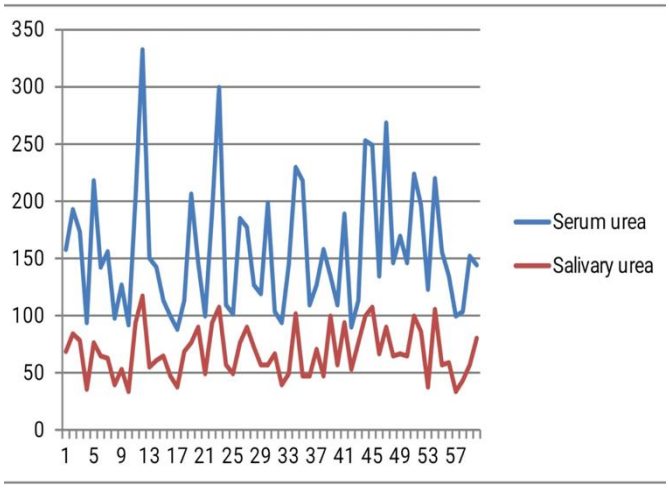

Figure. 3 Correlation between salivary & serum urea in CKD patients

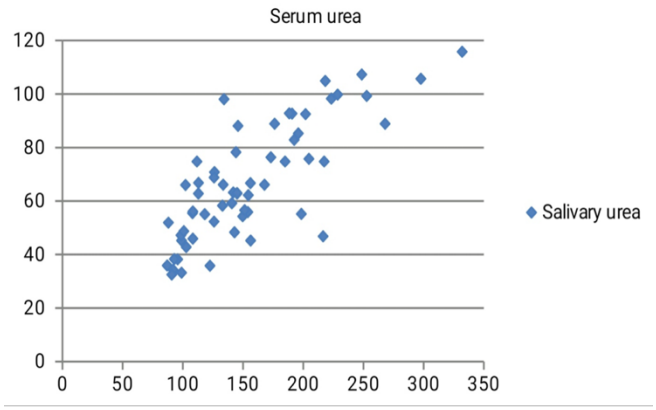

Figure. 4 Scatter plot between salivary & serum urea level of CKD patients

DISCUSSION:

In our study, patients with CKD showed increased levels of salivary urea and creatinine when compared with healthy individuals, also the salivary levels of creatinine and urea showed positive correlation with the levels in blood. Our findings are consistent with previous studies**(7)(8)** which also supports possibility of using salivary estimation of urea & creatinine as a marker for diagnosis of CKD. Kidneys regulate the volume of the extracellular and intracellular fluid to maintain homeostasis of the body by constant processing of the plasma through filtration, reabsorption and secretion of the substances**(9)**. Whenever there is an increase in the blood urea there is simultaneous increase in salivary urea also because the kidneys are not able to excrete urea in the

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renal failure and its concentration in blood increases with increased concentration in saliva because of increased serum urea which creates an increased concentration gradient in turn increasing the diffusion of urea from serum to saliva**(10)**.Creatinine is unable to diffuse easily across the cells of the salivary gland in the healthy state under normal conditions. But in patients of CKD, creatinine value increases in saliva possibly due to an alteration in the permeability of salivary gland cells and the high serum creatinine levels in CKD patients create a concentration gradient that facilitates diffusion of creatinine from serum in to saliva.

Our study showed a significantly high creatinine and urea level both in serum and saliva of CKD patients when compared with controls. Similar observation was made by Davidovich et al**(11)** and Xia et al**(12)**. The positive correlation between serum and salivary urea & creatinine observed in this study could be explained by the increased concentration of creatinine and urea in patients with CKD which creates a concentration gradient that facilitates increased diffusion of creatinine and urea from serum into saliva**(13)**. Blicharz**(14)** and colleagues suggested that, measurement of biomarkers in saliva may be an effective & alternative method for monitoring & diagnosing CKD patients. Monitoring of markers in saliva instead of serum is advantageous because saliva collection is noninvasive, simple and inexpensive approach with minimal risk of infection.

Our study supported that there was a significant linear relationship between serum urea and creatinine and salivary urea and creatinine levels respectively. The correlation coefficient for serum urea and salivary urea was 'r' = 0.79 and for serum creatinine and salivary creatinine was 'r'= 0.55 which is statistically highly significant ($p < 0.001$). Therefore, salivary creatinine and urea levels correlate well with the serum creatinine and urea respectively so that saliva can be used as a non-invasive diagnostic tool**(15)**. Lloyd JE et al.**(3) also** conducted a study on 26 renal disease patients and 23 healthy volunteers and found a statistically significant relationship between salivary and serum creatinine concentrations for the patients and salivary creatinine concentrations are 10-15% of those in blood. Similar results were obtained by Cardoso EML et al.**(16)** concluding that salivary urea estimation is a harmless and useful diagnostic tool. Venkatapathy R et al.**(17)** and Seethalakshmi C et al.**(18)** also showed a significant positive correlation for serum urea & salivary urea and also for serum & salivary creatinine. Tomas I et. al **(19)** in 2008, analysed the alterations of saliva in different stages of chronic renal failure (CRF) and statistically significant ($p < 0.01$) positive correlation was seen between blood and salivary urea concentrations ($r = 0.572$) in chronic renal failure patients and a statistically significant ($p < 0.01$) positive correlation was detected between the serum and salivary creatinine concentrations ($r = 0.40$). Lasisi TJ. et al **(20)** conducted a similar study in 2016 estimating urea and creatinine levels in blood and in whole unstimulated saliva for patients with end-stage CKD and healthy controls and established a positive correlation between blood and salivary creatinine as well as urea levels in patients undergoing dialysis treatment.

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CONCLUSION:

On the basis of our findings, we can say that saliva has a strong potential to be used as an alternative to blood for monitoring and also for diagnosing patients with Chronic kidney disease. Being a noninvasive method of collection saliva can reduce discomfort & risks associated with blood collection method.Our study reveals the most important finding is that saliva can play an important role in measuring serum creatinine and urea levels in individuals with CKD.

Abbreviations:

CKD: Chronic kidney disease, GLDH: glutamate dehydrogenase, SD: standard deviation

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