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STUDY OF SALIVARY CREATININE AS AN ALTERNATIVE TO SERUM CREATININE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Abstract : INTRODUCTION - Chronic Renal Failure is a progressive decline of the glomerular filtration rate, leading to an increase of serum creatinine and blood urea nitrogen levels. Frequent sampling of blood is mandatory to diagnose and monitor the progression of the disease. Collection of blood for serum analysis is an invasive procedure which often causes discomfort and anxiety to the patients. Saliva, a multi-constituent biologic fluid secreted by the salivary glands, is the major contributor of oral health. This study was done to determine the diagnostic ability of saliva as an alternative to serum to estimate creatinine in CKD patients. AIM OF THE STUDY - 1.To estimate serum and salivary creatinine levels among patients with Chronic Kidney Disease 2.To compare and correlate the serum and salivary creatinine levels among CKD cases and to evaluate the role of saliva as a noninvasive alternative to serum for creatinine estimation in CKD patients. MATERIALS AND METHODS - This case-control study was done by selecting 50 patients with Chronic Kidney Disease (CKD) and 50 age and sex-matched healthy controls. GFR was estimated by Cockcroft-Gault formula. Blood and whole unstimulated saliva samples were simultaneously obtained from all the patients. The samples were assayed immediately using creatinine estimation kit by Jaffe kinetic reaction. Statistical analysis was done using t -test. Correlation between serum and salivary creatinine was obtained in cases and controls using Pearsons correlation. RESULTS - Serum as well as salivary creatinine levels were found to be significantly higher in CKD patients than controls (p value less than 0.001).The correlation between serum and salivary creatinine in controls was not found to be significant, Pearson correlation coefficient, 0.168, whereas in CKD patients a significant positive correlation was found, 0.952. CONCLUSION - Saliva can be used as a non-invasive diagnostic tool as an alternative to serum, for estimating creatinine among Chronic Kidney Disease patients.

Keyword : Chronic Kidney Disease, Serum creatinine, Salivary creatinine.

INTRODUCTION:

Chronic Renal Failure is defined as the progressive and usually irreversible decline of the glomerular filtration rate, leading to an increase of serum creatinine and blood urea nitrogen levels.1 Chronic Kidney Disease (CKD) encompasses a spectrum of different pathophysiologic processes associated with abnormal kidney function, and a progressive decline in glomerular filtration Rate (GFR). Chronic Kidney Disease (CKD) is a worldwide public health problem, with increasing prevalence and adverse outcomes. Diabetes and Hypertension are the leading causes of CKD.2 Frequent sampling of blood is mandatory to diagnose and monitor the progression of the disease and for monitoring the therapeutic outcomes. Creatinine which is a waste product of metabolism of muscle is excreted primarily by the kidneys and its serum levels are used to assess the renal functions.3 Collection of blood for serum analysis is an invasive procedure which often causes discomfort and anxiety to the patients. CKD patients undergo dialysis which is inevitably associated with some amount of blood loss amounting to about 4 to 20mL. Additional blood loss in these patients results from frequent blood sampling.4 Hence, a simple diagnostic test which provides a reliable evaluation of disease status and stages would be of value to both clinicians as well as patients. Saliva, a multi-constituent biologic fluid secreted by the salivary glands, is the major contributor of oral health. It has potential advantage over serum because saliva collection is a, simple, noninvasive, and economic procedure that can be performed by the patient with minimal involvement from medical personnel.

A repeat sample can be easily obtained when required and is suitable for all age groups. It also provides a cost-effective approach for the screening of large populations. Saliva as a diagnostic medium will also be a boon to patients suffering from clotting disorders like haemophilia and in patients with compromised venous access.5-9

Aims and Objectives

1. To estimate serum creatinine and salivary creatinine levels among patients with Chronic Kidney Disease
2. To compare and correlate the serum and salivary creatinine levels among CKD cases and to evaluate the role of saliva as a noninvasive alternative to serum for creatinine estimation in CKD patients.

CHRONIC KIDNEY DISEASE:

Chronic Kidney Disease (CKD) is an international public health problem affecting about 5- 10% of the population and the expected incidence every year is approximately 5-8%.10 In South India, the main causes of CKD in decreasing order of prevalence are diabetic nephropathy (29.6%), chronic interstitial nephritis (20.4%), chronic glomerulonephritis (17.4%), and hypertensive nephropathy(11%). The Kidney

Disease Outcomes Quality Initiative (KDOQI) of the National Kidney Foundation (NKF) defines “Chronic Kidney Disease as either kidney damage or a glomerular filtration rate (GFR) of less than 60mL/min/1.73 m2 for 3 or more months”.11 In 2002 : KDOQI published its classification of the stages of Chronic Kidney Disease as follows:12

· Stage 1 : Kidney damage with normal or increased GFR (>90mL/min/1.73m2)
· Stage 2 : Mild reduction in GFR (60 -89mL/min/1.73m2)
· Stage 3 : Moderate reduction in GFR (30-59mL/min/1.73m2)
· Stage 4 : Severe reduction in GFR (15-29mL/min/1.73m2)
· Stage 5 : Kidney failure (GFR <15mL/min/1.73m2)

Patients with chronic kidney disease stages 1-2 are generally asymptomatic. Clinical manifestations typically appear in stages 3-5. Approximately 1 million nephrons are present in each kidney, contributing to the total GFR. In the case of renal injury, the kidney has an ability to maintain GFR by hyperfiltration and compensatory hypertrophy of the remaining healthy nephrons. Thus, adaptability of nephrons allows for continued normal clearance of plasma solutes. Plasma levels of substances such as creatinine start to show significant increases as the GFR decreases. Frequent sampling of serum for creatinine is done to assess the stage and progression of the disease. There are many studies with promising results which show that saliva can be used to detect lung cancer, pancreatic cancer, breast cancer, and type II diabetes.13-15 With this background, this study was done to determine the diagnostic ability of saliva as an alternative to serum to estimate creatinine in CKD patients.

MATERIALS AND METHODS

The study population comprised of 50 patients enrolled from Nephrology OPD and ward, who were already clinically diagnosed with Chronic Kidney Disease (CKD). 50 healthy volunteers (age and gender matched) who had no complaint or major illness in recent past were selected as controls. GFR was estimated by calculating creatinine clearance using Cockcroft-Gault formula: eCrCl = (140 – Age) × Weight(kg) × 0.85 if female 72 × Serum Creatinine(mg/dL) The patients in this study were found to be in stage 4 and stage 5 CKD. The patients were either under medical management alone or were also undergoing hemodialysis/ peritoneal dialysis. After obtaining a written informed consent, a clinical examination of the oral cavity was performed and the case details were recorded. Blood and whole unstimulated saliva samples were simultaneously obtained from all the patients. To minimize the effect of diurnal variation, all the samples were collected between 9:00 and 11:00 a.m..Two mL of blood was drawn from antecubital vein under aseptic condition. Two mL of whole saliva was obtained under restful conditions, in a sterile graduated container by spitting method. The participants were instructed to refrain from eating and drinking at least 90min before collection and thoroughly rinse mouth with distilled water prior to the collection to void the mouth of saliva. They were asked to sit in a comfortable position with head tilted slightly forward and to avoid swallowing and oral movements during collection and to pool the saliva in the floor of the mouth and spit every 60 seconds or when they experience an urge to swallow the fluid accumulated. This was done until 2mL of whole saliva was obtained. All collected samples were centrifuged at 3000RPM for 10 minutes. Salivary supernatant and serum were separated. The samples were assayed immediately using creatinine estimation kit by Jaffe kinetic reaction. Student’s t-test was employed for the statistical analysis of data. The data were expressed in terms of mean and standard deviation. ‘P’ value less than 0.05 was taken as the significant value. Pearson’s correlation coefficient was used to test the correlation between serum and salivary creatinine levels.

STATISTICS AND RESULTS

The control group comprised of 50 healthy volunteers. There were 28 males and 22 females. The mean age of the controls was 38.5 years with a standard deviation of 13.97. The minimum age was 22 and the maximum was 62 years. 50 patients with chronic kidney disease were enrolled. Among cases there were 31 males and 19 females. The mean age of this group (cases) was 44.5 years with a standard deviation of 13.6. Their ages ranged from 27 to 70.0 years. Based on their estimated GFR, 22 patients were classified into stage 4 CKD (GFR: 15–30 mL/min) and 28 patients into stage 5 CKD (GFR: <15 mL/min). Majority of the patients being referred to nephrology department were in late stages of CKD and thus patients selected in our study happened to be in stage 4 and stage 5. Among controls the serum creatinine values ranged from 0.6 to1.2mg/dL with a mean of 0.81mg/dL (SD 0.167) and the salivary values ranged between 0.05 and 0.19mg/dL with a mean of 0.107mg/dL (SD 0.04). Among CKD patients the serum creatinine level ranged from 3.2 to 12.6mg/dL with a mean of 8.43mg/dL (SD 2.46) and range of the salivary creatinine level was found to be between 0.36 an 1.4mg/dL with a mean of 0.879mg/dL (SD 0.279).

TABLE:1:

DESCRIPTIVE STATISTICS OF CONTROL AND STUDY GROUP:

	CONTROLS		CASES	
	MEAN	SD	MEAN	SD
AGE	38.5	13.97	44.5	13.6
WEIGHT	66.32	8.74	66.13	7.99
eGFR	96.63	17.01	14.66	11.44
SERUM CREATININE	0.81	0.167	8.43	2.46
SALIVARY CREATININE	0.107	0.04	0.879	0.29

Student t-test was employed to assess if salivary creatinine levels are also elevated at par with serum creatinine, in cases with CKD. The mean serum as well as the salivary creatinine concentration were found to be significantly higher in CKD patient compared to controls.

TABLE 2:

COMPARISON OF SERUM AND SALIVARY CREATININE LEVELS BETWEEN CKD PATIENTS AND CONTROLS USING -TEST

	GROUP	N	MEAN	SD	Statistical significance
Serum creatinine	Controls	50	0.81	0.167	p<0.001 significant
	Cases	50	8.43	2.46	
Salivary creatinine	Controls	50	0.107	0.04	p<0.001 significant
	Cases	50	0.879	0.29	

To assess if there was any association between serum and salivary creatinine and if changes in serum creatinine are accompanied by changes in salivary creatinine, a correlation analysis was performed using Pearson’s correlation, among both cases and controls. The correlation between serum and salivary creatinine in controls was not found to be significant, r = 0.168, and in CKD patients a significant positive correlation was found, r = 0.952.

DISCUSSION

Creatinine is a metabolic waste product that is excreted primarily by kidneys. It is present in all body fluids and secretions. Creatinine that is filtered at the glomerulus is excreted without undergoing tubular reabsorption and hence its level in the blood is used as an index of renal function3. The normal range of serum creatinine is 0.72–1.18mg/dL in men and 0.55-1.02mg/dL in women.16 Whole saliva is a mixed oral fluid derived from the major and minor salivary glands. In addition, saliva contains constituents of non-salivary gland origin, including a variety of microorganisms and their products, blood cells, desquamated epithelial cells, and food debris.

Saliva also contains serumderived components resulting from passive diffusion via gingival crevices; therefore, saliva has been proposed to be a good surrogate of blood for diagnostic purposes. The normal range of salivary creatinine is 0.05–0.2 mg/dL17. In the present study, the range of serum and salivary creatinine obtained were in accordance with the above mentioned reference ranges. In this study, a significantly high creatinine level was observed both in serum and saliva of CKD patients compared with controls. Similar observation was made by Xia et al.18 This is due to inability of the kidneys to excrete creatinine in renal failure and hence its concentration in blood increases3. The increased concentration in saliva may be because of increased serum creatinine which creates an increased concentration gradient which in turn increases the diffusion of creatinine from serum to saliva in CKD patients. It is also possible that saliva may be an attempted alternative route of excretion by the body in a compromised renal function state.19 To evaluate the association between serum and salivary creatinine and to see if changes in serum creatinine are accompanied by changes in salivary creatinine, a correlation analysis of both study groups (CKD cases and controls) was performed and it was found that there was no correlation among controls, whereas there was a significant positive correlation among CKD patients. Creatinine being a large molecule, with high molecular weight (MW 113Da) is maintained at constant plasma levels by kidneys. Also creatinine has a low lipid solubility.

Thus owing to its physical properties in a healthy state under normal conditions it is unable to diffuse easily across the cells through the tight intercellular junctions of the salivary gland.3,19,20 Hence, no significant correlation was obtained in controls. But in the diseased state there is an altered permeability of the salivary gland cells.21 Also the increased serum creatinine levels in CKD patients create a concentration gradient that facilitates increased diffusion of creatinine from serum in to saliva.19 Hence, a significant positive correlation was obtained in CKD patients. It will therefore be possible to substitute unstimulated whole saliva samples in place of serum samples for creatinine estimation in situations such as anemia, small blood volume, difficulties in access for sampling, preservation of major veins for future arterio-venous shunts, where it may be deemed desirable to reduce the frequency of venipuncture or blood samplings. Furthermore, saliva can be collected noninvasively and more easily by minimally trained personnel.1 Also, analysis of saliva may provide a cost-effective approach for the screening of large populations.6 Salivary creatinine estimation dramatically reduces anxiety and discomfort associated with blood sampling procedures and also increases their willingness to undergo frequent health check up that will greatly increase the opportunity to monitor their general health over time. Sampling saliva instead of blood is suitable for all age groups and also reduces the occupational risks to laboratory personnel. Thus the results of the present study suggest that the saliva can be used as alternative diagnostic medium for estimating serum creatinine among chronic kidney disease patients

CONCLUSION

Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes. Whole saliva contains locally produced as well as serum-derived markers that have been found to be useful in the diagnosis of a variety of systemic disorders. Whole saliva can be used as a non-invasive diagnostic tool for estimating creatinine in CKD patients. This test can be performed by individuals with modest training, including patients. This facilitates the development and introduction of screening tests that can be performed by patients at home. Analysis of saliva can offer a cost-effective approach for the screening of large populations, and may represent an alternative for patients in whom blood drawing is difficult, or when compliance is a problem. Among CKD patients, it is thus possible that routine biochemical work using blood in chronic dialysis patients may be done at less frequent intervals by more frequent monitoring of salivary parameters.

LIMITATIONS

One limitation of the study was that the sample size was small. Another limitation was that the study group consisted only of stage 4 and stage 5 CKD patients. To say that saliva can be used to diagnose CKD, a study comprising patients in all the stages of CKD and healthy controls should be performed.

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