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COMPARATIVE EVALUATION OF CREATININE IN SERUM AND SALIVA OF CHRONIC KIDNEY DISEASE PATIENTS

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ABSTRACT

Background & objectives: Chronic kidney disease (CKD) is progressive reduction in renal function. Renal excretory function can be assessed by measurement of creatinine concentrations in plasma which illustrates the filtration capacity of the glomerulus. The increased serum creatinine levels in CKD patients create a concentration gradient that facilitates increased diffusion of creatinine from serum into the saliva. Saliva has many advantages as a diagnostic media over serum, which includes non-invasive collection, good patient compliance, minimal risk of contracting infection, repeated sampling possible and is cost effective. The purpose of this study was to estimate and correlate serum and salivary creatinine in patients with CKD and to evaluate use of salivary creatinine as an alternative biomarker to serum creatinine in CKD patients. **Methods:** A total sample of 40 subjects aged 25 -65yrs, inclusive of both genders comprising 20 cases of healthy controls (Group I) and 20 diagnosed cases of CKD (Group II) were selected. 2ml of blood and unstimulated whole saliva was collected from both the groups. The blinded samples were processed in autoanalyzer using Jaffe's kinetic reaction. The collected data were subjected to Statistical analysis. **Results:** In the present study we found significant correlation of serum creatinine and salivary creatinine in both controls and CKD. Serum creatinine (6.21 ±4.02) and Salivary creatinine (0.70± 0.61) were both increased in CKD cases compared to Controls Serum creatinine (0.83±0.21) and Salivary creatinine (0.11± 0.07). Serum creatinine and salivary creatinine were xii both increased as CKD progressed. A sensitivity of 75% and specificity of 90% of salivary creatinine was found. **Conclusion:** We therefore recommend salivary creatinine as a biomarker in CKD and also as an alternative biomarker to serum creatinine in detecting and staging CKD, for assessing disease progression, therapeutic modalities and in screening of large population.

KEYWORDS : Chronic Kidney Disease, Creatinine, Serum, Saliva.

INTRODUCTION

Renal diseases are the major cause of global morbidity and mortality. The prevalence of Chronic Kidney Disease (CKD) is estimated to be 8-16% worldwide.[1] In India there is a rising burden of chronic diseases like hypertension and diabetes. The increase in number of CKD patients can be partially attributed to the epidemic of chronic diseases and the aging population. It is estimated that 25-40% of these patients are likely to develop CKD. [2] Chronic Kidney Disease is defined as structural or functional abnormalities of the kidney, with or without decreased Glomerular Filtration Rate (GFR), manifested by pathological abnormalities or markers of kidney damage, including abnormalities in the composition of the blood or urine or abnormalities in imaging tests[3]. This condition requires frequent serum analysis to diagnose and monitor therapeutic outcomes and to ascertain prognosis.[4] Renal excretory function can be assessed by measuring serum levels of compounds excreted by kidneys commonly the product of protein catabolism[5].

With impaired renal function, a decreased GFR, and the accumulation and retention of various products of renal failure, the oral cavity may show a variety of changes as the body progresses through an azotemic to a uremic state. Some of the presenting signs are ammonia-like taste and smell, stomatitis, gingivitis, decreased salivary flow, xerostomia, and parotitis. Thus oral health care professional should be able to recognize these oral symptoms as a part of the patient's systemic disease and not as an isolated occurrence.[3] Many constituents of the blood make their way into saliva, thus making saliva an indicator of the current state of the blood and of the rest of the body. Many biomarkers can be readily found in saliva.[6] The increased serum creatinine levels in CKD patients create a concentration gradient that facilitates increased diffusion of creatinine from serum into the saliva.[4]

Creatinine is a 113-d end product of muscle catabolism, derived by the metabolism of phosphocreatine in muscle as well as dietary meat intake or creatine supplement.[7] Virtually all the creatinine that is filtered at the glomerulus is excreted without reabsorption into the tubules. Thus measurement of creatinine concentrations in plasma and urine samples illustrates the filtration capacity of the glomerulus, ie. glomerular filtration rate (GFR). These characteristics make creatinine a useful endogenous marker for creatinine clearance and so its level in the blood is used as an index to renal function.[4]

Repeated blood investigations are required for monitoring a chronic systemic condition as in case of chronic renal failure.[8] Sampling blood for serum analysis is an invasive procedure. Saliva has got many advantages as a clinical tool over serum, which includes non-invasive collection of the sample by individuals with limited training, no special equipment required, fewer compliance problems as compared with collection of blood, minimal risk of contracting infection, repeated samples can be easily obtained and is cost effective for screening of large population.[9] Serum urea (>200mg/dl) and creatinine (>10mg/dl) are indicators for initiation of dialysis. Considering that such parameters will need to be tested regularly, an equally accurate noninvasive, rapid test if available would be beneficial.[8] With this background this study was undertaken to estimate and correlate creatinine levels in serum and saliva of CKD patients and to evaluate the use of salivary creatinine as an alternative biomarker to serum creatinine in CKD.

METHODOLOGY

SOURCE OF DATA This study was conducted at the Department of Oral Medicine and Radiology M.R. Ambedkar Dental College and Hospital, B.R. Ambedkar Medical College and Hospital, Bangalore.

Method Of Collection Of Data

The present study comprised of a total sample size of 40 subjects with age 25 -65yrs, inclusive of both genders. This

study was approved by the Ethical Review Board M.R. Ambedkar Dental College and Hospital, Bangalore. The duration of the study was 1 year. Detailed case history was recorded and clinical examination was carried out. Diagnosed cases of CKD confirmed by renal function tests were selected for the study. Written informed consent from selected patients was taken for the procedures to be carried out on them subsequently. The subjects included for the study were divided into 2 groups: Group I : 20 subjects of age and sex matched healthy subjects as controls. Group II : 20 subjects of diagnosed cases of Chronic Kidney Disease. Exclusion criteria was subjects who were dehydrated, pregnant, suffering from liver diseases and/or suffering from salivary gland disorders.

Method Of Collecting Saliva Sample

The saliva sample was collected prior to dialysis in patients undergoing hemodialysis. The patients were instructed to refrain from eating and drinking at least 90mins before collection and thoroughly rinse mouth with distilled water prior to collection of the saliva. 2ml of unstimulated whole saliva was collected in a sterile graduated container by spitting method from patients with CKD and controls. Samples were labelled, blinded and centrifuged at 3000rpm for 10 mins, salivary supernatant was separated and stored in cold storage unit at -20˚c.

Method Of Collecting Blood Sample

The blood sample was collected prior to dialysis in patients undergoing hemodialysis. 2 ml of blood was drawn from median cubital vein of patients with CKD and controls following aseptic procedures. Serum sample was obtained by centrifuging whole blood at 3000rpm for 10mins and stored in cold storage unit at -20˚c.

The blinded sample were processed in autoanalyzer (Roche Integra 400+) using Jaffe's kinetic reaction. The concentration of creatinine levels in serum and saliva was determined using spectrophotometric (520nm) technique.

Statistical Analysis:

By using patients demographic features statistical analysis was calculated with Student t test (two tailed, independent). Comparison between serum and salivary creatinine were considered for a significant p- value of less than 0.001. ROC curve was established.

RESULTS

OBSERVATION

The present study was undertaken to estimate and correlate creatinine levels in serum and saliva of CKD patients and to evaluate the use of salivary creatinine as an effective alternative biomarker to serum creatinine in CKD patients. A total 40 subjects were included in the study in which 20 were normal healthy subjects and 20 were diagnosed cases of Chronic Kidney Disease.

Study Sample

The study sample comprising of total of 40 subjects was divided into 2 groups. Group I : 20 subjects of age and sex matched healthy subjects as controls. Group II : 20 subjects of diagnosed cases of Chronic Kidney Disease. The data was collected, tabulated and subjected to Statistical analysis. Student t test (two tailed, independent) was applied to compare creatinine serum and saliva. ROC curve was established.

In group I (controls), the mean serum creatinine (0.83 ± 0.21) was found to be higher than mean salivary creatinine (0.11 ± 0.07). This correlation was statistically significant. ($p < 0.001$)
In group II (cases), the mean serum creatinine (6.21±4.02) was found to be higher than mean salivary creatinine (0.70±0.61). This correlation was statistically significant. ($p < 0.001$)

The mean serum creatinine level in Group I (controls) was 0.83±0.21 SD and in Group II(CKD cases) was 6.21±4.02 SD. The mean serum creatinine level was found to be higher in Group II (CKD cases) when compared to that of Group I (controls). The difference was found to be statistically significant ($p < 0.001$).

The mean salivary creatinine level in Group I (controls) was 0.11±0.07 SD and in Group II(CKD cases) was 0.70±0.61 SD. The mean salivary creatinine level was found to be higher in Group II (CKD cases) when compared to that of Group I (controls). The difference was found to be statistically significant ($p < 0.001$).

The mean serum creatinine levels in Stage 3 was 1.54±0.34, Stage 4 was 2.94±0.93 and Stage 5 was 7.91±3.58. The mean serum creatinine level in Stage 5 was found to be higher as compared to Stage 3 and Stage 4. Mean serum levels increased significantly as the stages of CKD progressed.

The mean salivary creatinine level in Stage 3 was 0.10±0.03, Stage 4 was 0.24±0.12 and Stage 5 was 0.93±0.59. The mean salivary creatinine level in Stage 5 was found to be higher as compared to Stage 3 and Stage 4. Mean salivary creatinine levels increased significantly as the stages of CKD progressed.

Sensitivity and specificity for different values of salivary creatinine were established and a cut-off value of >0.19 mg/dL was determined. In the present study a sensitivity of 75.00% and specificity of 90.00% was found. The total area under the curve obtained was 1.000 for serum creatinine and 0.879 for salivary creatinine.

DISCUSSION

Chronic renal failure is the progressive loss of function of kidney. It is usually a result of complications from chronic debilitating diseases and progressing age of population.[10]

Renal failure leads to a state of intoxication known as uremia, which is associated with accumulation of metabolic waste products and multi organ involvement. Hematologic, electrolyte, endocrine and skeletal disorders are the main changes.[11]

Creatinine is a waste product of creatine and phos phocreatine and is found almost exclusively (90%) in skeletal muscle tissues. Serum creatinine concentration is maintained by the balance between its generation and excretion by the kidneys. Since creatinine is generated in a steady manner and can be measured very simply from blood samples, it has become a useful test to estimate glomerular filtration rate (GFR), a measurement of kidney function. The normal reference range of serum creatinine for men is 60 to 110 micromol/L (0.7 to 1.2 mg/dL) and for women is 45 to 90 micromol/L (0.5 to 1.0 mg/dL). Estimated GFR (eGFR) equations, based on serum creatinine, are generally utilised for the systematic staging of chronic kidney disease (CKD).[12]

Saliva is a multiconstituent biologic fluid secreted by the salivary glands and plays an important role in oral and systemic health.[4] Various components of saliva are either passively diffused or actively transported directly from the serum into the saliva through the oral mucosa and/or gingiva,[13] and salivary glands. Saliva as a diagnostic fluid has a cutting edge over serum because it can be collected non-invasively and does not require special equipment for collection and storage as unlike blood, saliva does not clot. Advantageous for people in whom blood drawing is difficult as in obese, hemophiliacs, compromised venous access and in patients who are fearful of prick.[14] Anemia is one of the clinical and laboratory manifestations of CKD and repeated

serum sampling is desired for estimation of creatinine levels to stage the disease and monitor the therapeutic outcome. This will further intensify the anemic state in CKD. Hence salivary sampling will be beneficial for CKD patients for whom repeated sampling is required. Saliva remains a largely untapped source of medical information that can enhance diagnostic accuracy while saving the patient from some of the discomfort associated with a blood test or other more invasive procedures. [6]

This study comprised of a total sample size of 40 subjects with age 25 -65yrs, inclusive of both genders. Comparison between serum and salivary creatinine were considered for a significant p- value of less than 0.001.

Group I. Comparison Of Mean Serum Creatinine And Mean Salivary Creatinine Of Controls

In group I (controls), the mean serum creatinine (0.83 ± 0.21) was found to be higher than mean salivary creatinine (0.11 ± 0.07). This correlation was statistically significant. ($p < 0.001$). In the present study there was correlation in serum creatinine and salivary creatinine in controls which was statistically significant ($p< 0.001$). Our findings are consistent with the findings of Venkatapathy et al[4], Bader et al[6], Yajamanam N et al[15], Lasisi et al[16].

Group II. Comparison Of Mean Serum Creatinine And Mean Salivary Creatinine Of CKD Cases

In group II (cases), the mean serum creatinine (6.21±4.02) was found to be higher than mean salivary creatinine (0.70±0.61). This correlation was statistically significant. ($p < 0.001$) In the present study there was correlation of serum creatinine and salivary creatinine in CKD which was statistically significant ($p<0.001$)

Our findings are in agreement with the findings of Tomas et al[17], Venkatapathy et al [4], Yajamanam N et al [15], Lasisi et al [16], Divya Pandya et al[18], Renda. R[19]. Creatinine is a large molecule with a high molecular weight that exhibits low lipid solubility. In healthy individuals, it is unable to diffuse across the cells and the tight junction of the salivary gland, however in CKD patients, possibly, there is an alteration in the permeability of salivary gland cells, and also as serum creatinine increases, a concentration gradient occurs, and creatinine diffusion increases from serum to saliva. [17] Another factor could be that saliva is an alternative route of excretion when renal function is impaired.[4]

Comparison Of Mean Serum Creatinine Between Group I And Group II

The mean serum creatinine level in Group I (controls) was 0.83±0.21 SD and in Group II(CKD cases) was 6.21±4.02 SD. The mean serum creatinine level was found to be higher in Group II (CKD cases) when compared to that of Group I (controls). The difference was found to be statistically significant ($p<0.001$).

Our findings are consistent with findings of Venkatapathy et al [5], Yajamanam N et al [14], Lasisi et al [15], Divya Pandya et al [16], Renda. R [17]. Because of the decrease in GFR in renal disease, creatinine clearance via the renal system is compromised. The reduced GFR will then lead to an increase in plasma creatinine concentration.[20]

COMPARISON OF MEAN SALIVARY CREATING BETWEEN GROUP I AND GROUP II

The mean salivary creatinine level in Group I (controls) was 0.11±0.07 SD and in Group II(CKD cases) was 0.70±0.61 SD. The mean salivary creatinine level was found to be higher in Group II (CKD cases) when compared to that of Group I (controls). The difference was found to be statistically significant ($p<0.001$).

Our findings are in accordance with findings of Tomas et al[17], Venkatapathy et al [4], Bader et al [6], Yajamanam N et al [15], Lasisi et al [16], Divya Pandya et al [18], Renda. R [19]. Creatinine is a large molecule with a high molecular weight that exhibits low lipid solubility. In healthy individuals, it is unable to diffuse across the cells and the tight junction of the salivary gland, however in CKD patients, possibly, there is an alteration in the permeability of salivary gland cells, and also as serum creatinine increases, a concentration gradient occurs, and creatinine diffusion increases from serum to saliva.[19] Another factor could be that saliva is an alternative route of excretion when renal function is impaired.[4]

Group II. Comparison Of Mean Serum Creatinine Among Different Stages Of CKD

The mean serum creatinine levels in Stage 3 was 1.54±0.34, Stage 4 was 2.94±0.93 and Stage 5 was 7.91±3.58. The mean serum creatinine level in Stage 5 was found to be higher as compared to Stage 3 and Stage 4. In the present study we found that the mean serum creatinine levels increased significantly as the stages of CKD progressed. Our findings are in concordance with the findings of Tomas et al [17], Renda. R[19]. Elevation in serum creatinine concentration often signifies a substantial reduction in GFR, which declines with progression of CKD.[21]

Comparison Of Mean Salivary Creatinine Among Different Stages Of CKD

The mean salivary creatinine level in Stage 3 was 0.10±0.03, Stage 4 was 0.24±0.12 and Stage 5 was 0.93±0.59. The mean salivary creatinine level in Stage 5 was found to be higher as compared to Stage 3 and Stage 4. In the present study we found that the mean salivary creatinine levels increased significantly as the stages of CKD progressed. Our findings are in agreement with the findings of Tomas et al [17], Renda. R.[19]. With elevation in serum creatinine as the CKD stage progresses, there is also increase in saliva concentration, because of increased concentration gradient which in turn increases the diffusion of creatinine from serum to saliva. Saliva may also be an attempted alternative route of excretion by the body in a compromised renal function state.[4]

Receiver Operating Characteristics Curve For Serum And Salivary Creatinine Levels.

Sensitivity and specificity for different values of salivary creatinine were established and a cut-off value of >0.19 mg/dL was determined. In the present study a sensitivity of 75.00% and specificity of 90.00% was found. The total area under the curve obtained was 1.000 for serum creatinine and 0.879 for salivary creatinine.

Our findings are consistent with Venkatapathy et al[4]. who found (0.2mg/dl) cutoff value of salivary creatinine. Our finding are in contrast with Yajamanam N et al [15] (0.16mg/dl), Lasisi et al[16] (0.55mg/dl) and Renda. R[19] who found (0.125mg/dl) cutoff value of salivary creatinine.

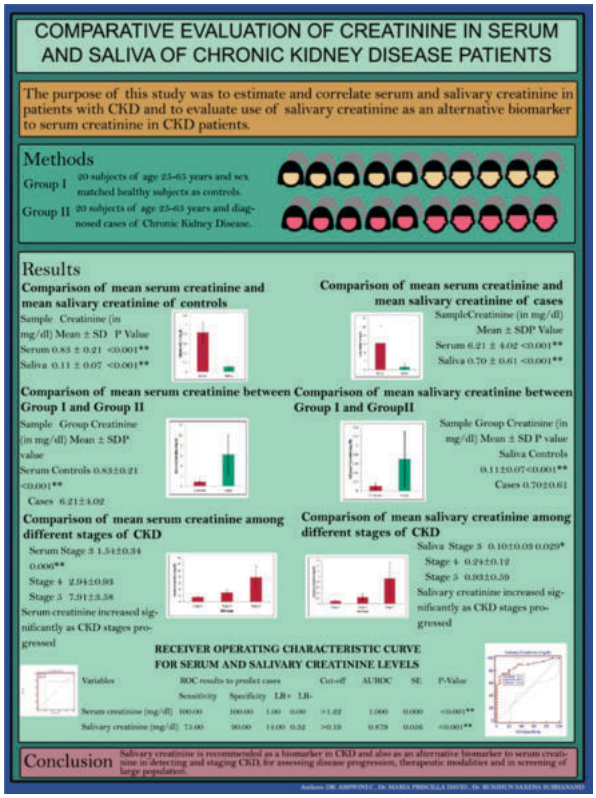
In the present study we found significant correlation of serum creatinine and salivary creatinine in both controls and CKD. Serum creatinine and salivary creatinine were both increased in CKD cases. Serum creatinine and salivary creatinine were both increased as CKD progressed. A sensitivity of 75% and specificity of 90% of salivary creatinine was found. Therefore we recommend salivary creatinine as an alternative biomarker to serum creatinine in CKD.

CONCLUSION

Monitoring of biomarkers in saliva instead of serum is advantageous because saliva collection is non-invasive, inexpensive, ease and feasibility of multiple sampling, painless, can be performed by minimally trained personnel, can be tested at home, thus avoiding visit to the clinic or hospital, less infectious, safer to handle, easy storage and

transport, and good patient compliance.[6] We therefore recommend salivary creatinine as a biomarker in CKD. We also recommend salivary creatinine as an alternative biomarker to serum creatinine in detecting and staging the disease, for determining the need for dialysis and in monitoring the effectiveness of dialysis in CKD. It can also be used for assessing disease progression, therapeutic modalities and in screening of large population. Identifying salivary creatinine in patients with chronic kidney disease is a simple , inexpensive, non-invasive and safe approach for disease detection and management. This will undoubtedly remain a major focus of routine investigation in the future and possess a high prospective to upgrade the next generation of diagnostics. This would subsequently improve access to care and escalate the efficacy of health care delivery.

Conflict of Interest- None



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