VOLUME - 12, ISSUE - 08, AUGUST - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Oral Medicine and Radiology

COMPARATIVE EVALUATION OF UREA NITROGEN LEVEL IN SERUM AND SALIVA OF CHRONIC KIDNEY DISEASE PATIENTS

Dr. Shilpa Kuravatti	MDS Oral Medicine and Radiology Consultant Dental Surgeon Aarohi Dental Clinic M. R. Ambedkar Dental College, Bangalore
Dr. Maria Priscilla David	MDS Oral Medicine and Radiology Former Professor and HOD Department of Oral Medicine and Radiology M.R. Ambedkar Dental College, Bangalore
Dr. Runjhun Saxena*	MDS Oral Medicine and Radiology Teeth Wellness Dental ClinicM.R. Ambedkar Dental College, Bangalore *Corresponding Author

ABSTRACT

Background and Objectives: Kidney disease is a worldwide public health problem, with increasing incidence and prevalence, high costs, and poor outcomes. Chronic Kidney Disease involves an irreversible loss of renal function. This results in increased Blood Urea Nitrogen (BUN) levels which may lead to high concentration of salivary urea nitrogen (SUN) levels. BUN is directly related to the excretory function of the kidney. Salivary based investigation provide an attractive alternative for blood, as it is a safe, noninvasive, cost effective technique for screening, detection and monitoring of disease with good cooperation by patients. This study was undertaken to estimate and correlate urea nitrogen levels in serum and saliva of CKD patients and to evaluate the use of salivary urea nitrogen as an effective alternative biomarker to serum urea nitrogen in CKD patients. **Methods:** A total of 40 subjects comprising of 20 healthy controls (Group I) and 20 cases of CKD (Group II) were selected. The selected cases were clinically diagnosed and confirmed cases of various stages of chronic kidney disease. 2ml of stimulated saliva and blood samples were collected from both the groups. Using urease/glutamate dehydrogenase method, the concentration of urea nitrogen levels in serum and saliva was evaluated. **Results:** In the present study we found significant correlation of serum urea nitrogen and salivary urea nitrogen in both controls and CKD cases. Serum urea nitrogen and salivary urea nitrogen were both increased in CKD cases. Serum urea nitrogen and salivary urea nitrogen increased as CKD progressed. **Conclusion:** We therefore recommend, salivary urea nitrogen as a biomarker in CKD. We also recommend the use of salivary urea nitrogen as an alternative biomarker to serum urea nitrogen in CKD. We suggest the use of salivary urea nitrogen to diagnose, stage and monitor management of CKD. Salivary urea nitrogen can also be used as a prognostic indicator in evaluating adequacy of dialysis and monitoring renal disease status.

KEYWORDS : Biomarker; Urea nitrogen; Chronic Kidney Disease; Saliva; Serum

INTRODUCTION

The kidneys are vital organs for maintaining a stable internal environment i.e homeostasis. Kidney have many functions like regulating the acid-base and fluid electrolyte balance of the body by filtering blood, reabsorbing water and electrolytes and excreting urea and other toxic metabolites.[1] Renal diseases are the major cause of global morbidity and mortality. In India approximately 7.85 million people are suffering from chronic renal failure making it a devastating medical social and economic problem.[2]

Chronic Kidney Disease is defined as structural or functional abnormalities of the kidney, with or without decreased 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. (GFR <60ml/min/1.73m2 for three months or more, with or without kidney damage).[3]

The plethora of oral manifestations observed in chronic renal failure and associated therapies are altered taste, gingival enlargement, xerostomia, parotitis, enamel hypoplasia, delayed eruption, various mucosal lesions like hairy leukoplakia, lichenoid reactions, ulcerations, angular chelitis, candidiasis etc.[4] Saliva has hundreds of components that may serve to detect systemic diseases or as evidence of exposure to various harmful substances, as well as provide biomarkers of health and disease status.

Nowadays, the saliva research field is rapidly advancing due to the use of novel approaches including metabolomics, genomics, proteomics and bioinformatics.[5] Evaluation of salivary parameter is an alternative to serum examination as frequent drawing of blood is troublesome and have added psychological trauma to the patient.[6] Saliva has got many advantages as a clinical tool over serum, plasma or tissue including simplicity of its collection, storage and shipping, cost effectiveness, easy to get larger volume of samples and real-time repeated sampling. Its procurement is non-invasive that is stress free and reduces patient anxiety and discomfort. It is easy and safe to handle; thus reducing manipulation for biochemical analysis.[7] Salivary concentrations of many metabolites, drugs and hormones also represent the free fractions of serum in many instances, with good correlations with the respective total concentrations in serum. Serum urea (>200mg/dl) and creatinine (>10mg/dl) are indicators for initiation of dialysis. Measurement of pre and post dialysis blood urea nitrogen (BUN) is a gold standard test for evaluating dialysis efficacy. Considering that such parameters will need to be tested regularly, an equally accurate noninvasive, rapid test if available would be beneficial. It can also be used as an epidemiological tool for the evaluation of prevalence of renal failure in community.[6]

With this background this study was undertaken to estimate and correlate urea nitrogen levels in serum and saliva of CKD patients and to evaluate the use of salivary urea nitrogen as an effective alternative biomarker to serum urea nitrogen in CKD patients.

METHODOLOGY

Source of Data: This study was conducted at the Department of Oral Medicine and Radiology M. R.A D. C, Bangalore and Nephrology Centre of Multispeciality hospital, Nidan Pathology Laboratory, Kolhapur. Method of collection of Data: The present study consisted of a total of 40 subjects, with ages 25 -65yrs, inclusive of both the genders. This study was approved by the Ethical Review Board of M.R Ambedkar Dental College and Hospital, Bangalore. Written informed consent from selected patients was taken for the procedures to

be carried out on them subsequently. Relevant case history was recorded, including their oral habits, frequency and duration. Detailed clinical examination was carried out. Subjects with clinically diagnosed Chronic Kidney Disease were included. The study samples were divided into two groups: Group I: 20 cases of age and sex matched healthy individuals with as controls. Group II: 20 cases of clinically diagnosed and confirmed cases of various stages of Chronic Kidney Disease. Inclusion Criteria: Group I – Healthy Subjects having no obvious oral lesions. Group II - Patients with clinically diagnosed and confirmed cases of various stages of Chronic Kidney Disease. Materials and Methods 69 Exclusion criteria 1. Subjects who are dehydrated 2. Subjects who are pregnant 3. Subjects suffering from liver disease 4. Subjects suffering from salivary gland disease 5. Subjects with history of myocardial infarction.

Method Of Collecting Saliva Sample: The subjects were clinically examined with standardized questionnaire to obtain the relevant medical history and informed consent was obtained. Clinical diagnosis was confirmed by renal function tests. Saliva sample was collected by spitting method. Subjects were instructed to chew paraffin wax for five minutes.2ml of saliva was collected over 5 minutes from patients with CKD and controls. Samples were labelled, blinded and centrifuged at 3600 rpm, supernatant was separated and transferred to osmolality vials which was stored in the cold storage unit at -20 degree Celsius.

Method Of Collecting Blood Sample: 2 ml of blood was drawn from median cubital vein of patients with CKD and controls following aseptic procedures. Serum samples were processed by centrifuging whole blood at 3600rpm for10 minutes and stored in cold storage unit at -20 degree Celsius. The blinded samples were processed in autoanalyzer (RXL dimension system) using urease/glutamate dehydrogenase method. The samples were diluted with 277microLitre of distilled water at 37 degree Celsius. To the diluted sample 90 microliter of Siemen's dimension flex cartridge reagent was added. The concentration of urea nitrogen levels in serum and saliva was analyzed using bichromatic(340-388nm) rate technique.

Statistical Analysis: By using patient demographic features statistical analysis was calculated with Student t test (two tailed, independent) for continuous measures. Differences were considered for p-values less than 0.001.

RESULTS AND OBSERVATIONS:

The results are illustrated in the form of tables and graphs in the image.

DISCUSSION

Kidneys are vital organs for maintaining homeostasis and are the main excretory organs of the body.[8] Renal failure refers to a condition where the kidneys lose their normal function of excreting metabolic waste products from the body. Renal diseases are life threatening in nature next to cardiovascular diseases. The incidence of renal diseases continues to rise worldwide and as a consequence, increasing number of renal patients will probably require oral health care. In these patients there will be impaired glomerular filtration, which leads to the accumulation of various metabolic waste products in the blood such as BUN.[9]

Renal failure is abrupt deterioration of renal function sufficient to result in failure of urinary elimination of nitrogenous waste products (urea nitrogen and creatinine). This deterioration of renal function results in elevations of blood urea nitrogen and serum creatinine concentrations. [10] Urea nitrogen is a small, uncharged molecule that is not protein bound, and as such, it is readily filtered at the renal glomerulus. Urea nitrogen undergoes renal tubular reabsorption by specific transporters. This tubular reabsorption limits the value of BUN as a marker for glomerular filtration. However, BUN usually correlates with the symptoms of uremia.[11] Hence it is used as one of the standard investigations for patients with renal disease.

AGE: The present study included subjects with ages 25 years and above with mean age of 47.35 years ± 9.60SD (standard deviation) for Group I and 46.85 years ± 9.06 SD. for Group II. We found middle aged and elderly subjects in group II. Subjects for group I were age matched. Our findings are in accordance with the findings of Sein KT, Arumainayaga G [12] & Akal T et al.[13] At an early stage, many chronic kidney diseases are asymptomatic. Aging, in addition to impaired function of organs such as the heart, also causes a gradually declining renal function and other physiologic changes. With the rise in the older adult population, the incidence and prevalence of CKD will subsequently increase as older adults are diagnosed with chronic medical conditions such as Diabetes Mellitus (DM) Type II, cardiovascular disease, and obesity. Furthermore, older adults can be at risk for CKD due to decreased awareness and a knowledge deficit regarding CKD. [14]

GENDER: In our Study, in Group I out of 20 (100%) subjects 15 (75%) subjects were Males and 5 (25%) were Females. In Group II out of 20 (100%) subjects 15 (75%) subjects were Males and 5 (25%) were Females In this study we found male preponderance over females in group II. Subjects for group I were gender matched. Our findings are in agreement with the findings of Akal T et al.[13] Males are commonly affected with chronic medical conditions such as Diabetes Mellitus (DM) Type II, cardiovascular disease, and obesity which are the main risk factors to develop CKD.[14]

CKD STAGE: In group II out of 20 (100%) subjects, 1 (5%) subject was Stage I, 2 (10%) were Stage II, 4 (20%) were Stage III, 7 (35%) were Stage IV, 6(30%) were Stage V. In this study we found more patients in advanced stages of CKD. Our findings are consistent with the findings of J.G. Raimann et al.[15] This can be ascribed to lack of awareness in rural areas and low socio economic status resulting in late presentation of this disease.[14]

GROUP I.COMPARISON OF MEAN UREA NITROGEN IN SERUM AND SALIVA OF CONTROLS. In group I (controls), the mean urea nitrogen in Serum (11.05±2.86) was found to be higher than Saliva (8.65±2.85). In the present study there was correlation of serum urea nitrogen and salivary urea nitrogen in controls which was statistically significant ($p < 0.001$). Our findings are in accordance with the findings of Peterson et al [16] and J.G. Raimann et al.[15] Our findings are in contrast with the findings of. Akal T et al[13] who found mean urea nitrogen to be higher in saliva than serum by using dipstick method in predialysis patients. This is due to high absorption of urea and nitrogen in the blood compared to other body fluids.[16]

GROUP II.COMPARISON OF MEAN UREA NITROGEN IN SERUM AND SALIVA OF CKD CASES In group II (CKD Cases), the mean urea nitrogen in Serum (40.00±26.28) was found to be higher than Saliva (35.05±25.05). In the present study there was correlation of Serum urea nitrogen and Salivary urea nitrogen in CKD cases which was statistically significant($p<0.001$). Our findings are in accordance with the findings of. Peterson et al [16] and J.G. Raimann et al.[15]

Our findings are in contrast with the findings of Akal T et al[13] who found mean urea nitrogen to be higher in saliva than serum by using dipstick method in predialysis patients. BUN is directly related to the excretory function of the kidney. If renal function is impaired urea nitrogen level will be increased in serum and saliva. Saliva contains analytes in concentrations that are 1000-fold less than those in blood.[15]

COMPARISON OF MEAN SERUM UREA NITROGEN BETWEEN GROUP I & GROUP II. The mean serum urea nitrogen level in Group II (CKD cases) was 40.00±26.28 SD and in Group I (controls) was (11.05±2.86) SD. In the present study the mean serum urea nitrogen level was found to be higher in group II (CKD cases) when compared to that of Group I (controls).This difference was found to be statistically significant (P<0.001). Our findings are in accordance with the findings of J.G. Raimann et al.[15] and Akai T.et al.[13] In renal patients there will be impaired glomerular filtration or obstruction that interferes with urinary excretion of urea nitrogen, which leads to the accumulation of various metabolic waste products in the blood such as BUN.[13,15]

COMPARISON OF MEAN SALIVARY UREA NITROGEN BETWEEN GROUP I & GROUP II. The mean salivary urea nitrogen level in Group II (CKD cases) was 35.05±25.05 SD and Group I (controls) (8.65±2.85)SD.

In the present study the mean salivary urea nitrogen level was found to be higher in group II when compared to that of Group I. This difference was found to be statistically significant, (P<0.001). Our findings are in accordance with the findings of J.G. Raimann et al[15] and Akai.T et al.[13] Elevation of BUN in renal diseases may result in high concentration of urea and nitrogen in saliva due to diffusion of nitrogenous waste into the saliva. It is also possible that saliva may be an attempted alternative route of excretion by the body in compromised renal function state.[13,15]

GROUP II.COMPARISON OF MEAN SERUM UREA NITROGEN AMONG DIFFERENT STAGES OF CKD. The mean serum urea nitrogen level in Stage I was 14.00. Stage II was 14.50±0.71, Stage III was 22.50±2.65, Stage IV was 40.86±18.60 and Stage V was 63.50±30.01. The mean serum urea nitrogen level in stage IV and stage V was found to be higher as compared to stage III, stage II and stage I. In this study we found that the mean serum urea nitrogen level increased significantly as the stage of CKD progressed. Our findings are in accordance with the findings of J.G. Raimann et al.[15] Among individuals with chronic kidney disease, the stage is defined by the level of GFR, with higher stages representing lower GFR levels. Lower GFR leads to excessive accumulation of urea nitrogen which will be seen at progressive stages of CKD.[15]

TABLE IX. GROUP II .COMPARISON OF MEAN SALIVARY UREA NITROGEN AMONG DIFFERENT STAGES OF CKD The mean salivary urea nitrogen level in Stage I was 12.00. Stage II was 12.00±0.00, Stage III was 18.00±4.69, Stage IV was 36.14±18.60 and Stage V was 56.67±28.92. The mean salivary urea nitrogen level in stage IV and stage V was found to be higher as compared to stage III, stage II and stage I. In the present study we found that the mean salivary urea nitrogen levels increased significantly as the stage of CKD progressed. Our findings are in accordance with the findings of J.G. Raimann et al.[15] CKD staging depends on level of GFR. As the stage progresses GFR will be low. So elevation of BUN in renal diseases may result in high concentration of urea and nitrogen in saliva due to diffusion of nitrogenous waste into the saliva. It is also possible that saliva may be an attempted alternative route of excretion by the body in compromised renal function state.[15] Human saliva is a biological fluid with enormous diagnostic potential. Because saliva can be non-invasively collected, it provides an attractive alternative for blood. Use of saliva as a diagnostic media also prevents unnecessary and periodic withdrawal of blood in patients who are already anemic as in CKD, which is not only cumbersome but also exposes them to infections.

CONCLUSION

In the present study we found significant correlation of serum urea nitrogen and salivary urea nitrogen in both controls and CKD cases. Serum urea nitrogen and salivary urea nitrogen were both increased in CKD cases. Serum urea nitrogen and salivary urea nitrogen increased as CKD progressed. We therefore recommend, salivary urea nitrogen as a biomarker in CKD. We also recommend the use of salivary urea nitrogen as an alternative biomarker to serum urea nitrogen in CKD.

We suggest the use of salivary urea nitrogen to diagnose, stage and monitor management of CKD. Salivary urea nitrogen can also be used as a prognostic indicator in evaluating adequacy of dialysis and monitoring renal disease status. Added benefits of saliva as a diagnostic media when compared to serum, is that salivary investigation is noninvasive and simple, which can be performed by unskilled personnel with less time consumption and excellent patient compliance. On the whole, evaluating urea nitrogen by saliva-based diagnostic tests will play an increasingly important role in the early detection and progression of renal diseases. Accordingly, monitoring of urea nitrogen concentrations should certainly be considered as a part of routine sialometric assessment in chronic kidney disease patients. Thus we believe that this is a stepping-stone and has the potential to revolutionize the diagnostic protocol for patients with Chronic Kidney Disease.

REFERENCES

1. Picken M. Atlas of renal pathology. Arch Pathol Lab Med 2000. 124(6): p. 927.
2. Narula AS. Chronic kidney diseases: the looming threat. Med. J. Arm forces India 2008;64:2-3.
3. Johnson AC, Leway AS, Coresh J, Levin A, Lau J, Eknoyan G: Clinical practical guidelines for Chronic Kidney Disease in adults: Part I. Definition, Disease stages, Evaluation, Treatment, and Risk factors. American Family Physician 2004;70(5):869-876.
4. Proctor R, Kumar N, Stein A, Moles D, Porler S. Oral and dental aspects of chronic renal failure. J Dental Res 2005;84(3):199-208.
5. Motamayel AF, Davoodi P, Dalband M. Saliva as a Mirror of the Body Health; DJH 2010;1(2).
6. Hofman LF. Human saliva as a diagnostic specimen. J Nutr 131;1621S-5S, 2001.
7. Khyani IAM .Saliva as Diagnostic Biofluid Pakistan Journal of Otolaryngology 2014; 30: 35-36.
8. Suresh.G, RaviKiran A, Samata.Y, Purnachandrarao N, VijayKumar.A. Analysis of blood and salivary urea levels in patients undergoing haemodialysis and kidney transplant; Journal of Clinical and Diagnostic Research. 2014 :8(7): 18-20.
9. Goli S, Alaparthi RK , Yalamanchili S, Talla H, Nunsavathu PN, Ambaldhage VK et al: Oral and Salivary Changes among Renal Patients Undergoing Hemodialysis and Renal Transplant: A Cross Sectional Study: BEMS Reports.2015: 1(1):20-22.

10. Dwinnell BG, Anderson RJ. Diagnostic Evaluation of the Patient with Acute Renal Failure, Atlas of Diseases of the Kidney. ISN Informatics Commission and NKF cyber Nephrology. 2012.
11. Kamal A. Estimation of blood urea (bun) and serum creatinine level in patients of renal disorder; Indian Journal of Fundamental and Applied Life Sciences.2014;4(4) ;199-202.
12. Sein KT, Arumainayagam G Correlation between Serum Urea and Salivary Urea.clinical chemistry,1987 :33(12): 2303-2304.
13. Akai T, Naka K, Yoshlkawa C, Okuda K, Okamoto T, Yamagami S et al; Salivary Urea Nitrogen as an Index to Renal Function: A Test-Strip Method: Clin. Chem.1983: 29(10);1825-1827.
14. Kasper DL,Braunward E,Fauci AS,Hauser SL,Longo DL,Jameson JL.Harrison's principles of Internal Medicine.16th ed. McGraw-Hill Companies;2005.
15. Raimann JG. Kirisits W, Gebetsroither E, Carter M, Callegari J, Rosales L,et al; Saliva urea dipstick test: application in chronic kidney disease; Clinical Nephrology,2011;76(1); 23-28.
16. Peterson SJ, Woodhead J, Cram J. Caries resistance in children with chronic renal failure: plaque ph, salivary ph, and salivary composition. Pediatric Research 1985;19(8):796-799.