International Journal of Advances in Nephrology Research

3(1): 21-25, 2020; Article no.IJANR.59674

Assessment of Creatinine Levels in Blood and Saliva of Heamodialysed Subjects

C. Amadi, Fyneface[1], Konne, Joel Burabari[2*] and Konne, Felix Eedee[1]

[1]Medical Laboratory Science, River State University, Port Harcourt, Nigeria.
[2]Department of Medicine and Surgery, School of Medicine, V. N. Karazin Kharkiv National University, Kharkiv, Ukraine.

Authors' contributions

This work was carried out in collaboration among all authors. Author CAF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KJB and KFE managed the analyses of the study. Author KFE managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Darko Nozic, Military Medical Academy, Serbia.
Reviewers:
(1) Jatmiko Susilo, Universitas Ngudi Waluyo, Indonesia.
(2) Manisha Mathur, Post Graduate Institute of Veterinary Education and Research (PGIVER), India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59674

Original Research Article

Received 28 May 2020
Accepted 03 August 2020
Published 15 August 2020

ABSTRACT

The aim of this study was to determine if salivary creatinine responds to changes in concentrations in health, disease and treatment, and how these changes relate to changes in blood creatinine levels. Creatinine was assayed in both blood and saliva of 29 haemodialysed subjects; before haemodialyis and after haemodialysis; and 21 healthy individuals who made up the control group. Creatinine was assayed using Jaffe method. The mean±SD concentrations of salivary creatinine in pre and post haemodialysed subjects as well as control group were 143.3±21.3umol/l, 56.6±8.8umo1/1 and 15.7±1.7umol1/1 respectively. The mean±SD concentrations of blood creatinine in pre and post haemodialysed subjects as well as control group were 646.3±29.0umo1/1, 211.1±7.7umo1/1 and 78.5±2.4umo1/1 respectively. The correlation coefficient between blood and salivary creatinine in pre-haemodialysed subjects was -0.12 while that for post haemodialysed subjects was 0.11 and for the control group was 0.02. The salivary creatinine in the three groups (pre, post and control) was statistically significant (F=23.85; P-value <0.05). The blood creatinine in the three groups (pre, post and control) was statistically significant (F=291.98; P-value <0.05). From the various results obtained, salivary creatinine responds to changes in concentration after therapeutic administration. Salivary creatinine may be considered along with other parameters a supportive marker for diagnosis of kidney disease.

**Corresponding author: E-mail: globalattention@yahoo.com;*

Keywords: Pre-haemodialysed (Pre-HD); Post-haemodialysed (Post-HD); kidney failure; saliva; blood; creatinine.

1. INTRODUCTION

Kidney failure is a condition of impaired kidney function in which the kidney fails to adequately excrete wastes from the blood. Chronic renal failure is a fast growing, silent disease that has affected every part of the world with increasing urbanization. Increasing urbanization has brought along with it changes in lifestyle, and diet, which have contributed today to the major diseases such as diabetes and hypertension that are the background causes to chronic kidney disease in all parts of the world. Currently, blood has been the body fluid of choice in disease diagnosis; however, saliva can be used as an alternative biofluid because it has numerous advantages. Saliva is a clean, tasteless, colourless slightly acidic viscous fluid, consisting of secretions from the parotid, sublingual, submandibular salivary glands and other glands of oral cavity. Every human salivary gland secrets about 600ml of saliva, 99.5% of it is water and antibacterial compounds such as secretory immunoglobulin and lysozyme. Saliva contains microorganisms, oral epithelial cells and food debris. The numerous functions of saliva include lubrication of the mouth, aiding food swallowing and digestion of starch, enhancing food taste and many more. In addition, it possesses diagnostic uses for both local and systemic diseases. Due to the remarkable relationship between mouth and general health, interests are developing in the study of saliva as a diagnostic fluid for systemic diseases, which kidney disease is one of them [1]. Considering the breakthrough of oral thermometer in measuring temperature in detecting fever and its consequent victory over its former redal thermometer has substantiated the fact that oral or salivary diagnosis promises a remarkable breakthrough in medicine. Saliva has biomarkers for the determination of renal function with well explained mechanisms of how and why these markers are found in saliva. Saliva assay has opened the path with multiple interests and research areas in virology, immunology, microbiology, endocrinology, epidemiology, forensics, genomics and clinical chemistry. Other researchers added that monitoring blood biomarkers for renal function at frequent intervals causes unnecessary discomfort and mental trauma to the patient, therefore, a much simpler and non-invasive technique for the diagnosis and management of renal function is very desirable. Other biological fluids are utilized for the diagnosis of kidney disease but saliva offers some distinctive advantages [2]. Studies have reported that saliva could be better because the procedure reduces anxiety, physical and psychological trauma; therefore patient's compliance during specimen collection is easier. Complications due to blood collection are not seen and moreover, blood collection requires trained personnel unlike in saliva [3]. Whole saliva can be collected non-invasively and by individuals with limited training. No special equipment is needed for the collection of the fluid or specimen. Some researchers contributed that non-invasive approach is obviously important in several situations such as in pediatric and geriatric clinics where invasive approach is usually difficult or when access to healthcare is unrealistic and in remote geographic areas where phlebotomists are unavailable [4]. However, this study will focus on the determination of creatinine in blood and saliva of haemodialysed subjects; before and after dialysis to ascertain if salivary creatinine allows for changes in concentration after therapeutic administration and perhaps an additional renal biomarker for kidney function.

2. MATERIALS AND METHODS

2.1 Study Area/Population

The study was conducted at Hilton Clinic, 2 Ejekwu Wike Street, Opposite Former Silver Spoon Hotel, off Ada-George Road, Rumuepirikom, Port Harcourt among haemodialysed subjects who have been diagnosed of renal failure, between the ages of 18 and 60, attending the Urology Clinic.

2.2 Sampling Method

Samples collected from the participants were done using randomization techniques.

Each dialysis bed was labeled 0 or 1 so that the number of "0" –labeled beds were equal to "1"-labeled beds. All subjects who used "0" labeled-bed were recruited for the study while subjects who used bed labeled "1" were not selected.

All control subjects were recruited among hospital staff who were registered with the hospital and do not have any history of kidney disease. This was confirmed from their clinical

folders. Control subjects were asked to pick a number from a container having a numbering system of "0" and "1". All control subjects that picked "1" were recruited for the study while control subjects that picked "0" were not selected.

2.3 Eligibility Criteria

The following are the inclusion criteria:

- Subjects registered with the hospital
- Subjects diagnosed with kidney failure
- Subjects attending urology clinic for haemodialysis
- Subjects between the ages of 18 and 60

The following are the exclusion criteria

- Subjects less than 18years old
- Subjects greater than 60years old
- Subjects with oral infection. In addition to physical examination, their clinical folders were checked for any history of oral infection.

2.4 Sample Collection Method

2.4.1 Saliva

Saliva was collected from the subjects using spitting method. The subjects were asked to spit 1ml of saliva into a plain bottle [5,6]. Prior to that, subjects were asked to wash their mouths with distilled water and to spit two or three times into a disposable plastic container, after which they were told to spit 1ml of saliva into a plain sample collection container. This procedure was performed before and after haemodialysis [7].

2.4.2 Blood

Blood was collected using venipuncture technique into a heparin bottle. The sample was collected first before dialysis and then after dialysis.

2.5 Sample Preparation

2.5.1 Saliva

The collected saliva sample was centrifuged for 5minutes at 4000rpm, after which the supernatant was separated and used for the analysis. In situations where the biofluid supernatants were not assayed immediately, the samples were stored at -20^{o}C. [6].

2.5.2 Blood

The blood collected was spun at 4000rpm, the supernatant was separated and used immediately for the analysis. In situations where the biofluid supernatants were not assayed immediately, the biofluids were stored at -20^{o}C. [6].

2.6 Laboratory Method

Jaffe method was used in the laboratory analysis of creatinine in blood and saliva. The principle of the test is based on the fact that under alkaline conditions, creatinine in plasma reacts with picric ions to produce a reddish complex; the absorbance of this complex was determined using a spectrophotometer at a wavelength of 520 mm [8].

2.7 Statistical Analysis

The correlations coefficient between blood and salivary creatinine levels were determined using Pearson's correlation analysis to determine the relationship between blood and salivary creatinine levels. ANOVA was done to determine if there was a significant difference in the means of the groups (Control group, pre-haemodialysed subject and post-haemodialysed subject). The level of statistical significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows demographic parameters. The mean±SD age of haemodialysed patients was 43±12yrs and the mean±SD age of the control group (health individuals) was 40±5yrs. Also, the total number of males and females recruited for the study was presented.

Table 2 shows the mean±SD concentration of creatinine in blood and saliva of haemodialysed subjects before and after dialysis. The correlation coefficient of creatinine between blood and saliva was also presented in the table along with ANOVA results.

Table 1. Demographic parameters

	Haemodialysed subjects	Control subjects
Age (yrs)	43±12	40±5
Males	17	10
Females	12	11

Table 2. Blood and Salivary Creatinine Levels (umol/1) in Pre-HD, Post-HD and Control groups

	Blood	Saliva	r^2
Pre-HD	646.3±29.0	143.3±21.3	-0.12
Post-HD	211.1±7.7	56.6±8.8	0.11
Control	78.5±2.4	15.7±1.7	0.02
P-value	<0.05	<0.05	
Remark	SS	SS	

N=29; Pre-HD = Pre-haemodialysis; Post-HD = Post-haemodialysis; SS = Statistically significant; r^2 = correlation coefficient

3.2 Discussion

An ideal biomarker should not only provide differential clinical information or diagnosis in health and disease, but should also provide suitable clinical data after treatment to ascertain therapeutic success or failure. Creatinine is a biomarker often evaluated in blood and urine for laboratory assessment of kidney function and staging of chronic kidney disease. It is used because it provides to a large extent clinic data required for the management of kidney disease. Therefore, the question; "like blood, can saliva also provide differential laboratory diagnosis for normal kidney function, kidney disease and can it provide clinical information to ascertain therapeutic achievement or failure?" From the result presented in Table 2, the mean concentration of creatinine in blood was 646±29.0umol/1 in pre-haemodialysed subjects while that in saliva was143.3±21.3umol/l. The mean concentration of blood creatinine in post-haemodialysed subjects was 211.1±7.7umol/1 while that in saliva was 56.6±8.8umol/1. There was a decrease in the concentration of creatinine after haemodialysis in both biofluids and this is in agreement with other study [5]. By this finding, it draws the fact that creatinine is not only found in saliva but it also decreases in concentration after therapeutic administration. That is to say that salivary creatinine responds to changes in concentration after therapeutic administration. The mean concentrations of creatinine in blood and saliva of control group were 78.5±2.4umol/1 and 15.7±1.7umol/1 respectively. Taking into consideration of the creatinine concentrations in the three groups (pre-haemodialysis, post haemodialysis and control groups) which depicted disease condition, treatment and health, it supports the definition of a good biomarker. Like blood, salivary creatinine level was lowest in healthy individual which made up the control group but peaked at disease condition (pre-haemodialysed subjects) [9] and concentrations dropped following treatment (post-haemodialysis). In saliva, there was a significant difference in creatinine level among the mean of the groups with P-value<0.05. Also, in blood there was a significant difference in creatinine level among the groups with P-value<0.05. Looking at the correlation analysis of creatinine in blood and in saliva, there was a weak negative correlation in pre-haemodialysed subjects. The correlation coefficient between blood and salivary creatinine concentrations in Post-haemodialysed subjects was a weak positive correlation. The correlation coefficient between blood and salivary creatinine concentrations in control group was 0.02. By interpretation, increase or decrease in blood creatinine has slight impact on the saliva because the level of relationship or association between blood creatinine and salivary creatinine is generally weak in all groups. To support these findings, a study conducted reported that about 10-15% of creatinine in blood diffuses into the saliva because of its high molecular weight compared to urea that diffuses higher due to its low molecular weight [10]. The increased molecular weight of creatinine may have contributed to the reduced rate of diffusion of creatinine across the basement from blood to saliva which may be the reason for the weak relationship or correlation between blood and salivary creatinine. This finding is not in agreement with many studies where there were contrary findings over the correlation of blood and salivary creatinine. Some works found negative correlation in control group and positive correlation is case group [11], although some studies revealed that creatinine had strong positive correlation in all groups [12,13,14].

4. CONCLUSION

This work has shown that salivary creatinine responds to changes in concentration in health, disease and treatment in kidney disease but the levels of relationship with changes in blood creatinine levels were weak. However, more studies on this parameter are required if salivary creatinine would be a future alternative or supportive diagnostic tool for kidney disease.

CONSENT AND ETHICAL APPROVAL

All subjects who met the eligibility criteria for the study and gave their written consent were recruited for the study

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yu-Hsiang L, Wong DT. Saliva: An emerging biofluid for early detection of disease. American Journal of Dentistry. 2009;22(4):241-248.
2. Sanjeev M, Vikram B, Sushant G, Gaurav A, Sanjay B. The diagnostic role of saliva - A Review. Journal of Clinical and Experimental Dentistry. 2011;3(4):314-320.
3. Sun F, Reichenberger EJ. Saliva as a source of genomic DNA for genetic studies: Review of current methods and applications. Oral Health Dentistry Management. 2014;13:217-22.
4. Daniel M, Isaac RR. Saliva as a diagnostic fluid. Dental Clinics of North America Journal. 2011;55(1):159-178.
5. Klassen JT, Krasko BM. The dental health status of dialysis patients. Journal of the Canadian Dental Association. 2002;68:34-38.
6. Nurkka A, Obiero J, Kaythy H, Sscott AG. Effects of sample collection and storage methods on antipneumococcal immunoglobulin A in saliva. Clinical Diagnosis Laboratory Immunology. 2003;10(3):357-61.
7. Fyneface CA, Onengiyeofori I, Davies T. Evaluation of saliva for monitoring renal function in haemodialysis patients at university of port Harcourt teaching hospital. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2018;1(2):1-6.
8. Toora BD, Rajagopal G. Measurement of creatinine by jaffe reaction- Determination of concentration of sodium hydroxide required for maximum colour development in standard, urine and protein free filtrate of serum. Indian Journal of Experimental Biology. 2002;40(3):352-354.
9. Tomas I, Marinho JS, Limeres J, Santos MJ, Araujo L, Diz P. Changes in salivary composition in patients with renal failure. Archives Oral Biology. 2008;53:528-532.
10. Venkatapathy R, Govindarajan V, Oza N, Parameswaran S, Dhanasekaran BP, Prashad KV. Salivary Creatinine Estimation as an Alternative to Serum Creatinine in Chronic Kidney Disease Patients. International Journal of Nephrology. 2014;74(27):120-124.
11. Lloyd JE, Broughton A, Selby C. Salivary creatinine assays as a potential screen for renal disease. Annual Clinics Biochemistry. 1996;33(5):428-31.
12. Bhavana SB, Mohankumar KP, Madhushankari GS, Mandana D, Puneeth HK. Diagnostic accuracy of salivary creatinine, urea, and potassium levels to assess dialysis need in renal failure patients. Dental Research Journal. 2017;14(1):13-18.
13. Taye JL, Yemi RR, Babatunde LS. Salivary creatinine and urea analysis in patients with chronic kidney disease: A case control study. Biomedical central. 2016;10:16-22.
14. Xia Y, Peng C, Zhou Z. Clinical significance of saliva urea, creatinine, and uric acid levels in patients with chronic kidney disease. Journal of Central South University Medical Sciences. 2012;37(11): 1171-1176.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59674