Evaluation of Saliva for Monitoring Renal Function in Haemodialysis Patients at University of Port Harcourt Teaching Hospital

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ABSTRACT

In this study, the concentrations of urea were assayed in both blood and saliva of 130 haemodialysis patients; before haemodialyis(pre-haemodialysis) and after haemodialysis (post haemodialysis); and 60 healthy individuals who made up the control group. The method used for urea assay was urease method. The mean±SD concentrations of salivary urea in pre and post haemodialysis patients, as well as control group, were 17±0.6mmol/l, 9.1±0.5mmol/l and 4.0±0.3mmol/l respectively. The mean±SD concentrations of blood urea in pre and post haemodialysis patients, as well as control group, were 21.6±0.5mmol/l, 9.1±0.4mmol/l and 4.2±0.2mmol/l respectively. The correlation coefficient between blood and salivary urea in pre-haemodialysis patients is 78.8% while that for post haemodialysis patients is 60.6% and for the control group is 90%. The ANOVA results of salivary urea in the three groups (pre, post and control) showed a significant difference with P-value <0.05. The ANOVA results of blood urea in the three groups (pre, post and control) showed a significant difference with P-value <0.05. From the various results obtained, saliva can serve as a diagnostic biofluid for renal disease especially with the salivary urea as the biomarker. Also, the salivary renal biomarker (urea) responds to changes in concentrations after therapeutic consideration. This study is in consonance with other literature that saliva is a diagnostic fluid for kidney disease; however, there is a need to carry out more research works to continually unveil the diagnostic potential of saliva in kidney disease.

Keywords: Pre-haemodialysis (Pre-HD); post-Haemodialysis (Post-HD); kidney failure; saliva; blood; urea.

1. INTRODUCTION

Kidney failure, also called renal insufficiency is a condition of impaired kidney function in which the kidney fails to adequately excrete wastes from the blood. Chronic kidney 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 renal failure or kidney disease in all parts of the world. Obviously, blood has been the body fluid of choice in disease diagnosis; however, saliva promises to be an attractive alternative over serum with numerous advantages over blood. Saliva is a clean, tasteless, odourless slightly acidic viscous fluid, consisting of secretions from the parotid, sublingual, submandibular salivary glands and other glands of oral cavity. Saliva is a

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collection of multiple salivary glands secretion as mentioned above lying beneath the oral mucosa. Every human salivary glands secret about 600ml of saliva, 99.5% of it is water and antibacterial compounds such as secretory immunoglobulin and lysozyme. It also contains microorganisms, oral epithelial cells and food debris. This is the rationale behind why saliva specimen needs to be prepared first by centrifugation before use. 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 oral or saliva 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]. Saliva has biomarkers for the determination of kidney function with well explained mechanisms of how and why electrolytes, urea, and creatinine are found in saliva. These mechanisms also explain why increased biomarker level in blood leads to corresponding increase 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. 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]. To patients, saliva which employs a non-invasive approach or method is better for them 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. This 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 in remote geographic areas where phlebotomists are unavailable [4]. 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. This study will be based on the use of saliva in the determination of kidney function with the view also of establishing the response of salivary renal biomarker to treatment as seen in blood.

2. MATERIALS AND METHODS

2.1 Study Area/Setting

The research study was conducted in Port Harcourt at University of Port Harcourt Teaching Hospital (UPTH). UPTH is a tertiary hospital located along East West Road, sharing boundaries with Choba, Alakahia, Aluu and Rumuekini communities in Obio/Akpor Local Government Area, Rivers State, Nigeria. It holds as a reference hospital to many hospitals in the state and neighbouring states.

2.2 Sampling Method

The study began in 10th May, 2017 and among haemodialysis patients who have been diagnosed of kidney failure, between the ages of 18 and 60, attending the Urology Clinic. The mode of haemodialysis was centre-based haemodialysis and Diasafe Plus Filter was used. All subjects who met the eligibility criteria for the study and gave their written consent were recruited for the study. Samples were collected from the participants in a simple randomization technique.

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

All control subjects were recruited from UPTH 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:

- Patients registered with the hospital (UPTH)

- Patients diagnosed with renal failure
- Patients attending urology clinic for haemodialysis
- Patients between the ages of 18 and 60

The following are the exclusion criteria:

- Patients less than 18years old
- Patients greater than 60years
- Patients not diagnosed with renal failure
- Renal failure patients not coming for haaemodialysis
- Patients with oral or mouth infection. Besides, checking their clinical folders for history of oral infection, the patients' mouths were physically observed for signs of mouth injury or infection before they were recruited for the study recruitment.

2.4 Sample Collection Method

2.4.1 Saliva

The main three ways to collect whole saliva are the draining method in which saliva is allowed to drip off the lower lip [5]. The second method is spitting technique where the patient was asked to spit saliva into a plain bottle [6]. In this study, the method used for saliva collection was spitting method. Patients 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.

2.4.2 Blood

The method used for blood collection was venipuncture. The sample was collected into a heparin bottle before dialysis and after dialysis.

2.5 Sample Preparation

2.5.1 Saliva

The whole saliva 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 used immediately for analysis, they were stored at -20^0C. [6].

2.5.2 Blood

The blood collected was spun at 4000rpm, after which the supernatant was separated and used immediately for the analysis. In situations where the biofluid supernatants were not used immediately for analysis, they were stored at -20^0C. [6].

2.6 Laboratory Methods

Urease method, an enzymatic method was used in the laboratory analysis of salivary and blood urea. Urease hydrolyzes urea to ammonia and Carbondioxide. The ammonia formed further reacts with a phenolic chromogen and hypochlorite to form a coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample. Absorbance was read using a spectrophotometer at a wavelength of 540nm.

2.7 Statistical Analysis

Correlation coefficient between plasma and salivary urea levels was calculated using Pearson's correlation analysis to determine the relationship between blood and salivary urea. ANOVA was also done to determine if there was a significant difference in the means of the groups (Control group, pre-haemodialysis subject and post-haemodialysis subject). The level of statistical significance was set at α=0 .05.

3. Results, Discussion and Conclusion

3.1 Results

Table 1 shows the demographic parameters. The mean±SD age of haemodialysis patients was 55±7 and the mean±SD age of the control group (healthy individuals) was 47±12.

Table 1. Demographic parameters

	Haemodialysis subjects	Control
Age (yrs)	55±7	47±12
Males	73	24
Females	57	36

3.2 Discussion

Out of a total of 130 sample size of patients recruited for the study, 73 were males and 57 were females. The control group comprised 24 males and 36 females, giving a total of 60 participants that made up the control group. From the result presented in Table 2, the mean concentration of urea in blood was found to be 21.0±0.5mmol/l in pre-haemodialysis patients while that for saliva was 17.3±0.6mmol/l. By this, there was a clear indication that urea was found

in saliva. The mean concentration of blood urea in post-haemodialysis patients was found to be 9.1±0.4mmol/l while that in saliva was 9.1±0.5mmol/l. there was a decrease in the concentration of urea after haemodialysis and this is in agreement with a study conducted by Klassen in 2002 [7]. By this finding, it draws the fact that urea was not only found in saliva but it also responded to therapeutic management. Therefore, salivary urea could serve as a diagnostic and management tool in kidney disease. The mean of urea concentrations in blood and saliva of the control group were found to be 4.2±0.2mmol/l and 4.0±0.3mmol/l respectively. Taking into consideration of the various urea mean concentrations (pre-haemodialysis, post haemodialysis and control groups), it draws to the fact that urea could be diagnostic because the level of urea was low in control group both in saliva and blood but peaked at the disease group without treatment (pre-haemodialysis group) [8] and concentrations drop following treatment (post-haemodialysis). Looking at the correlative analysis between urea concentration in blood and that in saliva, there was a significant positive correlation or relationship between blood urea and salivary urea levels. A correlation coefficient (r^2) of 78% between blood and salivary urea in pre-haemodialysis patients was presented in Table 2. This implies a significant positive correlation between blood urea and salivary urea in haemodialysis patients. A work conducted showed that there was a strong positive correlation between blood urea and salivary urea in kidney disease subjects [9]. This study continued by determining the level of relationship between blood urea and salivary urea in post haemodialysis patients. Table 2, presented a correlation coefficient of 60.6% which means a significant positive correlation between blood urea and salivary urea in post-haemodialysis patients. Furthermore, a correlative study was done between blood urea and salivary urea in healthy individuals (control group) and the correlation coefficient was found to be 90%. Other studies agree to this finding that there is a strong relationship between blood urea and salivary urea in the healthy group [10]. By interpretation, there is a significant positive correlation between the two groups. Therefore, urea is not only present in saliva but there is a strong relationship between blood urea and salivary urea so that increase in blood urea will lead to increase in salivary urea and a decrease in blood urea will lead to decrease in salivary urea. By this strong positive correlation between blood and salivary urea, salivary urea can serve as a resourceful diagnostic tool in the diagnosis and management of kidney disease. The study also subjected the data of salivary urea concentrations of three groups; pre-haemodialysis, post-haemodialysis and control to the analysis of variance (ANOVA). Table 2 showed that there was a significant difference in the mean of the groups at P-value < 0.05; by interpretation, there was a significant difference among the means of the groups under study. Also, the mean of blood urea of the three groups (pre-haemodialysis, Post-Haemodialysis and control) were subjected to ANOVA evaluation at α=0.05. From Table 2, the results revealed p-value < 0.05. By interpretation, there was a significant difference among the means of the groups under study. Urea in blood and saliva do not only hold strong positive correlation but are diagnostic in kidney disease because the level of urea varies from one case study to another, providing healthcare providers the course for diagnosis and in arriving at clinical decision in the treatment and management of kidney disease. That is to say that salivary urea level increases with disease progression [8] and decreases after treatment like haemodialysis [7].

Table 2. Comparing results of blood and salivary urea levels in mmol/l in Pre-HD, Post-HD and Control

	Blood	Saliva	r^2
Pre-HD	21.0±0.5	17.3±0.6	78.8%
Post-HD	9.1±0.4	9.1±0.5	60.6%
Control	4.2±0.2	4.0±0.3	90%
P-value	<0.05 SS	<0.05 SS	

N=130, Pre-HD = Pre-haemodialysis, Post-HD = Post-haemodialysis, SS = Statistically significant, r^2 = correlation coefficient

4. CONCLUSION

This work has showed that other non-invasive diagnostic approaches like the use of saliva can be used for the the diagnosis and management of kidney disease.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s)

ETHICAL APPROVAL

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.

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