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Saliva based analysis of biochemical factors in patients with chronic kidney disease undergoing hemodialysis

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Abstract

Background: Use of saliva as alternative to blood in monitoring systemic diseases is still subject to continued research. Hence, this study evaluated changes in biochemical composition of saliva and plasma before and after hemodialysis and also determined the correlation between these factors in saliva and plasma of patients with chronic kidney disease (CKD).

Methods: A cross sectional study that included 50 patients with CKD undergoing hemodialysis. Whole saliva and blood samples were collected from the participants before and after dialysis. Samples were analyzed for urea, creatinine, total protein, sodium, potassium, calcium, chloride, and bicarbonate. Data were compared using Related Samples Wilcoxon Signed Rank test. Correlation between plasma and salivary parameters was determined using Spearman's correlation test.

Results: Levels of salivary urea and creatinine were reduced in the post dialysis state in consistence with reduced plasma levels. Salivary and plasma bicarbonate levels were elevated in the post dialysis state compared to pre-dialysis while both salivary and plasma levels of total protein, sodium, potassium, calcium and chloride did not show significant change. There were positive correlations between salivary and plasma creatinine and potassium in the post dialysis state as well as calcium in both pre and post dialysis states.

Conclusion: Findings of this study suggest that saliva reflects plasma levels of biochemical factors in patients with CKD in the pre and post dialysis states. Hence, saliva may be an alternative to blood in monitoring patients with CKD undergoing dialysis.

Keywords: *Saliva; blood; chronic kidney disease; dialysis*

Résumé

Contexte: L'utilisation de la salive comme alternative au sang dans la surveillance des maladies systémiques fait toujours l'objet de recherches continuées. Par conséquent, cette étude a évalué les changements dans la composition biochimique de la salive et du plasma avant et après l'hémodialyse et a également déterminé la corrélation entre ces facteurs dans la salive et le plasma des patients atteints d'insuffisance rénale chronique (IRC).

Méthodes : Une étude transversale qui a inclus 50 patients atteints d'IRC sous hémodialyse. Des échantillons entiers de salive et de sang ont été prélevés chez les participants avant et après la dialyse. Les échantillons ont été analysés pour l'urée, la créatinine, les protéines totales, le sodium, le potassium, le calcium, le chlorure et le bicarbonate. Les données ont été comparées à l'aide du test du Rang Signé Wilcoxon des échantillons reliés. La corrélation entre les paramètres plasmatiques et salivaires a été déterminée en utilisant le test de corrélation de Spearman.

Résultats : Les niveaux d'urée salivaire et de créatinine ont été réduits dans l'état post dialyse en accord avec des taux plasmatiques réduits. Les taux salivaires et bicarbonatés plasmatiques étaient élevés dans l'état post dialyse par rapport à la pré-dialyse, tandis que les taux salivaires et plasmatiques de protéines totales, de sodium, de potassium, de calcium et de chlorure n'ont pas montré de changement significatif. Il y avait des corrélations positives entre la créatinine salivaire et plasmatique et le potassium dans l'état post dialyse ainsi que le calcium dans les états de dialyse pré et post.

Conclusion : Les résultats de cette étude suggèrent que la salive reflète les taux plasmatiques de facteurs biochimiques chez les patients atteints d'IRC avant et après la dialyse. Par conséquent, la salive peut être une alternative au sang dans la surveillance des patients atteints d'IRC subissant une dialyse.

Mots-clés: *Salive; sang; maladie rénale chronique; dialyse*

Introduction

Chronic kidney disease (CKD) is one of human diseases that have global impact for which diagnosis and monitoring require supplementing clinical evaluation with laboratory testing [1]. The increasing global burden of CKD and resultant end-stage renal disease (ESRD) continues to present serious challenges for the entire world [1, 2]. Management of CKD includes renal replacement therapy (RRT) and the available options are dialysis or kidney

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transplantation. Hemodialysis as a form of RRT requires frequent blood collection for adequate monitoring [3]. The frequent blood sampling associated with hemodialysis may result in blood loss [4]. In addition, the individuals involved in the management of patients with CKD are at more risk of blood borne diseases because of their frequent contact with blood. Hence, a non-invasive diagnostic test with minimal risk as well as ability to provide a dependable evaluation of disease condition and adequacy of hemodialysis would be invaluable to both the healthcare professionals and the patients.

It had been reported in several studies that changes occur in salivary secretions in some systemic diseases for which saliva has been suggested to be an alternative to blood in monitoring these diseases [5-7]. Also, the diagnostic potential of salivary urea and creatinine analyses in patients with CKD had been reported [8-11]. Saliva has great potential as a diagnostic fluid and offers many advantages over blood and other biological fluids because of its economic and noninvasive method of collection which promotes its use for monitoring systemic diseases [12, 13]. In addition, salivary analysis holds great promise as an effective tool for the diagnosis, prognosis and monitoring adequacy and effectiveness of dialysis in patients with CKD [11]. Other advantages of saliva as a clinical tool over blood and other body fluids include use of smaller aliquot samples, good cooperation from patients, cost effectiveness, easy storage and transportation, good sensitivity and correlation with levels of some factors in blood [14, 15]. Saliva is indeed a very useful diagnostic fluid. However, its potential for clinical medicine is not used as much as it should. Hence, its use as alternative to blood in monitoring of systemic diseases as well as efficacy of dialysis therapy is still subject to continued research. This study was therefore designed to evaluate changes in salivary biochemical factors before and after hemodialysis and to determine correlation between plasma and salivary biochemical factors before and after hemodialysis.

Methods

Study design

This was a prospective study of patients with CKD who were being dialyzed in a tertiary hospital. Following ethical approval from the institution Research Ethics Committee, the study was carried out among consecutive patients with CKD that required hemodialysis. Chronic kidney disease was defined as estimated glomerular filtration rate eGFR< 60mls/min/1.73m^2. Inclusion criteria were age >18 years, diagnosis of CKD and presence of indication for hemodialysis. Exclusion criteria were age < 18 years, diagnosis of acute kidney injury, patient who did not require hemodialysis, history suggestive of upper gastrointestinal bleeding, unconsciousness and inability to produce saliva.

This study included 50 patients with CKD composed of 35 males and 15 females with a mean age of 49.5 ± 16.07 years. The patients were in stages 3, 4 or 5 and there clinical data are as shown in table 1.

Table 1: Demographic and clinical data of participants

Variables	Mean ± SD / Value (%) N = 50
Age (years)	49.5 ± 16.07, Range: 19 to 77
Gender	
Male	35 (70%)
Female	15 (30%)
CKD grading	
Stage 3	6 (12%)
Stage 4	24 (48%)
Stage 5	20 (40%)

Note. CKD: chronic kidney disease

Each participant completed a self-administered questionnaire to obtain demographics, stage of CKD and dialysis history. Whole saliva samples were collected by asking patients to spit into a graduated universal bottle until about 3ml of saliva was produced just before and after dialysis when the patient was stable. Samples were transferred aseptically to sterile tubes and stored at -20°C until the time for laboratory analysis. Simultaneously, venous blood samples (5ml each) were collected in sample tubes with lithium heparin. Whole saliva and plasma (separated from the blood samples) were used for the biochemical analysis.

Following manufacturer's instructions (RANDOX Creatinine Reagent, UK), estimation of creatinine was done using modified Jaffe's method [16] while, estimation of urea was done using the method of Marsh *et al.*, [17] (RANDOX Urea Reagent, UK). Estimation of total protein was done using Biuret method [18]. Sodium and potassium levels were determined using flame emission spectrophotometry, while estimation of calcium was done using indirect colorimetric method [19]. Concentrations of chloride and bicarbonate were determined by Schales method using mercuric nitrate [20].

Statistical analysis

All statistical analyses were performed using IBM - Statistical analysis Software Package (SPSS Statistics) Version 22. 0. Armonk, New York. The main quantitative variables were salivary and plasma levels of urea, creatinine, total protein, sodium, potassium, calcium, bicarbonate and chloride before and after dialysis. Data are presented using descriptive statistics such as mean, median and interquartile range. Data were tested for normality using Kolmogorov-Smirnov test. Serum and salivary levels of most analytes were not normally distributed; hence the Related Samples Wilcoxon Signed Rank test was used to compare median values of the factors before and after dialysis. Correlation between plasma and salivary biochemical parameters was determined using Spearman's correlation test. All analyses were done at p-value < 0.05.

Results

Salivary levels of urea and creatinine were significantly reduced in the post dialysis samples compared to the pre dialysis samples ($p < 0.001$ and $p < 0.001$ respectively). These findings were similar to what was observed in plasma samples (Figs. 1 and 2). Levels of bicarbonate were significantly elevated in saliva and plasma in post dialysis samples compared to the pre dialysis samples ($p = 0.04$ and 0.02 respectively). Salivary levels of total protein, sodium, potassium, calcium, chloride and bicarbonate before and after dialysis are shown in table 2.

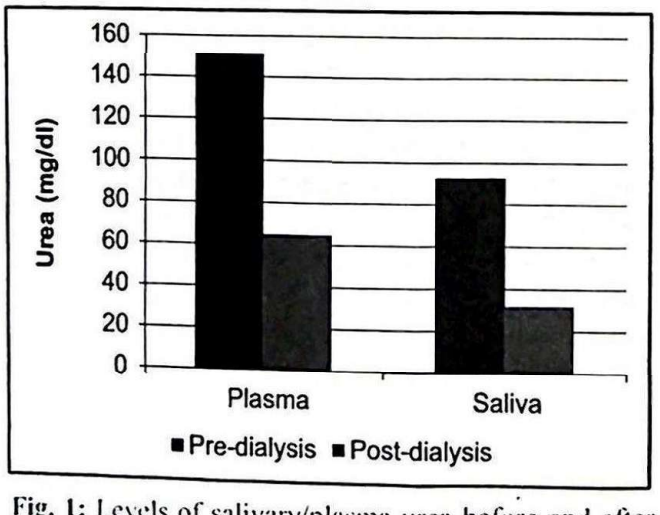
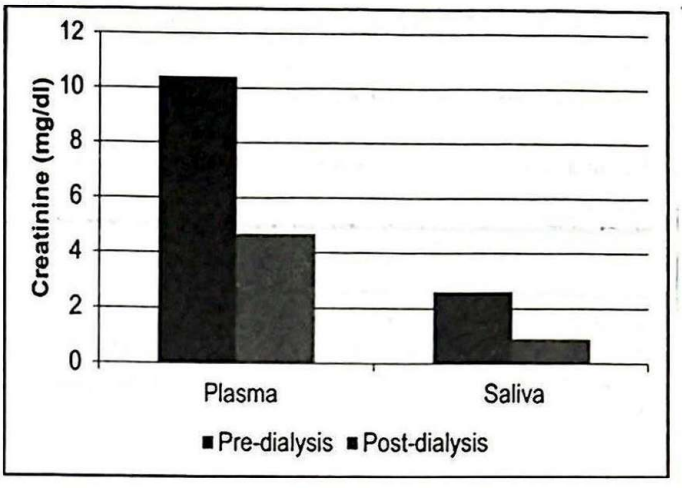


Fig. 1: Levels of salivary/plasma urea before and after dialysis

Plasma levels of total protein, sodium, potassium, calcium, chloride and bicarbonate before and after dialysis are shown in table 3. As shown in figures 1 and 2, plasma levels of urea and creatinine were significantly reduced in the post dialysis



Data are presented as median values

Fig. 2: Levels of salivary/plasma creatinine before and after dialysis

samples ($p < 0.001$ and $p < 0.001$ respectively). The mean percentage reductions of urea and creatinine in plasma were 57% and 55% respectively while the mean percentage reductions of urea and creatinine in saliva were 66% and 65% respectively.

Table 2: Salivary biochemical factors before and after dialysis

	Before dialysis	After dialysis	p-value
Sodium (mEq/L)	4.3(3.9)	5.4(4.6)	0.29
Potassium (mEq/L)	18.7(13.4)	19.2(8.5)	0.40
Chloride (mEq/L)	12(12)	14.1(11.8)	0.13
Bicarbonate (mg/dl)	14(16)	18(14)	0.04*
Total Protein (mg/dl)	17(21)	21(14)	0.32
Calcium (mg/dl)	7.5(3.4)	7.2(5.8)	0.26
Urea (mg/dl)	93(141)	32(58)	<0.001**
Creatinine (mg/dl)	2.6(2.5)	0.9(1.4)	<0.001**

Data are presented as median (Interquartile range)

Table 3: Plasma biochemical factors before and after dialysis

	Before dialysis	After dialysis	p-value
Sodium (mEq/L)	125(12)	129 (6)	0.06
Potassium (mEq/L)	5.3(2.2)	5.1 (3.6)	0.08
Chloride (mEq/L)	97(10)	99 (9)	0.24
Bicarbonate (mg/dl)	17(4)	18 (3)	0.02*
Total Protein (mg/dl)	60(10)	64 (14)	0.11
Calcium (mg/dl)	8.1(3.3)	8.7 (3.8)	0.37
Urea (mg/dl)	151(126)	65 (36)	< 0.001**
Creatinine (mg/dl)	10.4(7.18)	4.7 (3.6)	< 0.001**

Data are presented as median (Interquartile range)

There were positive correlations between salivary and plasma creatinine and potassium in the post dialysis state as well as calcium in both pre and post dialysis states (table 4).

Table 4: Correlation between salivary and plasma factors before and after dialysis

	Before dialysis	After dialysis
Sodium	-0.26 (0.08)	0.26 (0.09)
Potassium	-0.05 (0.75)	0.30 (0.04*)
Chloride	-0.27 (0.08)	0.17 (0.28)
Bicarbonate	-0.17 (0.27)	0.15 (0.34)
Total Protein	0.25 (0.08)	0.16 (0.28)
Calcium	0.39 (0.01*)	0.46 (< 0.01*)
Urea	-0.16 (0.26)	0.08 (0.59)
Creatinine	0.07 (0.63)	0.34 (0.02*)

Data are presented as the correlation coefficient (p value)

Discussion

The main findings from this study were reduced levels of salivary urea and creatinine and elevated levels of salivary bicarbonate after dialysis consistent with their levels in plasma. In addition, similar to what was observed in plasma, that there was no significant difference in the levels of salivary sodium, potassium, chloride, phosphate and total protein post dialysis compared to their pre dialysis levels.

In agreement with previous studies, our findings showed elevated concentration of salivary urea and creatinine in the patients with CKD before dialysis [21-23]. . In the present study, the salivary concentrations of urea and creatinine decreased substantially (66% and 65%, respectively) after haemodialysis probably because saliva reflected adequate changes in concentrations of urea and creatinine in the blood. Furthermore, the presence of urea and creatinine in the saliva indicates their passive diffusion from plasma to saliva through the salivary glands [24]. These findings also suggest that the salivary concentrations of urea and creatinine could be useful in monitoring the efficacy of dialysis. In addition, a good correlation was observed between the salivary and plasma levels of creatinine after dialysis. The usefulness of salivary creatinine in monitoring dialysis in patients with CKD is also corroborated by the positive correlation between the salivary and plasma levels after dialysis.

The post dialysis concentration of salivary bicarbonate was higher compared to what was observed in plasma. This finding may be explained by the contribution of the bicarbonate dialysate commonly used to correct the metabolic acidosis which is often associated with advanced CKD [25]. However, there was no appreciable difference in the concentrations of other salivary electrolytes (sodium, potassium and chloride), calcium and total protein. This finding is similar to what was observed in plasma. The lack of change in the levels of the salivary sodium after dialysis is similar to the report by previous studies [23, 26]. These observations in the concentrations of salivary electrolytes before and after dialysis could be explained in many ways. This could be because majority of the participants undergoing hemodialysis had more of uremia rather than electrolyte derangements and thus the pre and post dialysis levels of these parameters were similar. Also, the lack of change in the salivary concentrations of the electrolytes (sodium, potassium, calcium, chloride and phosphate) may be explained by the concurrent lack of change in their plasma levels although, the salivary concentrations of these ions do not depend exclusively on their plasma concentrations [27]. Sodium and potassium ions undergo active transport along the salivary duct via Na-K-Cl symporters apart from passive diffusion [28]. Contrary to our finding, Shasha *et al.* observed that salivary concentration of sodium and potassium level in hemodialysed patients fell until it reached values close to those of the controls in the post-dialysis state [29].

There were positive correlations between salivary and plasma levels of calcium in the pre and post dialysis states as well as concentrations of potassium in the post dialysis state. Contrary to our finding, a previous study reported no correlation between plasma and salivary levels of calcium in patients with CKD [30]. The differences in the findings could be explained by the variations in the study population as well as laboratory methods used.

In conclusion, saliva based biochemical analysis in patients with CKD showed reduced salivary concentrations of urea and creatinine while bicarbonate concentration was elevated after dialysis similar to what was observed in plasma. In addition, there was positive correlation between salivary calcium in the pre and post dialysis states as well as creatinine and potassium in the post dialysis state. However, there was no significant change in the salivary as well as plasma concentrations of sodium, potassium, calcium and total protein after dialysis. Also there was no correlation between saliva and plasma concentrations of sodium, potassium, and total protein. Findings of this study indicate that saliva reflects levels of the assessed biochemical factors in the plasma of patients with CKD in the pre and post dialysis states, with some of the saliva

factors showing correlation with the plasma counterparts. Hence, saliva may be an alternative to blood in monitoring chronic kidney disease especially in patients undergoing dialysis.

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References

1. Kidney disease outcome quality initiative: Clinical guidelines for chronic kidney disease. Evaluation, classification and stratification. Am J Kidney Dis 2002; 39:S1-246.
2. Laysaght MJ. Maintenance dialysis population dynamics: current trends and long term implications. J Am Soc Nephrol 2002; 13:37-40.
3. El Nahas AM and Bello AK. Chronic kidney disease: the global challenge. Lancet 2005; 365:331-340.
4. Vaziri ND, Kalantar-Zadeh K and Wish JB. New options for iron supplementation in maintenance hemodialysis patients. Am J Kidney Dis 2016; 67:367-375.
5. Ayadin SA. Comparison of ghrelin, glucose, alpha amylase and protein levels in saliva from diabetes. J Biochem Mol Biol 2007; 40:29-35.
6. Lasisi TJ and Fasanmade AA. Saliva composition in diabetic and non- diabetic patients. Niger J Physiol Sci 2012; 27:79-82.
7. Abrão AL, Falcao DP, de Amorim RF, *et al.* Salivary proteomics: A new adjuvant approach to the early diagnosis of familial juvenile systemic lupus erythematosus. Med Hypotheses 2016; 89:97-100.
8. Lloyd JE, Broughton A and Selby C. Salivary creatinine assays as a potential screen for renal disease. Ann Clin Biochem 1996; 33:428-431.
9. Zúñiga ME, Estremadoyro LO, León CP, Huapaya JA and Cieza JA. Validation of the salivary urea test as a method to diagnose chronic kidney disease. J Nephrol 2012; 25:431-436.
10. Venkatapathy R, Govindarajan V, Oza N, *et al.* Salivary creatinine estimation as an alternative to serum creatinine in chronic kidney disease patients. Int J Nephrol 2014; 742724:1-6.
11. Lasisi TJ, Raji YR and Salako BL. Salivary creatinine and urea analysis in patients with chronic kidney disease: A case control study. BMC Nephrol 2016; 17:1-6.
12. Galloway JW, Keijser BJ and Williams DM. Saliva in studies of epidemiology of human disease: the UK Biobank project. Periodontol 2000. 2016; 70:184-195.
13. Malathi N, Mythili S and Vasanthi HR. Salivary diagnostics: a brief review. ISRN Dent 2014; 158786:1-8.
14. Deepa T and Thirrunavukkarasu N. Saliva as a potential diagnostic tool. Indian J Med Sci 2010; 64:293-306.
15. Javaid MA, Ahmed AS, Durand R and Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. J Oral Biol Craniofac Res 2016; 6:66-75.
16. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scandinavian J Clin Lab Invest 1965; 17:381-387.
17. Marsh WH, Fingerhut B and Miller H. Automated and manual direct methods for the determination of blood urea. Clin Chem 1965; 11:624-627.
18. Jenzano JW, Brown CK and Mauriello SM. Temporal variations of glandular kallikrein, protein and amylase in mixed human saliva. Arch Oral Biol 1987; 32:757-759.
19. De Loureiro JA and Janz GJ. Iodometric and colorimetric methods for the estimation of calcium in serum based on the use of an improved permanganate solution. Biochem J 1944; 38:16-19.
20. Schales O and Schales SS. A simple and accurate method for the determination of chloride in biological fluids. J Biol Chem 1941; 140:879-882.
21. Dhalberg WH, Sreebny LM and King B. Studies of parotid saliva and blood in haemodialysis patients. J Applied Physiol 1967; 23:100-108.
22. Goll RD and Mookerjee BK. Correlation of biochemical parameters in serum and saliva in chronic azotemic patients and patients on chronic hemodialysis. J Dialysis 1978; 2: 399-414.
23. Seethalakshmi C, Koteeswaran D and Chiranjeevi V. Correlation of serum and salivary biochemical Parameters in end stage renal Disease patients undergoing hemodialysis in pre and post-dialysis state. J Clin Diagn Res 2014; 8: 12-14.

24. Garrett JR. Movements of organic molecules from blood to saliva and from glands to blood; in Garrett JR, Ekström J, Anderson LC (eds). Glandular mechanisms of salivary secretion. Front Oral Biol. Basel, Karger, 1998, 10th Edition, pp 153-166.
25. Kirschbaum B. The effect of hemodialysis on electrolytes and acid–base parameters. Clinica Chimica Acta 2003; 336:109-111.
26. Bots CP, Poorterman JHG, Brand HS, *et al*. Oral health status of dentate patients with chronic renal failure undergoing dialysis therapy. Oral Dis. 2006; 12:176-180.
27. Schneyer IH, Young JA and Schneyer CA. Salivary secretion of electrolytes. Physiol Rev 1972; 52:720-777.
28. Catalán MA, Nakamoto T and Melvin JE. The salivary gland fluid secretion mechanism. J Med Invest 2009; 56:192-196.
29. Shasha SM, Ben Aryeh H, Angel A and Gutman D. Salivary content in hemodialysed patients. J Oral Med 1983; 38:67-70.
30. Yajamanam N, Vinapamula KS, Sivakumar V, Bitla AR and Rao PS. Utility of saliva as a sample to assess renal function and estimated glomerular filtration rate. Saudi J Kidney Dis Transpl 2016; 27:312-319.