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Salivary Electrolytes, Total Protein and Immunoglobulin A in Patients with Chronic Kidney Disease: A Case Control Study

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Summary: The objectives of this study were to compare levels salivary electrolytes, total protein and immunoglobulin A (IgA) in patients with chronic kidney disease (CKD) and healthy individuals; and to determine the relationship between the salivary and blood levels of these factors between the two groups. Ninety-eight participants consisting of 48 patients with CKD and 50 healthy individuals (age and gender matched) were included. Whole saliva and blood samples were collected and analyzed for concentrations of electrolytes (K^+, Na^+, Ca^{2+}, Cl^-, and HCO_3^{2-}), total protein and IgA. Data were analyzed using Independent-Samples t-test and Pearson correlation test. Concentrations of salivary K^+, Ca^{2+}, Cl^-, and total protein were higher; while concentrations of salivary Na^+, HCO_3^{2-} were lower in patients with CKD compared with healthy individuals. There was no difference in the salivary IgA levels in patients with CKD compared with healthy individuals. Salivary calcium level showed linear correlation with the plasma calcium level while salivary chloride level showed negative correlation with plasma chloride level among patients with CKD. These findings indicate that saliva and plasma from patients with CKD are characterized by higher potassium, chloride, and lower sodium concentrations than their levels in healthy individuals; thus, suggesting a possible increased adrenal-cortical activity in patients with CKD.

Keywords*:* Saliva, electrolytes, total protein, correlation, chronic kidney disease

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INTRODUCTION

Chronic kidney disease (CKD), a progressive disorder marked by a loss of kidney function over time has become major problem worldwide (Meguid and Bello, 2005; Brosnahan and Fraer, 2010). The early stages of CKD are characterized by kidney damage and are generally asymptomatic. As the kidney disease aggravates, kidney function begins to worsen, leading to end-stage kidney disease, which requires kidney transplantation or hemodialysis (Levey et al., 2003). Individuals with CKD manifest diverse systemic alterations including oral complications and variations in the flow and composition of the saliva (Nandan et al., 2005; De la Rossa-Garcia et al., 2006; Davidovich et al., 2011; Fregoneze et al., 2013; Oyetola et al., 2015).

Saliva is a unique fluid that can be used to monitor oral and systemic diseases. It has many advantages over serum because its collection is non-invasive, simple, and requires minimal skill. Saliva sampling is appropriate for all age groups and can be repeated more frequently. It also offers a cost-effective method for the screening of large populations (Mandel, 1987; Samaranayake, 2007; Liu and Duan, 2012).

Saliva plays an essential role in the maintenance of oral homeostasis. Its constituents are the important factors responsible for its physiological functions (Mandel, 1987; Tabak, 2001; De Almeida *et al.*, 2008). Various salivary functions, including lubrication, cleansing action, antibacterial activity, buffering action, maintenance of tooth integrity, taste sensation and digestion may be disturbed by changes in salivary flow and biochemical properties. Parameters in saliva can be affected by many factors including diet and genetics; hence its usage as a biomarker or diagnostic fluid is still subject to continuous research.

Few articles in the literature have specifically analysed salivary biochemical changes in patients with CKD, and their findings are contradictory (Earlbaum and Quinton, 1981; Davidovich et al., 2011; Anuradha et al., 2015; Rodrigues, 2016). This study was therefore designed to compare levels of salivary electrolytes, total protein and IgA in patients with CKD and healthy individuals; and to determine the relationship between salivary and plasma levels of these factors.

MATERIALS AND METHODS

Design: A cross sectional case control study of patients with CKD and healthy individuals as controls (age and gender matched).

Study population: The study received ethical approval from the institution Research Ethics Committee (UI/UCH EC/13/0099). Patients with CKD were individuals attending the University College Hospital, Ibadan, Nigeria, diagnosed of the disease having estimated GFR of < 60 ml/min/1.73 m2 and stages 4 and 5 of National Kidney Foundation – Kidney Disease Outcome Quality Initiative (NKF-KDOQI) staging. The etiologies of CKD in the patients were hypertension, chronic glomerulonephritis, obstructive uropathy and diabetes mellitus. Most of the patients with CKD were undergoing dialysis treatment because of their late stage presentation. Also included in the study were the healthy individuals who were volunteers within the community and had no history of kidney disease, systemic or oral disease. Participants were provided information regarding risks and benefit of the study and verbal consent was taken. Participants had oral examination before saliva collection.

Saliva and blood sampling: This was performed according to the method previously described (Lasisi et al., 2016). Saliva collection was between 8.00 am and 10.00 am and participants had not taken meal for at least 2 hours before saliva collection. Whole saliva was collected by spitting method. Participants were asked to spit (after rinsing the mouth with distilled water) into calibrated universal plastic bottles until about 3 mls of saliva was collected. Simultaneously, 5mls of blood samples were taken from the participants by venipuncture into lithium heparin bottles and the plasma was used for the analysis. Saliva and plasma samples were stored at -20 °C until laboratory analysis. Saliva samples were defrosted at room temperature and then centrifuged at 3000 rpm for 10 min in order to remove contaminants before being used for the analysis.

Determination of total protein and electrolytes concentrations: Estimation of total protein was done using Biuret method (Jenzano et al., 1986). Sodium and potassium levels were determined using spectrophotometry, while estimation of calcium was done using indirect colorimetric method (De Loureiro, 1944). Concentrations of chloride and bicarbonate were determined by Schales method using mercuric nitrate (Shales and Shales, 1941).

Determination of salivary IgA levels: Immunoglobulin A level in the saliva samples was quantified using the Enzyme Linked Immunosobent Assay (ELISA) method (Salimetrics®, UK) according to the kits manufacturers' instructions. Briefly, each test sample was diluted 1:10,000 and 100 µL of test or standard was dispensed in duplicate with pipette into pre-designated wells. The micro titre plate was incubated at room temperature for thirty minutes and the contents of the wells were aspirated. Each well was filled with diluted Wash Solution and aspirated. This was repeated three times. The wells were filled again with undiluted wash buffer, drained by inversion and blotted (striking the wells on absorbent paper). This was repeated 3 times. Enzyme-Antibody Conjugate (100 µL) was pipetted to each well. The plate was covered and incubated in darkness for thirty minutes. The wells were thereafter, washed and blotted. TMB Substrate Solution (100 µL) was pipetted into each well, incubated in the dark at room temperature for 10 minutes after which 100 µL of Stop Solution was added. The plate reader was calibrated and the absorbance of the content of each well was determined at 450 nm.

Statistical analysis: Data are presented as mean ± standard deviation (SD) and compared using Independent student t-test. Correlation between plasma and salivary biochemical parameters was determined using Pearson correlation test. All analyses were done using IBM SPSS Statistics (version 22) at 5% level of significance.

RESULTS

The demographic data of the patients with CKD and the healthy controls are shown in table 1. Salivary levels of sodium and bicarbonate were significantly lower, whereas level of potassium was higher in patients with CKD compared to healthy individuals (table 2).

Similarly, plasma levels of sodium and bicarbonate were significantly lower, whereas level of potassium was higher in patients with CKD compared to healthy individuals (table 3).

Salivary levels of total protein, chloride, and calcium were significantly higher in patients with CKD compared to their levels in healthy individuals (table 2). There was no significant difference comparing levels of salivary IgA between patients with CKD and healthy individuals.

There was positive correlation between saliva and plasma calcium levels (figure 1); whereas, saliva and plasma chloride levels showed negative correlation in patients with CKD (figure 2).

Table 1: Demographic distribution of participants

	Healthy control (N = 50)	Patients with CKD (N = 48)
Age (yrs)	39.1 ± 7.34	39 .82 ± 11.07
Male	20	18
Female	30	30

Table 2: Salivary levels of electrolytes, total protein and IgA in patients with CKD and healthy control

	Healthy control	Patients with CKD	P value
Sodium (mmol/L)	15.79 ± 3.5	5.79 ± 4.41	< 0.001
Potassium (mmol/L)	18.50 ± 3.94	21.88 ± 8.63	0.02
Chloride (mmol/L)	3.72 ± 1.85	12.83 ± 8.51	< 0.001
Bicarbonate (mmol/L)	22.69 ± 2.42	15.61 ± 8.03	< 0.001
Calcium (mg/dL)	1.68 ± 1.09	6.82 ± 3.23	< 0.001
Total protein (mg/dL)	6.64 ± 4.71	17.81 ± 13.33	< 0.001
IgA (µg/mL)	491.40 ± 26.21	490.87 ± 43.52	0.96

Table 3: Plasma levels of electrolytes and total protein in patients with CKD and healthy control

	Healthy control	Patients with CKD	P value
Sodium (mmol/L)	137.87 ± 9.39	126.14 ± 12.45	< 0.001
Potassium (mmol/L)	3.89 ± 0.55	6.51 ± 4.09	< 0.001
Chloride (mmol/L)	105.17 ± 7.71	96.50 ± 10.56	< 0.001
Bicarbonate (mmol/L)	20 ± 1.78	17.38 ± 3.45	< 0.001
Calcium (mg/dL)	6.21 ± 2.16	7.57 ± 2.65	0.01
Total protein (mg/dL)	81.72 ± 7.88	57.26 ± 21.42	< 0.001

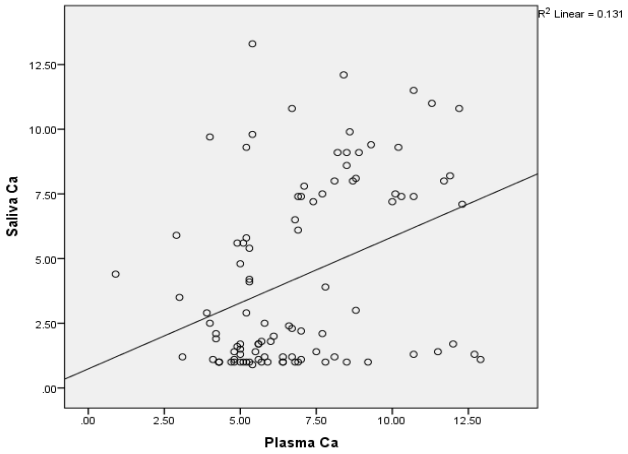

Figure 1: Correlation between salivary and plasma calcium concentrations in patients with CKD

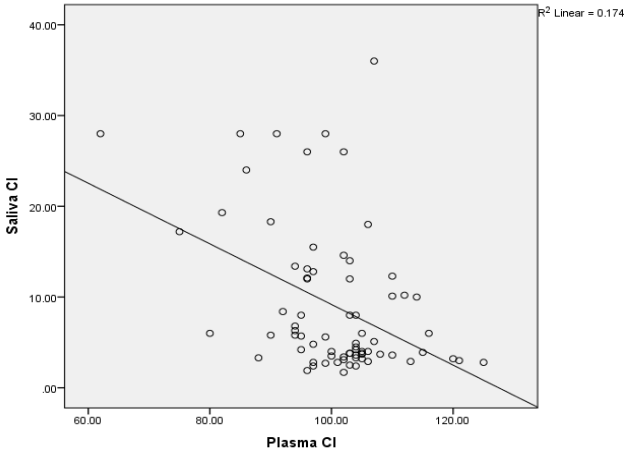

Figure 2: Correlation between salivary and plasma chloride concentrations in patients with CKD

There were no correlations between saliva and plasma sodium, potassium and bicarbonate in patients with CKD as well as the healthy controls (table 4 and 5).

Table 4: Relationship between plasma and salivary factors in patients with CKD

	Pearson correlation coefficient (r)	P value
Sodium	-0.20	0.19
Potassium	0.12	0.45
Bicarbonate	-0.14	0.36
Total protein	0.16	0.28

Table 5: Relationship between plasma and salivary factors in healthy controls

	Pearson correlation coefficient (r)	P value
Sodium	0.12	0.52
Potassium	-0.04	0.84
Chloride	0.16	0.40
Bicarbonate	-0.08	0.69
Calcium	-0.31	0.03
Total protein	-0.14	0.34

DISCUSSION

The comparatively lower concentration of salivary sodium in patients with CKD observed in the present study might have been related to the lower plasma level. Hyponatremia is a common complication of CKD due to many factors. Patients with CKD are at additional risk of hyponatremia due to compromised capacity to dilute or concentrate urine (Berl, 2008). Furthermore, diet restriction and use of multiple drugs are common and can contribute to the sodium derangements (Dhondup and Qian, 2017). However, our finding is contrary to the reports of higher salivary sodium concentrations (Anuradha et al., 2015; Bagalad et al., 2017) and lack of difference in the concentration of salivary sodium (Davidovich et al.,

2011) in patients with CKD compared with healthy controls. The differences in the findings are probably due to the occurrence of both hypo and hypernatremia in patients with CKD. Bagalad et al., (2017) also reported elevated serum levels of sodium with positive correlation with salivary levels in patients with CKD.

In this study, the higher concentration of salivary potassium in the patients with CKD is in agreement with the previous reports (Thomas et al., 2008, Davidovich et al., 2011; Bagalad et al., 2017). Similar to the report of Bagalad et al., (2017), the elevated salivary potassium level in the present study reflected the blood level. Hyperkalaemia is one of the complications of chronic CKD, usually developing when glomerular filtration rate (GFR) falls below 20% of normal (Gennari and Segal, 2002; Muso, 2004a; 2004b). The hyperkalaemic state in patients with CKD occurs for several reasons that include high dietary potassium intake relative to reduced renal function; extracellular shift of potassium caused by metabolic acidosis and treatment with renin–angiotensin–aldosterone system blockers that inhibit renal potassium excretion (Einhorn et al., 2009).

Low level of sodium, corresponding to high level of potassium, in the saliva relative to the plasma is a reflection of the active transport mechanism that has been reported in the literature (Poulsen, 1998; Turner and Sugiya, 2002). Furthermore, the lower salivary sodium concentration with higher potassium concentration in patients with CKD might be related to the reduced salivary flow rate previously reported in individuals with CKD (Hong-Seop et al., 1999; Thomas et al., 2008; Oyetola et al., 2015). The salivary electrolytes vary with the flow, such that a decrease in flow rate could explain, for example, a decrease in the concentration of sodium and an increase in the concentration of potassium. It has been reported that the reduced salivary flow rate in patients with CKD could be attributed to the underlying mechanisms that were suggested as direct glandular damage and/or the inadequacy in fluid intake (Epstein et al., 1980; Gavalda, 1999).

In the present study, despite reduction in the salivary concentration of sodium, salivary concentration of chloride was higher in patients with CKD. Dissociation between changes in salivary sodium and chloride has been reported previously by McCance, (1938) who observed that acute salt deficiency in five normal subjects produced a consistent fall in the concentrations of salivary sodium while the corresponding changes in chloride were inconsistent. Also, similar to our finding Davidovich et al., (2011) reported elevated salivary chloride in patients with CKD. Changes in salivary chloride levels were similar to changes in potassium levels, probably reflecting an increase in corresponding anion secretion, due to the active secretion of cation (in this case, potassium).

The salivary concentration of calcium in the patients with CKD in the present study was higher than in the control which is in contrast to previous reports of lower concentration of salivary calcium in patients with CKD (Anuradha et al., 2015; Bagalad et al., 2017). The plasma concentration of calcium was also higher in the patients with CKD compared with control and this may account for the elevated level in saliva. The higher level of salivary calcium in patients with CKD may suggest increased protection against dental caries in these individuals. A systematic review by Andrede et al., (2014) has documented lower dental caries prevalence in individuals with CKD. Similarly, Sewon et al., (1998) reported that high salivary calcium was associated with low DMF scores (caries index).

In the present study, despite lower plasma protein, salivary total protein was significantly higher in patients with CKD compared with healthy control. This finding corroborates the recent report of higher salivary total protein in experimental CKD in rat by Romero et al., (2017). It was suggested that the higher level of salivary total protein in chronic kidney disease could be attributed to amylase level. Amylase accounts for approximately 50% of the total protein produced by the salivary glands (Schenkels et al., 1995). In the experimental study by Romero et al., (2017), a significant increase in salivary amylase activity was observed in CKD group in response to isoproterenol stimulation. The higher concentration of salivary total protein in patients with CKD in this study might explain the increased buffering as well as reduced caries prevalence in these patients.

Lack of difference in the salivary IgA levels in patients with CKD compared with healthy control in the present study may suggest that components of the local humoral immune response (of which salivary IgA predominates) are not impaired in these individuals. Secretory immunoglobulin A (sIgA) is the most frequently found immunoglobulin in mixed saliva and is considered to be a secretory factor for acquired immunity in the oral cavity. Through restriction of microbial adhesion, salivary IgA forms part of the first line of defense and also participates in the preservation of the integrity of oral tissues (Bokor-Bratic, 2000; Dodds, 2005). Thus, sIgA plays an important role in oral homeostasis and is an important indicator of the defensive status of the oral cavity (Bernimoulin, 2003).

In conclusion, the findings from the present study indicate that the electrolyte content of whole saliva from patients with CKD is characterized by higher potassium, chloride, and lower sodium concentrations than saliva of healthy individuals. These findings are similar to the changes observed in the blood electrolyte concentrations; thus, suggesting a possible increased adrenal-cortical activity in patients with CKD. In addition, the finding of higher levels of salivary total

protein and calcium in patients with CKD in this study suggest their role in the reduced caries prevalence in these individuals.

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