RESEARCH ARTICLE

Saliva as an Alternative Diagnostic Fluid to Blood in Chronic Kidney Disease (CKD) Patients.

Muhammad Sajid[1], Nasir Iqbal[2], Abdul Razzaq[3]

[1, 2, 3] Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore, Pakistan.
[1]***Correspondence:*** *msajid1189@gmail.com*

Abstract

Chronic kidney disease (CKD) is impairment of kidney's function over time that ultimately results in slow progression of renal failure. In present study serum and salivary biochemical changes in chronic kidney disease patients were analyzed and evaluate as non-invasive diagnostic markers. For this purpose serum calcium, phosphate, urea, creatinine and total proteins and salivary calcium, phosphate and alpha amylase were analyzed. Overall high serum calcium levels were found in chronic kidney disease patients. Serum phosphate levels were found high as compare to normal individuals. Serum creatinine and urea levels were also elevated. Salivary calcium levels increased significantly as compare to normal individuals. While the salivary phosphate and salivary alpha amylase levels decreased in CKD patients. It is suggested that saliva can be used as surrogate of blood to evaluate the progression of chronic kidney disease and oral health of these patients.

Keywords: Chronic Kidney Disease, CKD, Renal Failure, Non-Invasive Diagnostic Markers, Surrogate.

Introduction

The main function of the kidneys is to remove the wastes and excess water from the body. Chronic kidney disease (CKD) is the slow loss of kidney function over time. This disease slowly gets worse over time (Tonelli et al, 2010). The early symptoms of CKD occur with or without any other illnesses. These symptoms may be the indication of kidney disease as the conditions are more progressive (Tolkoff et al, 2007).

Saliva is a very good surrogate of blood that reflects the concentrations of different parameters in blood. Blood tests measure the level of waste products like concentration of calcium, phosphate, alpha amylase, urea, creatinine and electrolytes in the body that should be removed by the kidneys during normal function. In case of abnormal kidneys the level of these waste products and electrolytes become high in blood that indicates the chronic kidney disease (Barry et al, 2007). The concentration of these wastes was found to be altered in saliva as well (Epstein et al, 1980). So, CKD patient's saliva was proposed as a diagnostic fluid

Blood urea nitrogen (BUN) measures the amount of urea nitrogen, a waste product of protein metabolism, in the blood and formed by the liver and carried by the blood to the kidneys for excretion. Because urea is cleared from the blood stream by the kidneys, a test measuring how much urea nitrogen remains in the blood can be used as a test of renal function (Palevsky, 2006). Creatinine is a break down product of creatine phosphate in muscle, and is usually produced at a moderately constant rate by the body, depending on muscle mass. Creatinine is primarily filtered out of the blood by the kidneys, glomerular filtration (Davidovich et al, 2009). If the filtration of the kidneys was poor, creatinine blood levels were increased. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine

clearance, which indicate the glomerular filtration rate (GFR), and stage of kidney disease (Gross et al, 2005).

Protein is essential as it is a part of every organ and tissue in the body. It is however, continually broken down and therefore, we need to ingest more to replace what is lost. Protein helps maintain and repair muscle mass and normal body functioning (Gornall et al, 1949). In chronic kidney disease the kidneys cannot filter out wastes, but cannot reabsorb the protein. If an individual have kidney disease, their kidneys may not be able to leave the protein in the blood as a result low protein levels in blood (Abboud et al, 2010).

With the progression of chronic kidney disease (CKD), disorders of mineral metabolism appear. The sequence of events begins with a deficit of calcitriol synthesis and retention of phosphorus (Davidovich et al, 2009). As a result of this, serum calcium decreases and parathyroid hormone (PTH) is stimulated, disturbances in phosphorus, calcium, and vitamin D metabolic pathways develop early during chronic kidney disease patients. Serum concentrations of phosphorus, calcium and parathyroid hormone provide complementary data for evaluating whether these metabolic pathways might contribute to the development of clinical disease outcomes in humans (Lorenzo et al, 2008).

Hyperphosphatemia is an important factor to cardiovascular calcification in chronic renal failure (CRF) patients. Although dietary phosphate reduction and treatment with phosphate binders, serum phosphorus level may be reduced. Phosphate may be secreted in the saliva, which is then swallowed (Tonelli et al, 2010) and this provides a source of endogenous phosphate and thus contributes to the hyperphosphatemia in CRF. Both hemodialysis (HD) and chronic renal failure (CRF) patients had significantly higher salivary phosphate levels as compared with healthy control individuals (Savica et al, 2008).

Hyperamylasemia is a common finding in chronic renal failure (CRF) patients. It has been suggested that the diagnosis of acute pancreatitis in these patients is confirmed when serum amylase activities are greater than three times the upper normal limits (Rafael et al, 2008). the hyperamylasemia levels in patients with chronic renal failure, the total serum amylase (Ta), and salivary (Sa) types of serum isoamylases, as well as the urine isoamylases (Tu, Pu, Su) was higher in chronic renal failure patients than the normal subjects (Tsianos et al, 1994).

Material and Methods

Total of 40 individuals of chronic kidney disease were included in group A that were undergoing hemodialysis regularly in "Mayo Hospital Lahore". 40 healthy individuals were selected as control in group B. Blood and saliva samples were collected from the chronic kidney disease patients and normal individuals. All the biochemical analysis was done at "Institute of Molecular Biology and Biotechnology" The University of Lahore. Urea, creatinine, total protein, calcium and phosphate were estimated in blood serum of chronic kidney disease patients and normal individuals by enzymatic kit method.

Urease enzyme catalysis the conversion of urea into ammonia. The ammonium ions react with a mixture of salicylate, hypochlorite and nitro-prusside to yield a blue-green dye (indophenols). The intensity of this dye is proportional to the concentration of urea in the sample. Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the colored complex formed is directly proportional to concentration of creatinine in sample. Absorbance was taken at 492nm through spectrophotometer.

In alkaline medium the copper reacts with the peptide bonds of proteins to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation, and potassium iodide prevents the auto reduction of copper. The absorbance of standard and sample was taken at 546nm within 30 minutes as described by (Gornall et al, 1949)

Calcium ions form a violet complex with ortho-cresolphthalein complex in alkaline medium. The intensity of the color complex is directly proportional to the concentration of calcium in the sample. The absorbance was measured at 578nm spectrophotometrically. Phosphorus in serum reacts with ammonium molybdate to form phosphorus molybdate, which is then reduced by stannous chloride and hydrazine sulphate to molybdenum blue.

The intensity of the color complex is directly proportional to the concentration of phosphate in the sample. The absorbance was measured at 640nm.

Salivary alpha amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-1-galacto-pyranosylmaltoside (GALG2-CNP) to glucose polymers and p-nitro phenyl oligosaccharide at short chain producing 2-chloro-4-nitrophenol. The increased destruction of glucose can be measured at 405nm.

Statistical Analysis

The statistical analysis was done by T-Test using SPSS (version 16) software. The difference in values was indicated in the form of probability ($P \leq 0.05$) values.

Results and Discussion

The present study was designed to investigate the biochemical changes in saliva and blood serum of chronic kidney disease patients. Overall calcium levels in saliva and serum of both groups were studied and significantly ($p<0.05$) high serum calcium levels were observed in chronic kidney disease patients (Figure 1). Outcomes were in line with the work of (Tsianos et al, 1994).

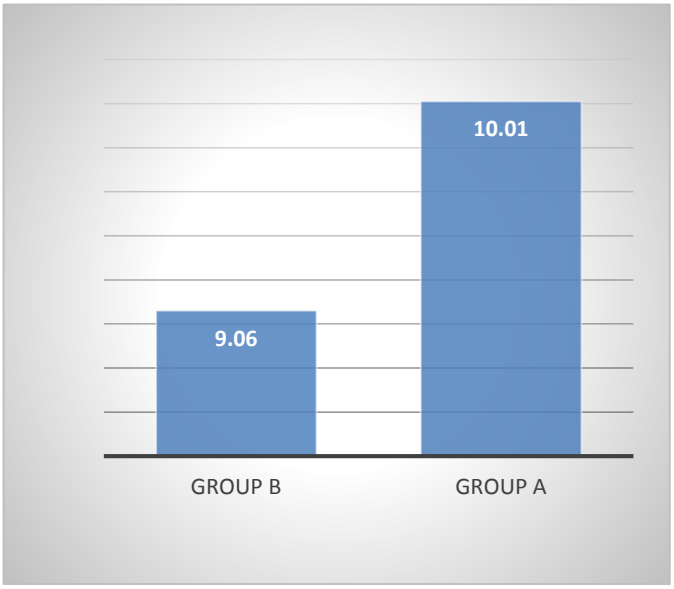

Figure 1: *Serum calcium levels in Group A (CKD) and Group B (control)*

They observed increased serum calcium levels in chronic kidney disease patients.

Salivary calcium levels (Figure 2) were also found to be increased but statistically was insignificant ($p> 0.05$). Increase in salivary calcium levels in chronic kidney disease patients as compare to normal individuals (Figure 2). The outcomes were correlated with the study of Davidovich E. (Davidovich et al, 2009). Where they recorded increased salivary calcium levels effecting the oral health of chronic kidney disease patients and pre-dialytic patients.

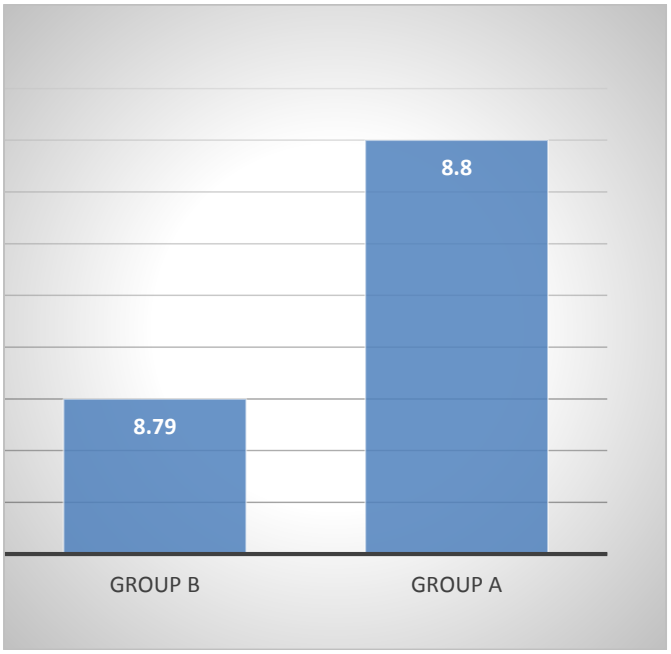

Figure 2*: Salivary calcium levels in Group A (CKD) and Group B (control)*

Phosphate levels in serum and saliva were also measured of both group A and group B (Figure 3). Significant increase ($p<0.05$) in serum phosphate levels were observed in chronic kidney disease patients (group A). These results were consistent with findings of (Savica et al, 2008). During their study studied the increased levels of serum phosphate in chronic kidney disease patients. In Saliva we recorded significantly ($p<0.05$) decreased levels of salivary phosphate in chronic kidney disease patients (group A) and the results are similar as reported by the (Rafael et al, 2008) Rafael and colleagues also observed low phosphate levels in saliva of chronic kidney disease patients during an oral health study (as shown in Figure 4)

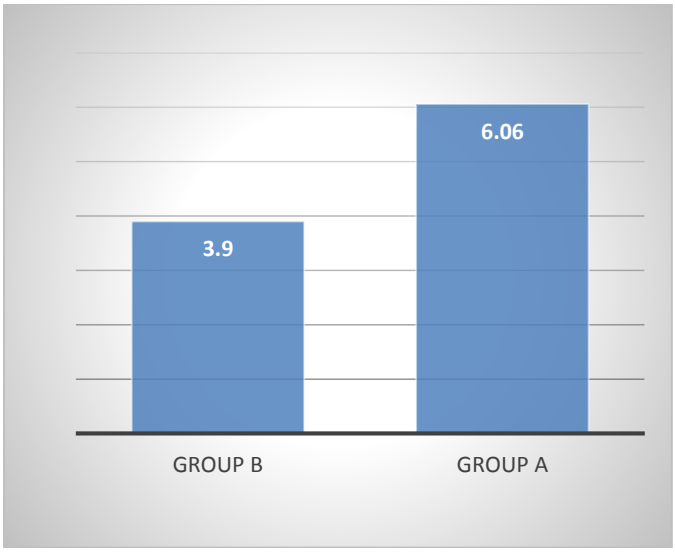

Figure 3: *Serum phosphate levels in Group A (CKD) and Group B (control)*

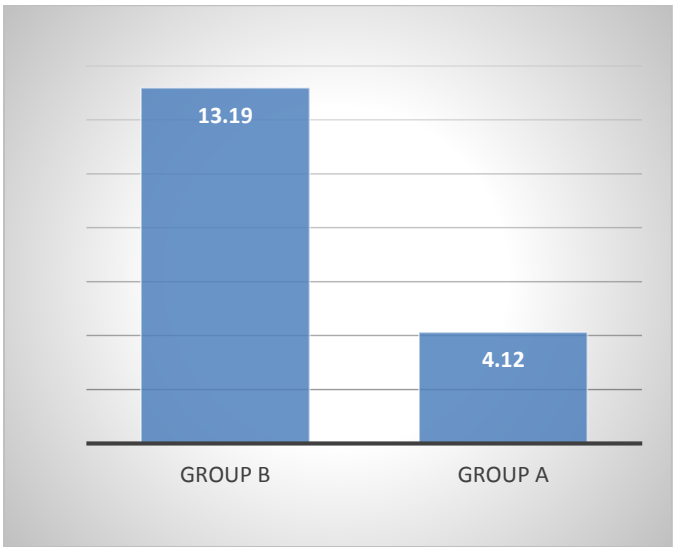

Figure 4: *Salivary phosphate levels in Group A (CKD) and Group B (control)*

Total protein levels in serum of chronic kidney disease patients were measured and also found significantly decreased ($p<0.05$) in CKD patients as compare to normal individuals (Figure 5). Our findings were also in agreement with the findings of (Safaei et al, 2009). They reported similar findings during their study of hypoproteinemia in end stage renal disease patients.

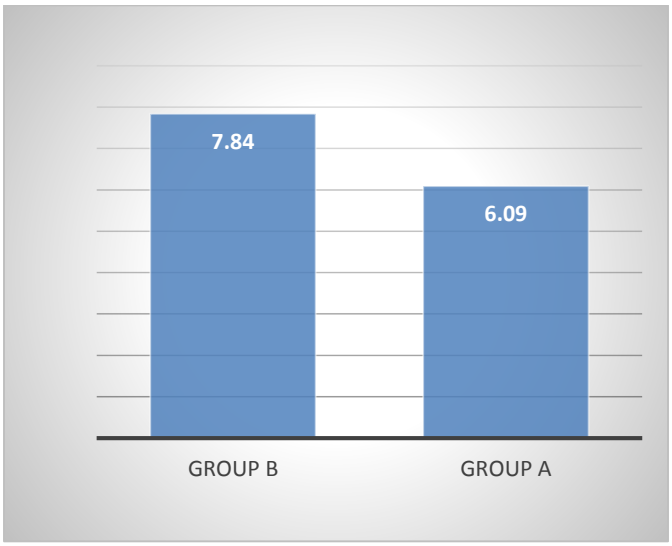

Figure 5: *Serum total protein levels in Group A (CKD) and Group B (control)*

Present study revealed that serum urea levels (Figure 6) were significantly ($p<0.05$) high in chronic kidney disease patients as compare to normal subjects. The findings were in line with the study of Jose et al, and Palevsky.

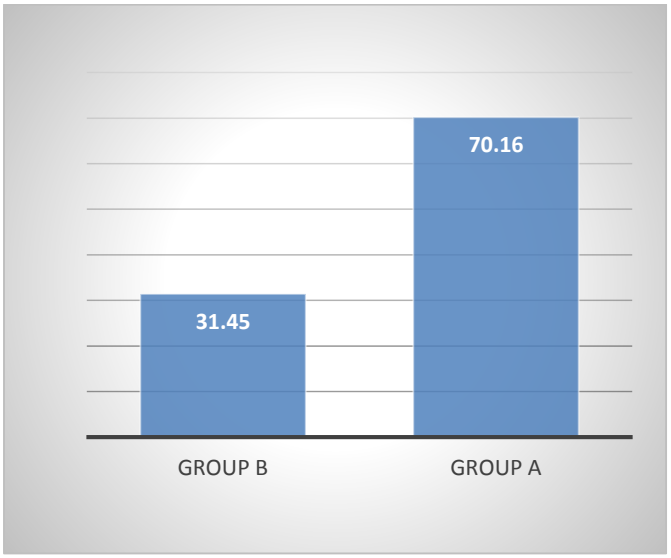

Figure 6: *Serum urea levels in Group A (CKD) and Group B (control)*

They observed the high serum urea levels in chronic kidney disease patients. Significantly ($p<0.05$) elevated levels of creatinine were also observed in chronic kidney disease patients as compare to normal individuals which was correlated with the work of Jose et al, Where they observed glomerulus filtration rate and high creatinine levels

in chronic kidney disease patients. As kidneys become impaired the creatinine levels was elevated and thus an indicator of malfunction or failure of kidneys (Figure 7).

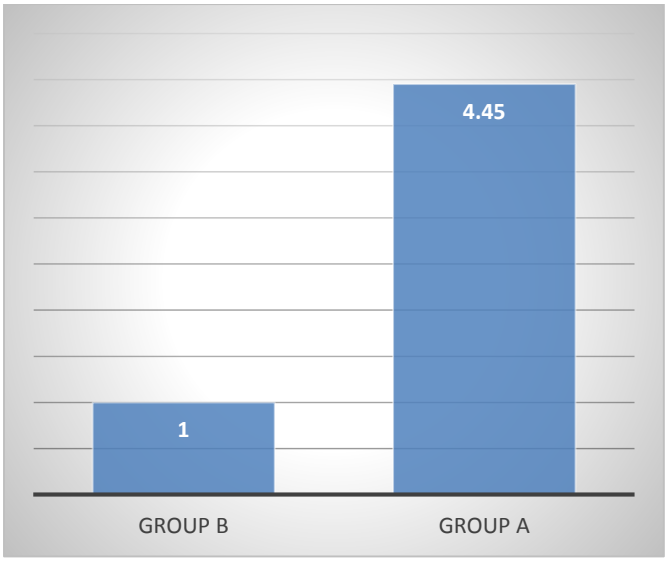

Figure 7: *Serum creatinine levels in Group A (CKD) and Group B (control)*

Significant ($p<0.05$) increase in creatinine levels were observed in chronic kidney disease patients (group A). Creatinine cannot be filtered out by impaired kidneys and their levels raised in blood. The similar outcomes were observed with the work of Tsianos et al, Where they also observed high creatinine levels in chronic kidney disease patients.

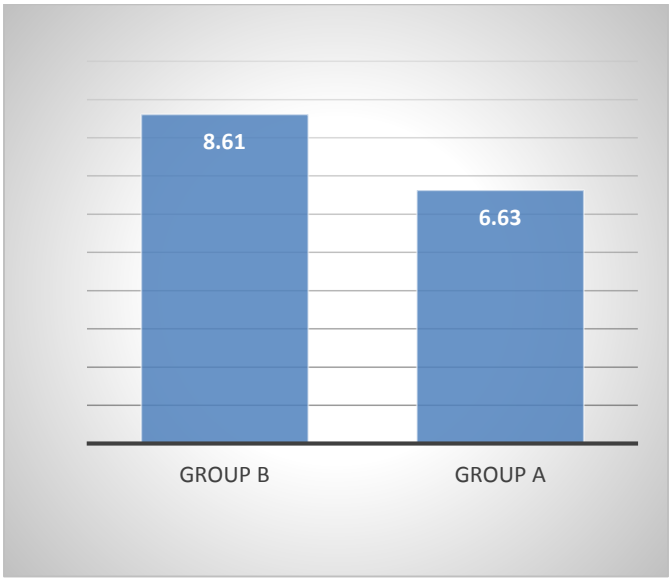

Figure 8: *Salivary alpha-amylase levels in Group A (CKD) and Group B (control)*

Figure 8 shows overall decreased levels of alpha amylase were observed in chronic kidney disease patients significantly ($p<0.05$). The outcomes were correlated with the work of Tsianos et al, Where they observed low salivary alpha amylase levels in chronic kidney disease patients.

Conclusion

It was concluded from the outcomes of the present study that significant biochemical changes observed in serum calcium, phosphate, urea, creatinine and total protein in kidney disease patients. Significant biochemical changes in salivary phosphate and alpha amylase was also observed in kidney disease patients except salivary calcium levels where insignificant alteration was observed. It was suggested that salivary biochemical changes can be used to observe progression of chronic kidney disease and to evaluate their oral health.

References

Abboud H, and Henrich WL. 2010. Clinical practice on Stage IV chronic kidney disease. N Engl J Med. 362; 56-65.

Barry JM, and Campbell W. 2007. Renal transplantation. Campbell-Walsh Urology Philadelphia. 9(2); 1295-1324.

Chaney AL, and Marbach G. 1962. BUN levels in end stage renal disease patients. Clinical chemistry. 8; 130.

Davidovich E, Davidovits M, Peretz B, Shapira J, and Doron JA. 2009. The correlation between dental calculus and disturbed mineral metabolism in paediatric patients with chronic kidney disease. Nephrology. Dial. Transplant. 24(8); 2439-2445.

Epstein SR, Mandel I, and Scopp IW. 1980. Salivary composition and calculus formation in patients undergoing hemodialysis. J Periodontol. 51; 336–338.

Gornall AG, Bardawill CJ, and David MM. 1949. Determination of serum proteins by means of biuret reagent. J Biol Chem. 177; 751.

Gross JL, Azevedo MJ, Silveiro SP, Canani LH, Caramori ML and Zelmanovitz T. 2005.

Diabetic nephropathy, diagnosis, prevention, and treatment. J Diabetes Care. 28(1); 164–76.

Jose P, Valdivieso**,** Maira B, Rastrollo**,** Pablo Monedero**,** Jokin de, and Francisco JL. 2007. Prognosis and serum creatinine levels in acute renal failure at the time of nephrology consultation. *J*BMC Nephrology. 8; 14.

Lorenzo SV, and Torregrosa V. 2008. Changes in mineral metabolism in stage 3, 4 and 5 chronic kidney disease (not on dialysis). J. Nefrologia. 28(3); 67-78.

Palevsky PM. 2006. Dialysis modality and dosing strategy in acute renal failure. J Seminars in Dialysis. 19; 165.

Rafael M, and Nagler Y. 2008. Saliva Analysis for Monitoring Dialysis and Renal Function. J clinical chemistry. 54(9); 1415-1417.

Safaei A, and Maleknejad. 2009. Spectrum of childhood nephrotic syndrome in Iran. Indian J Nephrol. 19(3); 87–90.

Savica V, Calo L, Santoro D, Monardo P, Granata A, and Bellinghieri G. 2008. Salivary phosphate secretion in chronic kidney disease. J Renal Nutr. 18(1); 87-90.

Tolkoff RN, and Ausiello D. 2007. Treatment of irreversible renal failure. Philadelphia Medicine. 23; 1571-1585.

Tonelli M, Pannu N, and Manns B. 2010. Oral phosphate binders in patients with kidney failure. N. Engl J. Med. 362; 13121-13241.

Tsianos EV, Dardamanis MA, Elisaf M, Vasakos S, and Siamopoulos KC. 1994. The value of alpha-amylase and isoamylase determination in chronic renal failure patients. Int. J. Pancreatol. 15(2); 105-11.