

Original Research Article

Salivary Electrolytes as Bio-Analyte for Diabetes Mellitus

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Abstract

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In diagnostic medicine, most processes that assaying metabolites/biomarkers are often cumbersome and invasive; requiring in most cases, medics-client co-understanding for success to be achieved. This has reportedly exacerbated ailments in subjects due to fright and/or fear of syringes, especially in tympanophobics. This study was therefore undertaken to investigate the possibility of using saliva electrolytes as alternative means (besides serum metabolites) to assessing the human blood glucose levels without need for actual invasion. To achieve this, a total of four hundred and twenty six (426) humans, comprising of three hundred and forty six (346) Diabetics (Experimental group) and eighty (80) Non-diabetic (Control group) individuals were ethically sourced from the central hospital, Warri, Delta State. Irrespective of gender, study obtained and compared selected serum and saliva electrolytes [Na^+ , K^+ , Cl^- , Ca^{2+} and Mg^{2+}]. Upon statistical comparison of mean differences between groups [Using the student t-test or one way analysis of variance (ANOVA) where appropriate], study found a statistically significant increase (at $p > .05$) for salivary electrolytes of diabetics compared to non-diabetics. Following comparison between groups, study observed a decrease in all but serum Cl^- concentration of diabetics when compared to non-diabetics. For all assayed parameters, study found a significant difference in salivary and serum electrolytes levels across sampled groups; implicating saliva as alternative means for which human blood glucose status may be assayed by non-invasive means. We recommend further sophisticated approach with higher sample size to this study.

Keywords: Saliva. Serum, Electrolytes, Bioassay, Diabetes Mellitus

INTRODUCTION

In humans, the salivary Glands are specialized exocrine glands that secrete saliva, a somewhat alkaline fluid that moistens the mouth, softens food, and aids in digestion (Ira et al., 2008). The submaxillary glands are located around the mouth under the lower jaw, the sublingual glands are located beneath the tongue, and the parotid glands are found in front of each ear. The buccal glands, in the cheeks near the front of the mouth, also secrete saliva (Negrato and Tarzia, 2010). The saliva of the parotid gland contains enzymes called amylases, one of

which, known as ptyalin, aids in the digestion of carbohydrates (Mohan et al., 2007; Giusti et al., 2007a).

The salivary glands, especially the parotid, are affected by the disease called mumps, a viral disease most common among children, although adults can also be infected (Lucacchini, 2007b; Peluso et al., 2007).

Studies have shown glucose as an important component and player in saliva and body fluids (Proteomic study of salivary peptides and proteins in patients with Sjögren's syndrome before and after

pilocarpine treatment. Arthritis and Rheumatism (Giusti et al., 2007a; Lucacchini, 2007b). Though several electrolytes (Na^+ , K^+ , Cl^- , and HCO_3^- , Mg^{2+} and Ca^{2+}) may also account for the osmolarity of saliva in buffering and maintenance of constancy and pH, however, keeping the epithelial integrity of the mouth is incomplete without salivary glucose and protein composition as they are known to form a major component of the glycoproteins and glycolipids in mucous surfaces; making them flexible, fluidy and polarized [6]. Even with normal saliva production, Abusharha and Pearce in 2012 found that absence of such glycolipids in oral epithelium would soon result in hypo-salivation (Mayooran et al., 2000; Araya, 2003; Yan et al., 2008).

Long standing hyperglycemia has also been shown to impair salivary gland functions, which leads to reduction in the salivary flow and changes in saliva's composition. As a consequence various dental as well as mucosal alterations can occur which include proliferation of various pathogenic microorganisms, taste alterations, burning mouth, dental caries, coated tongue and halitosis (Mealey, 2003).

Knowledge of the effects of hyperglycemia on salivary composition and function remains equivocal, with oral cavity acting as the mirror of systemic disease and saliva. It is the key feature that involves various functions from digestive to immune system; making saliva a diagnostic marker for systemic diseases (Bennick, 1982).

Evaluation of salivary parameters has been shown to be cost-effective and non-invasive for screening, diagnosis and monitoring of diabetes when compared to blood investigations which are painful and causes physical trauma and mental stress to patients, hence the need for this study. The noninvasive nature and simple collection of saliva allows for its ease of repetition, compliance and multiple collections; a potential aid in early diagnosis, monitoring of disease progression, or treatment responses with minimally trained personnel. This advantage of using saliva attract investigator who are looking for an alternative body fluid to simplify diagnostic procedures (Amithanavalkar et al., 2011; Aydin et al., 2007; Sert and Caylak, 2005; Marder et al., 1975; Moore et al., 2001).

Studies have shown that saliva compositions in humans are closely linked to those of body fluid electrolytes and glucose, and may be helpful in estimating their blood glucose status without need for actual invasion (Odigie et al., 2016). Available findings also show the non-invasive possibility of envisaging human blood groups from "saliva prints". However, the possibility of doing same for blood glucose status remains debatable; given cost intensiveness and the relative alternative to assaying blood glucose from a known volume of saliva. In conditions of hypo-salivation for instance, saliva flow rates and is known to remarkably decrease due to hyper-osmolarity (Chavez et al., 2001), keeping in mind that Saliva fluid instability and hyper-

osmolarity are core mechanisms that precipitate dry mouth. Howbeit, no attempt yet establishes a link between same (saliva electrolyte constituents) and any possible range of blood glucose.

Aim of Study

Study aimed at investigating saliva electrolytes as alternative means to diagnosing diabetes mellitus. Specifically, Study;

- i. Compared saliva and serum electrolyte levels in diabetics to those of non-diabetic individuals.
- ii. Evaluated changes in salivary and serum electrolytes activity for the two sets (Diabetics and non-Diabetics).
- iii. Ascertained available records on the possibility of estimating diabetes from a given volume of saliva.

MATERIALS AND METHODS

Study Design

Study was designed to be experimental in nature. It was targeted at humans, with a view to harnessing a cross section of diabetic sufferers, who regularly visits the general hospital, Warri, Delta State for checkups.

Adopting standards from the American Diabetes Association, 40 healthy subjects (control) of between the aged groups of 25-65 years were investigated. A hundred and seventy six (176) diabetics (Experimental group) were also ethically recruited for the exercise. For each subject, saliva fluids were assayed for constituent electrolyte, proteins, and sugar levels, and mapped against their corresponding serum levels for same variables as follows;

Table 1. Summary of study design and groups

Groups	Sub-Groups	Obtained Samples	Total
Experimental	Diabetic Saliva (Exp. Grp. I)	173	346
	Diabetic Serum (Exp. Grp. II)	173	
Control	Control Saliva (Con. Grp. I)	40	80
	Control Serum (Con. Grp. II)	40	
Total Participants			426

Study Location

Study was conducted at the central hospital, Warri, Delta State; southern Nigeria. Delta state is a 16,842 square kilometer (6,503 sq meters) area of land located

approximately between longitude $5^{\circ} 00'$ and $6^{\circ}.45'$ East and latitude $5^{\circ} 00'$ and $6^{\circ}.30'$ North of the equator. It is a densely populated area of an estimated population of 4,112,445 (2,069,309 males and 2,043,136 females) (NPC, 2006).

Population of Study

An estimated total of 346 adult diabetics (DM sufferers) reportedly visit the central hospital, Warri weekly. This amounted to about 17,992 yearly ($346 \times 52 = 17992$) of diabetics who visit the clinic as reported from availed record. Thus, study population targeted the total DM sufferers available per annum (17,992) at the central hospital, Warri, DM unit.

Sample and Sampling Technique

Using the random sampling technique, a total of 426 participants comprising of 346 diabetics (Experimental group) and 40 Non Diabetics (Control group) were drafted from aforementioned population. The decision to sample subjects (sample size) was informed by the statistical relation (below);

$$SS = \frac{Z^2 P (1 - P)}{C^2}$$

Where →

SS = Sample Size

Z = confidence level as z-score (95% = 1.96 from z-table)

P = Population proportion variance. (Maximal at 0.5 from binomial distribution table)

C = Confidence interval or margin of error (0.05).

Upon substitution, above relation yielded

$$SS = \frac{(1.96)^2 \times 0.5 \times (1 - 0.5)}{(0.05)^2}$$

$$SS = \frac{3.8416 \times 0.5 \times (0.5)}{0.0025}$$

$$SS = 380$$

Thus, three hundred and eighty (380) was minimal permissible sample size for a population as that of Warri (study location) for which confidence level appeared reasonable.

Inclusion Criteria

Subjects who voluntarily wheels to participate in the study

were selected. This selection was based on the knowledge that selected subjects were certified diabetic by their physicians, must be between the ages of 40-75 years, irrespective of gender.

Exclusion Criteria

Patients suffering from type I diabetes mellitus, or certified pregnant, physically and/or mentally challenged were excluded from the study. Individuals with chronic renal failure, hyperthyroidism, pancreatitis, pancreatic cancer or hypercholesterolemia were also excluded from the research. Those under medications like steroids, tricyclic antidepressants, epinephrine, diuretics, estrogen, lithium, and salicylates, were also be excluded.

Procedure

Saliva Collection

Two millilitres (2ml) of unstimulated saliva samples were taken between the hours 7.00am and 8.00am from selected subjects after an overnight fast (Pal et al., 2003). Participants were asked to spit (after rinsing their mouths with deionized water) into plastic vials (Lopez et al., 2003). Obtained saliva samples were then centrifuged at 6000 rpm for 10 minutes before being use. This was necessitated by the need to rid sample of any available contaminants; such as micro-organisms, food debris, etc. the supernatant were then analysed immediately.

Serum Collection

Five milliliters (5ml) of subjects' intravenous blood was obtained from the median cubital vein of the cubital fossa in their forearm, just by using a 5ml disposable syringe. The blood sample was collected and transferred into a fluoride oxalate tube and a plain container for centrifugation at 6000 rpm for 10minutes and analyzed.

Determination of Serum and Saliva Electrolyte Levels

Serum and Saliva Calcium (Ca²⁺) ion Determination

This was done using calcium test kit (A) end point method. The calcium combined specifically with arsenazole 111 at natural pH to formed blue purple complex. The intensity of formed color was directly proportional to the amount of calcium present in the sample. The serum and saliva samples were then labeled with 0.1ml of reagent solution pipetted from it into each of 3 test tubes labeled 'blank' and 'standard' and 'test' of serum and saliva. Then, 20µl standard was added to the

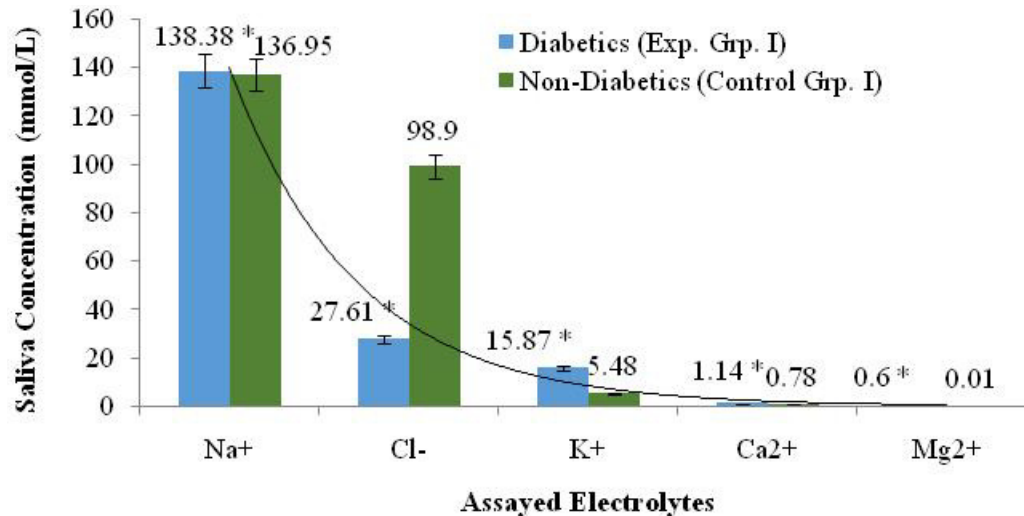


Figure 1. Comparative Changes in Average Saliva Electrolyte Compositions of Diabetics and Non-Diabetics Subjects

test tube marked standard, followed by 20 μ l of the test sample to the test tube. All the test tubes were kept in an incubator at 37°C for 2min before aspiration. The calcium concentration was then measured in both serum and saliva sample by automatic analyzer.

Serum and Saliva Sodium (Na⁺) ion Determination

In principle, Sodium is usually precipitated as the triple salt from sodium magnesium uranyl acetate. This excess uranium was then reacted with Ferro-cyanide to produce chromophore, whose absorbance varies inversely with the concentration of sodium in the test sample. All test tubes were then mixed and transferred into cuvette. Absorbance of all final mixture were read and recorded at 550nm using the spectrophotometer. Na⁺ was then determined using;

Calculation

Sodium conc. (mEq/L) =
$$\frac{\text{Absorb of Blank} - \text{Absorb of test sample}}{\text{Absorb of Blank} - \text{Absorb of standard}} \times 150 \text{ (conc. of std)}$$

Serum and Saliva Potassium (K⁺) Determination

The amount of potassium (K⁺) was determined with sodium Tetraphenylboron, a specifically prepared mixture that produced a colloidal suspension. With 10 μ l of potassium reagent pipetted into all tubes, 10 μ l of the standard was added to the test tube. After 3 minutes, the

wavelength of spectrophotometer was set at 500nm and zeroed with the reagent blank. The absorbance was read and recorded for all test tubes.

Calculation

Potassium (K⁺) conc. (mEq/L) =
$$\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times 4 \text{ (conc. of std)}$$

Serum and Saliva Chloride (Cl⁻) Determination

The test tubes were arranged appropriately as blank, standard and test. 1.5ml of chloride reagent was dispensed into each test tube. 10 μ l (0.01ml) of sample was added to the respective tubes and mixed properly. It was then incubated for 5 minutes at 37°C temperature. The spectrophotometer was regulated at 500nm wavelength and zeroed with reagent blank. Absorbance readings of all tubes were read and recorded.

Calculation

Chloride (Cl⁻) conc (mEq/L) =
$$\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times 100 \text{ (conc. of std)}$$

Determination of Serum and Saliva Magnesium (Mg²⁺) Concentrations

Saliva and serum magnesium (Mg²⁺) levels were determined by the Molybdenum Blue-Briggs method. This method is anchored on the principle that Mg²⁺ is

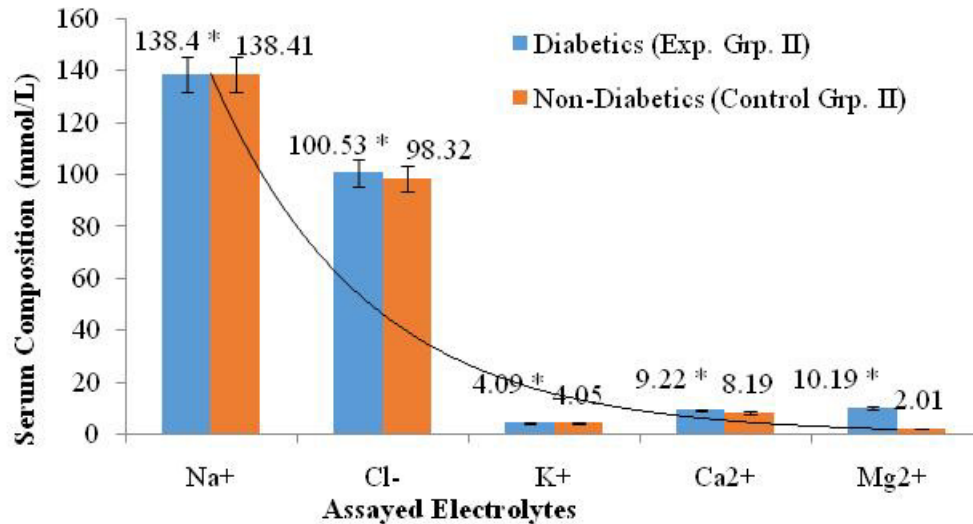


Figure 2. Comparative changes in Serum Electrolyte Compositions of Diabetic and Non-Diabetics Participants

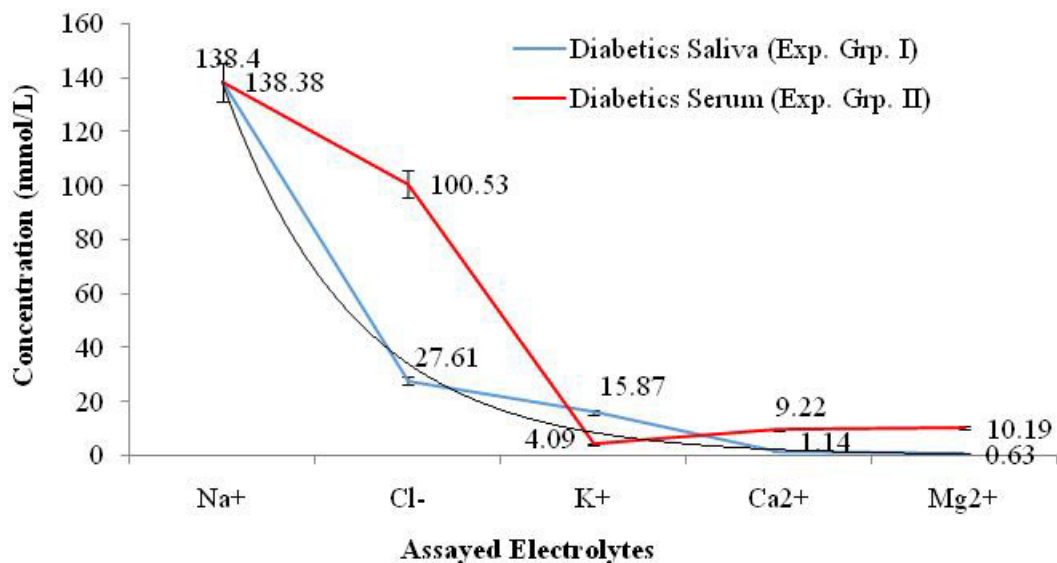


Figure 3. Comparison of Saliva and Serum Electrolytes for Diabetics

precipitated as magnesium ammonium phosphate, with subsequent estimation of the phosphate by the molybdenum blue method. The above principle was employed with modifications for phosphate determination used by Kuttner and Cohen (1927) and Youngburg and Youngburg (1930). In any case (be it serum or saliva), the magnesium component was estimated by the yellow color produced upon addition of vanadate and molybdate to the precipitate, just after removing any possible Ca²⁺ present.

Analytical Approach

Statistical measure of central tendencies (means) and

dispersion (standard deviations) were performed to compare and contrast between measured variables. Confidence levels for statistical based calculations were set at p values < 0.05. Where necessary, one-way analysis of variance (ANOVA) and student t-test were performed to check differences in mean between assayed groups.

RESULTS

Above Figure (figure 1) compares saliva electrolyte levels of diabetics to Non-diabetic subjects. Here, student t-test returned a statistically significant increase for all electrolytes in Diabetics when compared with Non-Diabetics (Control group I.). * = Significant at p > .05

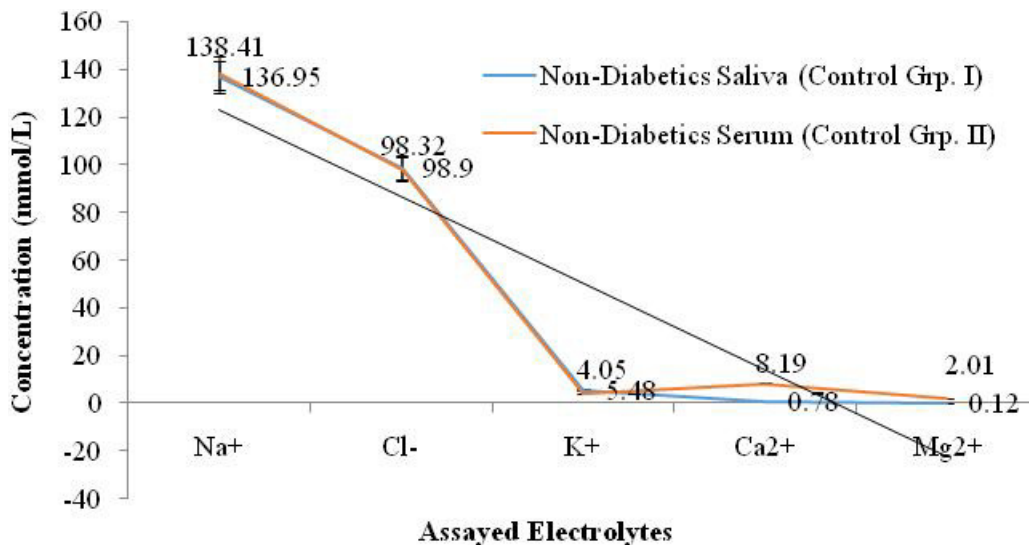


Figure 4. Comparison of Saliva and Serum Electrolytes for Non-Diabetics

Above figure 2 compares average serum electrolyte levels of diabetics (Experimental Group I) with those of Non-diabetics (Control Group II). t-test proved to be statistically significant for all assayed electrolytes except Cl⁻ when compared with Non-Diabetics (Control group II). * = significant at $p > .05$

DISCUSSION

Diabetes mellitus (DM) is an endocrine disease characterized by relative or absolute insufficiency in insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues (Pal et al., 2003; Lopez et al., 2003). It is a systemic disease that affects every system of the body (American Diabetes Association, 2012). Knowledge of the effects of hyperglycemia on saliva composition and functions remain equivocal, with oral cavity acting as the mirror of systemic disease. It is the key feature that involves various functions from digestive to immune system; making saliva a diagnostic marker for systemic diseases (Henskens et al., 2006). Studies have shown glucose as an important component and player in saliva and body fluids. Though several electrolytes (Na⁺, K⁺, Cl⁻, and HCO₃⁻, Mg²⁺ and Ca²⁺) may also account for the osmolarity of saliva in buffering and maintenance of constancy and pH, however, keeping the epithelial integrity of the mouth is incomplete without salivary glucose and protein compositions as they are known to form a major component of the glycoproteins and glycolipids in mucous surfaces; making them flexible, fluidy and polarized.

Presently, the diagnosis of DM is done by measuring serum blood glucose level by standardized methods which are invasive, physically and psychotically traumatic

to the patients (Van der Velden and Nieuw Amerongen, 1996). Hence, the current method discourages the individuals from undergoing investigations and results in lack of sufficient evidences for diagnosis of DM (Van der Velden and Nieuw Amerongen, 1996). In recent years, efforts have been made to replace blood investigations with other biological material sample that could be collected by non- invasive procedures (Bradford, 1976). Saliva has put forth as a potential diagnostic tool for surveillance of disease due to its several advantages, it clearly offers an inexpensive, simple and easily used screening method (Laemli, 1970).

Figure 1 (above) compares the average saliva electrolyte compositions for Diabetics (138.38) with those of Non-Diabetics (136.9). Seen from here is an increased mean value of sodium for diabetics than non-diabetics. This increase may have resulted from glucose capacity to act as an osmotically active substance, pulling sodium in symport fashion across concentration gradient. More so, glucose is known to increase osmolality of tissues to electrolytes, specifically Na⁺, resulting in the movement of water out of cells (Darwazeh et al., 1991). Also observed in figure 1 is that, Salivary Cl⁻ shows a lower mean value in diabetics (27.61 than non-diabetics (98.90) subjects, with salivary K⁺ level also showing higher mean value in diabetics (15.87) when compared to non-diabetics individuals (.48). physiologically, incremental levels of potassium ion (K⁺) concentration in the saliva of diabetics can be traceable to the decrease in salivary fluid output as a result of potassium efflux. Recall that for any excitable tissue, 3 atoms of K⁺ gets to the Extracellular fluid in exchange for any 2 atoms of Na⁺ that gets pumped in. this situation may interfere with the availability of K⁺ needed for the synthesis of saliva in the parotid gland. Thus, in diabetics, less than necessary concentration of K⁺ is possibly availed in the salivary

gland due to the “Repolarization deficit” of K^+ over Na^+ from the $Na^+ - K^+$ ATPase activity. This finding is in concordance with that of Meta et al (2004). Also from this study, salivary Ca^{2+} had a mean value of 1.14 in diabetics as against 0.78 seen in non-diabetic individuals upon comparison. Calcium, which is the fifth most abundant element in the human body (Darwazeh et al., 1991), is known to play a very crucial role in bone mineralization. Proline rich proteins are known to prevent enamel demineralization with special support from calcium. In the salivary glands, calcium is also a key functional electrolyte that drives and partakes in the biosynthesis of saliva. Therefore, higher calcium concentration could be a risk factor in the development of periodontal diseases. This possibly explains the reason for lower salivary Ca^{2+} concentration in Non-diabetics than Diabetics as seen from Figure 4.1. Salivary Mg^{2+} levels are also seen (from current study – fig. 4.1) with a higher mean value (0.60) in diabetic individuals than non-diabetics, upon comparison. This finding concurs with that of Malamud, who reported a similar result from their work in 2007 (Malamud, 2006).

Figure 2 compares serum electrolyte composition of diabetics to Non-diabetic subjects. From the figure, a slightly higher mean value of electrolyte concentration is generally seen in diabetics (138.41) than the non-diabetic fellows (138.40). In accordance with previous findings, here, emphasis rests with the fact that hypotonic renal losses (of water in excess of sodium) due to osmotic diuresis may result in hypernatremia (increased serum Na^+ concentration) due to high plasma tonicity and water loss. This caused an insufficient replaced [29]. serum K^+ electrolyte show a mean value (4.09) when compared to non-diabetics (4.05) the incidence of hyperkalemia is higher in diabetics due to redistribution of potassium from the intracellular to extracellular compartment (Palmer *et al.*, 2004). Serum chloride shows a mean difference (100.53) when compared with non-diabetics. The decrease in serum chloride and serum calcium show a mean of (9.22) when compared to non- diabetes (8.19) the increase in calcium is related to long term insulin resistance, it is thought that elevated intracellular free calcium concentration (by decreasing normal insulin stimulated glucose transport) increases the requirement for insulin resulting in hyperparathyroidism mediated insulin resistance (Aitken- Saavedra J et al., 2015). Serum Mg^{2+} in diabetics has a higher mean value (10.19) when compared with non-diabetics (2.01).

Figure 3 is a line graph that compares saliva and serum electrolytes for diabetics. From the graph, diabetic serum electrolyte is seen to deviate slightly from serum electrolyte levels; surging highest for Na^+ (138mmol/L) with minimal concentration of 4.09mmol/L for K^+ ion, which came least. Diabetics however shown a lowest at line graph with Na 138mmol/L, followed by Cl 27mmol/L, Ca^{2+} 1.14 mmol/L and Mg^{2+} 0.63mmol/L.

The line graph of Figure 4 shows similarities in trends

for saliva and electrolytes compositions of non-diabetics upon comparison. However, very slight difference in mean was seen for Ca^{2+} whose concentrations were .78 mmol/L and 8.19mmol/L for non-diabetic saliva and serum respectively. Ca^{2+} is mainly absorbed in the proximal tubule. Its reabsorption is coupled to Na absorption and it appears to compete with Mg^{2+} for transport in the loop of Henle. The Resnick hypothesis suggested that metabolic disorder such as hypertension, metabolic syndrome and diabetes, share a common altered intracellular condition, characterized by decreased Mg^{2+} level and reciprocally elevated free intracellular Ca^{2+} level (Abikshyeet et al., 2012).

CONCLUSION

From current study, a statistically significant difference in saliva and serum electrolyte concentrations was established across sampled groups (diabetic and non-diabetic). This observation is suggestive of saliva as an alternative means for which human blood glucose status may be assayed by non-invasive means.

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