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Variations in Electrolyte and Salivary Amylase (Ptyalin) Levels in HIV-Positive Subjects

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Authors' contributions

This work was carried out in collaboration between all authors. Author LOO designed the study, and wrote the protocol. Authors BOP and GTO managed the analyses and literature searches of the study. Author PRCE supervised the experimental protocol. All authors read and approved the final manuscript.

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ABSTRACT

Not only has studies shown oral saliva as veritable in the maintenance of health state of the oral cavity, it has been asserted that salivary fluid composition levels may be important diagnostic tools for numerous ailments, including the Human Immunodeficiency Virus (HIV). Current study investigated the changes in Salivary Amylase (ptyalin) and selected electrolyte (Na+, K+, Cl-, HCO^{3-} and Ca^{2+}) levels due to HIV infection. A hundred (100) human participants; comprising of fifty (50) HIV positive subjects (Group A) and fifty non-positive individuals (control group B) were ethically sought for the study. Saliva was then obtained from each sample with electrolyte and alpha amylase (ptyalin) levels assayed. Statistical analysis was performed on obtained variables (using the one-way analysis of variance - ANOVA) to obtain differences in mean, and comparisons

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made between groups. Following analysis, study found a decrease in salivary alpha amylase levels amidst HIV sufferers (Group A) upon comparison with control, implicative of a decrease in alpha amylase activity in HIV+ subjects. Salivary Sodium (Na^+) and Chloride ion (Cl^-) levels were also seen to statistically decrease upon comparison between groups. This decrease was also occasioned by an increase in potassium (K^+) and bicarbonate (HCO^{3-}) ion levels for HIV sufferers as compared to control (HIV negative) subjects. Similar but advanced studies are recommended to ascertain if the reason for these changes can be traceable to the continued use of anti-retroviral therapy, or by the mere presence of the HIV itself. Study has therefore shown that HIV alters saliva composition, possibly by decreasing alpha amylase, Na^+ and Cl^- levels.

Keywords: HIV; salivary electrolyte; alpha amylase.

1. INTRODUCTION

One of the most relegated, but valuable body fluid is the saliva. In practice, preservation and maintenance of good oral health, is incomplete without the salivary fluid; yet, it receives little or no attention until quantity or quality is diminished. Though in recent times, much research on the topic of salivary dysfunction have sprung up in relation to diseases and/or adverse effect on selected medications, saliva also has gain relevance as a noninvasive technique for systemic sampling measure to diagnosing ailments. A good knowledge base on the norms of salivary flow and function is therefore germane to the realization and extrapolation of results in future investigations relating to saliva [1-4].

Secreted by three pairs of major glands (parotid, submandibular and sublingual) and by many of minor salivary glands, Saliva is a mixed glandular secretion that constantly bathes the teeth and the oral mucosa. It is an alkaline fluid medium that moistens the mouth, softens foods, and aids digestion. It primarily sources from the secretory endpieces (acini cells) of salivary glands in the head [5,6]. Saliva is modified by serum exudates via tight junctions between several glandular cells (ultrafiltration) and via transcellular diffusion through these cells. It is also modified in the intercalated, striated and excretory (collecting) ducts that lead to the acini in the mouth.

It has been asserted that the average daily flow of whole saliva varies in health between 1 and 1.5 L [7]. Percentage contributions of the different salivary glands during unstimulated flow include 20% from parotid, 65% from submandibular, 7% to 8% from sublingual, and less than 10% from numerous minor glands. Stimulated high flow rates drastically change percentage contributions from each gland, with the parotid contributing more than 50% of total salivary secretions [7].

Even though saliva contains several important proteins, in humans, water accounts for 99.5% of its total composition; including electrolytes [2–21 mmol/L sodium (lower than blood plasma), 10–36 mmol/L potassium (higher than plasma), 1.2–2.8 mmol/L calcium (similar to plasma), 0.08–0.5 mmol/L magnesium, 5–40 mmol/L chloride (lower than plasma), 25 mmol/L bicarbonate (higher than plasma), 1.4–39 mmol/L phosphate, and Iodine (mmol/L, whose concentration is usually higher than plasma, but dependent variable according to dietary iodine intake)]. Saliva also contains mucus, antibacterial compounds and various enzymes [8].

In healthy people, the flow of saliva has been shown to average 1 – 1.5 L daily [9], with volume and constituents dependent on circadian rhythm [10], activity and stimulation of various saliva-producing glands [11], age, gender and blood type of patient [12], not neglecting also, physiological stimulus [13]. Salivary pH has also been shown to range from 6 to 7.4 at various levels [14,15]. Considering the inherent variability and instability of saliva, a number of difficulties are associated with the use of saliva for research and diagnostic purposes.

In HIV-positive individuals, swelling of the salivary gland (Xerostomia) has been reported to increase in a vast majority of adult population of sufferers [16,17]. This has been shown to affect quantitative changes in saliva composition, altering electrolyte levels and secretory rates of Na+, Ca2+, and Cl-; with preponderant changes in cystatin, lysozyme and total anti-oxidant levels/capacity of the oral fluid [18]. Typically, this HIV-induced salivary gland swelling presents a unilateral or bilateral diffuse soft swelling that result in facial disfiguration that is occasioned with severe pain. The situation is characterized by hyperplastic and intra-parotid lymph node infiltrates of predominantly CD8 T-Cells, indicative of memory cell phenotype [19]. Though

studies have implicated HIV in some salivary gland diseases, its frequency and infective capacity remains controversial [20]. Saliva has therefore shown significant potential for use in non-invasive diagnosis of ailments that can alter its electrolyte and amylase compositions. Current study is not exceptional as it aims at investigating the effect of HIV on saliva amylase and electrolyte compositions. Specifically, study examined the various changes in salivary Na+, K+, Cl-, HCO3- and Ca2+ ions of HIV+ subjects in comparison with healthy (HIV-) individuals.

2. MATERIALS AND METHODS

2.1 Study Design

A prospective study that involved 100 adult human subjects that were equally divided into two groups as;

Group I: HIV negative individuals (Control)
Group II HIV positive individuals (Experimental)

2.2 Study Area

Study was conducted at the HIV-care unit of the Central Hospital, Warri, and the Department of Human Physiology, Delta State University, Abraka, Delta State, Nigeria.

2.3 Study Population

Study employed a population of 100 human subjects, comprising of 50 HIV-positive and 50 HIV-negative individuals. The HIV+ subjects where known to regularly attend check-ups at their designated care units of the central hospital, warri, Delta State, with the non-positive patients (HIV-) drawn from any ward, and void of any record of HIV+ as such.

2.4 Sample and Sampling Technique

Purposive sampling technique was used to select participants based on their availability to the hospital where the study was conducted. The choice of hospital was based on the presence of treatment unit for people living with HIV. Sample size was estimated with the Statistical calculator, using module in Epi Info (version 3.5.1) at a study power of 80%, and a type 1 error of 5%. This yielded a total sample of 100 as adopted for this study.

2.5 Selection of Subjects for Participation

For reasons that certain medications may reduce saliva volume in hypo-salivation, patients with severe chronic ailments like diabetes mellitus and other systemic illnesses were exempted from the study. Subjects who were under medications for other diseases were also removed from participation in current study. Only those diagnosed of HIV with CD4 count of 350 cells/µl and below were included in the study. The non HIV+ group (control) consisted of healthy adults of age brackets 35-50 years.

2.6 Data Collection Instruments

Saliva, water, ion analyzer, saliva amylase test strip, chromogenic dye, spectrophotometer, refrigerator, biochemical analysis tool kits, ELISA test kit etc.

2.7 Procedure

2.7.1 Ethical approval

Ethical clearance was sought and obtained from the Bio-Research and Ethics committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State. Ethical approval was also granted by authority of the central hospital, warri, delta state; in HIV management and intensive care unit. Following brief explanation of study to participants, a written and informed consent was also sought and voluntarily approved.

2.7.2 Consent

Following brief explanation of study to participants, a written and informed consent was also sought and voluntarily approved.

2.8 HIV Conformation

Prior to commencement of study, the HIV sero-status of participants were re-confirmed. Plasma samples from each subject were obtained and tested with double rapid test kits (Capillus and Unigold; Trinity Bio-Tech, Ireland), and an ELISA method (Genie II HIV-1 & 2 kit).

2.9 Saliva Collection

In any case, saliva samples were collected in the early hours of the morning after making the subjects rinse their mouth thoroughly with water.

The samples were collected in sterile containers by instructing participants to allow it to be collected naturally, and expectorate it into the containers. Obtained samples were immediately taken for biochemical analysis.

2.10 α-Amylase Analysis

Salivary Amylase Estimation was performed by the direct substrate kinetic enzymatic method [21]. The procedure was done in accordance with the Bradford method using bovine serum albumin as a standard, and at a wavelength of about 595 nm [21]. To begin, the amylase strip containing starch substrate to which a chromogenic dye was attached to form an insoluble dye-substrate complex was employed. The saliva, which contains large quantity of amylase was first diluted to a ratio of 1:2. The reflotron plus instrument was switched on and allowed to boot for some time. After that, 32µ of saliva was placed on the test pad of the amylase strip. The instrument flap was opened with the strip placed inside, and then, closed. The starch-substrate-chromogenic dye complex within the amylase strip was hydrolyzed by the amylase in the saliva to form a color intensity that was then spectrophotometrically measured at 520nm. Obtained spectrophotometric result was then recorded from an LCD screen, with final result multiplied by 200.

2.11 Analysis of Saliva Electrolytes

Saliva samples were first converted to ice boxes, and then, each sample was centrifuged at 3500 rpm for 5 minutes. In each case, the supernatants were transferred to other test tubes and frozen at -4^0C before analysis proper. For determination of concentration of selected electrolytes, saliva was diluted at either 1/100 or 1/1000, with ionic concentrations determined with the flame emission spectrophotometry [21]. Concentrations of Cl- and HCO_3^{2-} were obtained by the Schale's method, using mercuric nitrate [12].

2.12 Determination of Salivary Electrolytes

Sodium (Na^+) is precipitated as the triple salt; sodium magnesium uranyl acetate, with the excess uranium being reacted with ferro-cyanide to produce chromophore, whose absorbance varies inversely as the concentration of test saliva sample. On the other hand, potassium ion (K^+) is determined by using sodium tetra-phenylboron, a specifically prepared mixture to produce colloidal suspension. Cl- was determined from the spectrophotometry.

2.13 Statistical Approach

Evaluation of result from statistical significance was done with the student t-test. With data expressed as mean± standard deviation, a p-value < .05 was accepted as statistically significant.

3. RESULTS

Result from this study described the effect of HIV infection on the level of salivary alpha amylase and electrolytes. Data from the HIV+ subjects were compared with those from the HIV- (control) individuals.

From Fig. 1, HIV+ subjects showed a statistically significant decrease in Na+ level ($p < .05$) upon comparison with HIV- individuals.

From Fig. 2 observed that HIV caused an insignificant increase in K+ level ($p < .05$) when compared with normal individuals.

Report from Fig. 3 shows that HIV statistically significant decrease in the mean salivary Cl- level ($p < .05$) upon comparison with control.

Data observed from Fig. 4 show a statistically significant increase in salivary bicarbonate ion level ($p < .05$). However, this proved insignificant following comparison with control subjects.

From Fig. 5, it is seen that HIV caused a statistically insignificant increase in salivary calcium ion level ($p < .05$). However, this proved insignificant following comparison with control subjects.

Result from Fig. 6 shows that HIV caused a statistically significant decrease in salivary alpha amylase level ($p < .05$) on comparison with control subjects.

4. DISCUSSION

Maintenance of optimal constancy in body fluids (intracellular, extracellular and interstitial fluids) electrolyte concentrations is inviolable to healthy living. This study examined the changes in saliva alpha amylase and electrolytes in sufferers of HIV.

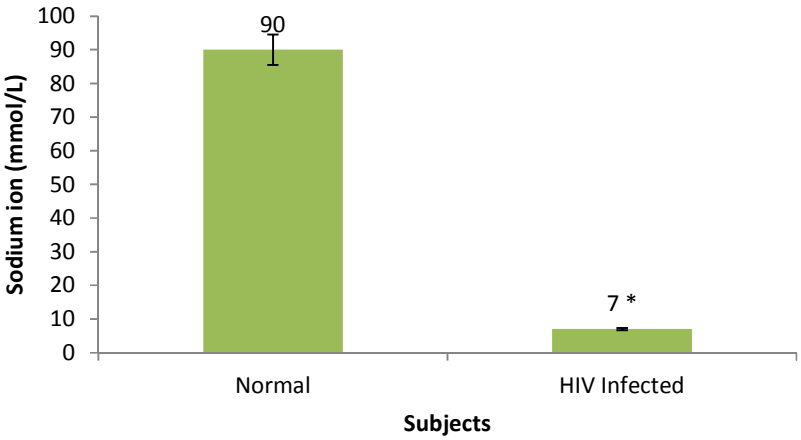

Fig. 1. Effect of HIV infection on salivary sodium ion level
**p < .05 with normal subjects*

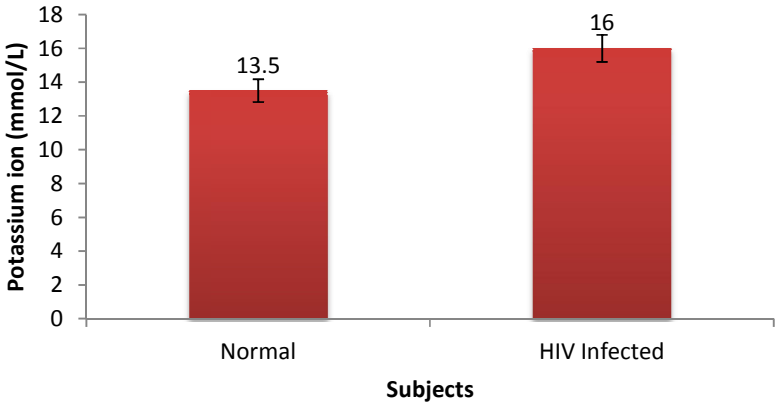

Fig. 2. Effect of HIV infection on Salivary Potassium ion Level

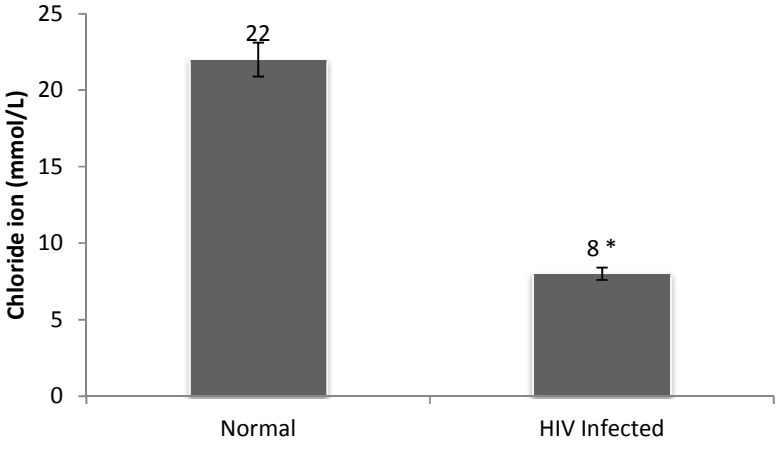

Fig. 3. Effect of HIV infection on salivary chloride ion level
**p < .05 with HIV+ subjects*

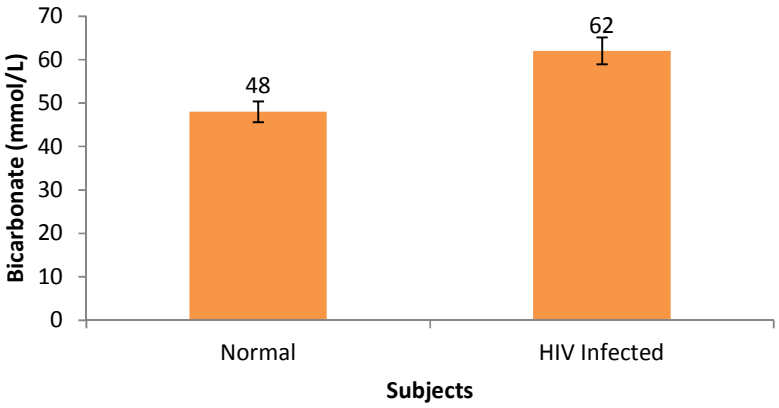

Fig. 4. Effect of HIV infection on salivary bicarbonate ion level

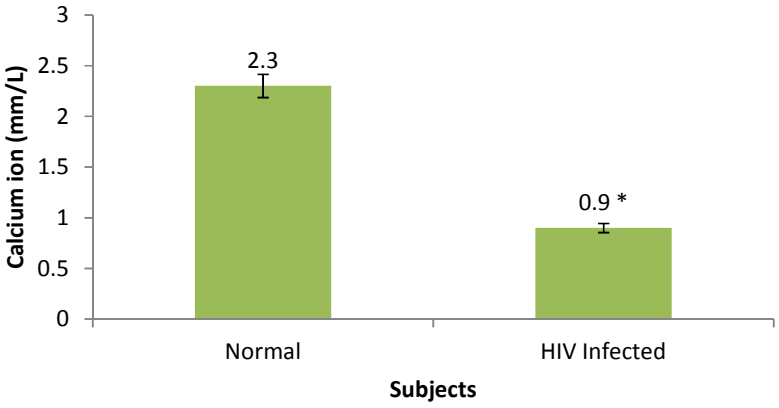

Fig. 5. Effect of HIV infection on salivary calcium ion level
**p < .05 with HIV+ subjects*

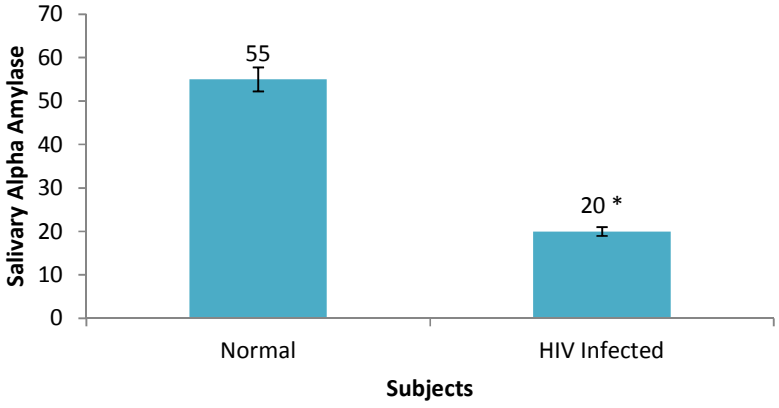

Fig. 6. Effect of HIV infection on Salivary Alpha Amylase
**p < .05 with HIV+ subjects*

Results from the study showed a significant ($p < .05$) decrease in salivary alpha amylase levels of HIV+ patients. A significant decrease was also observed in salivary Na^+, Ca^{2+}, and Cl^- of HIV sufferers upon comparison with healthy individuals (control). Meanwhile, salivary K+ and HCO_3^{2-} levels showed a statistically significant increase in HIV+ subjects with comparison to control (HIV-) individuals. In a similar study conducted in 2006, Isnard et al., found electrolyte disturbance to be associated with numerous morbid conditions, especially amongst HIV-infected individuals [22]. Apparently, much earlier studies have shown that low CD4 T-cell count (≤ 200 cells /mm^3) and nephropathy could be risk factors for such biological imbalances in HIV infected individuals [23]. Possible reason for increased salivary K+ and HCO_3^{2-} in HIV+ subjects is not clear. However, Winston et al., in 2009 had a similar experience in their work on HIV-associated nephropathy.

Furthermore, current study confirms similar reports on HIV-associated complications like hyperkalaemia, low serum bicarbonates, hypokalaemia, etc. in HIV+ patients [24]. It is also known, that duration-infected episodes as well as hyperkalaemia results from intravascular haemolysis or rhabdomyolysis, and occasionally, from decreased activity of Na^+, K^+-ATPase pump. Hyponatremia has also been known to frequently occur in infectious conditions as a result of increased levels of vasopressin, entry of Na+ into cells, Na+ loss, and resetting of osmoreceptors [25].

The decrease in salivary alpha-amylase observed in current study can be attributed to xerostomia, which is a known common disorder in HIV+ patients [16]. Previous studies have also asserted that salivary flow rates diminish with increased HIV disease severity for both mouth, as well as parotid saliva [26]. This could therefore be another reason for the drop in salivary alpha amylase level, and possibly failure in the exchange of Na+ and Cl- in the lumen of salivary duct that leads to their decrease. This finding concurs with that of Douglas, who posited that the main factor that affects salivary composition is the flow index that varies in accordance with the type, intensity, and duration of the stimulus [26]. Paradoxically, as the salivary flow rate increases, the concentration of total protein, Na^+, $Ca2^+$, Cl^- and HCO^{3-} as well as the pH rises to various levels. Whereas, concentrations of inorganic phosphate and magnesium ions diminished in preponderance [27-29].

5. IMPORTANCE OF STUDY

With HIV infections known to cause detrimental effects on several physiological parameters, current study validates the usefulness of saliva as a diagnostic tool for ailments, specifically HIV-AIDS. It presumably draws attentions on the need to manage salivary constituent derailments in the overall interest of diagnosing and prognosing HIV infections, plus widen the scope of clinically managing and enhancing the livelihood of sufferers for longevity and avoidance of supposed complications related to electrolyte depletion and xerostomia.

6. CONCLUSION

HIV disease is known to cause a variety of pathological disorders and imbalance in several physiological chemicals and electrolytes. In the light of this, this study has shown that HIV alters the salivary composition by decreasing salivary alpha amylase, Na^+ and Cl^- levels; while increasing K^+ and HCO^{3-} levels

7. RECOMMENDATIONS

We recommend that further studies be carried out with anti-retroviral drugs to establish facts on whether such drugs alleviate these complications resulting from electrolyte depletion on short and long term bases in HIV sufferers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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