COMPARATIVE STUDY ON VARIATIONS IN SELECTED TEAR ELECTROLYTES COMPOSITIONS AMONG HIV INFECTED PATIENTS

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ABSTRACT

Introduction: The human tear fluid produced in the lacrimal gland of the eye contains different electrolytes with the concentration varying in health and disease conditions. The focus of the study is to examine variations in selected tear fluid electrolytes (Sodium, Chloride, and Potassium ions) in HIV/AIDS sufferers.

Materials And Methods: A total of 27 subjects were recruited and used for the study; 16 subjects diagnosed to be Human Immuno-Deficiency Virus (HIV) positives served as experimental group while 11 samples obtained from HIV-negative subjects served as control group. Following collection of approximately 100µL of basal tear fluid through from lower conjunctiva sac in the right eyes of sampled subjects, selected electrolyte compositions (Na^+, K^+ and Cl^-) of obtained tears were assayed for biochemical estimation, also, blood samples were collected for bio-assay of immune variable (CD_4 count).

Results: Results obtained revealed that tears chloride ion (Cl-) concentration had significant difference when compared with healthy subjects (Mann Whitney U = 43.500. $p < 0.05$). A measure of association was also performed to establish a relationship between electrolyte variations and CD_4 levels. Result revealed a significant increase $p<0.05$ in chloride ion (Cl-) concentration for HIV sufferers as compared to control. Pearson product moment correlation coefficient (r) showed a weak negative relationship ($r = -0.260$) between Cl- concentration and CD_4 counts for experimental group (HIV sufferers), suggestive that tear fluid Cl- levels will decrease as immune marker cells (CD_4) or vice versa. Serum Na^+ concentration was higher in HIV seropositive subjects with K^+ proving an insignificant decrease as against control subjects.

Conclusion: Further studies are required on other body fluid electrolytes parameters to ascertain the veracity in electrolyte levels of HIV/AIDS sufferers.

Keywords: Tear Fluid, Electrolytes, CD_4, HIV/AIDS,

INTRODUCTION

Declining levels of electrolyte in HIV infected people are viable markers for decifying disease progression and severity, even before malnutrition becomes a sensitive factor [1]. Low levels of plasma electrolyte concentrations have been reported to have fundamentally connection with increased risk of death from HIV [1]. Accordingly, adequate electrolyte nutritional status may increase the resistance of HIV infection by improving the capacity of vital immune system cells known as T-cells and modifying their production of intracellular messengers known as cytokines. With appropriate information on its implications in diagnostic medicine, body fluid electrolytes can enhance proper immune functions. For instance, in HIV/AIDS sufferers, specific tear electrolyte homeostasis may help in the maintenance of the epithelial cells and osmotic pressure factor [2].

The HIV, which is the etiological agent that produces the immune-suppression resulting in AIDS belong to the lentivirus group of retroviruses; with two distinct variants identified, and HIV2 predominantly found in Africa than other parts of the world [3]. The virus has an innate enzyme called reverse transcriptase on its surface, a protein known as GP 20, which specifically recognizes and binds to the CD4 cells, T-lymphocytes and macrophages, allowing the transcription of the viral RNA to the host's DNA as such. In a recent report, HIV particles have been isolated from blood plasma, tears, urine, saliva, breast milk, as well as seminal and pre-ejaculated fluids and vaginal secretions [4, 5]. Although, tears and other body fluids such as urine and saliva contain the HIV virus, they are not certified as being infectious unless contaminated with blood. The HIV is transmitted by the percutaneous inoculation with virus by mucosa contamination and contamination of non-intact skin. In clinical practice, diagnosis of HIV/AIDS as well as its ocular associated diseases cannot be over-emphasized. The use of tears for diagnostic purpose has been proposed for drug monitoring in order to verify the dry eye syndrome, detect lysosomal storage disease such as Morlous Gauche, and in the detection of other bio-analytes of HIV antibodies [6].

There seems, by all accounts, to be an exceptional collaboration between human electrolytes and the HIV that cause AIDS. HIV replications in CD4 lymphocyte cells are critical in normal immune cell response to invading organisms. Nonetheless, as the CD4 cells are

explicitly destroyed during HIV infection, no further assistance is available for immune responses because of systemic immune failure, opportunistic infection thus arise, altering electrolyte balance in HIV/AIDS sufferers as such. The need for a correlative research as this on the electrolyte and CD4 changes is therefore a necessity [7].

The significant component of tear fluid includes; water, electrolytes, proteins, lipids and mucins. The aqueous layer of the tear film form 60% of the total composition of tear and it is made up of water, electrolytes, proteins, peptide growth factors, immunoglobulins, cytokines, vitamins, antimicrobials, and hormones [8]. Electrolytes present in tear film are primarily potassium, chloride, sodium, and bicarbonate with lower levels of magnesium and calcium ions [8]. The osmolarity of the lacrimal fluid is about 300 mosM and contains sodium ions (128.7 mM), potassium ions (17 mM), chloride ions (141.3 mM), and bicarbonate (12.4 mM) [9]. The electrolytes are responsible for the osmolarity of tears, acting as a buffer to maintain a constant pH and contribute to maintaining epithelial integrity of the ocular surface [10]. Tear fluid has about the same osmolarity as plasma but has lower sodium ions (140 mM plasma) and higher potassium ions (4 mM plasma) and much higher chloride ions (100 mM plasma). The higher potassium and chloride ions are a reflection of the way in which water is moved across the epithelium and into the gland lumen [11].

The ocular surface is highly exposed and as such, efficient tear production and turnover is essential for its continued health. Tear film parameter is not simply determined by only a reduced production or lack of tears, but by a complex ocular surface condition in which the tear film is unbalanced [12]. This can lead to an imbalance in electrolytes, proteins, lipids and mucins and permanent damage to the corneal and conjunctival epithelial cells and the corneal nerve fibers that trigger secretion. Hence, this study investigated the variations in selected tears electrolytes (Cl^-, Na^+ and K^+), specifically serum Cl- levels and CD_4 count in HIV/AIDS sufferers. Specifically, the study; determined the effects of immune marker (CD_4 count) on varying levels of serum electrolytes (Chloride, Sodium and Potassium ions) in HIV sufferers and investigated (using correlation), the relationship between serum electrolyte levels and CD_4 count in HIV patients.

MATERIALS AND METHODS

Study Design

Study adopted the experimental type of research design, a case-control clinical matching study. Sixteen (16) human subjects of sero-positive HIV status were ethically recruited from the department of chemical pathology, University of Benin Teaching Hospital (UBTH), a designated center for treatment of people living with HIV/AIDS. This formed the experimental group. Eleven (11) other samples were also obtained from sero-negative HIV subjects to serve as the control.

Selection Criteria

Exclusion Criteria

Subjects who were not sure of their HIV/AIDs status were excluded and the case files of subjects were consulted for confirmation, HIV/AIDs positive subjects who suffer dry eye were excluded from the study. Also, individuals who are on medications like topical atropine, anti-glaucoma or anti-inflammatory drugs were excluded because their tear film maybe compromised. Subjects with eye make-ups, systemic conditions such as hypothyroidism, diabetes mellitus were excluded as well. This is because these conditions reportedly lower tear fluid production and electrolyte levels by extension.

Inclusion Criteria

Non-contact lens wearing individuals and not crying, with no conjunctivitis symptoms and any other ocular complications were thus included in the study. Also, only subjects between 20 – 60 years old were included in the study as immune CD_4 cell count has been reported to decrease with increasing age. HIV/AIDs suffer CD4 count were also reported from case files and subsequently confirmed in the laboratory from collected samples analysis (see appendix).

Ethical Approval

Subjects gave consent as they were told the purpose for collection of tears fluid sample and were made to sign the consent form. Also, ethical approval was obtained from the Faculty of life sciences, University of Benin.

Tear Fluid Collection

Using the plain glass micro-capillary tubes, approximately 100 µL of basal (open-eye) tears was collected from the lower conjunctival sac of the right eyes of subjects. The time taken for tears to reach a specified point was recorded as flow rate (microliters per minute) though subjects were made to rest for 5 minutes between each collection, this was necessary to avoid stimulating reflex tears. Obtained samples were cooled immediately to +4°C for analysis. Before actual

collection of tear fluid, Clinical investigation was carried out for subjects. This was necessary in ruling out any sign(s) of ocular surface conditions.

Isolation of Tear Fluid Sodium (Na^+), Chloride (Cl^-) and Chloride (Cl^-)

The principle of ion selective methodology was used in isolation of electrolytes as stated by Beer's law which involves atomization of sample and absorption radiation from light from light source by the free atoms [13]. The two cations (sodium and potassium ions) and anion (chloride ion) were identified in this study. The tears fluid samples electrolyte composition was excited through collision with an active metastable gaseous species, and the excited sample components emitting a characteristic wavelength of light with a microwave cavity through which the electrolytes flow selectively including a microwave source coupled to the cavity that cause complete disassociation of the electrolytes.

A model 460 Atomic Absorption Spectrophotometer equipped with Deuterum AC Background corrector and an HGH-400 Graphite Heating Furnace (both from Perkin-Elmer Corp, Norwalk, CT 06856). The spectrophotometer was operated at 213.9nm, in the peak height mode, and with a 0.7-nm alternate slit width. The graphite furnace heating variables and carrier gas flow rates were determined epically to provide optimum sensitivity, accuracy and reproductivity for sodium, potassium and chloride respectively. The furnace heating procedure was; dry for 20 seconds, with 10 seconds, ramp to 110^0C, char for 20 seconds, with 10 seconds, ramp to 400^0C atomize for 4 seconds at 2100^0C; and expressed to maximum temperature 2700^0C, for 2 seconds pyrolytically coated graphite tube were used for all the determinations.

The values obtained were confirmed by using Perkin-Elmer model 460 and 306 Atomic Absorption spectrophotometer equipped with three-slot burner head (for air acetylene flame).

Determination of CD4 Count

CD_4 counts of participants were determined for control and experimental subjects were subjected base on laboratory test procedure under stringent protocol by expert technologists [14]

Statistical Analysis

Differences in mean between groups were tested with the one-way analysis of variance. Where a significant change exists, the Mann Whitney U test (post hoc) was used to ascertain the source of mean differences.

Relationship between test variables was performed with the Pearson Product Moment Correlation Coefficient (*r*). All test statistics were performed with the statistical package for social sciences (SPSS, version 21).

Results

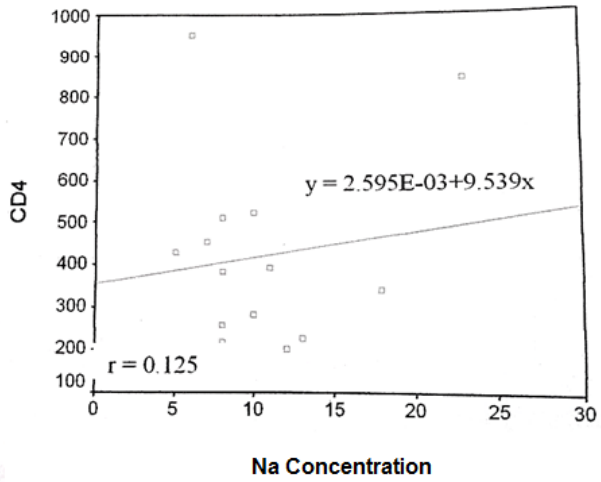

Fig 1: Relationship between CD4 count and Tears Sodium ion Concentration

Figure 1 shows the relationship between CD4 counts and tears Na^+ concentrations in HIV seropositive subjects. As seen, there was a weak positive relationship between CD4 count and Na^+.

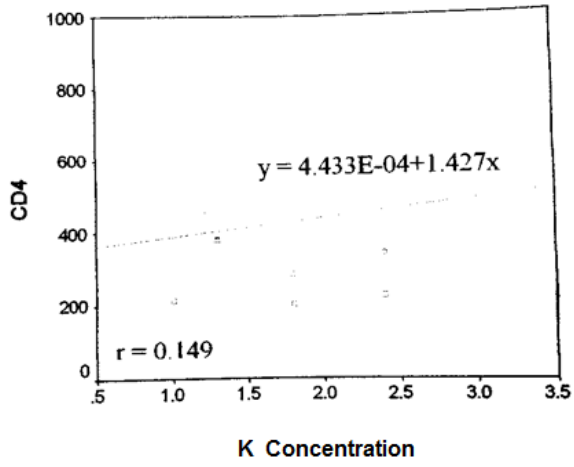

Figure 2: Relationship between CD4 count and Tears Potassium ion Concentration

As shown above Figure 2, there was a weak positive relationship (r = 0.149) between CD4 count and tears K^+ in HIV seropositive subjects.

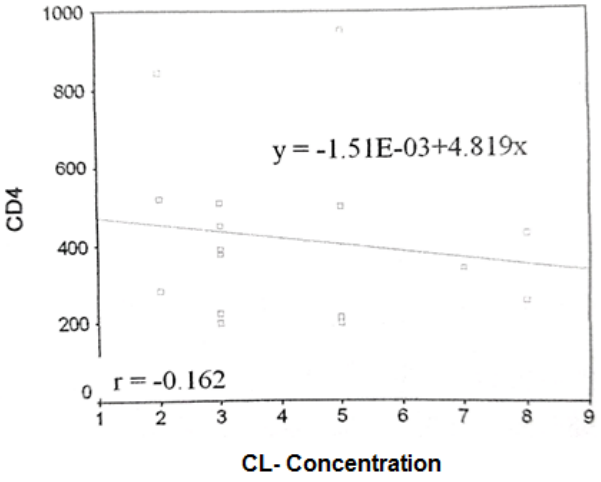

Figure 3: Relationship between CD4 count and Tears Chloride ion Concentration

From Figure 3 above, the relationship between CD4 counts and tears Cl^- concentration of HIV seropositive subjects is notably weak negative (r = -0.162).

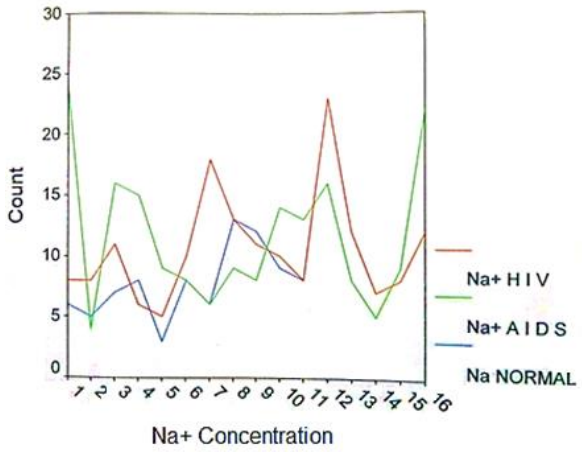

Figure 4: Changes in Tear fluid Sodium ion Concentration in HIV Seropositive Subjects

From above figure, serum Na+ concentration is observed to increase significantly ($p < 0.05$) in seropositive HIV subjects than normal (control) subjects.

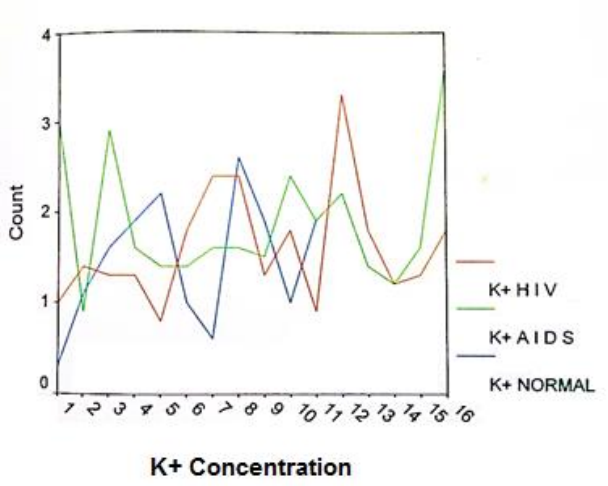

Figure 5: Changes in Tear fluid Potassium ion Concentration in HIV Seropositive Subjects

Above figure (Fig. 5) shows that K+ had a statistically significant increase ($p < 0.05$) for HIV seropositive subjects as against the control.

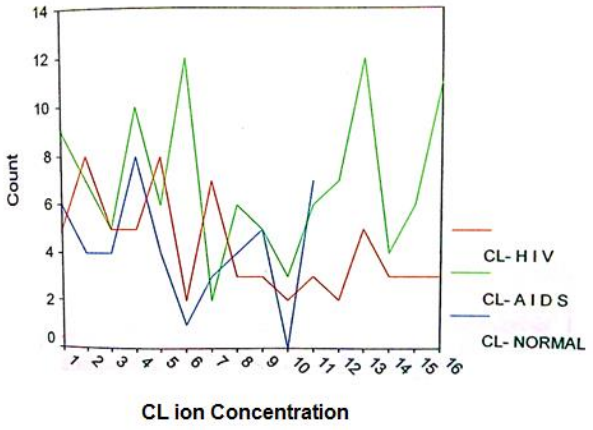

Figure 6: Changes in Tear fluid Chloride ion Concentration in HIV Seropositive Subjects

Above figure 6 shows that Cl- had a statistically significant increase ($p < 0.05$) for

HIV seropositive subjects compared to normal subjects.

DISCUSSION

This study comparatively investigated the variations in selected tear electrolytes among HIV sero-positive patients. The study stimulated and obtained tear fluid from HIV subjects and compared it for changes and relationship in sodium ion (Na), chloride ion (Cl-) and potassium ion (K) in the bid to establish an effect, and a relationship among tears electrolyte concentrations due to the presence the HIV virus. This finding agrees with previous studies conducted to examine water, electrolytes, and acid-base alterations in human immunodeficiency virus infected patients which revealed that HIV contaminated patients, especially those with AIDS, are inclined to a large group of various water, electrolyte, and acid base alterations [15]. These problems add to both morbidity and mortality in HIV contaminated patients.

From the result of current study, analysis of data revealed that HIV/AIDS has effect on tears electrolytes. The tears chloride ion (Cl-) concentration had a statistically significant difference when compared with healthy subjects (Mann Whitney U = 43.500. $p < 0.05$). This difference was further qualified as being higher (increased) as shown in figure 3. This implies that Cl- concentration was significantly higher in HIV positive subjects with a possible resultant effect in tears electrolyte imbalance and/or increased osmotic ocular pressure that may precipitate dry eyes. From this finding, though related literatures on plasma electrolytes are readily available, however, those for tears fluid are scarce. This finding is in line with previous studies who suggested that balanced composition of tears electrolytes is important in the maintenance of cornea epithelial cell integrity and osmotic pressure. Thus, the increased Cl- concentration observed is suggestive that cornea epithelial and osmotic pressure may have been compromised for HIV sero-positive subjects, implicative that tears Cl- had effect on serum CD4 count [16].

CONCLUSION

From this study, tear Cl- concentration is seen to have increased significantly in sero-positive HIV subjects, skewing towards reducing the CD4 immune marker cell count, thus increasing with decreased CD4 levels. Also, the relationship between CD4 counts and tears Na^+ concentrations in HIV seropositive subjects was weakly positive, implicative that they both have a direct relationship.

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