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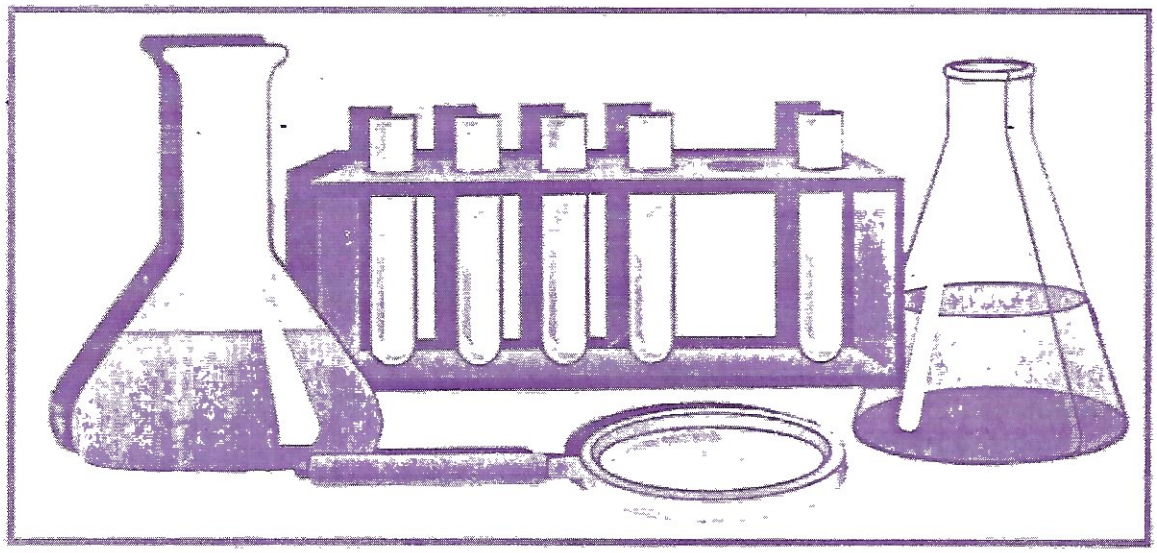

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JOURNAL OF BIOMEDICAL INVESTIGATION, DECEMBER, 2007, Vol. 5, No. 1

TABLE OF CONTENTS

Journal of Biomedical Investigation, 2007; 5(2):49-52.

Electrolyte and renal status of HIV seropositive individuals on ART: a short-term follow-up study.

[1]Onochie A.U, [2]Okaka. A.N.C., [3]Onyenekwe, C.C, [4]Meludu, S.C, [4]Ezegwunne I.P, [1]Ifemeje, J.C.

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ABSTRACT

The study was designed to assess the renal and electrolyte status of HIV seropositive subjects during antiretroviral therapy follow up for 4 months. For this study, 20 HIV seropositive subjects were randomly recruited before commencement of anti-retroviral therapy and followed-up into 4 months of anti-retroviral therapy. Blood samples were collected before and bi-monthly during anti-retroviral therapy. The result of the present study showed that before anti-retroviral therapy, the serum levels of potassium and bicarbonate were the only electrolytes that showed significant increase compared with corresponding values in the control subjects ($p<0.05$ in each case). However, the serum concentrations of urea and creatinine were consistently raised in the HIV seropositive subjects before and during the ART compared with values observed in the control subjects ($P<0.05$ in each case). The findings of the present study suggest impaired kidney functions in HIV seropositive subjects with no immediate improvement within 4 months of ART.

Keywords: Infection; drugs; organs.

INTRODUCTION:

About 4.8 million Nigerians are infected with HIV while only 50% of this population has access to Antiretroviral therapy (ART)[1]. HIV infection is known to progress to acquired immunodeficiency disease (AIDS) if ART is not administered. Both HIV infection and ART have been shown to result in functional impairment of some tissues/organs[2,3,4,5,6]. Some of these organs like the kidneys are actually involved in the regulation of acid-base balance and electrolyte balance in the body. Other complications of HIV infection such as proteinuria, excessive vomiting, and diarrhoea[7,8] among other things may also contribute to electrolyte imbalance in HIV infected subjects. Electrolytes are needed by all cells in the body for normal functioning and maintenance of body fluid. Hence evidence of imbalance in electrolytes may signal functional impairment. The present study was designed to assess the electrolytes and renal status of HIV infected subjects within few months of ART.

MATERIALS AND METHODS:

SUBJECTS: 20 HIV seropositive subjects were randomly recruited amongst those about to commence ART at the HIV clinic, Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and followed up bi-monthly for 4 months during ART and referred to as test subjects while 20 HIV seronegative subjects randomly recruited at the Voluntary Counseling and Testing (VCT) unit NAUTH served as the control subjects. The antiretroviral combination Stavudine, Lamivudine and Neviraprine were administered orally at recommended dosage of 40mg twice daily, 150mg twice daily and 200mg daily respectively. Blood sample was collected from the test subjects for analysis of serum electrolytes (Sodium, Potassium, Chloride bicarbonate), serum urea and creatinine during bimonthly visit while blood sample was collected once from the control subjects. The subjects gave informed consent and the ethical committee approved the study design.

METHODS:

Determinations of electrolytes such as Sodium and Potassium were by flame photometer, chloride by mercuric nitrate titrimetric method and bicarbonate by titrimetric method as described in Tietz[9].

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Determination of serum creatinine concentration: Serum creatinine was performed using VITROS Blood Chemistry Analyzer (USA). The procedure described briefly, unto DT 6011 module (slide) properly labeled for each participant was placed serum sample on the pipette location on the slide. The DT 6011 module was used to determine the serum creatinine value of the samples.

Determination of serum urea concentration: Serum urea was performed using VITROS Blood Chemistry Analyzer (USA). The procedure described briefly, unto DTSC module (slide) properly labeled for each participant was placed serum sample on the pipette location. The DTSC module was used to determine the serum urea value of the samples.

RESULTS:

The mean serum sodium concentration (mmo/l) in the test subjects during the pre- and post ART era was not significantly different from that observed in the control subjects ($p>0.1$). The mean serum potassium concentration (mmol/l) in Test subjects (7.8 ± 2.7) pre-ART was significantly raised compared with corresponding value in control subjects (4.1 ± 0.4) ($p<0.05$). However, the post ART serum concentration of potassium in the test subjects was similar to values observed in the control subjects. The mean serum concentration of thloride in test subjects during the pre- and post ART era was not significantly different from that observed in the control subjects ($p>0.1$). The mean serum concentration (mmol/l) of HCO_3^- in the test subjects of 24 ± 5 during pre-ART era was significantly higher than 21 ± 2 observed in control subjects ($P<0.05$). However, the post ART serum concentration of bicarbonate in the test subjects was similar to value observed in the control subjects ($p>0.1$). See table 1. The mean serum concentration (mmol/l) of urea in the test subjects during pre-ART (4.2 ± 3.2) was significantly raised compared to 2.4 ± 0.7 observed in the control subjects ($p<0.05$). By 2months and 4months into ART the mean serum concentrations of urea in the test subjects were 6.2 ± 10.2 and 3.8 ± 2.8; these values are significantly higher than that observed in the control subjects ($p<0.05$ in each case). Similarly, the mean serum creatinine concentration (nmol/1) in the test subject during pre-ART (110 ± 80) was significantly higher compared with 77 ± 7 observed in the control subjects ($p<0.05$). By 2 months and 4months into ART the mean serum concentrations of creatinine in the test subjects were 101 ± 53 and 99 ± 44; these values are significantly higher than that observed in the control subjects ($p<0.05$ in each case).

DISCUSSION

The present study observed mild alteration in serum potassium and bicarbonate concentration in HIV seropositive subjects. The high mean serum potassium level in these subjects could not be.., explained however the wide deviation from the mean thus suggested that some of the HIV seropositive subjects might at the time of presentation acquired some level of impairment in regulation of their electrolytes. Similarly, there was increase in serum bicarbonate level in these subjects. However, both alterations seem corrected within 2 months of antiretroviral therapy.

Studies have shown different reports regarding the electrolyte status of HIV infected subjects. Afhami et al[10] reported no alteration in electrolyte balance in HIV infected subjects. However, another study has reported evidence of alteration in electrolyte status of HIV infected subjects[11]. Considering the finding in the present study and other earlier reports, accurate definition of the clinical status of these HIV infected subjects and level of HIV associated complications at presentation may help to actually note possible variables that affect or control electrolyte balance in HIV infected subjects.

In the present study serum urea and creatinine were used as markers of renal function. The mean serum values of these markers were significantly raised in the HIV infected subjects. The mean serum value of urea was almost double in HIV infected subjects. Upon administration of ART a further three fold increase in serum urea was observed by 2 months into ART but dropped to two fold increase by 4 months into ART. Studies using different markers for assessment of renal function have shown either non-impairment in renal function[6,10] or impaired renal function in HIV infected subjects[3,6,12,13]

The present study concludes that there are possibilities of development of electrolyte imbalance and renal dysfunction in both HIV infected subjects with or without ART.

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Table 1: mean SD serum concentrations of sodium (mmol/l), potassium (mmol/l), chloride (mmol/l), bicarbonate (mmol/l), urea (mmol/l) and creatinine (nmol/l) in control subjects and HIV seropositive subjects pre-ART and during ART.

Parameters	+Control Subjects (n =20)	*pre-ART Subjects (n20)	**2 months ART subjects (n20)	***4months ART subjects (n20)	p-value
Sodium	134±5	137±13	135±11	135±11	+VS*p>0.1 +vs** p>0.1 +vs***p>0.1
Potassium	4.1±0.4	7.8±12.7	4.0±0.4	4.1±0.5	+vs * p<0.05 +vs ** p>0.1 +vs ***p>0.1
Chloride	100±4 -	101±13	101±8	99±4 -	+vs*p>0.1 +vs ** p>0.1 +vs*** p>0.1
Bicarbonate	21±2	24±5	22±5	22±2	+vs * p< 0.05 +vs **p <01 +vs p>0.1
Urea	2.4±0.7	4.2±3.2	6.2±10.2	3.8±2.8	+vs * p< 0.05 +Vs** p< 005 +Vs*** p< 0.05
Creatinine	77±7	110±80	101±53	99±44	+vs * p<0.05 +vs** p< 0.08 +vs*** p< 0.05

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Inter-relationship Between Malaria Parasitaemia And Widal Reaction In Febrile Patients

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ABSTRACT

The inter-relationship between malaria parasitaemia and widal reaction in febrile patients was investigated. Malaria and typhoid fever (febrile illnesses) were diagnosed by microscopic malaria parasite presence and Salmonella typhi/paratyphi antibodies screened by Widal reaction with titres greater than or equal to 160 regarded as positive. Of the 797 blood samples investigated. 125 (15.7%) were strictly malaria cases, 429 (53.9%) were mixed infection. 183 (23.0%) were strictly typhoid cases while 60 (7.5%) were negative for both febrile conditions. A co-infection rate of 52.7% and 58.9% was obtained for *Salmonella typhi* 'O' and 'H' respectively and this was closely followed by *S. paratyphi* B 'O' with a co-infection rate of 41.3%. This positivity rate of *S. paratyphi* B 'O' is significant ($P<0.05$) and may indicate a possible antigenic similarity with *S. typhi* and malaria parasite.

Key words: Malaria parasitaemia. Widal test, Paratyphi antigens.

INTRODUCTION

Fever, a clinical hallmark of inflammation and the common designation of systemic temperature elevation, is produced by a short polypeptide. interleukin-1 and Tumour Necrosis Factor-alpha (TNF-) derived from macrophages during infectious disease processes and inflammatory responses, apparently reset the body's "thermostat" to permit a higher body core temperature level[1]. Malaria and Typhoid are febrile illnesses resulting in Malaria and Typhoid fever respectively. World Health Organisation has recognized malaria, a mosquito - borne febrile illness, as causing more morbidity than any other disease and has been reported to be responsible for at least 1 million deaths a year, mostly in voting children in tropical countries[2]. It is the most common cause of outpatients visit to health care facilities and it is consistently reported as one of the five main causes of death in Nigeria[3]. Typhoid and Paratyphoid fever are clinically similar acute systemic illnesses caused by infection with *Salmonella tiphi* and *Salmonella paratyphi*. Both are generally termed enteric fever[4]. Typhoid fever is one of the leading preventable global causes of death due to infectious disease, accounting for over 600,000 annual deaths worldwide[5]. Schizogony in *Plasmodiun falciparum* infection has long been shown not to occur in the peripheral blood stream but in tissues and organs where there is massive multiplication in visceral capillaries[6]. Clark and Chaudhri[7] explained that the schizonts are not found in the peripheral blood but the tissue necrosis factor-α (TNF-α) released at the burst of schizogony, acts on the peripheral, unparasitized circulating red cells causing physical and chemical changes to the red cells rendering them susceptible to macrophage phagocytosis. Macrophages become activated and the release of interleukin-l inevitably ensues[8,9]. The clinical features of enteric fever tend to be more severe with *Salmonella typhi* (typhoid fever)[4]. The organisms penetrate the ileal mucosa and spread through the regional lymph nodes, the lymphatics and blood stream infecting mononuclear macrophages in lymph nodes, bone marrow, liver and spleen[4]. The infection of macrophages stimulates the production of interleukin-1 and Tumor Necrosis Factor-α (TNF--α) thereby causing the prolonged fever[10]. The pathogenesis of both malaria and typhoid fever centre on the production of the macromolecules Interleukin-1 and Tissue Necrosis Factor-alpha consequently producing fever. The

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definitive diagnosis of malaria is the presence of just a parasite in a blood film, which may be missed because of the cyclical nature of the parasite or the Microscopists skill. The isolation of *Salmonella typhi* or *paratyphi* organisms, which is diagnostic, may also be missed due to the stage of infection. Hence the need for this study.

MATERIALS AND METHODS

Subjects: These include 797 adults with febrile illness referred by some hospitals to Alees Diagnostic Medical Laboratory center, Lagos for laboratory investigations for malaria parasites and Widal reaction tests. 5ml of blood sample was collected from each subject and 2ml was dispensed into Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant tubes and mixed by inversion. The remaining 3ml was dispensed into clean plain tubes, allowed to clot, retract and serum separated for Widal tests.

Malaria Parasite examination: Thin and thick films were made on clean slides. The films were stained with Giemsa stain using a previously known method[11]. These were examined microscopically for malaria parasites and scored as described by Cheesbrough[12] as follows:

1-10 malaria parasites seen per 100 high power fields of examination = +.

11-100 malaria parasites seen per 100 high power fields of examination = ++.

1-10 malaria parasites seen per high power field of examination = +++.

More than 10 in every high power field of examination = ++++.

Widal Screening test: Rapid slide agglutination tests were done using Commercial Antigen kit (Biotech Reagents Ltd, UK). The Kit consists of *Salmonella typhi* (D) and *Salmonella paratyphi* A, B, and C for both the somatic (O) and flagella (H) antigens. Tube titrations were done on serum samples with doubling dilutions using normal saline ranging from 1 in 20 to 1 in 640. Titre values greater than or equal to in 160 were regarded as positive and less than 1 in 160 as negative for any of the antigens.

Statistical analysis: Results were analyzed using Microsoft excel Computer assisted programme. The test for significance was chi square with p values less than 0.05 regarded as significant.

RESULTS

Of the 797 blood samples examined, 554 (69.5%) had Malaria parasitaemia. One hundred and twenty-five (15.7%) of these were strict malaria cases while 429 (53.8%) had concurrent Widal positive titres. One hundred and eighty-three (23%) were Widal positive-malaria negative and 60 (7.5%) were malaria negative-Widal negative (Table 1). Various rates of cross-reaction of Salmonella antibodies were observed in patients positive for both Malaria and Widal tests. There was little rate of cross-reaction with most of the *S. paratyphi* antibodies ($\leq$10.0%) expect *S. paratyphi* B 'O' with a significant rate of

41.3% (x^2=8.386 p<0.0S).

The cross-reaction rates for *S. typhi* were 52.7% and 58.9% for 0 and H antibodies respectively (Table 2). The Widal test positive patients were found to have more of the *S. typhi* antibodies with both '0' and 'H' being almost equal with 46.4% and 45.4% respectively (Table 3). This was closely followed by *S. paratyphi* B 'O' with 43.7%. Other *S. paratyphi* species had less than 8.0% of patients being positive for Widal test only.

DISCUSSION

Typhoid and Malaria fevers are both endemic in Nigeria and in most cases both present with a common symptom of prolonged fever difficult to differentiate clinically except through laboratory diagnosis.

A significant proportion of 69.5% malaria positivity within the febrile conditions supports the report of Salako et al (1981) that malaria constitutes the major cause of outpatients visit to health-care facilities in Nigeria[3]. The prevalence of strictly malaria cases in this study is marginally but not significantly (p>0.05) lower than the findings of investigators from Enugu, South East Nigeria who reported a rate of 22.0% (12) but significantly (<0.05) lower than the report of other investigators who obtained 27% from Zaria in Northern Nigeria[14]. The rate of concomitant infection with *S. typhi*

'O' (52.7%) was similar to the 52.6% rate obtained by Ohanu et al.[13] but significantly higher than the 10% rate obtained by Mbuh et al.[14] using titres of greater than or equal to 160. Elsewhere in the world varying rates were also obtained; 47.9% from Cameroun[15] and 32.5% from India[16]. These variations can be attributed to the environmental sanitation, tradition and hygienic level of the sources of infection of the different localities of the studies.

Table 1: Summary of overall result

Test Result	No. (%) obtained
Malaria parasitaemia only	125 (15.7)
Malaria and Widal positive	429 (53.8)
Negative for Malaria parasite only	183(23.0)
Malaria negative, Widal negative	60(7.5)
TOTAL	797

Table 2: Occurrence of Salmonella diagnostic antibodies in Malaria positive patients (n=429)

Salmonella antigen	Positive for both MP and Widal [No. (%)].
S. typhi 'O'	226 (52.7)
S. typhi 'H'	253 (58.9)
S. paralyphi A 'O'	10 (2.3)
S. paratyphi A 'H'	34 (7.9)
S. paralyphi B 'O'	177 (41.3)
S. pararyphi B 'H'	43 (10.0)
S. paralyphi C 'O'	24 (5.6)
S. paratyphi C 'H'	32 (7.5)

Table 3: Distribution of Salmonella diagnostic antibodies among the Widal positive/Malaria negative subjects (n = 183).

Salmonella antigen	Positive for Widal test only (%)]
S. typhi 'O'	85 (46.4)
S. typhi 'H'	83 (45.40
S. paratyphi A 'O'	9 (4.9)
S. paratyphi A 'H'	14 (7.7)
S. paratyphi B 'O'	80 (43.7)
S. paratyphi B 'H'	28 (15.3)
S. paratyphi C 'O'	10 (5.5)
S. paratyphi C 'H'	11(6.0)

Typhoid is transmitted through food and drinking water and so it is mainly hygiene and sanitary conditions that determine its spread[5]. The presence of concomitant infections in patients also enabled subclinical parasitaemia which could have been missed but for the malaria fever synergized by typhoid. This indicates that some individuals' habour malaria parasites without fever thus signifying effect of immune tolerance to the parasite in the locality. This observation is supported by the findings of investigators who reported that a large proportion of children in endemic areas have malaria parasitaemia without clinical symptoms[17]. The Malaria-negative, Typhoidal antibody-negative cases found in this study could have been due to other infections that can give rise to fever. These are referred to as pyrexia of unknown origin[18].

With the exception of *Salmonella paratyphi* B 'O' with a high rate of co-infection, the rates of co-infection of the other paratyphoid group were significantly low ($p<0.05$). The paratyphoid Salmonellae are known to cause milder forms of the enteric fever[5] and so their presence in Malaria patients may not have been responsible for the fever. The fact that the rate of co-infection of *S. paratyphi* B 'O' and *S. typhi* (Group D) were quite close could indicate a possible antigenic similarity between *S. paratyphi* B 'O', *S. typhi* (Group D) and malaria antigen. The manifestation of both infections is fever and before fever can be produced, the malaria parasite or typhoid bacteria must release certain chemical molecules to bring about the rise in temperature. These molecules, which are fever causing agents, have been identified to be Tumor necrosis factor - α (TNF-α) and interleukin-1[1]. Just as malaria parasites and typhi/paratyphi bacteria invoke antibody production, TNF-α and Interleukin-1 released during the course of these infections may also be immunogenic producing their antibodies. The high titre of *S. paratyphi* B 'O' along with *S. typhi*, which correlated with Malaria parasitaemia, may be due to antigen similarities with cross-reacting antibodies.

S. Paratyphi B O' has been shown to manifest itself in both infections and thus probably has antigenic similarity with *S. typhi* and malaria parasites and/or the TNF-α and Interleukin-1. *S. paratyphi* B 'O' antigen therefore can be used as an indicator or predicting factor for typhoid and malaria and the similarity linking them thus needs further investigation.

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Complete Venous Blood Counts In Healthy Term Nigerian Neonates.

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ABSTRACT

Serial complete blood counts were performed on venous blood of 104 healthy, normal term Nigerian neonates (55 male, 49 females). The mean weight of the neonates was 3465±398g in the first 10 days of life. There were significant depressions in the value of total leucocyte count (8.37±2.38x10^9/L) on day 7-10 compared to values on day 1-3 (10.57±4.10x10^9/L) ($p<0.0l$). Similarly, the value of neutrophils (3.09±1.59x10^9/L) on days 7-10 was significantly reduced compared to values obtained during days 1-3 (6.77±3.9x109/L) (<0.01). However, elevations of lymphocyte count (4.89±1.80 x109/L), eosinophils counts (0.22±0.20x10 IL) and platelets count (241±50x10/L) on days 7-10 were observed compared to values of lymphocytes count (3.58±1.40 x109/L), eosinophils count (0.12±0.17x109/L) and platelets count (208±48x109/L) on days 1-3 of life ($p<0.01$ in each case). The haematocrit, monocytes count and basophils count; were not significantly different within the study period ($p>0.1$). The present study concludes that Healthy normal term Nigerian neonates presents with varying proportions of total and differential white cell and platelets counts within the first 10 days of life. This may have implications for neonatal haematology in our environment.

Key words: haematological parameters, term neonates, Nigeria

INTRODUCTION

The first published data on neonatal haemogram dated back to 1924 when Lippman first reported the morphologic and quantitative characteristics of blood corpuscles in newborn period [1]. Since then other researchers [2-6] from different parts of the world have determined normal haematological values during neonatal period. Although it has been shown that some haematological parameters (haemoglobin level and total leukocyte and neutrophil counts) are significantly higher in neonates than in infants and adults [7, 8], great variations exist in blood parameters in the newborn infants. These variations depend on gender, race, and socio-economic status of parents and as well as on health status of the neonates, types of sample used (cord, venous, capillary blood), methods used for the analyses (automated or manual) and time of blood sampling [9-11]. Studies have shown that haematological parameters (total white blood cell, red blood cell, and differential white blood cell counts, haematocrit, haemoglobin e.t.c.) are indices for measuring neonatal well being and can as well be used to predict the presence of disease [5,6,12]. For example, perinatal brain damage, intrauterine growth restriction or maternal-foetal ABO blood group incompatibility are associated with an increase in nucleated red blood cells in cord blood [13, 14]. It has also been shown that viral and bacteria infections were associated with alterations in total leucocytes, lymphocyte population and platelets counts [15]. While haemoglobin levels in healthy and sick term babies have been found to be significantly higher than those of healthy and sick pre-terms babies, platelet counts in sick term newborns are significantly lower than those of healthy term newborns [8]. However, platelet counts in both healthy and sick pre-term babies are significantly lower than those of term babies. In addition to variation in absolute values of haematological parameters, newborn also show some morphological changes in blood picture. For instance, the red cells of the newborn have been reported to be markedly macrocytic at birth with mean cell volume and diameter falling after the first week of life and reaching adults' values by the ninth week [16]. Furthermore, blood films from new born have been found to show normochromic cells, polychromasia and 3-5% of the red cells may show fragmentation, target cell formation and distortion of shape with markedly elevated numbers nucleated in the presence of haemolysis or hypoxia [17].

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Studies of neonatal haematology have been very limited in Nigeria and most were done on capillary and/or cord blood [18-201. Although Ogala [2] in his study used venous blood, estimation of blood parameters was done electronically.

Electronic counters are fast and more convenient than manual methods in the estimation of blood parameters but the financial implications of running it has made it inaccessible to most laboratories in Nigeria. Assuredly, independent evaluation of manual and electronic counter methods has deposed that manual methods provide a satisfactory measurement of haematological parameters [21,22]. This study is therefore aimed to provide manually determined baseline data of venous blood parameters in the new born infants in their first ten days of life and to see how the results compare with previously determined values in capillary and cord blood.

MATERIALS AND METHODS

This study was conducted at Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria. Our subjects were neonates delivered to Nigerian parents of various ethnic groups and socio-economic status. Neonates were included if they were singleton, delivered at full- term, either by normal vaginal delivery or by lower segment Caeserean Section (LSCS), normal and healthy without perinatal aphyxia or any known disorder within the period of the study. Exclusion criteria include multiple deliveries, pre-terms and small for gestational age (SGA) babies. The Ethical Committee of Obafemi Awolowo University Teaching Hospital complex, Ile-Ife, approved the protocol for the study. Before enrolment, informed consents were obtained from the parents of the participating neonates. Mothers of neonates were interviewed to obtain sociodemographic data such as age, parity, level of education and occupation. Infants' birth weights and gestational ages at delivery were obtained from case notes of the mothers. In all, one hundred and four neonates were recruited into the study. Venous blood (1-2.5 ml) was taken from the neonates from the dorsal veins between day 1-3 and day 7-10 post delivery. The blood samples were collected in plastic EDTA bottles, gently but thoroughly mixed and the analyses done within two hours of collection.

Haematocrit was determined by micro-method using capillary tube as described by Dacie and Lewis [23].

Total white blood cell count was done as in a standard text [23]. Briefly whole blood was diluted 1:20 in Turcks' solution (2% glacial acetic acid tinted with methyl violet). With a capillary tube, this was applied on charged haemocytometer and counted under light microscope at 40x objective. For differential white blood cell counts, dried thin films made from thoroughly mixed whole blood samples were stained by standard Leishman staining method. Platelets counts were done by Brecher and Cronkite method [21], using 1% ammonium oxalate as diluent and a settling time of 40 minutes. The level of education and/occupation of the parents assessed socio-economic status of infants.

STATISTICAL ANALYSIS

Data were analysed for means and standard deviations and significant test was done by students' t-test. Level of significance was considered as $p<0.05$, and $p<0.01$.

RESULTS

A total of one hundred and four neonates comprising fifty-five (52.9%) males and forty-nine (47.1%) females participated in the study. By ethnic origin, sixty (57.7%) of the neonates were Yoruba, twenty-nine (27.9%) Igbo, eight (7.7%) Hausa, and seven (6.7%) were other tribes. At birth, the mean weight of infants was 3465 $\pm$ 398g (range 3112-3886g) and mean gestational age 39.8$\pm$1.1wks (range 38.2-40.9wks). Fifty-two (50%) of the neonates were from lower socio-economic background; thirty-four (32.7%) and eighteen (17.3%) were from middle and upper socioeconomic class respectively. Of the 104 neonates, thirteen (12.5%) were delivered through lower segment Caesarean section while 91 (87.5%) were through normal vaginal delivery. Records on parity showed that 29 (27.9%) of the mothers were primigravidae, 42 (40.4%) have upto three children while the remaining 33 (31.7%) have more than three children. Also, sixty-eight (65.4%) of the mothers completed normal antenatal visits while 36 (3 4.6%) did not (data not shown). From table 1, total white blood cell count significantly decreased from 10.57 $\pm$ 4.10x10^9/L on day 1-3 to 8.37$\pm$ 2.38x10^9/L on day 7-10 ($p < 0.001$). While haematocrit remained fairly constant within the study period platelets counts increased from 208 $\pm$ 48x10^9/L on day 1-3 to 241 $\pm$ 50x10^9/L by day 7-10 ($p < 0.001$). Table 2 shows that while lymphocytes and eosinophils were significantly raised on day 7-10 (4.89$\pm$ 1.80(58.9%) and 0.22 $\pm$ 0.20(2.7%) from their initial values of 3.58 $\pm$ 1.40(37.4%) and 0.12 $\pm$ 0.17(1.2%) on

day 1-3 ($p< 0.001$), neutrophils were significantly depressed [(6.77±3.9 (60.3%) vs. 3.09 ± 1.59(37.2%), $p<0.001$]. However, monocytes and basophils did not show significant difference on day 1-3 and day 7- 1(($p>0.001$)]. The predominant blood pictures were macrocytosis, normocytosis and spherocytosis (data no shown).

Table 1: Haematocrit, Total white blood cell and Platelets counts in the first 10 days of post-natal life.

Ages/days	Hct ± SD(%)	Twbc ±SD x 10^9/L	Platelets ±SDx10^9/L
1-3 (n=104)	46.0±5.4	10.57± 4.10	208±48
7-10 (n=104)	45± 5.4	8.37± 2.38*	241±50*

Twbc = Total white blood cell; Hct= Haematocrit; *p <0.01.

Table 2: Differential white blood cell counts in the first 10 days of post-natal life.

Ages/day	Neut. (%)	Lymph. (%)	Mono. (%)	Baso. (%)	Eosin. (%)
1-3(n=104)	6.77±±3.9	3.58±±1.40	0.11±±0.12	0.01±0.04	0.12±0.17
Mean±SDx10^9/L	(60.3)	(37.4)	(1.0)	(0.1)	(1.2)
7-10 (n= 104)	3.09±± 1.59	4.89±± 1.80	0.08±0.11	0.02±0.06	0.22±0.20
Mean±± SDx10/L	(37.2)*	(58.9)*	(1.0)	(0.2)	(2.7)*

Neut. = Nuetrophils; Lymph.= Lymphocytes; Mono.= Monocytes; Baso. = Basophils.
Eosin.= Eosinophils; *P < 0.01.

DISCUSSION

This study has shown the mean haematocrit, total leucocytes count and Platelets count of healthy normal full-term Nigerian neonates. The values obtained for these parameters in the present study were lower than those quoted for Caucasians (Hct 47.0 ± 6.0%, total leucocyte count 12.3 ± 4.8x10^9/L, platelet count 269.9± 57.7x10^9/L) [4,5]. However, the values were in agreement with that reported by Alur and his co-researchers [9] in Ohio, U.S.A. and Scott-Emuakpor et. al [24] in African neonates. These results were also higher than those reported by Abdurrahaman and Adekoje [20] in northern Nigeria except for platelets (Hct= 42 %, total leucocytes 9.25 x10^9/L, platelet 173 x10^9/L). It then implies that the haematological values quoted in most of the western textbooks may not apply to Nigerian neonates. For instance the packed cell volume in the present study (45.5 ± 5.4%) in the first 10 days of life is lower than 60-62% quoted elsewhere [16]. Haematocrit is a measure of haemoglobin concentration and has been shown to vary with nutritional status; state of hydration and the type of sample used for its estimation [25]. The lower haematocrit observed in the present study may be partly attributed to intrauterine shift of fluid in infants as non of the neonate showed evidence of dehydration during the period of this study. It has been reported that intrauterine fluid shift may be triggered by the last stage of labour in infants delivered vaginally [26, 27]. However, the presence of suboptimal nutrition cannot be ruled out as it has been shown that in Africa, maternal nutritional status is a predictor of intrauterine growth and well being [28]. Our data showed that half of the neonates were delivered of mothers from lower socio-economic class who by implication may have sub-optimal nutrition especially the micronutrients. Additionally, antenatal non-compliance and multiparity were found in higher proportion of the mothers. Hence, the lower Hct values observed in the present study may also be partly attributed to some mothers not receiving the routine haematinics given during ANC visits or as a consequence of maternal depletion syndrome which was consequently transferred to their foetuses. Cord blood haemoglobin has been found to be lower in the

presence of low maternal haemoglobin and in newborn infants delivered by Cesarean section [29].

However, we could not establish a relationship between neonatal Hct and maternal nutritional status or parity. Nevertheless, the comparable values of Hct for both day 1-3 and day 7-10 showed that Hct was relatively stable during neonatal period and may not be affected by immediate extrauterine factors. It has also been shown that capillary blood samples have higher Hb, Hct, red blood cell (RBC), white blood cell (WBC), and lymphocyte counts than venous blood [25] thus suggesting that the lower Hct recorded in the present study may be related to the sample used (venous blood) and this underline the importance of considering the sample source when using haematological reference ranges. The significantly raised leucocytes in the first 3-day of life in recent study is consistent with previous findings [25]. This may be partly attributed to labour induced stress. In this study, 12.5% of the neonates were delivered by caesarean section while majority were through normal vaginal deliveries. Even though we did not have data on labour events, prolonged labour has been associated with increased leucocyte counts [30]. It has also been shown that mode of delivery may affect neutrophlil function or lymphocyte subpopulation [31]. Significantly high WBC counts in cord blood has been reported after vaginal delivery or vacuum extraction than after elective caesarean delivery [32]. Additionally, subclinical infections cannot be ruled out as the possible cause of leucocytosis in the present study even though our subjects were apparently healthy, as raised WBC has also been associated with neonatal infections [33]. It has been earlier reported [3,34] that neutrophils and lymphocytes account for about 85% of the white blood cell population by day 7, which is in corroboration with the present findings. Although haemoconcentration has been given as a probable cause of the elevated values of neutrophils especially in the first 3 days of life, this has been countered in favour of increased population of this leucocyte subset [35]. Accordingly, increase in lymphocyte subset may be attributed to displacement of neutrophils from marginal layer vessels, which has been established to occur in adults after violent exercise [36]. In the newborn, violent exercise can be likeed to personal labour during childbirth. The rise in lymphocyte count on day 7-10 may also be due to increased immunogenic stimulation in the extrauterine environment; probably in response to BCG vaccines as this period coincide with the time the first dose of the vaccine is given. Eosinophilia in day 7-10 in this study is in accord with earlier report [35] and may be associated with recovery from hyperadrenalism or shock state of birth and subsequent neonatal hypoadrenalism [37]. The reported platelet count in both day 1-3 and day 7- 10 in the present study is lower than that reported by earlier researchers for the same neonatal age but was not to the level of thrombocytopaenia [10,25]. The blood film pictures of the neonates in this study were consistent with reports of previous study [38]. However, reporting of manual blood picture is subjective and objections have been raised against its usefulness as values vary greatly according to the expertise of individuals performing the test. The present findings show that:

- Healthy normal tern Nigerian neonates have lower haematologieal parameters than their Caucasian counterparts in the first 10 days of life with relatively stable Hct.
- Total leucocyte count (dominated by neutrophils) was higher in the first three days of life and decreased subsequently with lymphocytes predominating.
- Eosinophils and platelets increased with neonatal age being higher in day 7-10 than in day 1-3.
- Macrocytic, normocytic and spherocytic red blood cells were common blood picture in healthy normal term Nigerian neonates. In conclusion, the haematological values recorded in this study were lower than those observed for Caucasiati neonates of the same age but the patterns were the same for both races. These findings may be of relevance in interpreting complete blood counts of neonates in this environment.

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Prolactin Secretory Response During Academic Exercises in Young Adult Male Subjects.

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ABSTRACT

Academic stress involving examinations is known to induce alterations in prolactin (PRL) physiology in human subjects. in the present study, we investigated the PRL secretory response to academic exercise in 13 male medical students during regular university examinations. Blood samples were taken at 0 hour, 1 hour and 2½hours of the exercise during morning hours, beginning at 09.00 hours. Samples were stored under -20°C until assayed. Serum PRL concentration was estimated by a solid phase enzyme-linked immunosorbent assay techniques. Mean (± sem) serum PRL concentrations were 7.86 ± 1.55ng/ml; 19.73 ± 5.72ng/ml; and 13.65±2.72 ng/ml at 0 hour, 1 hour and 2½ hours of exercise, respectively. We conclude that academic exercise induced elevation in PRL secretion but in a non-uniform pattern. The PRL secretory response showed two distinct patterns or phases: an exponential phase during the first 1 hour of exercise and a declining phase thereafter.

Key words: Prolactin secretion, academic exercise, male subjects.

INTRODUCTION

Prolactin (PRL) is a polypeptide hormone that is involved in a multitude of physiological processes. Academic work is one of the higher functions of the brain and involves a great deal of the learning and memory processes; thought processes, analysis of sensory information, language and other functions of the mind. There is ample evidence[1] to show that PRL secretion is responsive to academic stress in humans. Interestingly, while PRL secretion is controlled through the hypothalamic pituitary axis; the brain triggers the release of neuroendocrine secretions needed to respond to stress through the hypothalamic pituitary adrenal axis. Regular university examinations impose appreciable degree of mental stress on students. One then, wonders the impact of this examination-induced stress on the endocrine system. Already, physical stress is known to increase pri-receptor expression on human lymphocytes[2], and plasma prolactin level is also known to increase both during stress and after acute aerobic exercise[3-6]. Nguyen, et al[7] reported a rise in plasma prolactin level during mental work involving examination but no alteration in prolactin rhythm during mental work involving only lectures. It is not clear whether the examination-induced prolactin increases were sustained throughout the exercise period before declining at the end of the exercise or not. The work of Johansson, et al[1] suggests that the examination-induced prolactin rise is depressed subsequently by prolonged psychological stress. In the present study, we investigated the prolactin response to academic exercise in young adult male medical students taking the exercise duration time-course of the response as dependent variables.

MATERIALS AND METHODS

Subjects: A total of 13 male subjects were recruited for the study. Their mean age and body mass index (BMI) were 23.2years (range: 21-25 years); and 22.6 (range: 18.7-26kg/m) respectively. All the subjects were adjudged healthy and they gave their informed consent for the study. The local ethical committee of Madonna University College of Medicine and Health Sciences, Okija, Nigeria approved the study.

Sample collection: Blood samples were collected from the cubital vein between 0.900 - 12.00 hours according to standard procedures. Baseline samples were obtained before the commencement of the examination exercise, and subsequently at 1 hour, and $2^1/_2$ hours interval during the examination exercise. Sera were extracted into sample tubes and stored under - 20C until assayed.

Hormone assay: Serum Concentrations of prolactin were measured by a solid phase enzyme-linked immunosorbent assay according to procedures described by Uotila, et al[8] with little modifications. The assays were performed on an automated micro-titter well reader (BioTek Instruments, Inc., Winooski, Vermont USA) using microwell ELISA enzyme immunoassay test kits for hPRL (Diagnostic Automation Inc., Calabasas, California, USA).

Data analysis: We analysed our data on SPSS program (version 11.0) and variables were expressed as mean (± s.e.m).

RESULT

Mean (± sem) basal serum prolactin concentration in the male subjects was 7.86ng/ml ± 1.55. Thereafter, the mean serum prolactin concentrations were: 19.73ng/ml ± 5.72 and 13.65ng/ml ± 2.72 at 1hour and $2^1/_2$ hours of academic exercise respectively. The prolactin secretory response showed a biphasic pattern with maximum amplitude of 19.7ng/ml within the first 1 hour of academic exercise (see fig.1).

DISCUSSION

Our studies indicate that endocrine function is altered to a reasonable degree by academic exercise. Our observed prolactin response to the academic exercise is similar, to a large extent, to previous reports[1,7]. However, we were able to observe that the rise in serum prolactin concentration was never sustained even with the sustained academic exercise. This response appears to agree at least, in part, with the views of Johansson, et al[1] on the effect of prolonged psychological stress on the endocrine system. We observed also that students become more stabilised at the middle of examinations than at the commencement. This decline in the psychological stress will obviously induce a corresponding decline in the level of neuroendocrine secretagoques associated with stress. However, this may not have been enough to return the serum prolactin level to the baseline as our result could indicate. We feel that the impact of the continued memory physiology with the sustained exercise could be an important factor sustaining the rise in serum prolactin above the pre-examination level during the declining phase of the prolactin response. The question is then, at what duration of academic exercise can one confirm the definitive prolactin/endocrine response to mental challenge or academic exercise?

We suggest further investigations in this field, possibly, using a protocol that extends academic exercise far beyond the normal duration of university examinations. Such studies could also elucidate among other possibilities, the impact of chronic academic activities on human endocrine physiology.

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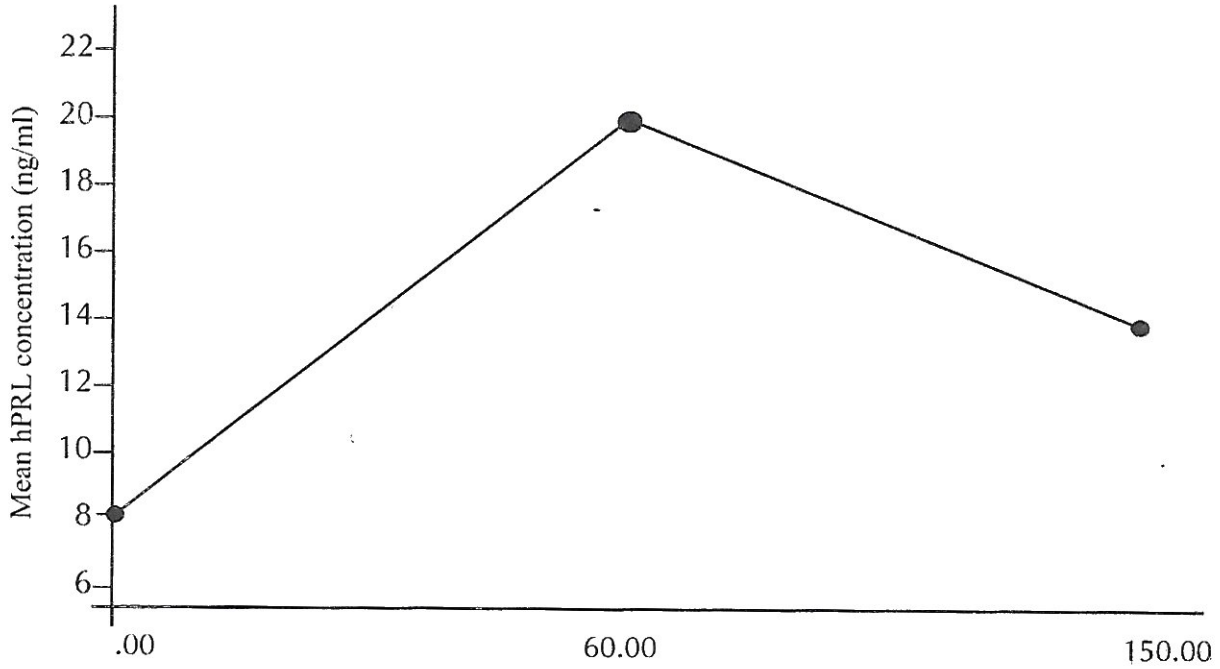

Duration of academic exercise (minutes)

Fig. 1: Bi-phasic response of prolactin secretion during academic exercise in young adult males.

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CD4 COUNTS IN HIV POSITIVE SUBJECTS BEFORE AND DURING ANTI-RETRO VIRAL THERAPY

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ABSTRACT

This study was designed to assess the impact of three drugs used in anti-retroviral therapy (ART), on the $CD4^+$T cell count and weight of the HIV seropositive participants. For this study, 50 Symptomatic HIV seropositive participants on combination anti-retroviral therapy (ART) of Starudine, Lamivudine & Nevirapine were recruited for the study. Similarly, 15 HIV seronegative subjects were recruited and they served as Control Subjects. Blood sample collected from the participants were used to determine $CD4^+$ T cell counts by magnetic field method while the weight of the participants were determined using weighing scale. The result showed that the mean ($\pm$SD) blood $CD4^+$T cell count before ART was 192 ± 109 while 2 months after commencing the therapy was 259 ± 108. However, the mean CD4T cell count in Control subjects was 844 ± 133. This showed that the $CD4^+$T cell count was significantly reduced in the HIV seropositive participants before and after 2 months ART compared with the control value ($P<0.05$ in each case). Amongst the HIV seropositive subjects, 92% had improved $CD4^+$T cell count, 6% had no difference in their $CD4^+$T cell count; while 2% had decreased $CD4^+$T cell count between pre and 2 months post ART. There was a significant reduction in weight of HIV seropositive participants compared with that of Control participants ($P<0.05$). 90% of the HIV infected participants gained weight, 6% lost weight while 4% had no difference in weight within the period under study. The finding in the present study showed the impact of HIV infection on both $CD4^+$T cell count and weight. An appreciation in both $CD4^+$T cell count and weight in this HIV seropositive subjects within 2 months of ART portends possible good prognosis. However, the failure of appreciation in some relevant percentage of studied participants calls for attention.

Key words: HIV; CD4 T cell; Weight; ART; Subjects.

INTRODUCTION

The Human Immunodeficiency Virus (HIV), which causes the disease Acquired Immunodeficiency Syndrome (AIDS) has caused a lot of concern the world over. A survey conducted by the Federal ministry of Health in Nigeria in 2003 showed that the AIDS epidemic was continuing to grow[1] hence the deep concern. The $CD4^+$ subset of T-lymphocytes is the prime target of the HIV on entry into a host. Cells bearing CD4 molecules on their cell membranes act as receptor for attachment of the envelope protein gp 120 (HIV-1)[2]. Although the CD4 subset of T-lymphocytes is the prime target on entry into a host, HIV also infects other cell lineages such as macrophages and monocytes, which express CD4 molecules at lower densities[3].

The infected cells undergo changes in Human Lymphocytes Antigen (HLA) Class II phenotype and are cleared from the circulation, which then leads to depletion of absolute $CD4^+$ cell numbers[4]. The depletion of $CD4^+$ leads to loss of normal function of the cells and thus contributes to the immunopathogenesis of AIDS, development of opportunistic infections which leads to full blown AIDS. Specific therapy to control viral replication has become a cornerstone in the management of HIV-infected patients[5,6]. Anti-retroviral therapy is undergoing continual evolution and current recommendation will change over time[7]. Highly active anti-retroviral Therapy (HAART) may offer the best hope for the control of HIV disease.[8,9,10] In this study, 3 drug combinations (Stavudine, Lamivudine & Nevirapine) were used in the management of the participants. The possible effect these drug combinations on the $CD4^+$ T cell counts and weight of the participants was assessed.

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SUBJECTS, MATERIALS AND METHODS

Subjects: A total of 50 HIV/AIDS subjects (24 males and 26 females) attending antiretroviral clinic at Nnamdi Azikiwe University Teaching Hospital were recruited for the study and followed up for 2 months. The HIV infected participants were in the category of symptomatic stage II. They were aged between 18-54 years. These HIV infected participants were on Lamivudine, Stavudine & Nevirapine. 15 Apparently healthy HIV seronegative subjects (8 males and 7 females) aged between 22-59 years old were also recruited to serve as control subjects. Blood samples collected from all the participants were screened for HIV and the blood CD4 counts of the HIV infected participants were measured before and during anti-retroviral therapy while the CD4⁺T cell count was determined once for the control participants using the magnetic field method. The weights of all the participants were determined at the same time of collection of blood for CD4 T cell' count. Ethical approval was obtained from the ethical committee of the Teaching Hospital. Informed consent was also obtained from the subjects.

METHODS

HIV screening: HIV screening was performed using the HIV kit (ACON Laboratories, San Diego, USA). The procedure is as described by the manufacturer and is an immunochromatographic method. In brief, a drop of serum was placed in the slot for sample and 2 drops of buffer provided was added. For a negative result, only one pink line appears (in the control line) but for a positive result, two pink lines appear, one in the control line and the other in the test line. For all analysis the internal control line must appear to validate the result.

Determination of CD4⁺ T cell count: The CD4⁺T cell count was determined by the magnetic field method (Dyna Bead Ltd. UK). The technique uses magnetic polymer beads coated with anti-CD4 monoclonal anti bodies to capture and isolate CD4 Tlymphocytes from whole blood. Briefly, 225µl of washing solution (PBS) was added to a tube containing 250 µl of blood. Diluted CD14 was then added. Tube was then capped, mixed by tilting and was then incubated for 10 minutes at room temperature. Magnet was placed in the tube for 2 minutes; after which 200µl was transferred into another appropriately labeled tube containing 200µl PBS. Into this solution was added 25µl anti-CD4, mixed and incubated for 10 mins. This was followed by the addition of the magnet and the supernatant solution discarded while 500µl of PBS was then added to the subnatant. Subsequently, 50µl of lysing solution was added, vortexed and left for 5 minutes at room temperature before addition of 50µl of Acridine orange. The solution was fed into the Improved Neubauer counting chamber and the CD4⁺T cells were counted.

Determination of weight: The weight of the subjects were measured bare feet standing upright by using a measuring scale calibrated in kilograms (kg) (HANA scale, China), before and during anti-retroviral therapy.

ARTs combination dosage: The antiretroviral combination Stavudine, Lamivudine and Neviraprine were administered orally to the 50 HIV/AIDS subjects for a period of 2 months. Informing the participants of the need and benefits to adhere strictly to dosage recommendation ensured strict compliance. The Dosage of the drugs were Stavudine tablets 40mg twice daily, Lamivudine tablet 150mg twice daily, Neviraprine tablets 200mg daily.

Statistical Analysis: Variables were expressed in mean and standard deviation while the difference in mean was compared using student t-test. Associations between variables were determined using correlation coefficient. Significant level was considered as $P<0.05$.

RESULT

The mean (±SD) CD4 T cell count before the anti-retroviral therapy was 192 ± 109 while 2 months post anti-retroviral therapy was 259 ± 108. The CD4⁺ count pre and post anti-retroviral therapy showed a significant difference ($P<0.05$) (Table 1).

The mean CD4 count in the control subjects was 844 ± 136 S.D. This was significantly higher than the values obtained in the HIV positive subjects pre and 2 months post anti-retroviral therapy ($P< 0.15$ in each case). This is shown in fig 1.

About 90% of subjects gained weight while 4% lost

weight and 6% had no change in weight for the period of 2 months. In the control subjects, no appreciable weight change was observed.

There were positive correlations between the weight at 2 months antiretroviral therapy and age ($r=0.285$; $p<0.05$); between weight at 2 months and CD4 count pre-antiretroviral therapy ($r=0.289$; $p<0.05$); and between weight at 2months and weight of subjects' pre-anti-retroviral therapy ($r=0.937$; $p<0.01$). The subjects who had increase in $CD4^+$ count were those that gained weight in this study. In this study the highest prevalence was between the age ranges of 32-38 years (36%).

DISCUSSION

According to the World Health Organization, $CD4^+$ count of between 400 and 1100 is considered within normal range. Of the 50 subjects studied only 8% had $CD4^+$ count within this range before commencing ART. This is not unexpected as most HIV/AIDS subjects whose count are still within the normal range are asymptomatic with less risk of infection thus they do not seek medical attention. If a patient is asymptomatic and has $Cd4^+$ count higher than 500cells/mm^3, no anti-retroviral therapy is recommended". In this case the subjects were symptomatic hence treatment was commenced. After 2 months of antiretroviral therapy, 92% of the subjects had increased $Cd4^+$ counts. This agrees with the work by Brechtl et al[10] and Pezzotti et al[12] who concluded from their work that HAART regimens appear to have positive effects on $CD4^+$ count, HIV viral load and body weight.

Table 1: Mean (±Sd) $CD4^+$T cell Count in Symptomatic HIV Positive subjects pre-ART and at 2 months-ART and Control Subjects.

Subjects	Mean $CD4^+$ T cell counts
Symptomatic HIV (pre-ART)	192 ± 109
Symptomatic HIV (2months ART)	259 ± 108
Control subjects	844 ± 108

Thus, ability of the HIV virus to infect $CD4^+$T cells was probably reduced due to HAART intervention. However, the drop in CD4 count in 6% of the symptomatic HIV positive subjects may suggest ART failure. Bretchtl et al noted that treatment failure was not uncommon. There could be a number of reasons for this. Individual reaction to the drugs, however, the biochemical toxicity was not estimated. Also, the $CD4^+$ count of the subject on reporting may affect response as well as ability to rebound. Also, short-term recovery may be difficult for some subject as we note the limitation of this investigation, which was for a short term of 2 months. Another important factor is the compliance to ART, which is considered a crucial determinant of treatment success[13]. According to Ickovics et al[14], non adherence to anti-retroviral therapy remains a formidable barrier in the management of HIV, resulting in the development of resistance and drug failure.

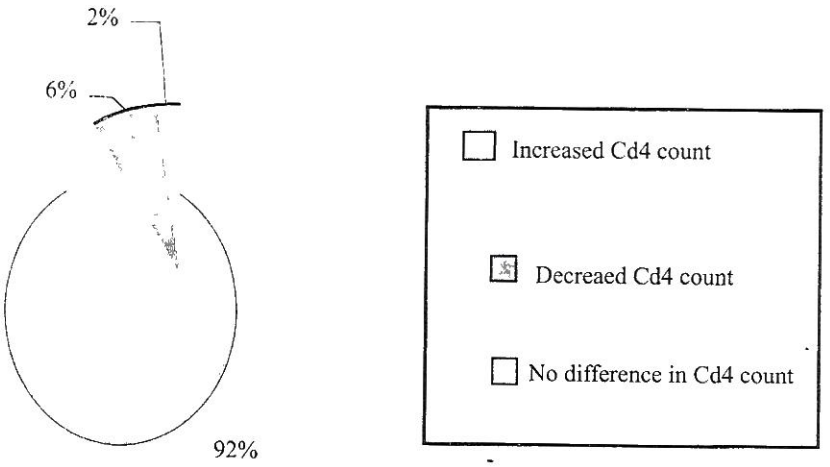
2% of the subjects showed no difference in their CD4 counts.

The subjects in this study also showed marked increase in body weight (90%) while 4% had decreased weight. 6% of the subject had static weights. Predominantly, those that gained weight were those that had increased $CD4^+$ count, post-ART showing improved prognosis. This might explain the positive association observed between the weight of the symptomatic HIV positive subjects by 2 months post-ART and pre-ART $CD4^+$T cell count. This agrees with the work by Hartshom and Cooley[7] and Bretchl[10] who reported improved body weight following ARTs.

CONCLUSION

The HAART gave marked improvement in CD4 count, weight and better prognosis in the 2 months of the study. Weight loss or gain may be used to monitor disease progression or improved prognosis prior to next hospital visit. It is important to note that this is a short term observation.

Fig 1: Subjects who had an increased CD4 count after retroviral therapy, those who had no effect or had counts decreased CD4



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Original article

Evaluation of CD4$^+$T CELL COUNT, Interleukin 6 (IL-6) and interferon gamma in HIV infected symptomatic and asymptomatic individuals.

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ABSTRACT

The study was designed to assess the effect of HIV infection on CD4$^+$T cell count, IL-6 and IFN-γ in HIV infected individuals. Forty-five HIV positive subjects (male 27, female 18), aged between 15-65 years were recruited for the study. 21 of this (male 10, female=11) were HIV symptomatic individuals, while the remaining 24 subjects (male=17, female=7), were HIV asymptomatic individuals; 22 HIV seronegative subjects served as control individuals. Blood samples collected from all the individuals that participated in the study were used for the determination of HIV status by immunochromatographic method, CD4$^+$T cell count by Flow Cytometry, IFN-γ and IL-6 by ELISA method. The result showed diminished CD4$^+$T cells count and serum concentration of IFN-γ in HIV symptomatic individuals ($P<0.05$ in each case). Meanwhile, the serum IL-6 concentration was similar amongst the three groups ($p>0.1$). The drop in both the CD4$^+$T cell count and serum concentration of IFN-γ in the HIV symptomatic individuals suggests possible progression in impairment of certain immunologic responses during HIV infection.

Keywords: cytokines; HIV; CD4

INTRODUCTION

HIV infection is known to alter expression of cellular protein via modulation of cytokine expression, which in turn could alter immune responses to viral infection. It is also known that cytokines play a crucial role in the pathogenesis of HIV infection[1]. Cytokines have complex effects on the replication of HIV, and conversely in infected individuals, also HIV directly affects cytokine production. Different report suggested that the progression of disease in HIV infected individual may be controlled by the balance between the levels of type 1 (Th1) and type2 (Th2) cytokine[2,3]. Some workers had suggested that progressive defects in interleukin 2 (IL-2), IL-12 and IFN-gamma production and the increased production of IL-4 (IL-4-driven helper IgE), IL-6 and IL-10 (Th2) could provide another correlate of disease progression[2,3,4]. Often a shift from a strong Th1/weak Th2 to a weak Th1/strong Th2 profile has been observed[4]. However, there is one report on the lack of evidence of switching of cytokine profile from Thito Th2[5]. But Prasad et al[6] reported increased levels of interleukin 6 (IL-6), interferon gamma and IL-2 RNAs and decreases macrophage inflammatory protein lB (MLP 1B) in infected thymocytes. Cytokine production from T lineage cells is also altered by HIV infection[6].

Anti CD3/anti-CD28-activated T - lymphocyte from HIV-1- infected individual showed a decrease in interferon gamma and IL-13 secretions compared with cells from uninfected individuals. Expression of these cytokines was increased when patients presents with bacterial or viral infections[7]. This study was designed to evaluate the CD4$^+$T cell count; serum concentrations of IL-6 and IFN-γ in HIV asymptomatic and symptomatic individuals.

STUDY APPROACH AND METHODOLOGY

Subjects: A total of forty-five HIV seropositive individuals were recruited for the study at the HIV Clinic, Nnamdi Azikiwe University Teaching Hospital Nnewi. 21 of these individuals were grouped according to WHO criteria for staging HIV into: HIV symptomatic (stage 2) group (n=21) and HIV asymptomatie (stage 1)

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group (n=24). Similarly, 22 apparently healthy HIV seronegative individuals served as control group for the study. The individuals that participated in the study gave informed consent and the NAUTH ethical committee approved the study design.

Sample collection: 4mls of Blood sample collected from each participant was dispensed respectively into EDTA tubes for CD4$^+$T cell count and into plain tubes for HIV screening, and determinations of serum IL-6 and IFN-γ concentrations.

METHODS

HIV Screening: Two different methods were used; Abbott determine^{Tm} 1&2 which is an in-vitro visually read immunoas say and immunochromato graphic test for the qualitative detection of antibodies to HIV- 1 and HIV-2 in human plasma. For the Abbott determine^{Tm} HIV- 1 & 2 the procedure as described by the manufacturer was used for the analysis. Briefly, 50iil of participants' plasma samples separated from corresponding whole blood samples in EDTA were applied to appropriately labeled sample pad. After 15 minutes of sample application, the result was read. This method has inherent quality control that validates the results. Two visible red colours in the region labeled control and patient represents HIV sero-positive reaction while a single red colour in the region labeled control represents HIV sero-negative reaction. For the immunochromato graphic method for HIV 1 & 2 it utilizes immobilized antigen for the detection of antibodies to HIV - 1 & 2 in the plasma. It is used as a point of care test and suitable for use in multi-test algorithms. The procedure as described by the manufacturer was used for the analysis. In brief, a 5ml plasma sample loop provided was used to collect the participants' plasma by touching it on the specimen and allowing the opening of the loop to fill with the liquid. The samples were dispensed into the sample wells in appropriately labeled sample pad. Three drops of the buffer supplied by the manufacturer was added drop-wise into the appropriately labeled sample wells. The results of the tests were read at 10mins after the addition of the running buffer. This method has inherent quality control that validates the results. The presence of two pink/purple lines in the region of test sample and control indicates HIV seropositive reaction while a single pink/purple line at the control region indicates HIV seronegative reaction. HIV sero-positive results' using these two methods was used to classify participants as presenting with HIV infection.

CD4$^+$T cell Count by Flow Cytometry Machine: 20ml of blood sample in EDTA tube was collected into Partec test tube. Then 20ml of CD4$^+$T antibody was added into the tube .The contents were mixed and incubated in the dark for 15 minutes at room temperature. 800ml of CD4 buffer was gently added into the mixture and mixed gently. Then the Partec tube was plugged on the Cyflow counter and the CD4$^+$T cells were displayed as peaks and the count determined.

Interferon gamma assay: The procedure was as described by the manufacturers of the interferon gamma assay kit[8]. 100µl of serum sample was added to wells of microtiter plate and 50µl of biotinylated detection antibody was also added to the wells. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated for 2 hours at room temperature and 100µl of streptavidin conjugate added to various wells, and were further Incubated for 30 minutes at room temperature. 100ml of ready to use TMB was added and the microtiter plate, incubated in the dark for 15 minutes for the colour to develop. 100µl H_2SO_4 was added in the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank.

Calculation:

Absorbance of test/absorbance of standard x concentration of standard = concentration of test.

HUMAN IL-6 BY ELISA

RESULTS

The mean IFN-γ of 40.8±8.3 in control group and 45.5±12.6 in HIV asymptomatic group, showed no significant difference ($p>0.01$). Similarly, the mean IL-6 of 15.1 ± 3.2 in control group and 15.4 ± 3.4 in HIV asymptomatic group were not significantly different ($p>0.1$). See table 1.

The mean IFN-γ of 25.5 ± 11.1 in HIV symptomatic group was significantly reduced compared to 48.8±8.3 observed in the control group ($p<0.05$). However the mean IL-6 value in HIV symptomatic and control group were not significantly different ($p>0.05$). See table 2.

The mean IFN-γ was significantly reduced in HIV symptomatic group compared with the HIV asymptomatic group 45.5±12.6 ($p<0.01$). However, there was no significant difference in IL-6 between the HIV symptomatic and HIV asymptomatic groups ($p>0.1$). Similarly the mean $CD4^+$ T cell counts was significantly reduced in HIV symptomatic group compared with HIV asymptomatic groups ($p<0.05$).

Table 1: Comparison of mean (±SD) Interleukin - 6, and Interferon--γ in HIV asymptomatic and control groups.

Parameters	Control group (n=22)	HIV asymptomatic group (n=24)	P -value
Interferon-γ	40.8 ±8.3	45.5 ±12.6	>0.1
Interleukin - 6	15.1 ±3.2	15.4 ± 3.4	>0.1

Table 2: comparisons of mean (±SD) Interleukin -6, and Interferon-γ in HIV symptomatic and control groups.

Parameters	Control (n=22)	Symptomatic HIV positive (n21)	P- value
Interferon-γ	40.8± 8.3	25.5±11.1	<0.01
Interleukin-6	15.1± 3.2	14.2± 5.6	>0.1

Table 3: comparison of mean (±SD) Interleukin-6, Interferon-γ and $CD4^+$T cell count in HIV asymptomatic and HIV symptomatic groups.

Parameters	HIV asymptomatic (n=24)	HIV Symptomatic (n=21)	P- value
Interferon-γ	45.5± 12.6	25.5± 11.1	<0.01
Interleukin -6	15.4 ±3.4	14.2±5.6	>0.1
$CD4^+$T cell count	397±164	162± 99	<0.01

DISCUSSION

The present study demonstrated decreased Interferon-g production in HIV symptomatic individuals. Thi cells usually secrete the interferon-γ when they are stimulated by specific antigen complex presented by macrophages. The possible decline in production of IFN- γ could be due to impaired antigen presentation or due to reduction in CD4 T cell count and function usually seen in progressive HIV infection.

Decrease in IFN-γ in symptomatic HIV subjects have been reported in several studies of HIV infected subjects[2,3,9,10]. The implication of these reports is defective cellular immunity. This may result in effective viral replication. Similarly, impaired nitroblue ingestion by neutrophils has been reported in HIV infected subjects suggesting possible defective antigen presentation. Hence defective secretion of IFN-γ seems to commence as early as stage 2 HIV infection but not observable in asymptomatic HIV individuals.

However, the serum concentration of IL-6 in both the HIV symptomatic and HIV asymptomatic individuals were similar to that observed in the apparently health individuals. This possibly an indication that HIV infection may not affect production of IL-6. In this study IL-6 was used as index for TH-2 cells secretions and possible functions. The finding of serum levels of IL-6 in both the HIV symptomatic and HIV asymptomatic individuals may suggest that at this stage of HIV progression antibody production by infected host might have not been compromised. IL-6 which is usually secreted by Th2 cells is known to be involved in B cells stimulation for antibody production against the HIV specific antigens and other opportunistic infections.

However, studies elsewhere did show increase in IL-6 concentration in HIV infected subjects. Thus confirming that antibody secreting ability of HIV infected subjects at this stage is still effective[3,10]. However, in another study, no significant change was observed in serum concentration of IL-6 in HIV infected

individuals as disease progresses The discrepancy in the findings for IL-6 may be an indication of progressive changes in immunological dynamics depending on possible pattern of secondary infections in the respective studied groups.

In the present study, mean $CD4^+$ T cell count was reduced in HIV symptomatic individuals and was just about half of the value recorded for the HIV asymptomatic individuals. The magnitude of drop in $CD4^+$ T cell count in HIV symptomatic individuals may be an indication of degree of collapse of the immune response particularly the TH-1 type as the disease progression is recorded. This massive drop in $CD4^+$ T cell count may also possibly reflect drop in CD 4 $^+$T cell functions. This may possibly explain the drop in IFN-γ as observed in the HIV symptomatic individuals.

Since cell mediated immunity has been shown to play an important role in controlling viral infection [12] such collapse may encourage viral replication in infected host. Using IFN-γ and IL-6 as markers of TH-1 and TH-2 immune responses in HIV infected individuals, this study suggest possible down-regulation of TH-1 pathway of immune mechanism which are necessary for effective immunity against HIV infection. However, it is important to the limitations of using single markers as index where there are multiple networks of cytokines that controls TH-1 or TH-2 immune mechanisms.

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JOURNAL OF BIOMEDICAL INVESTIGATION, DECEMBER, 2007, Vol. 5, No. 2

TABLE OF CONTENTS

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