Sjournals
Scientific Journal of Medical Science (2013) 2(9) 169-181
ISSN 2322-5025
doi: 10.14196/sjms.v2.i9.940

Contents lists available at Sjournals

Journal homepage: www.Sjournals.com

Original article

Evaluation of nutritional status of HIV infected females during menstrual cycle in Nnewi, Anambra state, Nigeria

N.R. Ukibe[a,*], C.C. Onyenekwe[b], J.E Ahaneku[c], S.N. Ukibe[d], S.C. Meludu[a], O.F. Emelumadu[e], C.O. Ifeadike[e], A.L. Ilika[e], M.O. Ifeanyichukwu[f], A.O. Igwegbe[g]

[a]*Department of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.*
[b]*Deparment of Medical Laboratory Science, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.*
[c]*Department of Chemical pathology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.*
[d]*Department of Prosthesis and Orthopedic Technology, School of Health Technology, Federal University of Technology Owerri*
[e]*Department of community medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.*
[f]*Department of immunology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.*
[g]*Department of Obstetrics and Gynecology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.*

*Corresponding author; Department of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.

ARTICLE INFO

Article history:
Received 31 August 2013
Accepted 08 September 2013
Available online 29 September 2013

Keywords:
Nutrition status
Hiv
Menstrual cycle
Premenopausal females

ABSTRACT

Background and Objectives: The present study was designed to evaluate the nutritional status of HIV infected females during menstrual cycle in Nnewi, Anambra State Nigeria. Materials and Methods: A total of 87 premenopausal females with regular menstrual cycle (27-31 days) and aged 15 - 45 years were randomly recruited for the study. 27 apparently healthy female participants were recruited and served as Control group while the remaining 60 participants were HIV infected females and were grouped according to WHO criteria for HIV staging into (i) Asymptomatic HIV infected females (n=20), (ii) Symptomatic HIV infected females (n=20), (iii)

Symptomatic HIV infected females on ART (n=20). Blood samples were collected at follicular and luteal phases of menstrual cycle for determination of Zinc, Copper, Total protein, Albumin, iron and Haemoglobin. Results: The result showed that the levels of Zn, Cu, Total protein, Albumin, Haemoglobin were significantly lower in Asymptomatic HIV infected females, Symptomatic HIV infected females and Symptomatic HIV infected females on ART compared to Control at follicular and luteal phases of menstrual cycle (P<0.05). Zn, Cu, Total protein, Albumin, Fe and Hb were significantly higher in Symptomatic HIV infected females on ART compared to Asymptomatic and Symptomatic HIV infected females at follicular and luteal phases of menstrual cycle (P<0.05). Conclusion: The result showed significant nutritional deficiency in HIV infected females at follicular and luteal phases of menstrual cycle which was improved in the participants who were placed on ART.

© 2013 Sjournals. All rights reserved.

1. Introduction

The HIV/AIDS pandemic has become a major public health problem worldwide especially in sub-Saharan Africa where more than 80 percent of all people living with HIV/AIDS reside (UNAIDS 2012). Reports have shown considerable evidence that the rate at which HIV infection progresses in women is different from that in men (Farzadegan et al, 1998, USAID, 2012). In Nigeria, about 3.1 million people are living with HIV/AIDS while 58% (1.72 million) are females most of who are within reproductive age range (15-45 years) (UNAIDS 2011; NACA 2012). HIV is a leading cause of morbidity and mortality among women of reproductive age in Nigeria (UNAIDS, 2011; NACA 2012).

The place of nutrition in reproductive function cannot be overemphasized. Apart from the regular food components such as proteins, carbohydrates, fats and vitamins, which are taken daily in human diet, the human nutrition also involves the consumption of inorganic substances called minerals or trace elements. These substances are naturally found in rocks and soil where plants and vegetables absorb them as they grow. Some minerals are needed by the body daily in large quantities and are present in virtually all cells of the body. They maintain the body's normal physiological processes including menstruation. Micronutrients such as Zn, Cu and Fe are members of the nutritionally important elements commonly referred to as trace elements. In addition to their normal physiological roles, these elements also perform reproductive (Bedwal and Bahuguma, 1994) and immunological (Heresi et al., 1985; Dunlap et al., 1994; Percival et al., 1998; Prasad et al., 2007) functions. Zn and Cu are commonly obtained from human diet such as meat, vegetables and water (Jaryum et al., 2010). HIV disease has been associated with nutritional deficiencies. Akinola et al (2012) reported that trace elements such as Zn, Cu and Se were significantly reduced in HIV patients who were not on ART but became significantly improved especially Zn on the introduction of highly active anti-retroviral therapy (HAART).

The role of some selected nutrients in the ovarian cycle cannot be overemphasized. For instance, copper is closely associated with estrogen receptors and function and deficiency states may manifest with menstrual irregularity (Bedwal and Bahuguma, 1994). The plasma concentration of zinc and copper shows significant variations in different disease conditions (Constance et al., 1995; Arinola et al., 2008; Akhuemokan et al., 2010; Akinola et al., 2012). Also the activity of the metalloenzymes of which zinc and copper are essential components will be reduced thereby depressing cellular activity and functions. Zinc and selenium deficiencies have been associated with hypothyroidism (Kralick et al., 1996; Gartner et al., 2009), which is prevalent in HIV disease and may aggravate menstrual disorders in HIV.

Nutritional deficiencies in HIV infected subjects have been attributed to malabsorption, repeated concurrent infections and increased losses (Silberry et al., 2001) probably due to increased frequency of diarrhea and

vomiting. It could also lead to malnutrition, immune abnormalities, hypogonadism and hypospermia (Mc Clain et al., 1985; Keen et al., 1990; King and Keen, 1994; Ripa and Ripa 1995).

The present study was therefore designed to evaluate the nutritional status of HIV infected females within reproductive age group during menstrual cycle.

2. Materials and methods

A total of eighty seven (87) females within reproductive age group (15-45 years) with regular menstrual (between 27-31 days) cycle were randomly recruited for the study. The participants consisted of 27 apparently healthy females recruited among the hospital staff, which served as Control females while the remaining Sixty (60) female participants were recruited at Heart to Heart centre and HIV clinic of Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria. They were grouped according to WHO criteria for staging HIV infections as: (i) Asymptomatic HIV infected females (n=20), (ii) Symptomatic HIV infected females (n=20), (iii) Symptomatic HIV infected females on ART (n=20). A structured questionnaire was administered to each of these participants to ascertain their menstrual cycle and reproductive history and other biodata.

2.1. Blood sample collection

Six mls of Blood sample was collected from each participant at follicular (7-13th day) and at luteal (21-23rd day) phases of menstrual cycle. The blood samples were collected between 8 to 10am by venepuncture. Four mls was dispensed into dry plain bottles and allowed to clot, retracted and centrifuged. The serum was separated from the clot immediately and transferred into the well labeled container and stored frozen at -200C until assayed for Copper, total protein, iron and albumin. The remaining two mls of blood was dispensed into EDTA bottles and was used immediately for malaria parasite screening, HIV screening and confirmation while the plasma content was centrifuged, separated immediately and transferred into the well labeled containers and also stored frozen at -200C for determination of Zinc.

2.2. Ethical clearance and informed consent

The ethics committee of Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra state, Nigeria approved the study design. The participants were informed about the study design and only those who gave their consent were recruited for the study.

2.3. Exclusion and inclusion criteria

Participants with HIV Stage –3 and 4 were excluded from the study only those adjudged as HIV Stage-1 (Asymptomatic HIV and Symptomatic -HIV (stage-2) were included for the study. Participants with malaria parasite infection as at the time of study were also excluded. Participant with tuberculosis were excluded and subjects with known fertility problems before contracting HIV infections were also excluded. Hence the female participants used were those with no prior fertility problems until the existence of HIV infection

2.4. Detection of antibodies to HIV-1 and HIV-2 in human plasma using immunochromatographic method as described by (piot et al., 1988)

2.4.1. Principle

Determine HIV-1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV1 and HIV-2. Antibodies to HIV-1 and/or HIV-2 present in the sample, bind to the antigen-selenium colloid conjugate and to the immobilized recombinant antigen at the patient window to form a red line at the patient window site.

2.4.2. Procedure

The procedure as described by the manufacturer was used for the analysis. Briefly, 50μl 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 test represents HIV seropositive reaction while a single red colour in the region of control represents HIV seronegative reaction.

2.5. HIV screening by immunoassay method using trinity biotech unigold assay kit (trinity biotech plc, ireland)

2.5.1. Principle

The Recombinant proteins representing the immunochromatographic regions of all the envelope proteins of HIV-1 and HIV-2 glycoprotein gp41, gp120 (HIV- 1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of nitrocellulose membrane is also sensitized as a control region. Antibodies of any immunoglobulin class specific to the recombinant HIV-1 or HIV –2 proteins reacts with the colloidal gold linked antigens and moved chromatographically along the membrane to the test and control regions of the test device. A pink/red band in the test region of the device visualized a positive reaction. A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV.

2.5.2. Procedure

The reagent was first removed from the refrigerator and allowed to stand for at least 20 minutes to reach room temperature. Appropriate patients information was labeled on the Unigold test device. Using the disposable pipettes two drops of serum sample was dispensed on the sample port carefully. Two drops of wash reagent was added to the sample port and allowed to react for 10 minutes. The result was read immediately within 10 minutes after the incubation time. A pink/red band in the test region of the device visualized a positive reaction. A line in the control region only indicates a negative test result.

2.6. HIV screening by immunochromatographic method using (hiv 1 and 2 stat-pak assay kit (chembio diagnostic system, inc new york, usa)

2.6.1. Principle

The chembio HIV1/2 STAT-PAK assay employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particle, and HIV1/2 antigens, which is bound to the membrane solid phase. The antibodies present in the sample, bind to the gold conjugated antibody binding protein and the dye conjugated-immune complex which migrates on the nitrocellulose membrane and captured by the antigens immobilized in the TEST (T) area producing a pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink /purple line in the CONTROL (C) area containing immunoglobulin G antigens.

2.6.2. Procedure

The procedure as described by the manufacturer was used for the analysis. In brief, 5 μl plasma sample was dispensed into the sample wells in appropriately labeled sample pad. Three drops of the running buffer (as supplied by the manufacturer) was added drop-wise into the appropriately labeled sample wells. The results of the tests were read at 10 minutes after the addition of the running buffer. This method had 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 seropositive results’ using these two methods was used to classify participants as presenting with HIV infection.

2.7. Determination of full blood count (FBC) by sysmex K21N hematology analyzer

2.7.1. Principle

Sulfoanalyser is added to hemolyse the red blood cells and HB is converted into SLS-Hb, the concentration of SLS-Hb is measured as light absorbance and is calculated by comparison with absorbance of diluents measured before the sample added. SLS (Sodium Lauryl Sulphate) sulpholyser reagent is a cyanide free reagent. It lyses the RBC and acts upon the globin of HB to form a stable hemichrome.

Procedure:

Blood sample collected in EDTA anticoagulant. (50 μl) was diluted with cellpack in the WBC counting chamber. A fixed volume of stromatolyser WH solution (1 volume of stromatolyser – WH to 2 volume of cellpack) is added to them automatically to obtain a final dilution of 1:500. The addition of stromatolyser- WH lyses the RBC

and so the remaining cell stroma is at a level detectable by the instrument. At the same time, the WBC membrane is preserved and WBCs are stabilized at a level detectable by the instrument. They are then counted by direct current method. Hemoglobin is released during RBC lyses, and is converted to red methemoglobin and read photometrically at 555nm. A portion of this diluted sample is transferred automatically to the hemoglobin detector where the absorbance of the red pigment is measured to give blood hemoglobin level. When Sysmex KX21N is ready, the sample collected was mixed very well, the tube was held up to the sample probe and the KX 21N beeped twice. The sample was removed from the sample probe and then the machine automatically analyzed the specimen and printed the result on the thermal printer after 60 seconds. The WBC count, Absolute Lymphocyte count, Absolute Neutrophil count were recorded.

2.8. Determination of zinc and copper in serum by atomic absorption spectroscopy (AAS)

2.8.1. Principle

In the flame AAS, the principle is based on the dissociation of the element from its chemical bond. This is then placed in an unexcited or ground state (neutral atom). Thus, the neutral atom is at a low energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum. The amount of radiant energy absorbed at a characteristic wavelength in the flame is proportional to the concentration of the element present in the sample. Quantification is achieved by preparing standards of the element.

2.8.2. Procedure for Zinc (Zn)

The serum was diluted 1:4 with water and aspirated to AAS. Standards and blanks were prepared by diluting with 5% glycerin (series of standards 1, 3 and 6 were recommended, however, 1 and 3 ppm were enough which have comparable concentration with sample). The instrument was set at zero with 5% glycerol in deionized water then the samples and standard were serially aspirated and analyzed at 213.8nm in AAS, unexcited zinc atom absorbs light of the same wavelength as that emitted by zinc in the excited state. The amount of light absorbed is proportional to the concentration of zinc present in solution. The results displayed digitally part per million (ppm) and converted to µg/dl by multiplying each result by 100.

2.8.3. Copper (Cu)

The serum was diluted 1:1 with water, aspirated and read in AAS. Standards and blanks were prepared with 10% glycerin (recommended standards are 5 ppm and 15 ppm Cu; however, the lowest standard alone could be used). Selenium (Se): This element was read from samples prepared for Zn or Cu analysis. Standards and blanks were prepared accordingly.

2.9. Serum total protein estimation was done using biuret method as described by (tietz, 1976)

The peptide bonds of protein react with the copper II ions in alkaline solution to form blue

Violet complex (Biuret reaction). Each copper ion complexing with 5 or 6 peptide bonds. Tartarate is added as a stabilizer whilst Iodide is used to prevent auto reduction of the alkaline copper complex. The color formed is proportional to the protein concentration and is measured at 546 nm (520-560nm).

2.10. Serum total albumin estimation was done using bcg dye method as described by (kimberly, 2011)

Albumin binds with bromocresol green (BCG) at PH 4.2 causing a shift in absorbance of the yellow BCG dye. The blue green color formed is proportional to the concentration of albumin present, when measured photometrically between 580-630nm with maximum absorbance at 625nm.

2.11. Serum iron estimation was done by colorimetric method (chromatest barcelona spain)

The principle of the test is based on the dissociation of iron from the transferring complex by the addition of acidic buffer containing hydroxylamine.The procedure was as described by the manufacturer of the kit. In brief, 200 µl of sample from each participant was put in appropriately labeled test tube containing 1.0 ml of working solution (containing one part of chromogen solution (ferrozine 40 mmol/l and sodium acetate 400 mmol/l) and four parts of buffer/reductant solution (Guanidine chloride 1.0 mol/l; hydroxylamine 0.6 mol/l; acetate buffer 400 mmol/l, pH 4.0; teepol mixture). The reagent blank and standard test tubes were prepared similarly; however, the sample blank was prepared with addition of 1.0 ml of the buffer/reductant solution. The reactions were incubated

at room temperature for 5 minutes after which the absorbance of the sample blank was read at 560 nm against distilled water while the absorbance of the sample tests and standards were read at 560 nm against reagent blank. Subsequently the concentration of serum iron was calculated from the formula: Serum iron (µg/dl): [A2T-A1T/A2S-A1S] x conc. of STD.

2.12. Statistical analysis

The version 16 of SPSS package was used in statistical analysis. The variables were expressed as mean (±SD). The student t-test and analysis of variance (ANOVA) and post-hoc (LSD) were used to assess significant mean differences. Graph Pad Prism version 5.03 was used for graph presentations. The level of significance was considered at P<0.05.

3. Results

3.1. Zinc and copper in tests groups and control group at follicular and luteal phases of menstrual cycle

The mean (±SD) plasma zinc level (ppm) in Asymptomatic HIV females was not significantly different between follicular (0.769±0.096) and luteal (0.787±0.062) phases of menstrual cycle (P>0.05). Similarly, the mean (±SD) plasma zinc level (ppm) in Symptomatic HIV females was not significantly different between follicular (0.704±0.021) and luteal (0.728±0.040) phases of menstrual cycle (P>0.05). In Symptomatic HIV females on ART, no significant difference was also observed in the mean zinc level (ppm) between follicular (0.867±0.048) and luteal (0.845±0.072) phases of menstrual cycle (P>0.05). The mean (±SD) zinc level (ppm) in Control females was significantly higher at follicular (1.018±0.092) compared to luteal (1.014±0.090) phases of menstrual cycle (P<0.05).

However, the mean plasma zinc level (ppm) in Asymptomatic HIV females (0.769±0.096, 0.787±0.062), Symptomatic HIV females (0.704±0.021, 0.728±0.040) and Symptomatic HIV females on ART (0.867±0.048, 0.845±0.072) were significantly lower compared to Control females (1.018±0.092, 1.014±0.090) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively). Furthermore, the mean (±SD) zinc level (ppm) in Symptomatic HIV females on ART (0.867±0.048, 0.845±0.072) was significantly higher compared to Asymptomatic HIV females (0.769±0.096, 0.787±0.062) and Symptomatic HIV females (0.704±0.021, 0.728±0.040) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively).

The mean (±SD) serum copper concentration (ppm) in Asymptomatic HIV females was not significantly different between follicular (0.892±0.129) and luteal (0.929±0.128) phases of menstrual cycle (P>0.05). The mean (±SD) serum copper concentration (ppm) in Symptomatic HIV females was not significantly different between follicular (0.856±0.086) and luteal (0.870±0.121) phases of menstrual cycle (P>0.05). No significant difference was observed in the mean serum copper level (ppm) in Symptomatic HIV females on ART between follicular (0.951±0.134) and luteal (0.980±0.117) phases of menstrual cycle (P>0.05). There was no significant difference in the mean serum copper level (ppm) in Control females between follicular (1.138±0.187) and luteal (1.145±0.215) phases of menstrual cycle (P>0.05 respectively).

The mean (±SD) serum copper concentration (ppm) in Asymptomatic HIV females (0.892±0.129, 0.929±0.128), Symptomatic HIV females (0.856±0.086, 0.870±0.121) and Symptomatic HIV females on ART (0.951±0.134, 0.980±0.117) were significantly lower compared to Control females at (1.138±0.187, 1.145±0.215) phases of menstrual cycle (P>0.05). Similarly, The mean (±SD) serum copper concentration (ppm) in Symptomatic HIV females on ART (0.951±0.134, 0.980±0.117) was significantly higher compared to Asymptomatic HIV females (0.892±0.129, 0.929±0.128) and Symptomatic HIV females (0.856±0.086, 0.870±0.121) at follicular and luteal phases of menstrual cycle (P<0.05) (See Fig 1).

3.2. Total protein and albumin in tests groups and control group at follicular and luteal phases of menstrual cycle

The mean (±SD) serum total protein concentration (g/l) in Asymptomatic HIV females was not significantly different between follicular (83.02±8.09) and luteal (84.26±5.76) phases of menstrual cycle (P>0.05). Similarly, the mean (±SD) serum total protein (g/l) in Symptomatic HIV females was not significantly different between follicular (86.71±8.23) and luteal (89.26±6.69) phases of menstrual cycle (P>0.05). In Symptomatic HIV females on ART, no significant difference was also observed in the mean total protein level (g/l) between follicular (81.12±6.32) and luteal (83.80±6.41) phases of menstrual cycle (P>0.05). The mean (±SD) serum total protein level (g/l) in Control

females was significantly lower at follicular (70.33±4.48) compared to luteal (76.41±5.62) phases of menstrual cycle (P<0.05).

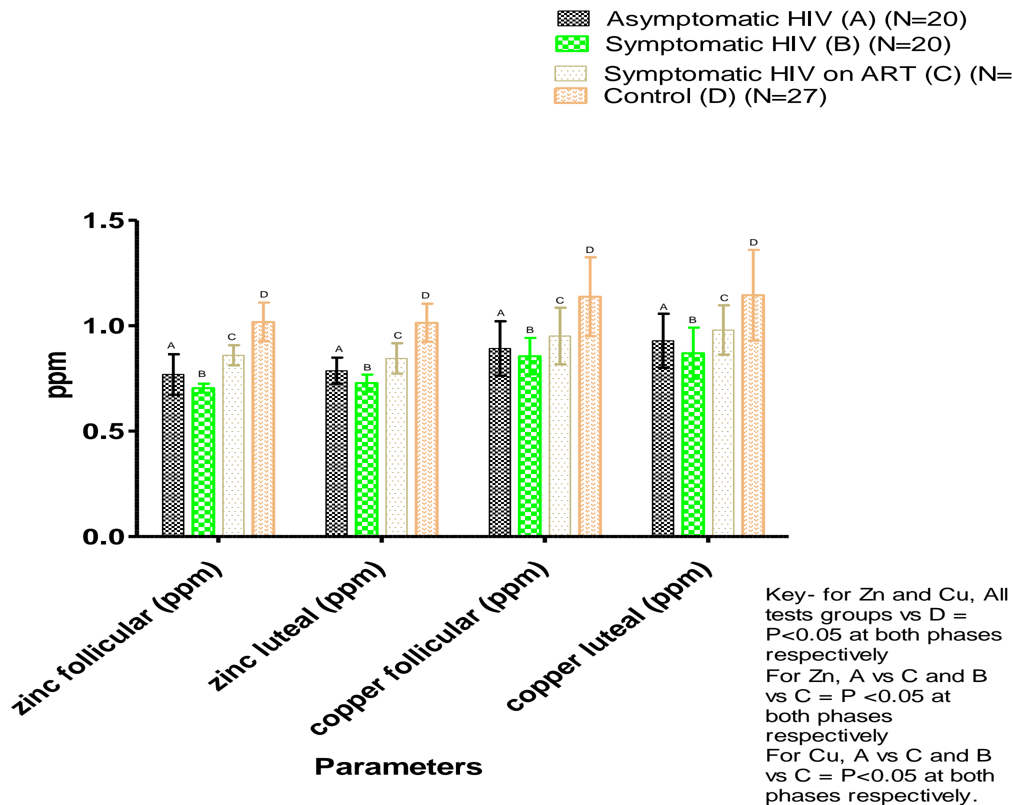

Fig 4.1: Comparison of (+SD) levels of Zn znd Cu in Tests groups and Control at follicular and lutealphases of menstrual cycle

However, the mean serum total protein level (g/l) in Asymptomatic HIV females (83.02±8.09, 84.26±5.76), Symptomatic HIV females (86.71±8.23, 89.26±6.69) and Symptomatic HIV females on ART (81.12±6.32, 83.80±6.41) were significantly higher compared to Control females (70.33±4.48, 76.41±5.62) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively). Similarly, the mean (±SD) serum total protein level (g/l) in Symptomatic HIV females on ART (81.12±6.32, 83.80±6.41) was significantly lower compared to Symptomatic HIV females (86.71±8.23, 89.26±6.69) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively).

Furthermore, the mean (±SD) serum albumin concentration (g/l) in Asymptomatic HIV females was not significantly different between follicular (35.68±2.18) and luteal (36.41±4.48) phases of menstrual cycle (P>0.05). Similarly, the mean (±SD) serum albumin (g/l) in Symptomatic HIV females was not significantly different between follicular (31.38±2.06) and luteal (32.49±3.02) phases of menstrual cycle (P>0.05). In Symptomatic HIV females on ART, no significant difference was also observed in the mean serum albumin level (g/l) between follicular (37.68±2.91) and luteal (36.52±3.39) phases of menstrual cycle (P>0.05). The mean (±SD) serum albumin level (g/l) in Control females was significantly higher at follicular (58.87±6.18) compared to luteal (53.53±6.13) phases of menstrual cycle (P<0.05).

However, the mean serum albumin level (g/l) in Asymptomatic HIV females (35.68±2.18, 36.41±4.48), Symptomatic HIV females (31.38±2.06, 32.49±3.02) and Symptomatic HIV females on ART (37.68±2.91, 36.52±3.39) were significantly lower compared to Control females(58.87±6.18, 53.53±6.13) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively). Furthermore, the mean (±SD) serum total protein level (g/l) in Symptomatic HIV females on ART(37.68±2.91, 36.52±3.39) was significantly higher compared to Symptomatic

HIV females (31.38±2.06, 32.49±3.02) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively) (See Fig 2).

3.3. Haemoglobin and serum iron concentrations in tests groups and control group at follicular and luteal phases of menstrual cycle

The mean (±SD) serum iron concentration (µg/dl) in Asymptomatic HIV females was not significantly different between follicular (55.12±5.29) and luteal (56.18±5.80) phases of menstrual cycle (P>0.05). Similarly, the mean (±SD) serum iron level (µg/dl) in Symptomatic HIV females was not significantly different between follicular (48.61±4.89) and luteal (47.46±4.50) phases of menstrual cycle (P>0.05). In Symptomatic HIV females on ART, no significant difference was also observed in the mean serum iron level (µg/dl) between follicular (61.84±9.21) and luteal (66.84±6.91) phases of menstrual cycle (P>0.05). The mean (±SD) serum iron level (µg/dl) in Control females was significantly lower at follicular (80.64±11.26) compared to luteal (98.77±15.14) phases of menstrual cycle (P<0.05).

However, the mean serum iron level (µg/dl) in Asymptomatic HIV females (55.12±5.29, 56.18±5.80), Symptomatic HIV females (48.61±4.89, 47.46±4.50) and Symptomatic HIV females on ART (61.84±9.21, 66.84±6.91) were significantly lower compared to Control females (80.64±11.26, 98.77±15.14) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively). Similarly, the mean (±SD) serum iron level (µg/dl) in Symptomatic HIV females on ART (61.84±9.21, 66.84±6.91) was significantly higher compared to Asymptomatic HIV females (55.12±5.29, 56.18±5.80) and Symptomatic HIV females (48.61±4.89, 47.46±4.50) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively).

The mean (±SD) Hb concentration (g/dl) in Asymptomatic HIV females was not significantly different between follicular (10.14±1.29) and luteal (10.56±1.35) phases of menstrual cycle (P>0.05). Similarly, the mean (±SD) Hb concentration (g/dl) in Symptomatic HIV females was not significantly different between follicular (9.15±1.52) and luteal (9.10±1.47) phases of menstrual cycle (P>0.05). In Symptomatic HIV females on ART, no significant difference was also observed in the mean Hb concentration (g/dl) between follicular (11.06±1.59) and luteal (11.15±1.62) phases of menstrual cycle (P>0.05). The mean (±SD) Hb concentration (g/dl) in Control females was significantly lower at follicular (12.24±1.69) compared to luteal (13.85±1.84) phases of menstrual cycle (P<0.05).

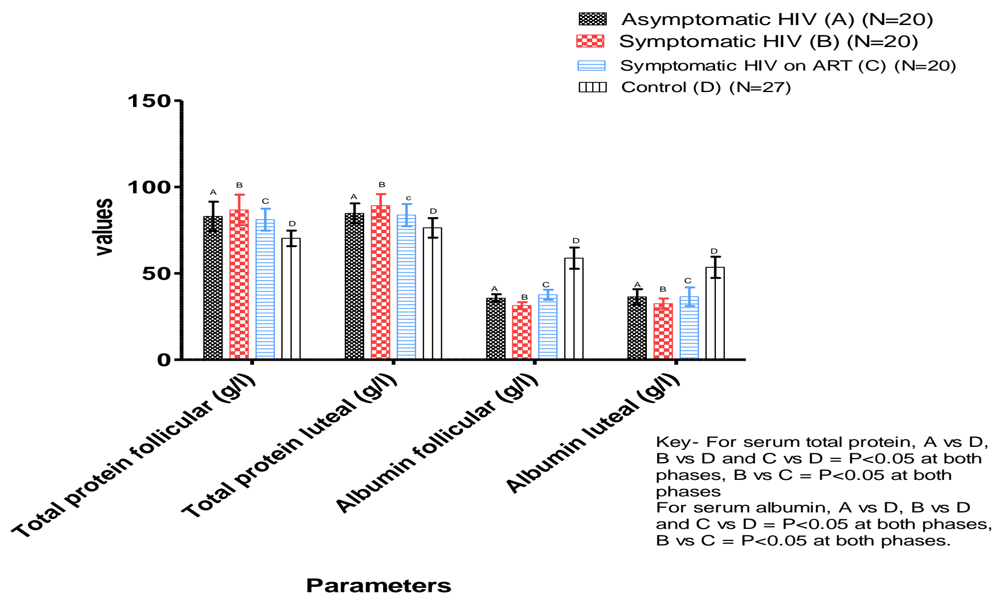

Fig 4.2: Comparison of mean (±SD) serum levels of Total protein and Abumin in Tests groups and Control group at Follicular and Luteal phases of menstrual cycle

However, the mean Hb concentration (g/dl) in Asymptomatic HIV females (10.14±1.29, 10.56±1.35), Symptomatic HIV females (9.15±1.52, 9.10±1.47) and Symptomatic HIV females on ART (11.06±1.59, 11.15±1.62) were significantly lower compared to Control females (12.24±1.69, 13.85±1.84) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively). Similarly, the mean (±SD) Hb concentration (g/dl) in Symptomatic HIV females on ART (11.06±1.59, 11.15±1.62) was significantly higher compared to Asymptomatic HIV females (10.14±1.29, 10.56±1.35) and Symptomatic HIV females (9.15±1.52, 9.10±1.47) at both follicular and luteal phases of menstrual cycle (P<0.05 respectively) (See Fig 3).

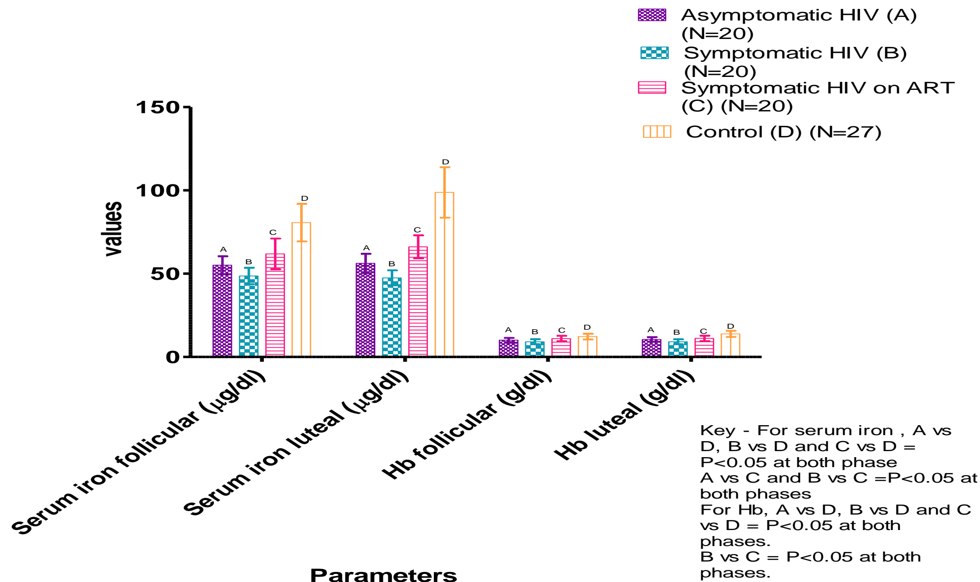

Fig 4.3: Comparison of mean (±SD) serum levels of iron and Haemoglobin concentration in Tests groups and Control group at follicular and luteal phases of menstrual cycle.

4. Discussion

The significantly low levels of zinc and copper in Asymptomatic and Symptomatic HIV infected females during menstrual cycle signifies imbalance in the metabolic and nutritional status of these patients and is consistent with previous reports (Constance et al., 1995; Akinola et al., 2012). Malnutrition has been documented to be an intriguing consequence of HIV infection (Fenton et al., 2008). Previous findings (Deuster et al., 1987; Das et al., 1997) reported significantly lower levels of plasma zinc at luteal phase of menstrual cycle of apparently healthy women which is consistent with the present study. This could have arisen as a result of nutritional deficiencies due to poor intake from anorexia, increased loss due to increased frequency of diarrhea and vomiting or poor renal function which could be prevalent in HIV infected subjects. There has been evidence of alteration in the nutritional status of HIV infected females among black people which may be attributed to HIV infection, malabsorption due to poor metabolism, opportunistic infection and the state of the host immune responses (Vorster et al., 2004). The implications of zinc and copper deficiencies in HIV infected women include further impairment of immune status of the affected women, increased prevalence of menstrual and reproductive dysfunction including complete absence of menstruation which could be due to hypogonadism (Heresi et al., 1985; Odeh, 1992; Bedwal and Bahuguma, 1994; King and Keen, 1994; Dunlop et al., 1994; Prasad et al., 2000). The significantly low level of zinc and copper observed in the present study could also be attributed to HIV disease progression and low CD4 T-cell count. This has been previously documented (Graham et al., 1991; Beach et al., 1992; Baum et al., 1995; 1997; Falutz et al., 1998; Van staden et al., 1999; Ukibe et al., 2012).

The insignificant difference in the mean levels of serum total protein and albumin in Asymptomatic and Symptomatic HIV infected females at follicular and luteal phases of menstrual cycle showed significant nutritional

and possible metabolic changes between the phases of menstrual cycle. This could be due to abnormal variation in progesterone level as a result of HIV infection. Malipatil and Shilpa (2013) reported significantly high level of serum total protein at luteal phase and high serum albumin at follicular phase of menstrual cycle in apparently healthy women. This is consistent with the finding in the present study in control females. The variations in the present study could be attributed to the influence of progesterone during menstrual cycle as a result of its anabolic effect on synthetic mechanism of the liver. Previous reports have been documented (Apseloff et al., 2000; Malipatil and Shilpa). Serum albumin has been reported to be used to indicate the degree of haemodilution (Waites et al., 1998). Previous reports have also documented that serum proteins bind sex steroids and regulate activity of menstrual cycle. Estrogen has anabolic effect causing positive nitrogen balance due to growth promoting effect which causes slight increase in the total body proteins (Indu, 2009) while Progesterone exerts anabolic effect which accounts for some of the weight gain (Pandubiri et al., 2012).

The significantly high levels of total serum protein with significantly lower levels of albumin components in Asymptomatic and Symptomatic HIV infected females is accounted for by increased level of the immunoglobulin components with reduced level of albumin possibly due to malnutrition, malabsorption and poverty. Reports of elevated levels of serum protein and reduced albumin concentrations in HIV and AIDS infected patients have been previously documented (Gramlich et al., 1995; Vorster et al., 2004; Hattings et al., 2009). Gramlich et al (1995) suggested that infections and changes in vascular permeability including hydration status can affect the circulating proteins in the affected individuals. Also high levels of immunoglobulin G has been reported in black people (Tollerud et al., 1995). Increase in some proteins and decrease in albumin as a reaction to acute phase response has also been implicated in increased incidence of mortality in HIV infected women (Feldman et al., 2000). The low level of albumin in HIV infected females in the present study may indicate acute phase response in these subjects. This has been documented previously (Van staden et al., 1999). However, Ludar et al (1999) reported insignificant difference in albumin levels between HIV infected patients and healthy people.

Furthermore, the insignificant difference in the mean levels of Hb and serum iron in Asymptomatic and Symptomatic HIV infected females between follicular and luteal phases of menstrual cycle is inconsistent with a previous report (Kim, 1993; Ofojekwu et al., 2013). In apparently healthy women with normal menstrual cycle, Hb and serum iron have been reported to be higher at luteal phase than follicular phase of menstrual cycle (Kim, 1993; Ofojekwu et al., 2013) which is consistent with our findings in Control females. However, the significantly higher levels of Hb and serum iron in Symptomatic HIV infected females on ART signifies some levels of stability at various phases as a result of the beneficial effect of ART. Ofojekwu et al (2013) observed a positive correlation between HB and serum iron concentration within the phases of menstrual cycle.

The significantly low levels of Hb and serum iron in HIV infected females with or without ART compared to Control signifies iron deficiency anaemia. This could be attributed to HIV progression, malnutrition, malabsorption and high incidence of opportunistic infections. Iron deficiency anaemia could also be as a result of possible depletion of iron store in HIV infected females thereby making more iron available in transport. This is said to be detrimental to HIV infected subjects (Asobayire et al., 2001; Onyenekwe et al., 2007; Ukibe et al., 2010). Onyenekwe et al (2008) have also reported a negative association between serum iron and PCV in HIV infected women in South Eastern Nigeria. Some other studies have documented reduced Hb concentration, transferrin and raised ferritin in malaria -HIV co-infected subjects (Das et al., 1997). The causes of iron deficiency anaemia in HIV infection is multifactoral and includes suppression of the bone marrow and erythropoiesis, nutritional deficiency and increased RBcs destruction as a result of increased incidence of malaria with loss of iron in urine (Sussan, 2009).

The improvement in the concentrations of zinc, copper, Hb, albumin and iron in Symptomatic HIV infected women on ART highlights the beneficial effects of treatment thus bringing about nutritional stability by improving appetite and intake and reducing viral load and loss through vomiting and diarrhea. These have far reaching effects on reversing menstrual irregularities and restoring fertility or reproductive function of these women. Adequate supplementation of zinc in HIV infected females on ART has been reported to stabilize weight and increases CD4 T-cell count with consequent reduction in the prevalence of opportunistic infections in the affected individuals (Baum et al., 1991; Mocchagiani et al., 1995; Kassu et al, 2006; Ukibe et al., 2012). However, excessive zinc administration may on the other hand contribute to HIV-1 disease progression as a result of interaction of copper and iron utilization which affects HDL cholesterol concentration (Fosmire, 1990; Schlesinger et al., 1993). It is believed that copper and ceruloplasmin levels are increased in hypozincaemic states and intestinal absorption of zinc decreases in oral iron administration (Prasad et al., 1997).

In conclusion, nutritional deficiencies involving proteins and micronutrients occur in HIV infected females. Adequate Zinc supplementation and early introduction of ART is therefore recommended to improve the condition of these subjects and to curb the mortality and morbidity associated with nutritional deficiency in HIV infected women within their reproductive age.

Acknowledgement

We are grateful to Tertiary Education Trust TFUND (TETFUND) Nnamdi Azikiwe University Awka for partly sponsoring the study. We are extremely grateful to the staff of Heart to Heart center and HIV clinic NAUTH Nnewi, Anambra State, Nigeria for their active supports in the collection of samples.

References

Akhuemokhan, K.I., Eregie, A., Fasanmade, O.A., 2010. Trace mineral status and glycemic control in Nigerians with type 2 diabetes. Afri. J. Diab. Med., 1(1), 20-22.

Akinola, F.F., Akinjinmi, A.A., Oguntibeju, O.O., 2012. Effect of combined antiretroviral on selected Trace elements and CD4+ T-cell count in HIV-positive persons in an African settings. J AIDS. Clin. Resear., 3(10), 185-190.

Apseloff, G., Bao, X., LaBoy-Goral, L., Friedman, H., Shah, A. , 2000. Am. J. Ther., 7(5), 297-302.

Arinola ,O.G., Charles-Davis, M.A., 2008. Micronutrient levels in the plasma of Nigerians females with breast cancer. Afr. J. Biotech., 7(11), 1620-1623.

Asobayire, F.S., Adon, P., Davidson, L., Cook, J.D., Hurrel, L.R.F., 2001. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalence of malaria and other infections: a study in Cote d'Ivoire Am J. Clin Nutr., 74(6), 776-82.

Baum, M. K., Shor-Posner, G., Lu, Y., Rosner, B., Sauberlich, H.E., Fletcher, M.A., Szapocznik, J., Eisdorfer, C., Buring, J.E., Hennekens, C.H., 1995. Micronutrients and HIV-1 disease progression. AIDS., 9,1051-1056.

Baum, M.K., Javier, J.J., Mantero-Atienza, E., Beach, R.S., Fletcher, M.A., Sauberlich, H.E., Feaster, D., Shor-Posner, G., 1991. Zidovudine-associated adverse reactions in a longitudinal study of asymptomatic HIV-1-infected homosexual males. J. Acquir. Immune. Defic. Syndr. Hum. Retro., 4, 1218-1226.

Baum, M.K., Shor-Posner, G., Zhang, G., Lai, H., Quesada, J., Miguez-Burbano, M., Fletcher, M.A., Sauberlich, H., Page, J.B., 1997. HIV-1 infection in women is associated with severe nutritional deficiencies. J. Acquir. Immune. Defic. Syndr. Hum. Retro., 16, 272-278.

Beach, R.S., Mantero-Atienza, E., Shor-Posner, G., Javier, J.J., Szapocznik, J., Morgan, R., Sauberlich, H.E., Cornwell, P.E., Eisdorfer, C., Baum, M.K., 1992. Specific nutrient abnormalities in asymptomatic HIV-1 infection. AIDS., 6, 701-708.

Bedwal, R.S., Bahuguna, A., 1994). Zinc copper and Selenuim in reproduction. Cell. Mol. Life Sci. 50(7): 626-640

Chandra, R.K., 1980. Acrodermatitis enteropathica. Paediatr. Scand., 66, 789-791

Chandra, R.K., 1997. Nutrition and the immune system: an introduction. Am. J. Clin. Nutr., 66(2), 460S-463S.

Constance, J., Peuchant, E., Pellegrin, J.L., Sergeant, C., Hamon, G., 1995. Fatty acids and plasma antioxidant in HIV-positive patients: correlation with nutritional and immunological status. Clin. Bioch., 28(4), 421-426.

Das, K., Chuwdhury, A.R., 1997. Metallic ion concentration during menstrual cycle in normally menstruating women. Ind. J.Med. Sci., 51(1), 52-54.

Deuster, P.A., Dolev, E., Bemier, L.L., Trotmann, U.H., 1987. Magnesium and zinc status during the menstrual cycle. Am. J. Obstet. Gynecol., 157(4), 964-8.

Dunlop, W.M., James, G.W., 1994. Hume DM. Anemia and neutropenia caused by copper deficiency. Ann. J. Intern. Med. 80(5), 470-476.

Falutz, J., Tsoukas, C., Gold, P., 1988. Zinc as a cofactor in human immunodeficiency virus-induced immunosupression. J. Am. Med. Assoc., 259, 2850-2851.

Farzadegan, H., Hoover, DR., Astemborski, J., 1998. Sex differences in HIV-1 viral load and progression to AIDS. Lancet., 352(12), 1510-1514.

Feldman, J.G., Burns, D.N., Gange, S.J., et al., 2000. Serum albumin as a predictor of survival in HIV-infected women in the Women's Interagency HIV Study. AIDS., 14(7), 863–70.

Fenton, M., Silverman, E.C., 2008. Medical nutrition therapy for Human Immunodeficiency Virus (HIV) infection and Acquired Immunodeficiency Syndrome (AIDS). In: Mahan L.K, Escott-Stump S, eds. Krause's Food, Nutrition & Diet Therapy. 12th ed. Philadelphia. W.B. Saunders., 2008, 991–1020.

Fosmire, G.J., 1990. Zinc toxicity. Am. J. Clin. Nutr., 51, 225-227.

Gatner, R., 2009. Selenium and thyroid hormone axis in critical ill states: an overview of conflicting view points. J. Trace. Elem. Med. Biol., 23(2), 71-74.

Graham GG, Cordano A., 1976. Copper deficiency in human subjects In: Prasad As, Oberleas D, eds. Trace elements in human health and diseas. Vol 1 Zinc and copper New York: Academic Press., Pp363-372.

Graham, N.M.H., Sorenson, D., Odaka, N., Brookmayer, R., Chan, D., Willett, W.C., Morns, J.S., Saah, A.J., 1991. Relationship of serum copper and zinc levels to HIV-1 seropositivity and progression to AIDS. J. Acquir. Immune. Defic. Syndr., 4, 976-980.

Gramlich, L.M., Mascioli, E.A., 1995. Nutrition and HIV infection. JNB., 6(1), 2–11.

Hattingh, Z., Walsh, C., Veldman, F.J., Bester, C.J., 2009. The metabolic profiles of HIV-infected and non-infected women in Mangaung, South Africa. S. Afr. J. Clin. Nutr., 22(1), 23-28

Heresi, G., Castill-Duran, C., Arevalo, M., Schlesinger, L., 1985. Phagocytosis and immunoglobulin levels in hypocupremic infants. J. Nutr. Resour., 5(1), 1327-34.

Indu Khurana., 2009. Text book of med. Physiol, Reprint 1St Ed, Elsevier publications., 859-860.

Jaryum, K.H., Dayok, O., Daniang, I.E., Longdet, T.Y., 2010. Trace elements Nutrition in the Developing World: A Rev J. Med. Appl. Bio. Sci. 2(1), 12-18

Kassu, A., Yabutani, T., Mahmud, Z.H., Mohammed, A., Nguyen, N., Huong, B.T., Hailmariam, G., Diro, E., Ayele, B., Wondmikun, Y., Motonaka, J., Ota, F., 2006. Alterations in serum levels of trace elements in tuberculosis and HIV infections. Eur. J. Clin. Nutr., 60(5), 580-586

Keen, C. L., 1990. Zinc deficiency and immune function. Annu. Rev. Nutr., 10, 415-431.

Kim, I., Yetley, E.A., Calvo, M.S., 1993. Am. J. Clin. Nutr., 58(5), 705-9.

Kimberly wonderly., 2011. J. Basic. Appl. Sci., 9, 71-75.

King, J. C., Keen, C. L., Shils, M.E., Olson, J.A., Shike, M., 1994. eds. Modern Nutrition in Health and Disease 8th ed. Lea & Febiger Philadelphia.

Kralik, A., Eder, k., Kirchgessner, M., 1996. Influence of zinc and selenium deficiency on parameters relating to thyroid hormone metabolism. Horm.Metabol. Resaer., 28(5),223-226

Luder, E., Godfrey, E., Godbold, J., Simpson, D.M., 1990. Assessment of nutritional, clinical, and immunologic status of HIV-infected inner-city patients with multiple risk factors. J. Am. Diet. Assoc., 5(6), 655–60.

Malipatil, B.S., Serum Electrolyte Status and Liver Functions in Different Phases of Menstrual Cycle. Resear. J. Pharm. Biol. Chem. Sci.

McClain, C. S., 1985. Zinc metabolism in malabsorption syndromes. J. Am. Coll. Nutr., 4,49-64.

Mocchegiani, E., Veccia, S., Ancarani, F., Scalise, G., Fabris, N., 1995. Benefit of oral zinc supplementation as an adjunct to zidovudine (AZT) therapy against opportunistic infections in AIDS. Int. J. Immunopharmacol., 17, 719-727.

National Agency for the Control of AIDS (NACA) (2012). Federal Republic of Nigeria, Global AIDS Response Country Progress Report Nigeria.

Odeh, M., 1992. The role of zinc in acquired immunodeficiency syndrome., 231, 463-469.

Ofojekwu, M.N., Nnanna, O.U., Okolie, C.E., Odewumi, L.E, Isiguzoro, I.O.U, Lugos, M.D., 2013. Hemoglobin and Serum Iron Concentrations in Menstruating Nulliparous Women in Jos, Nigeria. J.Labor. Med., 44, 121-124.

Onyenekwe, C.C, Ukibe, N.R., Meludu, S.C., Ilika AL., Ezeani, M.C., Ofiaeli, N., Onochie, A., Ilika, A.L., Aboh, N., 2007. Prevalence of malaria as co-infection in HIV infected individuals in a malaria endemic area of South Eastern Nigeria. J. Vector. Borne. Dis., 250-254.

Onyenekwe, C.C., Ukibe, N.R., Meludu, S.C., Ezeani, M.C., Ofiaeli, N., Onochie, A., Ilika, A.L., Ifeanyi, M.O., Aboh, N., Elee, P., 2008. Serum level of Anti- BCG, Albumin, packed cell volume and white blood cell count in subjects with HIV and Malaria Co-morbidity. J. Trop. Med. Health., 361 17-22.

Padubidri VG, Shirish N. Shaw's., 2012. Textbook of Gynaecology, Edn 15, Elsevier publication., 25-50.

Percival, S.S., 1998. Copper and immunity. Am. J. Clin. Nutr., 67, (5Suppl), 1064S-1068S.

Piot, P., Plummer, F.A., Mhalu, F.S., Lamboratory, J.L., Chin, J., Mann, J.M., 1988. An Inter. Perspec. Sci., 239(3), 573-579

Prasad, A.S., 2007. Zinc mechanisms of host defenses. J. Nutr., 137, 1345-1349.

Prasad, A.S., Beck, F.W.J., Endre, L., Handschu, W., Kukuruga, M., Kumar, G., 1996. Zinc deficiency affects cell cycle and deoxythymidine kinase (TK) gene expression in HUT-78 cells. J. Lab. Clin. Med., 128, 51-60.

Prasad, A.S., Fitzgerald, J.T., Bao, B., Beck, F.W.J., Candrasekar, P.H., 2000. Duration of symptoms and plasma cytokine levels in patients with the common cold treated with zinc acetate. Ann. Intern. Med., 133, 245-52.

Prasad, A.S., Kaplan, J., Beckn, F.W.J., Penny, H.S., Shamsa, F.H., Salwen, WA., Marks, S.C., Mathog, R.H., 1997. Trace elements in head and neck cancer patients: Zinc status and immunological functions. Otolaryngol Head Neck. Surg., 116, 624-629.

Ripa, S., Ripa, R., 1995. Zinc and immune function. Minerva. Med., 86, 315-318.

Schlesinger, L., Arevalo, M., Arredondo, S., Lonnerdal, B., Stekel, A., 1993. Zinc supplementation impairs monocyte function. Acta. Paediatr., 82, 734-738.

Siberry, G.K., Ruff, A.J., Black R., 2001). Zinc and human immunodeficiency virus infection. J. Nutr. Resear., 22(3), 527-538

Sussan, F., Clark., 2009. Iron deficiency anemia: diagnosis and management. Curr. Opin. Gastroenterol., 25(2), 122-128.

Tietz, N.W., 1976. Fundamentals of clinical chemistry, WB Saunders. Philadelphia., 919.

Tollerud, D.J., Brown, L.M., Blattner, W.A, et al., 1995. Racial differences in serum immunoglobulin levels: Relationship to cigarette smoking, T-cell subsets, and soluble interleukin-2 receptors. J. Clin. Lab. Anal., 9(1), 37–41.

Ukibe, N.R., Onyenekwe, C.C., Ahaneku, J.E., Meludu, S.C., Ilika, A.L., Ifeanyichukwu, M., Ezeani, M., Igewegbe, A.O., 2012. Use of absolute lymphocyte count or neutrophil ingestion rate of nitroblue tetrazolium (NBT) as alternative index to CD4+ T- cell count to initiate ART in the management of HIV/AIDS disease. Int. J. Biol. Chem. Sci., 6(1), 99-107.

UKibe, N.R., Onyenekwe, C.C., Ahaneku, J.E., Ukibe, N.R., Meludu, S.C., Ilika, A., Ifeanyi, M.O., Ezeani, M.C., Igwegbe, A.O., Onochie, A., Ofiaeli, N., 2010. Packed cell volume and serum iron in subjects with HIV-malaria co-infection in Nnewi, South Eastern Nigeria. Int. J. Biol. Chem. Sci., 4(2), 471-478.

UNAIDS., (2011, November) 'World AIDS Day Report 2011.

UNAIDS., (2012) 'Women Out Loud: How Women Living with HIV Will Help the World End AIDS'

USAID/Nigeria., (2012). HIV/AIDS and Tuberculosis.

Van Staden, A.M., Barnard, H.C., Nel, M., 1998. Nutritional status of HIV-1 seropositive patients in the Free State Province of South Africa – laboratory parameters. Centr. Afr. J. Med., 44(10), 246–50.

Vorster, H.H., Kruger, A., Margetts, B.M., 2004. The nutritional status of asymptomatic HIV-infected Africans: Directions for dietary intervention? Public. Health. Nutr., 7(8), 1055–64.

Waites, G.T., Wood. P.L., Walker, R.A., Bell, S.C., 1988. J. Reprod. Fert., 82, 665-672.

Williams, D., 1983. Copper deficiency in humans. Semin Haematol. 20(1), 118-28.