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Evaluation of Some Cytokines, CD4, Hepcidin, Iron, and Some Haematological Parameters of Patients Living with Human Immunodeficiency Virus in Southeastern Part of Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OEI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KO, OISI and EBN managed the analyses of the study. Authors UMC, OC and AEI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A study to evaluate the levels of interferon-gamma, interleukins 6 and 10, hepcidin, iron status and some haematological parameters in persons living with human immunodeficiency virus was carried out. A total of 150 subjects aged 18-60 years were enlisted for this study. The subjects were grouped into: Group A (50 control subjects) and Group B (100 HIV subjects, 50 subjects were non ART HIV patients, 50 subjects were on Lamivudine, Tenofavir and Efavirenz). About 7ml of venous blood were collected from each subject; 4.5 ml of blood were placed into plain tubes for assay of

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interferon gamma, interleukins (6&10), hepcidin and iron and 2.5ml for FBC, CD4 count and HIV screening. The cytokines and hepcidin were measured using Melsin ELISA Kits and Teco Diagnostics kits used for iron. Full blood count was determined by automation using Mindray BC-5300, China. The data was analysed with the statistical package for social science (SPSS) version 20 using ANOVA and the level of significance set at P<0.05. The results showed difference that was statistically significant (P<0.05) in IFN-γ (16.25±0.87 pg/ml, 29.31±1.44 pg/ml,18.49±1.48 pg/ml, P=0.000), IL-6(7.98±0.22 pg/ml, 11.08±1.21 pg/ml, 8.79±0.76 pg/ml, P=0.000), IL-10(8.52±0.62 pg/ml, 16.62±1.53 pg/ml, 10.39±1.06P g/ml P=0.000), CD4 (1045.54 ±247.24 Cells/L, 195.60 ±35.94 Cells/L,10.39±1.06 cells/L P=0.000), hepcidin (6.03±1.38 ng/ml, 39.59 ±4.50 ng/ml, 20.86±3.43 ng/ml, P=0.000), Iron (86.29±7.27 µg/dl, 73.43±5.45 µg/dl, 85.44±8.45 µg/dl, P=0.000), TIBC (345.56±28.40 µg/dl, 287.19 ±8.21 µg/dl, 305.46±18.82 µg/dl, P=0.000), %TSA (25.16±3.18%, 25.61±2.22%,28.08 ±3.42%, P=0.000) WBC (5.87 ±0.88 X 10^9/L, 4.69±0.72X 10^9/L,4.80±0.45X 10^9/L, P=0.000), Neutrophils (60.57±2.83%, 75.16±3.68%, 69.04±2.90%, P=0.000), Lymphocytes (30.69 ±2.84%, 17.24±2.50%, 24.46±2.60%, P=0.000), Monocytes (5.59 ±1.2%, 4.18±1.12%, 3.97±0.92%, P=0.000), Eosinophils (2.30 ±1.05%, 2.16±0.82%, 1.67±0.57%, P=0.000), Basophil 0.86 ±0.39%, 1.31±0.94%, 0.86±0.44%, P=0.018), RBC (4.92±0.30 X 10^{12}/L, 3.34±0.21 X 10^{12}/L, 3.60 ±0.18X 10^{12}/L, P=0.000), Haemoglobin (14.75±0.90,g/dl, 10.05±0.65 g/dl,10.80±0.53 g/dl, P=0.000), PCV (44.25±2.70%, 30.14±1.95,32.56±1.50%, P=0.000), MCV (89.92±2.3fl, 79.49±1.28fl,88.15±2.08fl, P=0.029), MCH (36.12±1.53 pg, 26.60±0.48P, P=0.002), Platelets (261.75±22.71 X 10^9/L, 246.16±9.93 X 10^9/L,189.32±17.00X 10^9/L, P=0.000), ESR (7.03 ±1.38mm/hr, 59.52 ±6.46mm/hr,43.34±4.82mm/hr, P=0.000) when compared among Control, Non ART HIV and ART positive subjects.g,28.57±1.78 pg, P=0.000), MCHC (368.46±12.28 g/l, 318.92±7.33 g/l, 333.56±22.61 g/l. The study shows that interferon gamma, interleukin 6, interleukin 10 and hepcidin are some of the biomarkers in the pathogenesis of HIV. The infection of HIV increases the levels of the cytokines. The cytokines and hepcidin can be used as prognostic and diagnostic markers as their levels decreased with treatment of the patients.

Keywords: Some cytokines; CD4; hepcidin; iron; some haematological parameters; human immunodeficiency virus.

1. INTRODUCTION

Cytokines are important immunomodulating agents of immune system. Human immunodeficiency virus infection has been suggested to alter blood cell populations and change Th1/Th2 balance [1].

Peptide hepcidin, is a key iron-regulatory hormone [2], which is released from hepatocytes in response to inflammation via iron and oxygen. Interestingly, inflammation induces hepcidin production, mediated by the inflammatory cytokine IL-6. This results in sequestration of Fe in the stores and Fe-limited erythropoiesis, and eventually anaemia of inflammation [2]. The study will determine hepicidin level and IL-6. This will help to discover the role of the HIV infection on these parameters which may be the major cause of anaemia in the patients.

Interleukin 6 (IL-6) is a proinflammatory cytokine that regulates various physiological processes [3]. It plays a key role in the acute phase response and in the transition from acute to chronic inflammation [4]. Evidence has accrued to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory diseases [3,5]. Human immunodeficiency virus (HIV) infection has long been shown to induce expression and secretion of IL-6 [6,7]. This study will find out the changes that may be associated to the IL-6 levels in patients with HIV infection. It will be important to determine the changes the infection could cause to these cytokines. The cytokines that were studied include interferon-gamma, interleukin 6 and interleukin 10. These are inflammatory and anti-inflammatory cytokines that are useful in the pathogenesis of the infection and the prognosis.

The study was done to determine the levels of interferon-gamma, interleukin 6, interleukin 10, iron status, hepcidin and haematological parameters of patients living with human immunodeficiency virus Southeast, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at the Federal Medical Centre, Umuahia, located in South-Eastern Nigeria.

2.2 Study Population and Enrolments

A total of one hundred and fifty (150) subjects aged 18-60 years were enlisted for this study. The participants were recruited by purposive sampling technique. The HIV subjects were recruited from HIV clinic based on HIV screening tests, while apparently healthy age and sex matched subjects were recruited as controls. The subjects were grouped into:

Group A: 50 control subjects.

Group B: HIV positive subjects (n=100)

(i) Naïve HIV positive subjects (n=50), (ii) ART HIV Positive subjects on Lamivudine, Tenofavir and Efavirenz therapy (n=50)

2.3 Selection Criteria

A. Inclusion criteria

(i) Subjects of both sexes aged 18-60 years positive for *Mycobacterium tuberculosis* and HIV screening and confirmatory tests were included in the study.
(ii) Those that gave consent were included.

B. Exclusion criteria

The following subjects were excluded

a. Those that tested negative for pulmonary tuberculosis and HIV
b. Pregnant women
c. Diabetes mellitus patients
d. Persons below 18 years and above 60 years
e. Those that did not give consent.

2.4 Sample Collection

Seven milliliters (7 ml) of venous blood was collected from each subject and 2.5 ml was dispensed into bottles containing di-potassium salt of ethylenediamine tetra-acetic acid (K_2-EDTA) at a concentration of 1.5 mg/ml of blood and was used for full blood count, CD4 count and HIV screening.

Also, 4.5 ml was dispensed into plain tubes. Serum was obtained after clotting by spinning at 3000 RPM for 10 minutes and was used for interferon gamma, interleukin-6, and interleukin-10, iron and hepcidin determination.

Three separate sputum samples (consisting of one early morning sample and two spot samples) were collected in a wide mouth container from the subjects for pulmonary tuberculosis diagnosis.

The whole samples was analysed in Links Laboratory, Owerri by Sandwich ELISA method for interferon gamma, interleukins (6 and 10) and hepcidin and and HIV tests CD4 count, Full Blood count analysed in the Diagnostic Laboratory Unit, University Health Services Department of Michael Okpara University of Agriculture, Umudike, Abia State. Ziel Nelosn and GeneXpert were done in Federal Medical Centre, Umuahia, Abia State, Nigeria.

2.5 Laboratory Procedures

All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly adhered to.

2.5.1 Determinations

A. Determination of CD4 count by flowcytometery (Partec Cyflow counter), Germany

Procedure: All required reagents was brought to room temperature and 850µl of the count check bead green will be analysed to ensure that the cyflow machine is working properly. The desired numbers of rohren test tubes was placed in a test tube rack. 20µl of CD4 easy count kits (CD4 Mab-PE) were pipetted into different test tubes labeled appropriately for the assay. Then, 20µl of blood sample was also pipette into each respective test tube and incubated in the dark for 15 minutes at room temperature after mixing properly. This was followed by the addition of 850 µl easy count. No lyse buffer was added to each test tube. This was mixed properly to avoid air bubbles and analysed on the Partec Cyflow. The result was displayed and copied from the screen.

B. Full blood count by automation using Mindray BC-5300, China

Procedure: The sample is EDTA bottle was placed in the spiral mixer and allowed to mix very well. Whole blood mode was activated in the LCD screen, the sample no (code) was inputted via key board and then the key will be selected. Then the sample was mixed very well again and the cap was removed and inserted into the probe

and the SART button was pressed. When the LCD screen displays ANALYSING; the sample was removed and recapped. The analyser was executed automatic analysis and displays the result on LCD screen.

C. Determination of serum iron concentration by Ferozine method Teco Diagnostics (Iron/TIBC) Laketiew Ave, Acashein, CA 92807

Procedure: Iron free clean tubes were labeled as test, blank and standard. The 2.5 ml of iron buffer reagent was added to all the labeled tubes. Also, 0.5 ml of the samples was added to the respective tubes and was mixed. The reagent blank was used to zero the spectrophotometer at 560nm. The absorbance of all tubes was read and value will be recorded (A1 reading). Then, 0.5 ml of iron reagent was added to all the tubes and was mixed properly. The tubes were palced in a heating bath at 37°C for 10 minutes. The reagent blank was also used to zero the spectrophotometer at 560nm and another absorbance of all the tubes was read and the value obtained was recorded (A2 reading).

Calculation

Serum iron (µg/dl) = A2 Test-A1 Test x Con of A2 std-A1 std

Where A1 Test= Absorbance of first reading of the test

A2= Absorbance of the second reading of the test
A1 std= Absorbance of the first reading of the standard
A2 std= Absorbance of the second reading of the standard

D. Determination of total iron binding capacityby Ferozine method of TECO Diagnostics (Iron/TIBC) Laketiew Ave, Acashein, CA 92807

Procedure: Iron free clean test tubes were labeled as test, blank and standard and 0.2 ml of unsaturated iron binding capacity buffer reagent was added to all the tubes according to the sample number, while 10 ml of iron free water was added to standard tube and was properly mixed. To the test 0.5 ml of sample and 0.5 ml iron standard were added to the test, and was properly mixed. The reagent blank was used to zero the spectrophotometer at 560nm wavelength. The absorbance of the samples was read and recorded as A1 reading. Also 0.5 ml iron standard tube and was properly mixed. To the test, 0.5 ml of sample and 0.5 ml iron standard were added to the test, and was properly mixed. The reagent blank was used to zero the spectrophotometer at 560nm wavelength. The absorbance of the samples was added to the tubes and was mixed properly and was placed in a heating bath at 37°C for 10 minutes. The reagent blank was used to zero the spectrophotometer at 560nm and another reading was taken as the A2 reading.

Calculation

UIBC (µg/dl) =
Conc. Of std-A2 Test-A1 Test x Conc. Of std
A2 std-A1 std

TIBC (µg/dl)= Iron + UIBC

Where A1 Test= Absorbance of first reading of the test
A2= Absorbance of the second reading of the test
A1 std= Absorbance of the first reading of the standard
A2 std= Absorbance of the second reading of the standard

E. Alere Determine HIV-1/2 Kit (Japan, Lot No: 84904k100a) for first Line HIV Screening test

Procedure: The desired numbers of test units from the test card were removed by bending and tearing at the perforation. The protective cover from each test was removed. About 50µl of sample (serum) was added to the sample pad and allowed to flow through the solid phase. The result was read within a 15 minutes.

F. Uni-Gold™ HIV (Trinity Biotech, Lot No: HIV7110042) for second line HIV Screening test

Procedure: Two drops of whole blood were applied to the sample port, followed by 2 drops of wash solution and was allowed to react. Antibodies of any immunoglobin class, specific to the recombinant HIV-1 or HIV-2 proteins reacted with the colloidal gold linked antigens. The antibody protein colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device.

G. Chembio HIV ½ Stat-Pak (USA, Lot No: 33020516) used as tie breaker

Procedure: With the sample loop provided, 5µl of the sample was taken and applied on the sample pad of the device. Then 3 drops (105µl) of the running buffer were added on the sample well also. The result was then read after 10 minutes.

H. Human Interferon-gamma (IFN-γ) ELISA Kit by Melsin Medical Co Limited, Catalogue Number: EKHU-0162

Procedure: Dilutions of standard was prepared to get a concentration of 240ng/l, 160ng/l, 80ng/l, 40ng/l and 20ng/l. 50µl of standards were pipette into the standard wells. 10µ of test serum were added into each well. 40µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, stop solution). 50µl of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30 minutes at 37^0C. It was washed for 4 times. 50µl of chromogen solution A and 50µl of chromogen solution B was added to each well. They were mixed and incubated for 10 minutes at 37°C. 50µl of stop solution was added to each well. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minutes taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

I. Interleukin 6 (IL-6) Assay

Human Interleukin 6 commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0140

Procedure: Dilutions of standard was prepared to get a concentration of 240 ng/l, 160 ng/l, 80ng/l, 40 ng/l and 20 ng/l. 50µl of standards were pipette into the standard wells. 10µ of test serum were added into each well. 40µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, stop solution). 50µl of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30 minutes at 37^0C. It was washed for 4 times. 50µl of chromogen solution A and 50µl of chromogen solution B was added to each well. They were mixed and incubated for 10 minutes at 37°C. 50µl of stop solution was added to each well. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minutes taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

J. Human Interleukin 10 (IL-10) Assay by commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0155

Procedure: Dilutions of standard was prepared to get a concentration of 240ng/l, 160ng/l, 80ng/l, 40ng/l and 20ng/l. 50µl of standards were pipette into the standard wells. 10µ of test serum were added into each well. 40µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, stop solution). 50µl of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30 minutes at 37°C. It was washed for 4 times. 50µl of chromogen solution A and 50µl of chromogen solution B was added to each well. They were mixed and incubated for 10 minutes at 37°C. 50µl of stop solution was added to each well. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minutes taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

K. Human Hepcidin (Hepc) ELISA Kit by MELSIN Medical Co Limited was used with Catalogue Number: EKHU-1674

Procedure: Dilutions of standard was prepared to get a concentration of 240 ng/l, 160 ng/l, 80 ng/l, 40 ng/l and 20 ng/l. 50µl of standards were pipette into the standard wells. 10µ of test serum were added into each well. 40µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen

solution A and B, stop solution). 50µl of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30 minutes at 37°C. It was washed for 4 times. 50µl of chromogen solution A and 50µl of chromogen solution B was added to each well. They were mixed and incubated for 10 minutes at 37°C. 50µl of stop solution was added to each well. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minutes taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

2.6 Statistical Analysis

Data was analysed using statistical package for social science (SPSS) version 20. Student t-test, ANOVA (Analysis of Variance), Pearson Product Moment and Chi-Square were the tools employed. Results were expressed as mean ± standard deviation and are presented in tables and significance level was set at P<0.05.

3. RESULTS

The results of the study are presented here in table below as mean ± standard deviation (SD) of the parameters studied from the subjects.

The results showed difference that was statistically significant (P<0.05) in IFN-γ (16.25±0.87 pg/ml, 29.31±1.44 pg/ml, 18.49±1.48 pg/ml, P=0.000), IL-6(7.98±0.22 pg/ml, 11.08±1.21 pg/ml, 8.79±0.76 pg/ml, P=0.000), IL-10(8.52±0.62 pg/ml, 16.62±1.53 pg/ml, 10.39±1.06 Pg/ml P=0.000), CD4 (1045.54 ±247.24Cells/L, 195.60 ±35.94Cells/L, 10.39±1.06cells/L P=0.000), hepcidin (6.03±1.38 ng/ml, 39.59 ±4.50 ng/ml, 20.86±3.43 ng/ml, P=0.000), Iron (86.29±7.27 µg/dl, 73.43±5.45 µg/dl,85.44±8.45µg/dl, P=0.000), TIBC (345.56±28.40 µg/dl, 287.19 ±8.21µg/dl, 305.46±18.82µg/dl, P=0.000), %TSA (25.16±3.18%, 25.61±2.22%,28.08 ±3.42%, P=0.000) WBC (5.87 ±0.88 X 10^9/L, 4.69±0.72X 10^9/L,4.80±0.45X 10^9/L, P=0.000), Neutrophils (60.57±2.83%, 75.16±3.68%, 69.04±2.90%, P=0.000), Lymphocytes (30.69 ±2.84%, 17.24±2.50%, 24.46±2.60%, P=0.000), Monocytes (5.59 ±1.2%, 4.18±1.12%, 3.97±0.92%, P=0.000), Eosinophils

Table 1. Mean±SD values of interferon-gamma, IL-6, IL-10, CD4, hepcidin, iron, and some haematological parameters of control, non art and art HIV positive subjects

Parameters	Control	NON ART	ART	F-value	P-value
IFN-γ(pg/ml)	16.25±0.87	29.31±1.44	18.49±1.48	1073.320	0.000*
IL-6(pg/ml)	7.98±0.22	11.08±1.21	8.79±0.76	154.677	0.000*
IL-10(pg/ml)	8.52±0.62	16.62±1.53	10.39±1.06	376.643	0.000*
CD4(cells/l)	1045.54±247.24	195.60 ±35.94	389.18±101.63	172.600	0.000*
Hepcidin(ng/ml)	6.03±1.38	39.59 ±4.50	20.86±3.43	374.510	0.000*
Iron(µg/dl)	86.29±7.27	73.43±5.45	85.44±8.45	67.946	0.000*
TIBC(µg/dl)	345.56±28.40	287.19 ±8.21	305.46±18.82	58.456	0.000*
%TSA(%)	25.16±3.18	25.61±2.22	28.08 ±3.42	14.181	0.000*
WBC(X 10^9/L)	5.87 ±0.88	4.69±0.72	4.80±0.45	12.264	0.000*
Neu(%)	60.57±2.83	75.16±3.68	69.04±2.90	102.695	0.000*
Lym(%)	30.69 ±2.84	17.24±2.50	24.46±2.60	165.695	0.000*
Mon(%)	5.59 ±1.2	4.18±1.12	3.97±0.92	137.764	0.000*
Eos(%)	2.30 ±1.05	2.16±0.82	1.67±0.57	6.246	0.000*
Bas(%)	0.86 ±0.39	1.31±0.94	0.86±0.44	16.626	0.018*
RBC(X 10^{12}/L)	4.92±0.30	3.34±0.21	3.60 ±0.18	126.293	0.000*
Hb(g/dl)	14.75±0.90	10.05±0.65	10.80±0.53	91.625	0.000*
PCV(%)	44.25±2.70	30.14±1.95	32.56±1.50	124.014	0.000*
MCV(fl)	89.92±2.3	79.49±1.28	88.15±2.08	168.021	0.000*
MCH(pg)	36.12±1.53	26.60±0.48	28.57±1.78	230.341	0.000*
MCHC(g/l)	368.46±12.28	318.92±7.33	333.56±22.61	3400.675	0.000*
Plt(X 10^9/L)	261.75±22.71	246.16±9.93	189.32±17.00	244.763	0.000*
ESR(mm/hr	7.03 ±1.38	59.52 ±6.46	43.34±4.82	486.218	0.000*

*Significant level-*P < 0.05,*
Ns Not significant (P > 0.05)

(2.30 ±1.05%, 2.16±0.82%, 1.67±0.57%, P=0.000), Basophil 0.86 ±0.39%, 1.31±0.94%, 0.86±0.44%, P=0.018), RBC (4.92±0.30 X 10^{12}/L, 3.34±0.21 X 10^{12}/L,3.60 ±0.18X 10^{12}/L, P=0.000), Haemoglobin (14.75±0.90,g/dl, 10.05±0.65g/dl, 10.80±0.53g/dl, P=0.000), PCV (44.25±2.70%, 30.14±1.95, 32.56±1.50%, P=0.000), MCV (89.92±2.3fl, 79.49±1.28fl, 88.15±2.08fl, P=0.029), MCH (36.12±1.53pg, 26.60±0.48P, P=0.002), Platelets (261.75±22.71 X 10^9/L, 246.16±9.93 X 10^9/L,189.32±17.00X 10^9/L, P=0.000), ESR (7.03 ±1.38mm/hr, 59.52 ±6.46mm/hr, 43.34±4.82mm/hr, P=0.000) when compared among Control, Non ART HIV and ART positive subjects.g,28.57±1.78pg, P=0.000), MCHC (368.46±12.28 g/l, 318.92±7.33 g/l, 333.56±22.61 g/l.

4. DISCUSSION

The levels of interferon gamma were higher in non ART HIV than ART HIV. This also implies that the level of interferon gamma is high in HIV before treatment which decreases in the course of treatment. On the other hand interferon gamma, a T helper type 1 (Th1) cytokine found at high concentrations at sites of pulmonary TB, promotes transcriptional activation of HIV through activation of other nuclear factors [8].

HIV non ART showed higher level of IL-6 than the control group but no significant difference to HIV ART. This shows that treatment in HIV has no serious effect on IL-6. This shows that IL-6 may have a major role to play in the pathogenesis of the infection. The treatment outcome may be affected by modifying the levels of IL-6 in HIV patients. Human immunodeficiency virus (HIV) infection has long been shown to induce expression and secretion of IL-6 by monocytes and macrophages [9]. Even when virologically suppressed, treated HIV-infected persons have higher plasma levels of IL-6 than well-matched controls [6,7] Activated inflammation, as demonstrated by persistently higher IL-6 levels, may have profound and far-reaching clinical implications. Interleukin 6 plays a major role in the acute phase response and in the transition from acute to chronic inflammation [4]. Evidence has occurred to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory [5]. This can be a biomarker in the diagnosis and prognostic monitor of the progress of the disease as well as the cure of the disease. Some reports involving both HIV-infected and HIV-uninfected persons have linked increased IL-6 levels to a variety of adverse clinical outcomes, including anaemia [10], cancer [11] and death [12].

The study showed higher level of IL-10 in non ART HIV group than ART HIV group. Interleukin 10 functions to limit the immune response to pulmonary TB and may contribute to pulmonary TB pathogenesis [13]. This shows that IL-10 may be stimulated by the release of cytokines induced by the causative organisms to tuberculosis and HIV to inflict pain on the patients.

CD4 is very important in accessing the immune level especially in HIV infection because HIV attacks CD4 exposing the body to a lot of opportunistic infections. Infection with the HIV is characterized by dysfunction in T cells which results from the combined effects of changes in antigen specific lymphocytes, and soluble factors such as chemokines and cytokines [14]. Among HIV- infected patients, low haemoglobin levels are strongly predictive for tuberculosis [15,16], and anaemia is one of the most common complications of both disease processs [17]. There was significant change in CD4 when compared among the groups. There was increase in ART HIV subject s compared to non ART HIV subjects. CD4 is a measure of immune status for HIV patients and there was increase in CD4 count of ART HIV showing that there was improvement in immune status of the HIV patients on antiretroviral therapy. HIV patients should be encouraged to be placed on ART on time to ensure improved well being of their health status by building robust immune response.

The increase in hepcdin in these patients may be associated with the anemia observed in the body and this may be a mechanism the body uses to counteract the anaemia seen in them by challenging the body to produce more iron to couple with globin to form haemoglobin to transport oxygen needed in the metabolism of the body.

There is also a decrease of hepcidin in HIV ART group compared to the HIV non ART group.

HIV ART group has higher level of iron than iron HIV non ART group. This may be linked to reduction in induced senescent red blood cells. Absorption of iron from the diet must have increase as there will be improved gastrointestinal tract of the patients.

HIV can directly infect bone marrow progenitor cells leading to bone marrow suppression, which

is associated with upregulated hepcidin expression [18].

The decrease in WBC count in patients with non ART HIV positive patients may be due to suppressive effects of HIV infection on the bone marrow, affecting production of WBC [19].

Platelets of non ART HIV patients showed no significant decrease among ART HIV. It also shows that antiretroviral therapy has suppressive effects on the platelets.

There are higher concentrations of IL-6 and IL-10 levels among HIV patients that are non ART, and the levels of IL-6 and IL-10 are reduced by ART [20].

5. CONCLUSION

The study shows that interferon gamma, interleukin 6, interleukin 10 and hepcidin are some of the biomarkers in the pathogenesis of HIV. The infection of HIV increases the levels of the cytokines. The cytokines and hepcidin can be used as prognostic and diagnostic markers as their levels decreased with treatment of the patients.

The study has shown wide variations in the haemtological indices studied. The red blood cell, packed cell volume and haemoglobin were suppressed but improved win treatment. Anaemia is a major factor causing morbidity and mortality in the patients living with HIV.

The haemtological parameters like haemoglobin, RBC and PCV increased significantly with treatment showing improvement in health status of the patients.

It is recommended that those involved with the management of HIV patients should monitor the levels of these cytokines studied together with iron status and hepcidin. The levels of these cytokines are critical to the prognosis of the patients receiving treatments.

CONSENT AND ETHICAL APPROVAL

With a well detailed research proposal and a letter of introduction from the Head of Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria, Consent form and an application letter were submitted to the Head, Health Research and Ethics Committee of the Institution was met. After their meetings and thorough perusal of the protocols of the research, an ethical approval was given for the study and consents were obtained from the subjects before participating in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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