

## Comparative Analysis of Interferon-Gamma, IL-6, IL-10, CD4, Hepcidin, Iron Status and Some Haematological Parameters of Control and Non ART HIV Positive Subjects

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DOI: [10.36347/sjams.2019.v07i07.023](https://doi.org/10.36347/sjams.2019.v07i07.023)

| Received: 30.06.2019 | Accepted: 17.07.2019 | Published: 21.07.2019

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### Abstract

### Original Research Article

HIV is a public health issue that has disturbed the existence of mankind especially in the developing countries. A study to evaluate the levels of interferon-gamma, interleukins 6 and 10, hepcidin, iron status and some haematological parameters in non ART HIV positive subjects was carried out. A total of 100 subjects aged 18-60 years were enlisted for this study. The subjects were grouped into: Group A (50 control subjects) and Group B (50 were non ART HIV subjects). About 4.5ml of blood were collected into plain tubes for assay of 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 determination. Full blood count was determined by automation using Mindray BC-5300. The data was analysed with the statistical package for social science (SPSS) version 20 using ANOVA and Pearson Product Moment method were statistical tools for the analysis and the level of significance set at  $P < 0.05$ . The results showed difference that was statistically significant ( $P < 0.05$ ) in  $IFN-\gamma$  ( $16.25 \pm 0.87$  pg/ml,  $29.31 \pm 1.44$  pg/ml,  $P = 0.000$ ), IL-6 ( $7.98 \pm 0.22$  pg/ml,  $11.08 \pm 1.21$  pg/ml,  $P = 0.000$ ) IL-10 ( $8.52 \pm 0.62$  pg/ml,  $16.62 \pm 1.53$  pg/ml,  $P = 0.000$ ), CD4 ( $1045.54 \pm 247.24$  Cells/L,  $195.60 \pm 35.94$  Cells/L,  $P = 0.000$ ), hepcidin ( $6.03 \pm 1.38$  ng/ml,  $39.59 \pm 4.50$  ng/ml,  $P = 0.000$ ), Iron ( $86.29 \pm 7.27$   $\mu$ g/dl,  $73.43 \pm 5.45$   $\mu$ g/dl,  $P = 0.000$ ), TIBC ( $345.56 \pm 28.40$   $\mu$ g/dl,  $287.19 \pm 8.21$   $\mu$ g/dl,  $P = 0.000$ ), WBC ( $5.87 \pm 0.88 \times 10^9/L$ ,  $4.69 \pm 0.72 \times 10^9/L$ ,  $P = 0.000$ ), Neutrophils ( $60.57 \pm 2.83\%$ ,  $75.16 \pm 3.68\%$ ,  $P = 0.000$ ), Lymphocytes ( $30.69 \pm 2.84\%$ ,  $17.24 \pm 2.50\%$ ,  $P = 0.000$ ), Monocytes ( $5.59 \pm 1.2\%$ ,  $4.18 \pm 1.12\%$ ,  $P = 0.000$ ), Basophil ( $0.86 \pm 0.39\%$ ,  $1.31 \pm 0.94\%$ ,  $P = 0.003$ ), RBC ( $4.92 \pm 0.30 \times 10^{12}/L$ ,  $3.34 \pm 0.21 \times 10^{12}/L$ ,  $P = 0.000$ ), Haemoglobin ( $14.75 \pm 0.90$  g/dl,  $10.05 \pm 0.65$  g/dl,  $P = 0.000$ ), PCV ( $44.25 \pm 2.70\%$ ,  $30.14 \pm 1.95$ ,  $P = 0.000$ ), MCV ( $89.92 \pm 2.3$  fl,  $79.49 \pm 1.28$  fl,  $P = 0.029$ ), MCH ( $36.12 \pm 1.53$  pg,  $26.60 \pm 0.48$  pg,  $P = 0.000$ ), MCHC ( $368.46 \pm 12.28$  g/l,  $318.92 \pm 7.33$  g/l,  $P = 0.002$ ), Platelets ( $261.75 \pm 22.71 \times 10^9/L$ ,  $246.16 \pm 9.93 \times 10^9/L$ ,  $P = 0.000$ ), ESR ( $7.03 \pm 1.38$  mm/hr,  $59.52 \pm 6.46$  mm/hr,  $P = 0.000$ ) and no significant difference ( $P > 0.05$ ) in Eosinophil ( $2.30 \pm 1.05\%$ ,  $2.16 \pm 0.82\%$ ,  $P = 1.000$ ), %TSA ( $25.16 \pm 3.18\%$ ,  $25.61 \pm 2.22\%$ ,  $P = 1.000$ ) when compared between Control and Non ART HIV positive subjects. The results of the correlations of hepcidin in control showed inverse relationship to lymphocytes ( $-0.118$ ,  $P = 0.749$ ), monocytes ( $-0.046$ ,  $P = 0.035$ ), RBC ( $-0.0299$ ,  $P = 0.036$ ), Hemoglobin ( $-0.297$ ,  $P = 0.038$ ), IL-6 to lymphocytes ( $0.0267$ ,  $P = 0.066$ ), monocytes ( $-0.099$ ,  $P = 0.494$ ), RBC ( $-0.007$ ,  $P = 0.912$ ), haemoglobin ( $-0.005$ ,  $P = 0.975$ ), IL-10 to lymphocytes ( $-0.220$ ,  $P = 0.124$ ),  $IFN-\gamma$  to RBC ( $0.225$ ,  $P = 0.116$ ), haemoglobin ( $0.224$ ,  $P = 0.118$ ). The correlations showed significant relationship when hepcidin was correlated to monocytes ( $-0.046$ ,  $P = 0.035$ ), RBC ( $-0.299$ ,  $P = 0.036$ ), Haemoglobin ( $-0.297$ ,  $P = 0.038$ ). The results showed negative correlations in hepcidin of non ART HIV positive subjects to monocytes ( $-0.083$ ,  $P = 0.757$ ), RBC ( $-0.065$ ,  $P = 0.884$ ), IL-10 to lymphocytes ( $-0.220$ ,  $P = 0.291$ ), monocytes ( $-0.243$ ,  $P = 0.242$ ),  $IFN-\gamma$  to monocytes ( $-0.125$ ,  $P = 0.550$ ), RBC ( $-0.139$ ,  $P = 0.509$ ), haemoglobin ( $-0.0098$ ,  $P = 0.641$ ) and positive correlations in hepcidin to lymphocytes ( $0.189$ ,  $P = 0.694$ ), haemoglobin ( $0.031$ ,  $P = 0.884$ ), IL-6 to lymphocytes ( $0.394$ ,  $P = 0.051$ ), monocytes ( $0.025$ ,  $P = 0.907$ ), RBC ( $0.094$ ,  $P = 0.654$ ), haemoglobin ( $0.020$ ,  $P = 0.926$ ),  $IFN-\gamma$  to lymphocytes ( $0.146$ ,  $P = 0.487$ ). There was no significant correlation in non ART HIV positive subjects. There was increase in the levels of cytokines studied, decrease in iron status of the Non ART HIV positive patients and wide variations in some of the haematological parameters studied. This shows that these cytokines could be a diagnostic marker as acute phase reactants that manifested in the course of the infection.

**Key words:** Interferon-Gamma, Comparative Analysis, hepcidin, Haematological Parameters of Control.

## INTRODUCTION

Human Immunodeficiency Virus (HIV) is reported as a lentivirus that leads to acquired Immunodeficiency Syndrome (AIDS) [1]. This is a situation in humans in which progressive failure of the immune system allows life challenging opportunistic infection and cancers to survive. Human Immunodeficiency Virus is a public health challenge that threatens the existence of human race especially in the developing countries where it has caused a lot of havoc in the lives of the populace.

Infection with HIV happens by the transfer of blood, semen, vaginal fluid, pre-ejaculate or breast milk of the infected person to HIV free person. Within these body fluids, HIV is present as both free virus particles and virus within infected immune cells. The 4 main channels of transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth [2].

HIV infects a lot of different tissues in humans including the marrow, lymph node, brain, skin and bowel [3]. Acute HIV is usually characterized by fever, malaise, lymphadenopathy and rash. These situations are subclinical. A chronic infection of AIDS that follows is asymptomatic in acute phases. If a person is infected with this virus, the virus works so fast damaging the immune system causing the person susceptible to little infections. HIV is pandemic infection and the long term consequences of this pandemic will affect every country one way or another over time. This is a progressive pandemic challenging world public health and health care provision, as well as political and economic stability [4].

A variation number of AIDs deaths happen in sub Saharan African slowing economic growth and increasing the burden of poverty [5].

The prognosis of HIV infected persons is quite different in many adults; the average incubation time to the development of AIDs is 10-11 years<sup>6</sup>. However, in certain persons (about 20%) AIDS is shown within 65 years of infection yet another 12% of persons remain free of AIDs for years<sup>7</sup>. A variable used for prognostication is the amount of HIV-1 RNA in the plasma soon after seroconversion. Shortly after entry into the body, there is a burst viremia.

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 [8].

Peptide hepcidin is a key iron-regulatory hormone [9], which is produced from hepatocytes in connection to iron and oxygen. Interestingly, inflammation stimulates hepcidin production, enhanced by the inflammatory cytokine IL-6. This leads to sequestration of Fe in the stores and Fe-limited erythropoiesis, and eventually anaemia of inflammation [9]. The study will determine hepcidin level and IL-6 and correlate them to haemoglobin and PCV. This will help to discover the role of the co 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<sup>10</sup>. It plays a key role in the acute phase response and in the transition from acute to chronic inflammation [11]. Evidence has accrued to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory diseases<sup>10, 12</sup>. Human immunodeficiency virus (HIV) infection has long been shown to induce expression and secretion of IL-6 [13, 14]. This study will find out the changes that may be associated to the IL-6 levels in HIV infection. IL-6 is known to exhibit multifactorial function. It will be important to determine the changes the infection could cause to this cytokine.

The study was done to determine the level of interferon-gamma, IL-6, IL-10, iron status, hepcidin, CD4 and some haemological parameters of Apparently healthy individuals and Non ART HIV positive subjects in South East, Nigeria.

## MATERIAL AND METHODS

### Study Area

This study was carried out at Federal Medical Centre, Umuahia, located in South-Eastern Nigeria and serve patients of high, middle and lower socio-economic status and with Ibo as the dominant tribe.

### Study Population and Enrolments

Group A: 50 control subjects.

Group (B): Naïve HIV positive subjects (n=50),

### Selection Criteria

#### INCLUSION

- Subjects of both sexes aged 18-60 years positive for HIV who were not on antiretroviral treatment were included in the study.
- Those that gave consent were also included.

#### EXCLUSION

The following subjects were excluded

- Pregnant women
- Diabetes mellitus patients
- Persons below 18 years and above 60 years
- Those that did not give consent.

### Sample Collection

Seven milliliters (7ml) of venous blood was collected from each subject and 2.5ml was dispensed into bottles containing di-potassium salt of ethylenediamine tetra-acetic acid (K<sub>2</sub>-EDTA) at a concentration of 1.5mg/ml of blood and was used for full blood count, CD4 count and HIV screening.

Also, 4.5ml 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 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 Nelsn and GeneXpert were done in Federal Medical Centre, Umuahia, Abia State, Nigeria.

### LABORATORY PROCEDURES

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

## DETERMINATIONS

### Determination of CD4 count by flowcytometry (Partec Cyflow counter)

#### 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.

### Full blood count by automation using Mindray BC-5300

#### 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.

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 2.5ml of iron buffer reagent was added to all the labeled tubes. Also, 0.5ml 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 eas read and value will be recorded (A1 reading). Then, 0.5ml of iron reagent was added to all the tubes and was mixed properly. The tubes were palced in a heating bath at 37<sup>0</sup>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) =  $\frac{A2 \text{ Test} - A1 \text{ Test} \times \text{Con of } A2 \text{ std}}{A1 \text{ std}}$

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

### 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 0.2ml of unsaturated iron binding capacity buffer reagent was added to all the tubes according to the sample number, while 10ml of iron free water was added to standard tube and was properly mixed. To the test 0.5ml of sample and 0.5ml 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 0.5ml iron standard tube and was properly mixed. To the test, 0.5ml of sample and 0.5ml 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

$$\text{UIBC } (\mu\text{g/dl}) = \frac{\text{Conc. Of std-A2 Test-A1 Test}}{\text{A2 std-A1 std}} \times \text{Conc. Of std}$$

$$\text{TIBC } (\mu\text{g/dl}) = \text{Iron} + \text{UIBC}$$

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

#### **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.

#### **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 immunoglobulin 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.

#### **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.

#### **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°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.

#### **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 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.

#### Human Interleukin 10 (IL-10) Assay by commercial ELISA Kit by MEL SIN 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<sup>0</sup>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<sup>0</sup>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.

#### Human Hecpidin (Hepc) ELISA Kit by MEL SIN Medical Co Limited was used with Catalogue Number: EKHU-1674

##### 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<sup>0</sup>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<sup>0</sup>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.

##### Statistical Analysis

Date 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.

## RESULT

**Table-1: Comparism of Mean±SD Values Of Interferon-Gamma, IL-6, IL-10, CD4, Hecpidin, Iron, and Some Haematological Parameters of Control and Non Art HIV Positive Subjects**

Parameters	Control	NON ART	P-value
IFN-γ(pg/ml)	16.25±0.87	29.31±1.44	0.000*
IL-6(pg/ml)	7.98±0.22	11.08±1.21	0.000*
IL-10(pg/ml)	8.52±0.62	16.62±1.53	0.000*
CD4(cells/l)	1045.54±247.24	195.60 ±35.94	0.000*
Hecpidin(ng/ml)	6.03±1.38	39.59 ±4.50	0.000*
Iron(µg/dl)	86.29±7.27	73.43±5.45	0.000*
TIBC(µg/dl)	345.56±28.40	287.19 ±8.21	0.000*
%TSA(%)	25.16±3.18	25.61±2.22	1.000 <sup>NS</sup>
WBC(X 10 <sup>9</sup> /L)	5.87 ±0.88	4.69±0.72	0.000*
Neu(%)	60.57±2.83	75.16±3.68	0.000*
Lym(%)	30.69 ±2.84	17.24±2.50	0.000*
Mon(%)	5.59 ±1.2	4.18±1.12	0.000*
Eos(%)	2.30 ±1.05	2.16±0.82	1.000 <sup>NS</sup>
Bas(%)	0.86 ±0.39	1.31±0.94	0.003*
RBC( X 10 <sup>12</sup> /L)	4.92±0.30	3.34±0.21	0.000*
Hb(g/dl)	14.75±0.90	10.05±0.65	0.000*
PCV(%)	44.25±2.70	30.14±1.95	0.000*
MCV(fl)	89.92±2.3	79.49±1.28	0.000*
MCH(pg)	36.12±1.53	26.60±0.48	0.000*
MCHC(g/l)	368.46±12.28	318.92±7.33	0.000*

Plt( $\times 10^9/L$ )	261.75 $\pm$ 22.71	246.16 $\pm$ 9.93	0.002*
ESR(mm/hr)	7.03 $\pm$ 1.38	59.52 $\pm$ 6.46	0.000*

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

The results showed difference that was statistically significant (P<0.05) in IFN- $\gamma$  (16.25 $\pm$ 0.87pg/ml, 29.31 $\pm$ 1.44pg/ml, P=0.000), IL-6 (7.98 $\pm$ 0.22pg/ml, 11.08 $\pm$ 1.21pg/ml, P=0.000) IL-10 (8.52 $\pm$ 0.62pg/ml, 16.62 $\pm$ 1.53pg/ml, P=0.000), CD4 (1045.54  $\pm$ 247.24Cells/L, 195.60  $\pm$ 35.94Cells/L, P=0.000), hepcidin (6.03 $\pm$ 1.38ng/ml, 39.59  $\pm$ 4.50ng/ml, P=0.000), Iron (86.29 $\pm$ 7.27  $\mu$ g/dl, 73.43 $\pm$ 5.45  $\mu$ g/dl, P=0.000), TIBC (345.56 $\pm$ 28.40  $\mu$ g/dl, 287.19  $\pm$ 8.21 $\mu$ g/dl, P=0.000), WBC (5.87  $\pm$ 0.88  $\times 10^9/L$ , 4.69 $\pm$ 0.72 $\times 10^9/L$ , P=0.000), Neutrophils (60.57 $\pm$ 2.83%, 75.16 $\pm$ 3.68%, P=0.000), Lymphocytes (30.69  $\pm$ 2.84%, 17.24 $\pm$ 2.50%, P=0.000), Monocytes (5.59  $\pm$ 1.2%, 4.18 $\pm$ 1.12%, P=0.000), Basophil 0.86

$\pm$ 0.39%, 1.31 $\pm$ 0.94%, P=0.003), RBC (4.92 $\pm$ 0.30  $\times 10^{12}/L$ , 3.34 $\pm$ 0.21  $\times 10^{12}/L$ , P=0.000), Haemoglobin (14.75 $\pm$ 0.90g/dl, 10.05 $\pm$ 0.65g/dl, P=0.000), PCV (44.25 $\pm$ 2.70%, 30.14 $\pm$ 1.95, P=0.000), MCV (89.92 $\pm$ 2.3fl, 79.49 $\pm$ 1.28fl, P=0.029), MCH (36.12 $\pm$ 1.53pg, 26.60 $\pm$ 0.48Pg, P=0.000), MCHC (368.46 $\pm$ 12.28g/l, 318.92 $\pm$ 7.33g/l, P=0.002), Platelets (261.75 $\pm$ 22.71  $\times 10^9/L$ , 246.16 $\pm$ 9.93  $\times 10^9/L$ , P=0.000), ESR (7.03  $\pm$ 1.38mm/hr, 59.52  $\pm$ 6.46mm/hr, P=0.000) and no significant difference (P>0.05) in Eosinophil (2.30  $\pm$ 1.05%, 2.16 $\pm$ 0.82%, P=1.000), %TSA (25.16 $\pm$ 3.18%, 25.61 $\pm$ 2.22%, P=1.000) when compared between Control and Non ART HIV positive subjects.

**Table-2: Correlation of Hepcidin, IL-6, IL-10 and IFN- $\Gamma$  to Lymphocytes, Monocytes, RBC and Haemoglobin among the Control**

Parameters	Analysis	Lymph	Mon	RBC	Hb
Hepcidin	r	-0.118	-0.046	-0.299	-0.297
	P-Value	0.749	0.035	0.036	0.038
IL-6	r	-0.267	-0.099	-0.007	-0.005
	P-Value	0.066	0.494	0.962	0.975
IL-10	r	-0.220	0.254	0.130	0.129
	P-Value	0.124	0.076	0.368	0.368
IFN- $\gamma$	r	-0.019	-0.116	0.225	0.224
	P-Value	0.897	0.421	0.116	0.118

Where, r= correlation coefficient

The results of the correlations of hepcidin in control showed inverse relationship to lymphocytes (-0.118, P=0.749), monocytes (-0.046, P=0.035), RBC (-0.0299, P=0.036, Hemoglobin (-0.297, P=0.038), IL-6 to lymphocytes (0.0267, P=0.066), monocytes (-0.099, P= 0.494), RBC (-0.007, P=0.912), haemoglobin (-

0.005, P=0.975), IL-10 to lymphocytes (-0.220, P=0.124), IFN- $\gamma$  to RBC (0.225, P=0.116), haemoglobin (0.224, P=0.118). The correlations showed significant relationship when hepcidin was correlated to monocytes (-0.046, P=0.035), RBC (-0.299, P=0.036), Haemoglobin (-0.297, P=0.038).

**Table-3: Correlation of Hepcidin, IL-6, IL-10 and IFN- $\Gamma$  to Lymphocytes, Monocytes, RBC and Haemoglobin among the Non Art HIV Positive Subjects**

Parameters	Analysis	Lymph	Mon	RBC	Hb
Hepcidin	r	0.189	-0.083	-0.065	0.031
	P-Value	0.694	0.757	0.884	0.884
IL-6	r	0.394	0.025	0.009	0.055
	P-Value	0.051	0.907	0.965	0.795
IL-10	r	-0.220	-0.243	0.094	0.020
	P-Value	0.291	0.242	0.654	0.926
IFN- $\gamma$	r	0.146	-0.125	-0.139	-0.098
	P-Value	0.487	0.550	0.509	0.641

The results showed negative correlations in hepcidin of non ART HIV positive subjects to monocytes (-0.083, P=0.757), RBC (-0.065, P=0.884), IL-10 to lymphocytes (-0.220, P= 0.291), monocytes (-0.243, P=0.242), IFN- $\gamma$  to monocytes (-.125, P=0.550), RBC (-0.139, P=0.509), haemoglobin (-0.098, P=0.641) and positive correlations in hepcidin to

lymphocytes (0.189, P=0.694), haemoglobin (0.031, P=0.884), IL-6 to lymphocytes (0.394, P=0.051), monocytes (0.025, P=0.907), RBC (0.094, P=0.654), haemoglobin (0.020, P=0.926), IFN- $\gamma$  to lymphocytes (0.146, P=0.487). There was no significant correlation in non ART HIV positive subjects.

## DISCUSSION

Interleukin 6 and IL-10 increased in HIV non ART compared to the control. This shows that IL-6 and IL-10 are part of the acute cytokines released in the course of HIV infection. Interleukin 10 (IL-10) is one of the most important anti-inflammatory cytokines reported to affect multiple cell types, including macrophages, monocytes, dendritic cells, CD4 T cells and CD 8 T cells [28]. The dominant function of IL-10 is to down-regulate the immune response and limit tissue injury. However, the excessive production of this cytokine directly inhibits CD4+ T cells responses which may result in a failure to control the infection [15].

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. Hepcidin increased in the HIV non ART patients compared to the control which is acute reactant protein that regulate the level of iron in the patients which affect the outcome of HIV infection. Hepcidin is the main hormone that regulates the synthesis and release of iron in the body. Hepcidin is an acute phase reactant peptide that is the central regulator of iron homeostasis, and its expression is modulated by several factors, including body iron status and hypoxia [16]. Similarly, infections and inflammation may stimulate hepcidin expression by hepatocytes, a process that is mediated via proinflammatory cytokines, usually interleukin 6 (IL-6), and signaling through the STAT-3 pathway [17, 18]. Hepcidin leads the process of ACD by causing iron to be diverted from the circulation and sequestered within cells of the reticuloendothelial system and by inhibiting duodenal absorption of iron. Thus, as a consequence of inflammation, hepcidin restricts the availability of iron for incorporation into erythroid progenitor cells [19].

Although diminished work capacity due to reduced haemoglobin levels is the best described functional consequence of poor iron status, other outcomes include diminished intellectual performance, altered body temperature regulation, and reduced immunity and resistance to infections [20]. Alterations in iron homeostasis have been shown in HIV infected subjects. Epidemiological works have reported a link between raised iron status, HIV progression and the challenge for opportunistic infections [21]. Elevated hepcidin levels limit iron availability to the bone marrow. HIV activates the progression of any of them to a serious stage with serious alterations in immunological and haematological systems of the patients. This may be associated to HIV-associated anaemia, which is a usual complication of progressed HIV infection with adverse effect on the prognosis and quality of life [22]. HIV can directly infect bone marrow progenitor cells leading to bone marrow suppression, which is associated with upregulated hepcidin expression [23].

The decrease in WBC count in patients with non ART HIV positive patients may be due suppressive effects of HIV infection on the bone marrow, affecting production of WBC [24]. This portrays that HIV has a direct attack on the white cells and improves with treatment. A reduction in lymphocyte may show advanced HIV infection, reflect depletion of CD4 T lymphocytes, enhanced immunosuppression and anaemia [25, 26].

The non ART HIV patients showed no significant decrease among ART HIV. The results of hepcidin showed negative relationship to lymphocytes, monocytes, RBC and haemoglobin in control subjects. This shows that hepcidin is regulated in negative feedback mechanism with lymphocytes, monocytes, RBC and haemoglobin. This process is highly needful in anaemia and inflammation as a regulatory factor of hepcidin. The same thing is applicable to IL-6 which showed inverse relationship with lymphocytes, monocytes, RBC and haemoglobin. This similar relationship of hepcidin and IL-6 could be as a result of the regulatory role of IL-6 on hepcidin in iron homeostasis that in turn regulates erythropoiesis.

This shows that in apparently healthy individuals, lymphocytes have negative relationship with hepcidin and other haematological variables like monocytes, RBC and haemoglobin maintain positive feedback on the association to IL-10. Interferon-gamma maintains negative feedback mechanism to lymphocytes and monocytes. This feedback mechanism could be seen in control as there changes could cause serious alterations in the immunological and haematological systems of the subjects.

The study in non ART HIV positive subjects revealed negative relationship when hepcidin was correlated to monocytes and red blood cells even though the relationship was not significant. The results also showed positive correlation to lymphocytes and haemoglobin. Increase in hepcidin has a corresponding increase in haemoglobin and lymphocytes likewise as increase in lymphocytes and haemoglobin increases in the level of hepcidin even though the relationships were not significant. The results of IL-6 showed positive relationships to all the haematological variables including lymphocytes, monocytes, red blood cells and haemoglobin unlike negative relationship observed in the control. This shows that HIV infection can adversely affect the levels of lymphocytes, monocytes, red blood cells and haemoglobin. Interleukin 6 (IL-6) is an important cytokine that is pleiotropic in nature. Interleukin 10 (IL-10) showed negative relationship when correlated to lymphocytes and monocytes. The cytokine could be produced in the lymphocytes and monocytes and should be regulated to avoid hyperactivity of the cytokine. The study showed positive relationship indicating a strong association in the level of IL-10 to red blood cells and haemoglobin.

The relationships were not significant. The results showed inverse relationships to monocytes, red blood cells and haemoglobin except to lymphocytes which is not the same pattern in control. The results showed negative correlations of IFN- $\gamma$  in non ART HIV positive subjects to monocytes, haemoglobin and positive correlations in IFN- $\gamma$  to lymphocytes. There was no significant correlation in non ART HIV positive subjects. IFN- $\gamma$  induces the transcription of more than 200 genes in macrophages, including those for the production of antimicrobial molecules such as oxygen free radicals and nitric oxide, which represent one of the best effector mechanisms for elimination of *M. tuberculosis* [27].

## CONCLUSION

The study shows that interferon gamma, interleukin 6, interleukin 10 and hepcidin are some of the biomarkers in the pathogenesis of HIV. The study has shown wide variations in the haematological indices studied. The red blood cell, packed cell volume and haemoglobin were suppressed. Anaemia is a major factor causing morbidity and mortality in the patients. These parameters should be monitored in the patients infected with HIV as they particularly the cytokines and the hepcidin were shown to be elevated in the patients.

## REFERENCES

- Weiss RA. How does HIV cause AIDS. *Nature*; 1993, 460(7256): 711-716.
- Fox CH, Cottler-Fox M. The pathology of HIV infection. *Immunology. Today*; 1992, 13: 353.
- Baker FJ, Silverton RE, Pallister CJ. Haemostasis in *Introduction to Medical Laboratory Technology*. 7<sup>th</sup>ed, Honeyland Resources limited. 2007, 387.
- Kuby J. The immune system in AIDS. In *Immunology: 3<sup>rd</sup> edition*; Freeman and Company. 1997, 523-541.
- Greener R. AIDS and macroeconomic input, 4<sup>th</sup>ed, *State of the Art: AIDS and economic IAEN*. 2002, 49-55.
- Hogg RS, Yip B, Chan KJ, Wood E, Craib KJ, O'Shaughnessy MV, Montaner JS. Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy. *Jama*. 2001 Nov 28;286(20):2568-77.
- Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, Hahn BH, Wolinsky S, Bhattacharya T. Timing the ancestor of the HIV-1 pandemic strains. *science*. 2000 Jun 9;288(5472):1789-96.
- Zhang DL, Hughes RM, Ollivierre-Wilson H, Ghosh MC, Rouault TA. A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. *Cell metabolism*. 2009 May 6;9(5):461-73.
- Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. *Annu. Rev. Nutr.*. 2006 Aug 21;26:323-42.
- Tanaka T, Kishimoto T. The biology and medical implications of interleukin-6. *Cancer immunology research*. 2014 Apr 1;2(4):288-94.
- Kaplanski G, Marin V, Montero-Julian F, Mantovani A, Farnarier C. IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends in immunology*. 2003 Jan 1;24(1):25-9.
- Atreya R, Mudter J, Finotto S, Müllberg J, Jostock T, Wirtz S, Schütz M, Bartsch B, Holtmann M, Becker C, Strand D. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo. *Nature medicine*. 2000 May;6(5):583-588.
- Neuhaus J, Jacobs Jr DR, Baker JV, Calmy A, Duprez D, La Rosa A, Kuller LH, Pett SL, Ristola M, Ross MJ, Shlipak MG. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *The Journal of infectious diseases*. 2010 Jun 15;201(12):1788-95.
- Armah KA, McGinnis K, Baker J, Gibert C, Butt AA, Bryant KJ, Goetz M, Tracy R, Oursler KK, Rimland D, Crothers K. HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. *Clinical Infectious Diseases*. 2012 Apr 24;55(1):126-36.
- Sharma SK, Mohan A, Kadiravan T. HIV-TB co-infection: epidemiology, diagnosis & management. *Indian J Med Res*. 2005 Apr 1;121(4):550-67.
- Piperno A, Mariani R, Trombini P, Girelli D. Hepcidin modulation in human diseases: from research to clinic. *World journal of gastroenterology: WJG*. 2009 Feb 7;15(5):538.
- Armitage AE, Eddowes LA, Gileadi U, Cole S, Spottiswoode N, Selvakumar TA, Ho LP, Townsend AR, Drakesmith H. Hepcidin regulation by innate immune and infectious stimuli. *Blood*. 2011 Oct 13;118(15):4129-39.
- Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2012 Sep 1;1823(9):1434-43.
- Weiss G, Goodnough LT. Anemia of chronic disease. *New England Journal of Medicine*. 2005 Mar 10;352(10):1011-23.
- Yip R, Dallman PR, Ziegler EE, Filer L. Present knowledge in nutrition. *Iron*, 7th ed.; Ziegler, EE, Filer, LJ, Eds. 1996:278-92.
- McDermid JM, Jaye A, van der Loeff MF, Todd J, Bates C, Austin S, Jeffries D, Awasana AA, Whittle HC, Prentice AM. Elevated iron status strongly predicts mortality in West African adults with HIV infection. *JAIDS Journal of Acquired Immune Deficiency Syndromes*. 2007 Dec 1;46(4):498-507.
- Wisaksana R, Sumantri R, Indrati AR, Zwitser A, Jusuf H, de Mast Q, van Crevel R, Van Der Ven A. Anemia and iron homeostasis in a cohort of HIV-

- infected patients in Indonesia. *BMC infectious diseases*. 2011 Dec;11(1):213.
23. Kanda J, Mizumoto C, Kawabata H, Tsuchida H, Tomosugi N, Matsuo K, Uchiyama T. Serum hepcidin level and erythropoietic activity after hematopoietic stem cell transplantation. *haematologica*. 2008 Oct 1;93(10):1550-1554.
24. Moyle G. Anaemia in persons with HIV infection: prognostic marker and contributor to morbidity. *AIDS reviews*. 2002;4(1):13-20.
25. Rajasekaran S, Jeyaseelan L, Vijila S, Gomathi C, Raja K. Predictors of failure of first-line antiretroviral therapy in HIV-infected adults: Indian experience. *Aids*. 2007 Jul 1;21:S47-53.
26. Kanya MR, Semitala FC, Quinn TC, Ronald A, Nyama-Meya D, Mayanja-Kizza H, Katabira ET, Spacek LA. Total lymphocyte count of 1200 is not a sensitive predictor of CD4 lymphocyte count among patients with HIV disease in Kampala, Uganda. *African health sciences*. 2004;4(2):94-101.
27. Cooper AM. Cell-mediated immune responses in tuberculosis. *Annual review of immunology*. 2009 Apr 23;27:393-422.
28. Moore M. Empowerment at last?. *Journal of International Development*. 2001 Apr;13(3):321-9.