



# **Studies on Some Cytokines, CD4, Hcpidin, Iron, and Some Haematological Parameters of Pulmonary Tuberculosis Patients Co-infected with Human Immunodeficiency Virus on Chemotherapy for 60 Days in Southeast, Nigeria**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author OE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OGU, EBN and AAA managed the analyses of the study. Authors AFN, EIS and OOC 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 2000 subjects aged 18-60 years were enlisted for this study. The subjects were grouped into: Group A (100 PTB-HIV subjects on baseline) and Group B (100 PTB-HIV subjects on treatment for 60 days). About 7ml of venous blood were collected from each subject; 4.5 ml of blood were placed into plain tubes for assay of interferon gamma, interleukins (6 &10), hepcidin and iron and 2.5 ml 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 significant difference in IFN- $\gamma$  ( $P=0.000$ ), IL-6 ( $P=0.000$ ) IL-10 ( $P=0.000$ ), hepcidin ( $P=0.000$ ), Iron ( $P=0.004$ ), TIBC ( $P=0.000$ ), WBC ( $P=0.029$ ), Neutrophils ( $P=0.011$ ), Lymphocytes ( $P=0.000$ ), Monocytes ( $P=0.000$ ), Basophil ( $P=0.013$ ), RBC ( $P=0.000$ ), Haemoglobin ( $P=0.000$ ), PCV ( $P=0.000$ ), MCV ( $P=0.029$ ), MCH ( $P=0.000$ ), MCHC ( $P=0.000$ ), Platelets ( $P=0.000$ ), ESR ( $P=0.000$ ) and no significant difference in CD4 ( $P=0.966$ ), %TSA ( $P=0.998$ ) and Eos ( $P=0.052$ ) when compared among PTB-HIV on baseline and 60 days on treatment respectively. The study shows that interferon gamma, interleukin 6, interleukin 10 and hepcidin are adjuncts to some of the biomarkers in the pathogenesis of pulmonary TB and HIV infection. The cytokines and hepcidin can be used as prognostic and diagnostic markers as their levels decreased with 60 days of treatment of the patients. The haematological parameters like haemoglobin, RBC and PCV increased significantly within 60 days of treatment showing improvement in health status of the patients.

**Keywords:** *Interferon-gamma; interleukin 6; interleukin 10; CD4; hepcidin; iron and some haematological parameters of pulmonary tuberculosis patients coinfecting with human immunodeficiency virus; chemotherapy on 60.*

## 1. INTRODUCTION

Pulmonary tuberculosis (TB) is a chronic bacterial disease caused by *Mycobacterium tuberculosis* (MTB) complex which commonly affects the lungs; (pulmonary TB (PTB), but can affect other sites as well; (extra-pulmonary TB (EPTB) as opined by Thumamo *et al.* (2012). *Mycobacterium tuberculosis*, the bacterium that causes human pulmonary tuberculosis disease, is an old enemy. Pulmonary tuberculosis (PTB) is a global public health problem and is the second leading cause of death. All inclusive, the disease takes a life every 20 seconds [1], Yang *et al.*, (2015). Although much progress has been made with regard to the control measures, the World Health Organization estimated that 9 million people developed tuberculosis in 2013 and that 1.5 million deceased, including 360,000 people who were infected with human immunodeficiency virus (Pai and Schito, 2015). Pulmonary tuberculosis is a major public health problem in Nigeria with an estimated prevalence of 616 cases per 100,000. Nigeria ranks first in Africa, and fourth among the 22 high pulmonary TB burden countries in the world, and no fewer than 460,000 cases of pulmonary

TB are reported annually in Nigeria (WHO, 2008).

Human immunodeficiency virus infection is the single most important factor for the resurgence of pulmonary TB globally and the major reason for failure to achieve set pulmonary tuberculosis control targets especially in areas with high prevalence (Okonkwo *et al.*, 2013). Hospital records show that pulmonary tuberculosis and HIV have synergistic interactions that speedily accelerate the decline of the host immune system, accentuating the progression of each other (Obeagu, E. I., et al., 2019). Pulmonary tuberculosis -HIV co-infection, the presence of the two diseases at the same time in a patient, presently poses serious and major public health challenges especially in the African region, including Nigeria. Globally, some 14 million people are estimated to have pulmonary TB- HIV co-infection with the dual epidemics being particularly pervasive in Africa due to the high incidence of HIV in this region [2].

The co infection has profound effects on the immune system, as it is capable of disarming the host's immune responses [2]. Human

Immunodeficiency virus co-infection is the most powerful known risk factor for progression of *Mycobacterium tuberculosis* infection to active disease, increasing the risk of latent pulmonary tuberculosis reactivation 20-fold [2] and that is why this study was done to consider some of these major cytokines to understand what happens to immunological system for 60 days of treatment which has direct or indirect effects on the haematological parameters.

There are few reports on host iron status at the time of pulmonary tuberculosis diagnosis [3]. Friis *et al.* [4] in their study reported iron limited erythropoiesis and anaemia of inflammation during infections. According to them, infection often precipitates a substantial acute protein which leads to the sequestration of iron. In this study, iron status was determined to find out the impact of pulmonary tuberculosis with HIV co infection on iron status for 60 days treatment which may have a role in the pathogenesis of the infection. Cytokines are important immunomodulating agents of immune system. Human immunodeficiency virus co infection has been suggested to alter blood cell populations and change Th1/Th2 balance (Zhang *et al.*, 2009), which affects the course of pulmonary tuberculosis, clinical presentation, signs and symptoms [5], leading to misdiagnosis or delay in diagnosis of pulmonary tuberculosis (Ngowi *et al.*, 2008).

Peptide hepcidin, is a key iron-regulatory hormone (Nemeth and Ganz, 2006), 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 (Nemeth and Ganz, 2006). The study will determine hepcidin level and IL-6. This will help to discover the role of the co infection for 60 days of treatment 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 (Tanaka and Kishimoto, 2014). Evidence has accrued to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory diseases (Tanaka and Kishimoto, 2014; [6]. Human immunodeficiency virus (HIV) infection has long been shown to induce expression and secretion

of IL-6 (Neuhaus *et al.*, 2010) [7]. This study will find out the changes that may be associated to the IL-6 levels in pulmonary tuberculosis patients with HIV coinfection for 60 days of treatment. It will be important to determine the changes the co infection could cause to this cytokine for a period of 60 days treatment. The lack of reliable biomarkers to indicate or predict the different clinical outcomes of *M. tuberculosis* infection has been given as a key reason for the failure of developing new diagnostic and prognostic tools, drugs and vaccines against tuberculosis [8].

The study was done to determine the levels of interferon-gamma, interleukin 6, interleukin 10, iron status, hepcidin and haematological parameters of patients with pulmonary tuberculosis co-infected with human immunodeficiency virus on 60 days chemotherapy in a tertiary hospital in Southeast, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This study was carried out at the directly observed treatment-short course Tuberculosis (TB DOTS) centre of Federal Medical Centre, Umuahia.

### 2.2 Study Population and Enrolments

A total of two hundred (200) subjects aged 18-60 years were enlisted for this study. The participants were recruited by purposive sampling technique. The HIV and pulmonary tuberculosis subjects were recruited from the tuberculosis directly observed treatment, short course (TB-DOTS) clinic and HIV clinic based on sputum smear acid fast bacilli by Ziehl Neelsen's stain and GeneXpert MTB/RIF assay and HIV screening tests (Obeagu, E. I., et al., 2019). The subjects were grouped into:

Group A: 100 PTB-HIV subjects on baseline

Group B: 100 PTB-HIV subjects on 60 days of treatment

### 2.3 Selection Criteria

#### 2.3.1 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.

### 2.3.2 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<sub>2</sub>-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 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 Nelson 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. Ziehl-Nelson Technique for *Mycobacterium tuberculosis* diagnosis [9]

###### Procedure

**Smear preparation:** A piece of clean stick was used to transfer and spread sputum materials evenly covering an area of about 15-20 mm diameter on a glass slide. The smear was air dried and labeled.

**Heat fixation:** The slide with the smear uppermost was rapidly passed three times through the flame of a Bunsen burner and was allowed to cool.

**Ziehl-Nelson Staining:** The slide containing the smear was placed on a slide rack and the smear covered with carbol fuschin stain. The stain was heated until vapour just begins to rise. The heated stain was allowed to remain on the slide for 5 minutes. The stain was washed off with clean water and then covered with 3% v/v acid alcohol for 5 minutes or until smear is sufficiently decolourised, that is pale pink. The slide was washed off with clean water. The smear was covered with Methylene blue stain for 2 minutes and then washed off with clean water. The back of the slide was wiped clean and placed in a draining rack for the smear to air dry.

***Mycobacterium tuberculosis* diagnosis:** The smear was examined microscopically using the X100 oil immersion objective. Scanning of the smear was done systematically and when any definite red bacillus is seen, it was reported as AFB positive.

##### B. GeneXpert method for detection of *Mycobacterium tuberculosis* and Rifampicin resistance (GeneXpert MTB/FIF)

###### Procedure

The assay consists of a single-use multi-chambered plastic cartridge pre-loaded with the liquid buffers and lyophilised reagent beads necessary for sample processing.

##### DNA extraction and hemi-nested real-time PCR

Sputum samples were treated with the sample reagent (containing NaOH and isopropanol). The sample reagent was added in the ratio of 2:1 to

the sputum sample and the closed specimen container was manually agitated twice during 15 minutes of incubation at room temperature. 2 ml of the treated sample was transferred into the cartridge, the cartridge was loaded into the GeneXpert instrument and automatic step completed the remaining assay steps.

The assay cartridge also contained lyophilized *Bacillus globigii* spores which served as an internal sample processing step and the resulting *B.globigii* DNA was amplified during PCR step. The standard user interface indicates the presence or absence of *Mycobacterium tuberculosis*, the presence or absence of rifampicin resistance and semi quantitative estimate of *Mycobacterium tuberculosis* concentration (high, medium, low and very low). Assays that are negative for *Mycobacterium tuberculosis* and also negative for *B.globigii* internal control was reported as invalid.

#### **C. Determination of CD4 count by flowcytometry (Partec Cyflow counter), Germany**

##### **Procedure**

All required reagents was brought to room temperature and 850 µl 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. 20 µl of CD4 easy count kits (CD4 Mab-PE) were pipetted into different test tubes labeled appropriately for the assay. Then, 20 µl 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.

#### **D. 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.

#### **E. 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 560 nm. 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 placed 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**

$$\text{Serum iron } (\mu\text{g/dl}) = \frac{\text{A2 Test} - \text{A1 Test} \times \text{Con of A2 std}}{\text{A1 std} - \text{A2 std}}$$

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

#### **F. Determination of total iron binding capacity by 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 560 nm 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 560 nm 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 560 nm and another reading was taken as the A2 reading.

#### Calculation

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

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

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

#### G. 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 50 µl 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.

#### H. 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.

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

##### Procedure

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

#### J. 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 240 ng/l, 160 ng/l, 80 ng/l, 40 ng/l and 20 ng/l. 50 µl of standards were pipette into the standard wells. 10 µ of test serum were added into each well. 40 µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, stop solution). 50 µl 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. 50 µl of chromogen solution A and 50 µl of chromogen solution B was added to each well. They were mixed and incubated for 10 minutes at 37°C. 50 µl of stop solution was added to each well. Optical density of the samples was read in a microtiter plate reader at 450 nm 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. 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, 80 ng/l, 40

ng/l and 20 ng/l. 50 µl of standards were pipette into the standard wells. 10 µ of test serum were added into each well. 40 µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, stop solution). 50 µl 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. 50 µl of chromogen solution A and 50 µl 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 450 nm 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. 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 240 ng/l, 160 ng/l, 80 ng/l, 40 ng/l and 20 ng/l. 50 µl of standards were pipette into the standard wells. 10 µ of test serum were added into each well. 40 µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, stop solution). 50 µl 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. 50 µl of chromogen solution A and 50 µl of chromogen solution B was added to each well. They were mixed and incubated for 10 minutes at 37°C. 50 µl of stop solution was added to each well. Optical density of the samples was read in a microtiter plate reader at 450 nm 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.

#### M. 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. 50 µl of standards were pipette into the standard wells. 10 µ of test serum were added into each well. 40 µl of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, stop solution). 50 µl 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. 50 µl of chromogen solution A and 50 µl 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 450 nm 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 was 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 showed significant difference ( $P < 0.05$ ) in IFN- $\gamma$  ( $54.04 \pm 3.54$  pg/ml,  $28.27 \pm 1.35$  pg/ml,  $P = 0.000$ ), IL-6 ( $20.68 \pm 1.47$  pg/ml,  $13.65 \pm 1.35$  pg/ml,  $P = 0.000$ ) IL-10 ( $30.38 \pm 1.59$  pg/ml,  $18.50 \pm 1.11$  Pg/ml,  $P = 0.000$ ), hepcidin ( $66.13 \pm 11.04$  ng/ml,  $45.90 \pm 4.77$  ng/ml,  $P = 0.000$ ), Iron ( $52.09 \pm 5.10$  µg/dl,  $66.16 \pm 4.95$  µg/dl,  $P = 0.004$ ), TIBC ( $230.80 \pm 17.00$  µg/dl,  $278.20 \pm 14.91$  µg/dl,  $P = 0.000$ ), WBC ( $5.13 \pm 0.28$  X  $10^9/L$ ,  $3.96 \pm 0.22$  X  $10^9/L$ ,  $P = 0.029$ ), Neutrophils ( $68.29 \pm 2.46\%$ ,  $63.54 \pm 2.08\%$ ,  $P = 0.011$ ), Lymphocytes ( $13.73 \pm 1.48\%$ ,  $24.80 \pm 1.40\%$ ,  $P = 0.000$ ), Monocytes ( $11.60 \pm 1.57\%$ ,  $6.52 \pm 1.06\%$ ,  $P = 0.000$ ), Basophil

**Table 1. Mean±SD values of interferon-gamma, IL-6, IL-10, CD4, hepcidin, iron, and some haematological parameters of PTB-HIV subjects on baseline and 60 days on treatment**

Parameters	Baseline	60 Days	P-value
IFN-γ(pg/ml)	54.04±3.54	28.27±1.35	0.000*
IL-6(pg/ml)	20.68±1.47	13.65±1.35	0.000*
IL-10(pg/ml)	30.38±1.59	18.50±1.11	0.000*
CD4(cells/l)	183.80±8.78	236.41±18.06	0.996 <sup>NS</sup>
Hepcidin(ng/ml)	66.13±11.04	45.90±4.77	0.000*
Iron(μg/dl)	52.09±5.10	66.16±4.95	0.004*
TIBC(μg/dl)	230.80±17.00	278.20±14.91	0.000*
%TSA(%)	22.59±1.69	23.83 ±2.09	0.998 <sup>NS</sup>
WBC(X 10 <sup>9</sup> /L)	5.13 ±0.28	3.96±0.22	0.029*
Neu(%)	68.29±2.46	63.54±2.08	0.011*
Lym(%)	13.73 ±1.48	24.80±1.40	0.000*
Mon(%)	11.60 ±1.57	6.52±1.06	0.000*
Eos(%)	4.76 ±1.24	10.27±8.85	0.052 <sup>NS</sup>
Bas(%)	1.62 ±0.58	0.87±0.25	0.013*
RBC( X 10 <sup>12</sup> /L)	2.90±0.35	3.91±0.24	0.000*
Hb(g/dl)	7.83±2.95	11.71±0.72	0.000*
PCV(%)	26.07±3.19	35.16±2.12	0.000*
MCV(fl)	71.17±2.06	76.39±3.27	0.000*
MCH(pg)	21.70±1.77	27.67±1.85	0.000*
MCHC(g/l)	299.80±13.00	352.00±6.50	0.000*
Plt(X 10 <sup>9</sup> /L)	119.86±16.98	154.00±27.63	0.000*
ESR(mm/hr)	60.54 ±8.94	40.37±2.52	0.000*

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

(1.62±0.58%, 0.87±0.25%, P=0.013), RBC (2.90±0.35 X 10<sup>12</sup>/L, 3.91±0.24 X 10<sup>12</sup>/L, P=0.000), Haemoglobin (7.83±2.95 g/dl, 11.71±0.72 g/dl, P=0.000), PCV (26.07±3.19%, 35.16±2.12%, P=0.000), MCV (71.17±2.06 fl, 76.39±3.27 fl, P=0.029), MCH (21.70±1.77 pg, 27.67±1.85 pg, P=0.000), MCHC (299.80±13.00 g/l, 352.00±6.50 g/l, P=0.000), Platelets (119.86±16.98 X 10<sup>9</sup>/L, 154.00±27.63 X 10<sup>9</sup>/L, P=0.000), ESR (60.54 ±8.94 mm/hr, 40.37±2.52 mm/hr, P=0.000) and no significant difference (P>0.05) in CD4 (183.80 ±8.78 Cells/L, 236.41±18.06 cells/L, P=0.966), %TSA (22.59±1.69%, 23.83 ±2.09%, P=0.998) and Eos (4.76 ±1.24%, 10.27±8.85%, P=0.052) when compared among PTB-HIV on baseline and 60 days on treatment respectively.

#### 4. DISCUSSION

Interferon gamma has been implicated as the major cytokine that is released in tuberculosis infection. The levels of interferon gamma decreased in the pulmonary TB-HIV subjects on 60 days treatment and were statistically significant. Decrease in interferon gamma levels were observed in this study on the subjects on 60 days 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 (Toossi *et al.*, 2011).

Interleukin 6 is a pleiotropic cytokine and regulates even hepcidin and in turn iron which is crucial to the pathogenesis of pulmonary TB. Transcriptional activation of HIV by proinflammatory cytokine (IL-6) is based on induction of nuclear factors, namely nuclear factor (NF)-KB and NF-IL-6 [10]. In the course of the management of tuberculosis, interleukin 6 is an important cytokine that should be monitored. Immunological response in these infections is very important as the interplay of these cytokines may have prognostic effects on the patients.

This shows that IL-6 may have a major role to play in the pathogenesis as seen in pulmonary TB 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 [11]. Even when virologically suppressed, treated HIV-infected persons have higher plasma levels of IL-6 than well-matched



controls (Neuhaus *et al.*, 2010;[7]. Activated inflammation, as demonstrated by persistently higher IL-6 levels, may have profound and far-reaching clinical implications. The levels of IL-6 in pulmonary TB-HIV on baseline were higher than the values among pulmonary TB-HIV on 60 days treatment. This shows that duration of treatment in pulmonary TB-HIV subjects has a decreasing impact on IL-6. Interleukin 6 levels should be monitored whenever pulmonary TB-HIV patients are required to improve the well-being of the patients. It has been shown that IL-6 is a proinflammatory cytokine that controls different physiological processes (Tnanka and Kishimoto, 2014). Interleukin 6 plays a major role in the acute phase response and in the transition from acute to chronic inflammation (Kaplanski *et al.*, 2003). Evidence has occurred to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory and autoimmune diseases [6]. This can be an adjunct biomarker in the diagnosis and prognostic monitor of the progress of the disease as well as the cure of the disease. It shows that a longer duration will have a more pronounced decreasing effect on the patients to avert drug induced haemolytic anaemia.

Some works have revealed that when tuberculosis infection occurs, a variety of pro and anti-inflammatory cytokines are produced at disease sites and then released into circulation (Munk and Emoto, 1995; [12]. 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 [13]. 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 (Sharma and Bose, 2001). Interleukin 10 is one of the most important anti-inflammatory cytokines reported to inhibit CD4 + T cell responses by inhibiting APC function of cells infected with mycobacteria (Rojas *et al.*, 1999). Interleukin 10 could be an indicator of the presence of these infections. This elevation of IL-10 helps to control the raised inflammatory cytokines to prevent tissue damage in the patients. The free radicals released in the course of these infections may affect the release of these cytokines and in turn affect the well-being of the patients.

The study revealed a decrease in IL-10 level which was statistically significant during

treatment. Another study has shown a consistent decrease in IL-10 levels in active pulmonary TB patients at all time points of therapy, suggesting that patients who maintain high IL-10 levels at the end of treatment are exposed to pulmonary TB-HIV recurrence [14]. Sahiratmadja *et al* also observed reduced IL-10 production during PTB-HIV therapy, suggesting that this cytokine may be a useful biomarker signature to assess the disease progression (Sahiratmadja *et al.*, 2007). Interleukin 10 functions to limit the immune response to pulmonary TB-HIV and may contribute to pulmonary TB-HIV pathogenesis [15]. 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. CD4 is a major immune arm that the body uses in defending itself against these agents.

There was no significant change observed in the CD4 level of pulmonary TB-HIV subgroups based on treatment. This implies that the CD4 level of pulmonary TB-HIV patients may not improve with the duration of treatment.

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 (Piperno *et al.*, 2009). 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 [16], Ganz and Nemeth [17]. 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 (Weiss and Goodnough, 2005). In addition to its central role in iron regulation, hepcidin has antimicrobial properties and appears to have an important role in the innate immune response against *Mycobacterium tuberculosis* (Sow *et al.*, 2011). Emerging evidence suggests a key role for the iron regulator hepcidin in the innate immune

response to *M. tuberculosis* infection (Shin and Jo, 2011).

The results showed a decrease in hepcidin that was statistically significant among pulmonary TB-HIV on baseline compared to PTB-HIV subjects on 60 days of treatment. Hepcidin is an acute phase protein with reduced level with increased duration treatment and that may be linked to the prognosis of treatment in the patients.

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 (Yip and Dallman, 1996).

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 [18]. Elevated hepcidin levels limit iron availability to the bone marrow. The treatment in pulmonary TB-HIV should be prolonged and properly administered as this may not cure the HIV and the presence of any of the infection can activate 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 (Wisaksana et al., 2011). Also, hepcidin-mediated iron accumulation in macrophages may raise the challenge for outgrowth of intracellular pathogens like *Mycobacterium tuberculosis*. Increased hepcidin concentrations in progressed HIV are mainly caused by HIV-associated inflammation. HIV can directly infect bone marrow progenitor cells leading to bone marrow suppression, which is associated with upregulated hepcidin expression [19].

The TIBC of pulmonary TB-HIV on 60 days was higher than pulmonary TB-HIV on baseline of treatment.

The ESR was higher in pulmonary TB-HIV on Baseline than pulmonary TB-HIV subjects on 60 days of treatment. The ESR decrease in the course of the treatment in all the patients. Erythrocyte sedimentation rate has been reported to be raised in infections and

inflammations which could be linked to elevated synthesis of acute phase proteins usually seen in chronic infections and release of proteins by *Mycobacterium tuberculosis* into the circulation. This increase in ESR raises plasma viscosity resulting to insufficient perfusion [20] and implies increased rheology of blood in tuberculosis.

For pulmonary TB-HIV, lymphocytes increased significantly during treatment. This shows that the increase in time level of duration of treatment improves the level of lymphocyte in pulmonary TB co infected with HIV infection. Pulmonary tuberculosis has been reported to independently suppress lymphocytes, thereby worsening HIV-related immunosuppression. Lymphocyte count could be used as a crude measure of disease progression during HIV infection [21]. Other researchers have shown that lymphocyte count could serve as an inexpensive laboratory indicator to help physicians predict treatment failure and also progression to AIDS (Rajasekaran et al., 2007; [22].

The red blood cells, PCV, haemoglobin improved with the 60 days treatment. The possible mechanisms for the development of anaemia during pulmonary TB infection may be due to nutritional insufficiency, impaired iron utilization, malabsorption, bone marrow granuloma and shortened duration of RBC survival [23].

There was no difference that was statistically significant in MCV of the pulmonary TB-HIV patients even during treatment. The study also showed increase in MCH among pulmonary TB-HIV at 60 days. Anaemia in pulmonary TB has been reported to normocytic normochromic [24]; Lomard and Mansuelt, 1989; [25] which is in agreement with this study from the results of MCV and MCH.

The platelets increased among pulmonary TB-HIV at 60 days of treatment. This shows that 60 days of treatment on pulmonary TB-HIV patients has increasing effect on the platelets. This finding contradicts a report from India which suggested that reduction in platelets may be due to the effect of anti-tuberculosis drugs and immune destruction of platelets (Nagu et al., 2014).

## 5. CONCLUSION

The study shows that interferon gamma, interleukin 6, interleukin 10 and hepcidin are adjuncts to some of the biomarkers in the

pathogenesis of pulmonary TB and HIV infection. The cytokines and hepcidin can be used as prognostic and diagnostic markers as their levels decreased with 60 days of treatment of the patients.

The study has shown wide variations in the haematological indices studied. The red blood cell, packed cell volume and haemoglobin were suppressed but improved in the course of treatment. Anaemia is a major factor causing morbidity and mortality in the patients especially pulmonary TB patients co infected with HIV. This will help the Physicians and all health care providers handling pulmonary TB-HIV patients in tackling the challenges of drug failure and enlighten the world on the level of improvement associated to the duration of treatment that are expected to occur in the patients.

### CONSENT AND ETHICAL APPROVAL

With a well detailed research proposal and a letter of introduction from the Head of Department, 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. patients' written consent has been collected and preserved by the author(s).

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

### REFERENCES

1. Divangahi M. The New Paradigm of Immunity to Tuberculosis *Nature*. 2013;4:294.
2. Getahun H, Gunneberg C, Granich R, Nuun. HIV infection-associated tuberculosis: the epidemiology response. *Clinical Infectious Disease*. 2010;50:201-207.
3. Gangaidzo IT, Moyo VM, Mvundura E, Aggrey G, Murphree NL, Khumalo H, Saungweme T, Kasvosve I, Gomo ZA, Rouault T. Association of pulmonary tuberculosis with increased dietary iron. *Journal of Infectious Diseases*. 2001;184:936-939.
4. Friis H, Range N, Kristensen CB, Kæstel P, Changalucha J, Malenganisho W, Krarup H, Magnussen P, Andersen AB. Acute-phase response and iron status markers among pulmonary tuberculosis patients: a cross-sectional study in Mwanza, Tanzania. *British Journal of Nutrition*. 2009;102:310-317.
5. Lado FL, Barriogo'mez E, Arceo EC, Carbarcosortizdebarro'n A. Clinical Presentation of Tuberculosis and the Degree of immunodeficiency in Transducer Glycoprotein 130. *Journal of Immunology*. 1999;164:273-282.
6. Atreya R, Mudter J, Finotto S. 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*. 2012;6:583-588.
7. Armah KA, McGinnis K, Baker J. HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. *Clinical Infectious Disease*. 201;255:126-136.
8. Mihret A, Abebe M. Cytokines and Chemokines as Biomarkers of Tuberculosis. *Journal of Mycobacteria Disease*. 2013;3:128.
9. Cheesbrough M. *District Laboratory Practice in Tropical Countries Part 2 (2<sup>nd</sup>ed.)*. Cambridge University Press. 2006;253-266.
10. Akira S, Isshiki H, Sugita T. A Nuclear Factor for IL-6 Expression (NF-1L6) is a Member of a C/EBP Family. *European Molecular Biology Organisation Journal*. 1990;9:1897-1906.
11. Breen EC. Pro and anti-inflammatory cytokines in human immunodeficiency virus infection and acquired immunodeficiency syndrome. *Pharmacology and Therapeutics Journal*. 2002;95:295-304.
12. Devenci F, Akbulut HH, Turgut T, Muz MH. Changes in Serum Cytokine Levels in Active Tuberculosis with Treatment. *Mediators of Inflammation*. 2005;5:256-262.
13. Moore KW, de Waal Malefyt R, Coff man RL, O'Garra A. Interleukin- 10 and the interleukin-10 receptor. *Annual Review of Immunology*. 2001;19:683-765.
14. Lago PM, Boechat N, Migueis DP, Almeida AS, Lazzarini LC, Saldanha MM. Interleukin-10 and Interferon-gamma Pattern during Tuberculosis Treatment:

- Possible Association with Recurrence. International Journal of Tuberculosis and Lung Disease. 2012;16(95):656-659.
15. Boussiotis VA, Tsai EY, Yunis EJ, Thim S, Delgado JC, Dascher CC. IL-10 Producing T Cells Suppress Immune Responses in Anergic Tuberculosis Patients. Journal of Clinical Investigations. 2000;105(9):1317-1325.
  16. Armitage AE, Eddowes LA, Gileadi U. Hepcidin Regulation by Innate Immunity and Infectious Stimuli. Blood. 2011;118:4129-4139.
  17. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochimica et Biophysica acta. 2012;1823:1434-1443.
  18. McDermid JM, Jaye A, Schim van der Loeff MF, Todd J, Bates C. Elevated Iron Status Strongly Predicts Mortality in West African Adults with HIV Infection. Journal of Acquired Immune Deficiency Syndromes. 2007;46:498-507.
  19. Kanda J, Mizumoto C, Kawabata H, Tsuchida H, Tomosugi N. Serum Hepcidin Level and Erythropoietic Activity after Haematopoietic Stem Cell Transplantation. Haematologica. 2008;93:1550-1554.
  20. Grigoleit HG, Lehrach F, Miller R. Diabetic angiopathy and blood viscosity. Acta Diabetologica. 1973;1:1311-1323.
  21. Godfrey F, Quigley P, Ayles M, Mwinga H, Hosp A, Porter M. Screening people living with HIV before tuberculosis preventative therapy. AIDS.1999;13:2190-2191.
  22. Kemya MR, Semitala FR, Quinn TC, Ronald A, Meya DN, Kizza HM, Katabira ET, Space 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 Science. 2004;4:94-101.
  23. Berkowitz FE. Hemolysis and infection: categories and mechanisms of their interrelationship. Review of Infectious Diseases. 1991;13(6):1151-62.
  24. Baynes R, Flax H, Bothwell T, Bezwoda W, MacPhail A, Atkinson P. Haematological and iron related measurements in active pulmonary tuberculosis. Scandinavian Journal of Haematology. 1986;36(3):280-7.
  25. Dosumu E. Pattern of some haematological indices in newly diagnosed pulmonary tuberculosis cases in Iwo, Nigeria: diagnostic and therapeutic implications. Nigerian Journal Medicine. 2000;10(1):18-20.

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