Studies on Some Cytokines, Hepcidin, Iron Status and Haematological Parameters of Patients with Pulmonary Tuberculosis in Southeast, Nigeria

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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 TB patients with pulmonary TB in Southeast, Nigeria. A total of 200 subjects aged 18 - 60 years were enlisted for this study. The subjects were grouped into: Group A (100 control subjects), Group B (100 TB Subjects. About 4.5 ml of blood were collected into plain tubes for assay of interferon gamma, interleukins (6 and 10), hepcidin and iron and 2.5 ml for FBC. 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. The data was analysed with the statistical package for social science (SPSS) version 20 using ANOVA and Pearson Moment method and the level of significance set at $P < 0.05$. The results showed significant increase ($P < 0.05$) in interferon-gamma of TB subjects (40.28 ± 8.99 pg/ml) compared to the control (16.25 ± 0.87 pg/ml), significant increase ($P < 0.05$) in IL-6 in TB groups (12.46 ± 3.29 pg/ml) compared to control (7.98 ± 0.22 pg/ml). The results of IL-10 were significantly higher in TB groups (16.42 ± 4.36 pg/ml) compared to control (8.52 ± 0.62 pg/ml). There was significant increase ($P < 0.05$) in monocytes of TB ($8.54 \pm 2.64\%$) compared to control. RBC showed significant decrease ($P < 0.05$) in TB ($4.01 \pm 0.49 \times 10^{12}$/L) compared to the control ($4.92 \pm 0.29 \times 10^{12}$/L). The cytokines and hepcidin can be used as prognostic and diagnostic markers as their levels decreased with increased duration of treatment of the patients. There was significant increase in RBC, Hb and PCV with increased duration of treatment showing improvements in the patients.

Keywords: Interferon; TB; Cytokines; Hepcidin

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* [1]. *Mycobacterium tuberculosis*, the bacterium that causes human pulmonary tuberculosis disease, is an old enemy. Historically, pulmonary tuberculosis (PTB) has a lineage that could be traced to the earliest history of mankind having been in existence since 150,000 - 200,000 years ago [2].

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 [3,4]. Although much progress has been made about 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

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human immunodeficiency virus [5]. 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 [6]. Ita and Udofia [7] reported the prevalence rate of 38.5% pulmonary TB in Ikot Ekpene and 17.6% in Itu Local Government area of Akwa Ibom State; they reported that male subjects had a higher incidence rate of pulmonary TB (35.6%) compared to 29.6% in female. Similarly, Nwanta *et al.* (2011) reported an overall prevalence rate of 37.9% pulmonary TB in Enugu state, Nigeria.

There are few reports on host iron status at the time of pulmonary tuberculosis diagnosis [8]. Friis *et al.* [9] 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. The alarming increase in the incidence of pulmonary tuberculosis in our country has been made worse by elevated occurrence of HIV/AIDS (WHO, 2013).

Peptide hepcidin, is a key iron-regulatory hormone [10] 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 [10].

Interleukin 6 (IL-6) is a proinflammatory cytokine that regulates various physiological processes [11]. It plays a key role in the acute phase response and in the transition from acute to chronic inflammation [12]. Evidence has accrued to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory diseases [11,13]. 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 tolls, drugs and vaccines against tuberculosis [14].

A research by Akpan *et al.* [15] agreed that the mean of the total WBC count in pulmonary tuberculosis patients is usually normal or not significantly elevated as compared to the normal mean of a population. Platelets are effector cells that play an important role in the inflammatory and immunological response and have the capacity to release cytokines and, thus acting as an immune regulator; therefore this direct relationship between platelet and WBC is logical because when there is an immune response at pulmonary tuberculosis infection, platelets tend to increase [16].

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 in Southeast, Nigeria.

Materials and Methods

Study area

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

Advocacy, mobilisation, pre-survey contacts and ethical considerations

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.

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Study population and enrolments

One hundred subjects each comprising the pulmonary tuberculosis patients and apparently healthy subjects as control were recruited for the study. The participants were recruited by purposive sampling technique. The pulmonary tuberculosis subjects were recruited from the tuberculosis directly observed treatment; short course (TB-DOTS) clinic based on sputum smear acid fast bacilli by Ziehl Neelsen's stain and GeneXpert MTB/RIF assay while apparently healthy age and sex matched subjects were recruited as controls. The subjects were grouped into:

- Group A: 100 control subjects.

- Group B: *Mycobacterium tuberculosis* positive subjects (n = 100).

Selection criteria

Inclusion criteria

- Subjects of both sexes aged 18 - 60 years positive for *Mycobacterium tuberculosis* and apparently healthy individuals were included in the study.

- Those that gave consent were included.

Exclusion criteria

The following subjects were excluded:

- Those that tested negative for pulmonary tuberculosis and HIV

- Pregnant women

- Diabetes mellitus patients

- Persons below 18 years and above 60 years

- Those that did not give consent.

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.

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 were analysed in Links Laboratory, Owerri by Sandwich ELISA method for interferon gamma, interleukins (6 and 10) and hepcidin, Full Blood count analysed in the Diagnostic Laboratory Unit, University Health Services Department of Michael Okpara

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University of Agriculture, Umudike, Abia State. Ziel Nelson 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

Ziehl-Nelson technique for Mycobacterium tuberculosis diagnosis [17]

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 could 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.

GeneXpert method for detection of Mycobacterium tuberculosis and rifampicin resistance (GeneXpert MTB/FIF)

Procedure: The assay consists of a single-use multi-chambered plastic catridge pre-loaded with the liquid buffers and lypholised 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. 2ml of the treated sample was transferred into the catridge, the catridge was loaded into the GeneXpert instrument and automatic step completed the remaining assay steps.

The assay catridge 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 negative for *B. Globigii* internal control was reported as invalid.

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 keyboard and then the key will be selected. Then the sample was mixed very well

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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 2.5 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 palced in a heating bath at 37°C for 10 minutes. The reagent blank was also used to zero the spectrophotometer at 560 nm 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

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 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

UIBC (μg/dl) = <u>Conc. Of std-A2 Test-A1 Test</u> 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

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A1 std = Absorbance of the first reading of the standard

A2 std = Absorbance of the second reading of the standard

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. 10µ 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). 50µl of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30 minutes at 37ºC. 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 37ºC. 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.

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. 50µl of standards were pipette into the standard wells. 10µ 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 37ºC. 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 37ºC. 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.

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. 10µ 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 37ºC. 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 37ºC. 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

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

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. 10μ 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.

Statistical analysis

Data was analysed using statistical package for social science (SPSS) version 20. Student t-tes, 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

Parameters	Control	TB	P-value
IFN-γ (pg/ml)	16.25 ± 0.87	40.28 ± 8.99	0.000[*]
IL-6 (pg/ml)	7.98 ± 0.22	12.46 ± 3.29	0.000[*]
IL-10 (pg/ml)	8.52 ± 0.62	16.42 ± 4.36	0.000[*]
Hepcidin (ng/ml)	6.03 ± 1.38	35.59 ± 10.68	0.000[*]
Iron (μg/dl)	86.29 ± 7.27	77.19 ± 12.94	0.001[*]
TIBC (μg/dl)	345.56 ± 28.40	313.48 ± 30.53	0.000[*]
%TSA (%)	25.16 ± 3.18	24.52 ± 4.41	0.743[NS]
WBC (X 10^9/L)	5.87 ± 0.88	5.40 ± 0.89	0.006[*]
Neu (%)	60.57 ± 2.83	58.77 ± 4.36	0.048[*]
Lym (%)	30.69 ± 2.84	30.33 ± 7.49	0.986[NS]
Mon (%)	5.59 ± 1.2	8.54 ± 2.64	0.000[*]
Eos (%)	2.30 ± 1.05	1.19 ± 0.69	0.384[NS]
Bas (%)	0.86 ± 0.39	1.17 ± 0.54	0.012[*]
RBC (X 10^{12}/L)	4.92 ± 0.30	4.10 ± 0.49	0.000[*]
Hb (g/dl)	14.75 ± 0.90	12.01 ± 1.49	0.000[*]
PCV (%)	44.25 ± 2.70	36.0 ± 4.46	0.000[*]
MCV (fl)	89.92 ± 2.3	80.20 ± 3.23	0.000[*]
MCH (pg)	36.12 ± 1.53	29.70 ± 2.54	0.000[*]
MCHC (g/l)	368.46 ± 12.28	196.22 ± 62.39	0.000[*]
Plt (X 10^9/L)	261.75 ± 22.71	169.20 ± 26.45	0.000[*]
ESR (mm/hr)	7.03 ± 1.38	34.28 ± 10.29	0.000[*]

Table 1: Comparison of mean ± Sd values of interferon-gamma, Il-6, Il-10, Cd4, hepcidin, iron and some haematological parameters of control and Ptb subjects.
Significant level -*P < 0.05. Ns - Not significant (P > 0.05).

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The results showed difference that was statistically significant (P < 0.05) in IFN-γ (16.25 ± 0.87 pg/ml, 40.28 ± 8.99 pg/ml, P = 0.000), IL-6 (7.98 ± 0.22 pg/ml, 12.46 ± 3.29 pg/ml, P = 0.000) IL-10 (8.52 ± 0.62 pg/ml, 16.42 ± 4.36 pg/ml, P = 0.000), CD4 (1045.54 ± 247.24 Cells/L, 264.24 ± 49.74 Cells/L, P = 0.000), hepcidin (6.03 ± 1.38 ng/ml, 35.59 ± 10.68 ng/ml, P = 0.000), Iron (86.29 ± 7.27 µg/dl, 77.19 ± 12.94 µg/dl, P = 0.001), TIBC (345.56 ± 28.40 µg/dl, 313.48 ± 30.53 µg/dl, P = 0.000), WBC (5.87 ± 0.88 X 10^9/L, 5.40 ± 0.89 X 10^9/L, P = 0.006), Neutrophils (60.57 ± 2.83%, 58.77 ± 4.36%, P = 0.048), Monocytes (5.59 ± 1.2%, 8.54 ± 2.64%, P = 0.000), Basophil (0.86 ± 0.39%, 1.17 ± 0.54%, P = 0.012), RBC (4.92 ± 0.30 X 10^{12}/L, 4.10 ± 0.49 X 10^{12}/L, P = 0.000), Haemoglobin (14.75 ± 0.90 g/dl, 12.01 ± 1.49 g/dl, P = 0.000), PCV (44.25 ± 2.70%, 36.0 ± 4.46%, P = 0.000), MCV (89.92 ± 2.3fl, 80.20 ± 3.23fl, P = 0.000), MCH (36.12 ± 1.53 pg, 29.70 ± 2.54 Pg, P = 0.000), MCHC (368.46 ± 12.28 g/l, 196.22 ± 62.39 g/l, P = 0.000), Platelets (261.75 ± 22.71 X 10^9/L, 169.20 ± 26.45 X 10^9/L, P = 0.000), ESR (7.03 ± 1.38 mm/hr, 34.28 ± 10.29 mm/hr, P = 0.000) and no significant difference (P > 0.05) in %TSA (25.16 ± 3.18%, 24.52 ± 4.41%, P = 0.743), Lymphocytes (30.69 ± 2.84%, 30.33 ± 7.49%, P = 0.986), Eosinophils (2.30 ± 1.05%, 1.19 ± 0.69%, P = 0.384) when compared between control and TB subjects respectively.

Discussion

The results showed that interferon gamma increased in pulmonary TB than the control which was statistically significant. This shows that interferon gamma is seriously involved in the pathogenesis of tuberculosis. This could be linked to inflammation and oxidation involved in these synthesis and release of interferon gamma as a defective mechanism by the body. Interferon gamma (IFN-γ) has key roles with inflammatory granuloma, organization, immune protection against intracellular mycobacterium and cell mediated immunity [18].

The study also showed that the levels of interleukin 6 (IL-6) were higher in pulmonary TB than the control group. 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. 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 [19,20]. 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 CD8 T cells (Moore., *et al.,* 2001). 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 [21]. The results showed that the levels of IL-10 were significantly higher in pulmonary TB patients compared to healthy subjects (P < 0.05). The findings are similar with previous studies that have shown higher levels of IL-10 in the active pulmonary TB group than in the control group [22-24].

Interleukin 10 can be found in the serum, plasma and bronchoalveolar larvage fluid of active pulmonary TB patients and may contribute to the anergy and failure of lymphocytes to proliferate in response to pulmonary TB [25,26]. It is the balance between the inflammatory and protective immune response that determines the outcome of tuberculosis infection [27].

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 [28]. 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 [29,30]. 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, because of inflammation, hepcidin restricts the availability of iron for incorporation into erythroid progenitor cells [31]. There were elevations of hepcidin in pulmonary TB compared to control. This shows that hepcidin is involved in the pathogenesis of pulmonary TB infection. The increase in hepcdin in these patients may be associated with the anaemia observed in

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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. Emerging evidence suggests a key role for the iron regulator hepcidin in the innate immune response to *M. tuberculosis* infection [32]. In patients with tuberculosis, higher hepcidin concentrations were strongly associated with more severe anaemia. Since hepcidin has a well described, central role in anaemia of chronic disease (ACD) as opined by Weiss and Goodnough [31] in which its expression is upregulated predominantly by IL-6 in response to infections such as tuberculosis [29], these results provide further evidence to suggest that ACD is the predominant mechanism underlying anaemia in patients with tuberculosis [33].

The results showed that the total iron binding capacity (TIBC) was reduced in pulmonary TB compared to the control. This shows that the level of entire iron in pulmonary TB. The study showed that the level of %TSA showed no significant difference between control and the test group. Distortions in iron availability are common in infectious diseases and most of these alterations may be associated to actions of the iron-regulatory hormone hepcidin [30].

Pulmonary tuberculosis has been reported to exert varieties of haematological effects [34]. The study showed a decrease that was statistically significant in red blood cells, PCV and haemoglobin of tuberculosis patients. This may be the reason anaemia is a major complication in tuberculosis. Nwankwo *et al.* [35] reported lower PCV compared to the control.

The erythrocyte sedimentation rate (ESR) values for pulmonary TB patient obtained in this study were higher than the control. This agrees with the works of Ibeneme *et al* [36]. 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 [37] and implies increased rheology of blood in tuberculosis. The increase in ESR as seen in this study has been reported in a study done in South Africa previously [38], which could result from inflammation, increase in viral load, stage of the disease, immune status, nutritional status and reduced PCV. Total white cell count (WBC) showed no significant difference between control and pulmonary TB Another study in Nigeria by Awodu [39] showed a decrease in WBC among pulmonary TB patients compared to control. This variation in the studies could be as the response of bone marrow to *Mycobacterium tuberculosis* infection varies in different individuals, one may have to attribute this to genetic status and ethnic variations of patients, as well as the infectious nature of the pathogens.

The results showed decrease that was statistically significant in Red blood cell, haemoglobin and packed cell volume which may be the cause of anaemia usually seen in pulmonary TB patients. These parameters improved with increased duration of 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 [40]. Weiss [41], Mean [42] and Nemeth *et al.* [43] explained the mechanism causing anaemia in pulmonary TB, saying that the invasion of bacteria leads to activation of T-lymphocyte and macrophages, which induce the production of the cytokines like interferon gamma (IFN-$^{\gamma}$) and interleukin 6 (IL-6) which with their products will divert iron into iron stores in the reticuloendothelial system resulting in decreased iron concentrations in the plasma thus limiting its availability to red cells for haemoglobin synthesis, inhibition of erythroid progenitor cell proliferation and inappropriate production and activity of erythropoietin which may lead to anaemia supported response of the bone marrow to anaemia [44-48].

Conclusion

The study shows that interferon gamma, interleukin 6, interleukin 10 and hepcidin are some of the biomarkers in the pathogenesis of pulmonary TB. The cytokines and hepcidin can be used as prognostic and diagnostic markers as their levels decreased with increased duration of 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 the course of treatment. Anaemia is a major factor causing morbidity and mortality in the patients

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especially pulmonary TB patients. This will help the Physicians and all health care providers handling pulmonary TB 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.

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

The cytokines studied such as interferon-gamma, interleukin 6 and interleukin 10 and hepcidin can be used as adjunct for diagnostic and prognostic markers to help in accurate and timely diagnosis to prevent misdiagnosis and delay in diagnosis that has been the major challenge of actualizing set controls of pulmonary tuberculosis. These cytokines, hepcidin and the haematological parameters studied can play role of predictive values.

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