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Studies on Haematological parameters of patients with pulmonary tuberculosis before treatment with different ranges of CD4 levels in Southeast, Nigeria

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Abstract

Pulmonary tuberculosis is increasing at alarming rate with high level of resistance to chemotherapy. The study was done to determine the changes in haematological parameters in relation to ranges of CD4 of patients with pulmonary tuberculosis before treatment in Southeast, Nigeria. A total of one hundred subjects were recruited for the study comprising 50 subjects each for Patients with pulmonary tuberculosis before treatment with CD4 count of 100-200 cell/L and 201-300 cells/L drawn from the Health institution. The study was done in a tertiary health institution in Southeast, Nigeria. Three milliliters (3ml) of venous blood was collected from each subject and was dispensed into bottles containing di-potassium salt of ethylenediamine tetra-acetic acid (K_2-EDTA) at a concentration of 1.5mg/ml of blood and was used for full blood count and CD4 count. Haematological parameters were analysed using Mindray BC-5300. The results were expressed as mean± standard deviation. The data were analysed with the statistical package for social science (SPSS) version 21 using t-test, ANOVA and the level of significance was set at $P<0.05$. The results showed decrease in neutrophil (61.85±1.94%, 63.94±2.99%, P= 0.046) and MCV (78.09±1.64fl, 79.50±1.02fl, P=0.015) and no significant difference in WBC (5.53±0.36 $X10^9$/L, 5.32±0.58 $X10^9$/L, P=0.285), lymphocytes (23.08±2.21%,21.16±3.16%, P=0.087), monocytes (11.37±0.81%,11.49±1.06, P=0.766), eosinophils (2.28±0.61%, 2.16±0.33, P=0.574), basophils (1.42±0.26%, 1.23±0.24%, P=0.126), RBC(3.64±0.20 $X10^{12}$/L, 3.63±0.18 $X10^{12}$/L, P=0.839), haemoglobin (10.93±0.60g/dl, 10.89±0.55g/dl), PCV (32.80±1.80%,32.66±1.64%, P=0.840), MCH (27.47±0.49pg, 27.55±0.32pg, P=0.646), MCHC (35.14±0.45g/dl, 35.31±0.36, P=0.306), Platelet (140.81±3.28 $X10^9$/L, 141.12±4.05 $X10^9$/L, P=0.833) and ESR (47.66±2.36mm/hr, 47.46±2.98mm/hr) of the of TB patients based on ranges of CD4 counts. The study shows that the range of CD4 count has no much changes in haematological parameters except decrease in neutrophil and MCV.

Keywords: *Pulmonary Tuberculosis patients, CD4, Haematological parameters*

Introduction

It has been documented that pulmonary tuberculosis (TB) is a chronic bacterial disease associated to *Mycobacterium tuberculosis* (MTB) complex which mainly 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 pathogen for human pulmonary tuberculosis disease, is an old enemy. By history; 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 (Okonkwo *et al.*, 2013). It is documented that pulmonary tuberculosis first observed in Europe and later got to the US, Africa and Asia via voyagers and early settlers (Okonkwo *et al.*, 2013). *Mycobacterium tuberculosis* is an acid fast facultative intracellular rod shaped bacterium. It is non- motile, obligates aerobe with stretched generation time and has affinity for macrophages (Zumla *et al.*, 2013Gupta-wright and Lawn, 2015).

It is reported that pulmonary tuberculosis (PTB) is a global public health challenge and is the second leading cause of death. All inclusive, the disease takes a life every 20 seconds (Divangahi, 2013; Yang *et al.*, 2015). A report has shown that in Nigeria, pulmonary tuberculosis is a major public health problem with an estimated prevalence of 616 cases per 100,000. Nigeria leads 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 documented yearly in Nigeria (WHO, 2008). Ita and Udofia (2005) opined the prevalence rate of 38.5% pulmonary TB in Ikot Ekpene and 17.6% in Itu Local Government area of Akwa Ibom State; they obserevd that male subjects had a higher prevalence of pulmonary TB (35.6%) compared to 29.6% in female. Similarly, Nwanta *et al.* (2011) opined an overall prevalence rate of 37.9% pulmonary TB in Enugu state, Nigeria. Some studies have shown that haeamtological parameters are good indicators of health status (Obeagu *et al.*, 2017, Obeagu *et al.*, 2018; Obeagu *et al.*, 2019).

The study was done to determine the changes in haematologial parameters in relation to CD 4 ranges in patients with pulmonary tuberculosis before treatment 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.

Subjects

A total of one hundred subjects were recruited for the study comprising 50 subjects each for Patients with pulmonary tuberculosis before treatment with CD4 count of 100-200 cell/Land 201-300 cells/L drawn from the Health institution.

Sample Collection

Three milliliters (3ml) of venous blood was collected from each subject and was dispensed into bottles containing di-potassium salt of ethylenediamine tetra-acetic acid (K_2-EDTA) at a concentration of 1.5mg/ml of blood and was used for full blood count and CD4 count.

Laboratory Procedures

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

Determinations

A. Ziehl-Nelson Technique for *Mycobacterium tuberculosis* diagnosis (Cheesbrough, 2006)

Procedure

Smear preparation: A piece of clean stick was used to transfer and spread sputum materials evenly covering an area of about 15-20mm 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.

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 also negative for *B.globigii* internal control was reported as invalid.

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

Procedure

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

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.

Ethical Consideration

The details of the research were explained to the subjects and written consents obtained from them and were assured of joining the study willingly and confidentiality also assured. The subjects who gave their consents were allowed to participate in the study.

Statistical Analysis

The results were expressed as mean± standard deviation. The data were analysed with the statistical package for social science (SPSS) version 21 using t-test, ANOVA and the level of significance was set at $P<0.05$.

Results

Table 1: mean ±SD values of haematological parameters of patients with pubmonary TB based on the range of CD4 count

Parameters	100-200 cell/L	201-300 cells/L	P-VALUE
WBC ($X10^9$/L)	5.53±0.36	5.32±0.58	0.285^{NS}
NEUTROPHIL(%)	61.85±1.94	63.94±2.99	0.046^{*}
LYMPHOCYTES(%)	23.08±2.21	21.16±3.16	0.087^{NS}
MONOCYTES(%)	11.37±0.81	11.49±1.06	0.766^{NS}
EOSINOPHILS(%)	2.28±0.61	2.16±0.33	0.574^{NS}
BASOPHILS(%)	1.42±0.26	1.23±0.24	0.126^{NS}
RBC($X10^{12}$/L)	3.64±0.20	3.63±0.18	0.839^{NS}
HAEMOGLOBIN(g/dl)	10.93±0.60	10.89±0.55	0.840^{NS}
PCV(%)	32.80±1.80	32.66±1.64	0.840^{NS}
MCV(fl)	78.09±1.64	79.50±1.02	0.015^{*}
MCH(pg)	27.47±0.49	27.55±0.32	0.646^{NS}
MCHC(g/dl)	35.14±0.45	35.31±0.36	0.306^{NS}
PLT($X10^9$/L)	140.81±3.28	141.12±4.05	0.833^{NS}
ESR(mm/hr)	47.66±2.36	47.46±2.98	0.848^{NS}

The results showed decrease in neutrophil (61.85±1.94%, 63.94±2.99%, P= 0.046) and MCV (78.09±1.64fl, 79.50±1.02fl, P=0.015) and no significant difference in WBC (5.53±0.36 $X10^9$/L, 5.32±0.58 $X10^9$/L, P=0.285), lymphocytes (23.08±2.21%,21.16±3.16%, P=0.087), monocytes (11.37±0.81%,11.49±1.06, P=0.766), eosinophils (2.28±0.61%, 2.16±0.33, P=0.574), basophils (1.42±0.26%, 1.23±0.24%, P=0.126), RBC(3.64±0.20 $X10^{12}$/L, 3.63±0.18 $X10^{12}$/L, P=0.839), haemoglobin (10.93±0.60g/dl, 10.89±0.55g/dl), PCV (32.80±1.80%,32.66±1.64%, P=0.840), MCH (27.47±0.49pg, 27.55±0.32pg, P=0.646), MCHC (35.14±0.45g/dl, 35.31±0.36, P=0.306), Platelet (140.81±3.28 $X10^9$/L, 141.12±4.05 $X10^9$/L, P=0.833) and ESR (47.66±2.36mm/hr, 47.46±2.98mm/hr) of the of TB patients based on ranges of CD4 counts.

Discussion

Studies have shown that pulmonary tuberculosis is a major infectious disease with very high prevalence in developing countries like Nigeria which continues to rise (Akpan *et al.,* 2012; Obeagu *et al.,* 2019). The study revealed decrease in neutrophils and MCV of the patients with pulmonary tuberculosis with CD4 count of 100-200 cells/L compared to pulmonary TB patients with CD4 count of 201-300 cells/L. Koyama *et al* in 2008 reported that the presence of bacterial pathogens in patients' blood stream often stimulates the production of plasma cytokines and other immunomodulatory proteins (Koyama *et al.,* 2008). Other haematological parameters when compared among the two groups of TB patients with different range of CD4 count showed no significant difference. CD4 count has been utilized as a measure of immune status and pulmonary tuberculosis is known to suppress immune status leading to high mortality rates if not promptly and well treated with appropriate anti-tuberculosis drugs. The decrease in neutophils in TB patients with CD4 of 100-200 cells/L may be due to bone marrow suppression and will affect the response to opportunistic infections. This will also affect cytokine release and other immune response. The study also showed decrease in MCV which could be linked to anaemia. Studies have shown that in patients with pulmonary TB, anaemia is strongly associated with morbidity and risk of death (Redig and Berliner, 2013; Isanka *et al.,* 2012; Kendon *et al.,* 2012).

Conclusion

The study shows that the range of CD4 count has no much changes in haematological parameters except decrease in neutrophil and MCV. The changes in neutrophils and MCV should be monitored in patients with pulmonary tuberculosis in relation to CD4 level to ensure stable immunity in the patients.

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