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Haematological Evaluation of *Mycobacterium tuberculosis* Subjects at Central Hospital Agbor, Nigeria

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Abstract
Tuberculosis (TB) is a severe public health problem in Nigeria that is caused by *Mycobacterium tuberculosis* and has its attendant effects. The aim of this study was to evaluate the haematological profile of 146 newly diagnosed patients with *Mycobacterium tuberculosis* attending Central Hospital Agbor, Delta State. A total of 38 *Mycobacterium tuberculosis* negative individuals were monitored as control. Five millilitres of venous blood were collected into EDTA containers. Complete blood count (Packed cell volume (PCV), haemoglobin, total white cell count, lymphocyte, platelet and neutrophil counts) was estimated using Sysmex XP 300 – haematology analyser. SPSS was used to analyse the data. There was no significant difference ($p>0.05$) observed in the total white blood cell counts between tuberculosis subjects and control subjects. Tuberculosis patients had significantly lower ($p<0.05$) haemoglobin levels (9.81 ± 0.24) than control (12.08 ± 0.42). There were significantly higher ($p<0.05$) platelet counts among tuberculosis patients (314.09 21.84) than controls subjects (279.35 ± 20.29). The lymphocyte (%) were significantly lower ($p < 0.05$) in tuberculosis than controls; and neutrophil (%) were significantly higher ($p < 0.05$) in tuberculosis (54.78 ± 2.64) than in controls (49.28 ± 2.73). There were no significant differences ($p>0.05$) in the mean cell volume and mean cell haemoglobin of tuberculosis and control subjects when compared. However, there was a significantly lower ($p<0.05$) mean cell haemoglobin concentration among the tuberculosis patients compared to controls. There was significantly higher ($p<0.05$) Neutrophil Lymphocyte Ratio (NLR) and Platelet-Lymphocyte Ratio (PLR) among the tuberculosis subjects when compared with controls. There were observed haematological abnormalities in tuberculosis subjects in the study area. The study identified that NLR and PLCR as novel biomarkers for monitoring systemic inflammation in tuberculosis. It is therefore pertinent to estimate haematological parameters early in tuberculosis subjects to help in informed clinical decision making in the management of these subjects.

Keywords: *Mycobacterium tuberculosis,* Biomarkers, Anaemia, Lymphocytes, T-Lymphocytes, Tuberculosis, Nigeria, Erythrocyte Indices.

Introduction

Tuberculosis (TB) remains a global public health problem and one of the top ten leading causes of death, worldwide, with developing countries bearing the highest-burden (WHO, 2020). Nigeria has the highest tuberculosis burden in Africa and one of the world's widest gaps between estimated and reported cases according to the recently released Global TB Report 2019. A comparison of the reports for 2018 and 2019 showed that the disease burden is increasing in Nigeria in sharp contrast with the improving global outlook (Adepoju, 2020).

Haematological changes have been reported in tuberculosis subjects and play a significant role in tuberculosis-associated complications. Therefore, this study aimed to assess the

haematological parameters of newly diagnosed tuberculosis patients at Central Hospital, Agbor, Delta State, Nigeria.

Materials and Method

A comparative cross-sectional study was conducted among 146 subjects with confirmed cases of tuberculosis selected from among 500 patients presenting to the outpatient's Clinics at the Central Hospital, Agbor. Delta State Nigeria with symptoms of tuberculosis. Socio-demographic, behavioural, and clinical data were collected using a structured questionnaire. Inclusion criteria included newly diagnosed case of pulmonary tuberculosis as per the case definitions of the National Tuberculosis Control Programme (Federal Ministry of Health, 2015), residence in Delta State and anti-TB chemotherapy naivety. Individuals who did not meet the inclusion criteria: non-residence in Delta State, those on anti-TB chemotherapy were excluded from participation in the study.

Ethical Approval

Ethical approval was obtained from the State Ministry of Health, Asaba and the Institutional Ethical Committee of Central Hospital, Agbor, Delta State. The participants were recruited after giving informed oral or written consent. Enrolment was purely voluntary.

Laboratory Analysis

Five millilitres (5mL) of venous blood were collected into a Vacutainer tube containing di-potassium salt of ethylene di-amine tetra-acetic acid (K2 EDTA). Full blood count was analysed within 3 hours of sample collection, using Sysmex XP- 300 Haematology analyser manufactured by Sysmex Corporation, Kobe, Japan. Haematological parameters analysed were red blood cell (RBC) count, mean cell volume (MCV), packed cell volume (PCV), white blood cell (WBC) count, neutrophil count, lymphocyte count, platelet count, mean platelet volume (MPV). Manual calculation was used to obtain absolute neutrophil count by multiplying the total white blood cell count by the percentage of neutrophils (Al-Gwaiz and Babay, 2007), while the absolute lymphocyte counts were calculated by multiplying the total white blood cell count by the percentage of lymphocyte respectively (Gogia *et al*., 2011). Neutrophil-Lymphocyte Ratio (NLR) was calculated as absolute neutrophil count divided by absolute lymphocyte count (Walsh *et al*., 2005); Platelet-Lymphocyte Ratio (PLR) was calculated as Platelet count divided by absolute lymphocyte count (Yang *et al*., 2017).

Statistical Analysis

Data entry, analysis was carried out with Statistical Package for Social Sciences (SPSS) IBM Chicago, version 21. The data obtained were subjected to mean ± SEM (Standard Error of Mean). Analysis of variance (ANOVA) with Tukey's Post Hoc test was used for the inferential analysis of data generated. A p-value of < 0.05 was considered significant.

Results

The subjects for this study were selected from among a total of five hundred (500) suspected TB subjects. Of this number, two hundred and eight one (56.2%) were male, while two hundred and nineteen (43.8%) were female subjects. A total of 146 patients tested positive for *Mycobacterium tuberculosis* using GeneXpert. The overall prevalence rate of tuberculosis was 29.5%. Their age ranges were between 8 – 75 years with a mean age of 35.41 ± 1.55 years. The patients in age groups 31-45, 16-30yrs represented the majority of the patients accounting for 40.4% and 33.2% of the entire patient population studied; while those in groups 0- 15 accounted for 18.4%, age groups 46-60yrs recorded 5.8%, while the remaining 2.2% were from patients in age groups 61- 75yrs.

Two hundred and ninety-one (58.2%) of participants were married, while 180 (36.0%) were single. Widows and divorcees accounted for 5.8% of the study population. Two hundred and thirty-nine (47.8%) of the participants had secondary education, while 126 (25.5%) had tertiary education. One hundred and thirty (130), representing 26.0%, had primary education, with five (1%) reporting that they never had any form of education. Three hundred and thirty-three representing 64.6% of participants were self-employed individuals; eighty (16.0%) were employed by the government, 33 (6.6%) were students, 23 housewives (4.6%), 24 farmers (4.8%), while others included 17 commercial sex workers represented 3.4% of the population studied as shown in table 1.

Table 1: Distribution of age, gender, educational status and occupation of suspected tuberculosis patients among the study population

Parameters	Attributes	Number of TB Suspected (%)	TB Cases	TB Prevalence (%)
Age (Years)	0 –15	92 (18.4%)	4	4.3
	16 – 30	166 (33.2%)	50	30.1
	31 – 45	202 (40.4%)	66	32.7
	46 – 60	29 (5.8%)	20	69.0
	61 – 75	11 (2.2%)	6	54.5
Gender	Male	281 (56.2%)	54	19.2
	Female	219 (43.8%)	92	42.0
Marital Status	Single	180 (36.0%)	49	27.2
	Married	291 (58.2%)	85	29.2
	Divorced	11 (2.2%)	7	63.6
	Widow	18 (3.6%	5	27.8
Education Level	No Education	5 (1.0%)	5	100.0
	Primary	130 (26.0%)	80	61.5
	Secondary	239 (47.8%)	45	18.8
	Tertiary Education	126 (25.5%)	16	12.7
Occupation	Civil Servant	80 (16.0%)	15	18.8
	Housewife	23 (4.6%)	10	43.5
	Farmers	24 (4.8%)	12	50.0
	Self-employed	323 (64.6%)	100	62.3
	Students	33 (6.6%)	7	21.2
	Sex Workers	17 (3.4%)	2	11.8
Total (%)		**500**	**146**	**(29.5)**

Table 2 shows the number of patients who were TB positive. A total of one hundred and forty-six (29.5%) subjects tested positive for *Mycobacterium tuberculosis* using GeneXpert. Male subjects constituted 37% and females 63% of the tuberculosis case. The majority (45.2%) of the tuberculosis cases were in the age group 31-45 years age bracket followed by 16 – 30 (37.7%) and 46-60 (10.3%) and 61- 75yrs (4.1%) with 15 years below (2.7%).

Table 2: Distribution of gender and age among positive tuberculosis subjects

Age (Years)	Males (n=54)	Females (n=92)	Total (n=146)
≤15	3 (5.6%)	1 (1.1%)	4 (2.7%)
16 – 30	18(33.3%)	37 (33.9%)	55 (37.7%
31 – 45	26 (48.1%)	40 (43.5%)	66 (45.2%)
46 – 60	7 (13.0%)	8 (8.7%)	15 (10.3%)
61 – 75	0	6 (6.5%)	6 (4.1%)

There was no significant difference (p>0.05) observed in the total white blood cell counts between tuberculosis subjects and controls. Tuberculosis patients had a significantly lower (p<0.05) haemoglobin levels (9.81 0.24) than control (12.08 0.42). There were a significantly higher (p<0.05) platelet counts among tuberculosis patients (314.09 21.84) than controls (279.35 20.29). The lymphocyte (%) were significantly lower ($p < 0.05$) in tuberculosis subjects compared to controls while the neutrophil (%) were higher significantly ($p < 0.05$) in tuberculosis (54.78 2.64) subjects compared to controls (49.28 2 .73). There was no significant difference (p>0.05) in the mean cell volume and mean cell haemoglobin of tuberculosis subjects compared to controls. However, there was a significantly lower (p<0.05) mean cell haemoglobin concentration among tuberculosis subjects compared to non-tuberculosis controls. There was significantly higher (p<0.05) NLR and PLR among the tuberculosis subjects compared to controls as shown in table 3.

Table 3: Comparison of Haematology Results between Tuberculosis Patients and Controls

Parameter	TB subject (n=146)	Control (n=38)	p value
WBC (10^3µ/l)	6.47 ± 0.42	6.45 ± 0.35	0.975†
Hb (g/dl)	9.81 ± 0.24	12.08 ± 0.42	0.000*
PCV (%)	30.34 ± 0.69	36.41 ± 1.14	0.000*
Platelets (10^3µ/L)	314.09 ± 21.84	279.35 ±2 0.29	0.018*
Lymphocyte (%)	32.54 ± 1.95	41.54 ± 2.69	0.009*
MXD (%)	12.69 ± 1.77	10.05 ± 1.04	0.024*
Neutrophils (%)	54.78 ± 2.64	49.28 ± 2.73	0.001*
MCV (fL)	81.97 ± 1.38	81.86 ± 1.61	0.961†
MCH (pg)	26.80 ± 0.62	27.19 ± 0.65	0.703†
MCHC (g/dL)	32.24 ± 0.23	33.18 ± 0.29	0.020*
NLR	2.91 ± 0.60	1.46 ± 0.19	0.003*
PLR	15.73 ± 3.11	7.88 ± 1.02	0.009*

***Significant; †Not Significant**

Key: *WBC=White Blood Cell counts; Hb= Haemoglobin concentration, PCV= Packed Cell Volume; MXD = Mixed cell population; MCV=Mean Cell Volume; MCH =Mean cell Haemoglobin; MCHC =mean corpuscular haemoglobin concentration; NLR= Neutrophil/Lymphocyte Ratio; PLR= Platelet/Lymphocyte Ratio.*

Table 4 shows the haematological parameters of tuberculosis subjects based on gender. There was a significantly higher ($p < 0.05$) haemoglobin, PCV, neutrophil, platelet, and lymphocyte count among females than males' subjects. No significant difference ($p > 0.05$) was observed in white blood cells between male and female subjects. There was a significantly ($p < 0.05$) higher MCV and MCH among male compared to female patients. There was no significant difference ($p > 0.05$) in MCHC and NLR among male and female subjects. However, PLR was significant ($p < 0.05$) in male and female tuberculosis patients.

Table 4: Comparison of Haematological profiles of tuberculosis patients based on Gender.

Parameter	Male (n=54)	Female (n=92)	*p*-value
WBC ($10^3\mu$/l)	6.80 ± 1.40	6.50 ± 0.40	0.856†
Haemoglobin (g/dl)	9.25 ± 0.15	10.40 ± 0.50	0.015*
PCV (%)	28.95 ± 4.05	32.25 ± 1.05	0.013*
Platelets ($10^3\mu$/L)	203.50 ± 10.50	490.00 ± 154.00	0.020*
Lymphocyte (%)	44.95 ± 8.85	34.20 ± 0.10	0.034*
Neutrophils (%)	47.35 ± 6.95	54.60 ± 1.80	0.041*
MCV (fL)	87.55 ± 11.65	74.60 ± 3.20	0.039*
MCH (pg)	28.90 ± 7.30	24.10 ± 1.40	0.005*
MCHC (g/dL)	32.50 ± 4.00	32.20 ± 0.50	0.947†
NLR	1.13 ± 0.38	1.60 ± 0.06	0.342†
PLR	4.76 ± 1.17	14.34 ± 4.54	0.017*

Key: *Significant; †Not Significant

Discussion

Tuberculosis is a disease of public health concern in Nigeria. World Health Organisation reports that Nigeria has the second-highest tuberculosis disease burden after South Africa; Nigeria is ranked fifth in the countries with high tuberculosis incidence worldwide as tuberculosis accounts for more than 10% of all deaths in Nigeria (WHO, 2014).

White blood cells (WBC) are an integral part of the immune system responsible for the protection of body infections and invading microorganisms and are indicators of systemic inflammation (Balta *et al*., 2016). They are responsible for protecting the body against infections and invading organisms. The study observed that total white blood cell counts in the tuberculosis subjects was higher but not statistically significant (p>0.05) when compared with non-tuberculosis controls. This is in agreement with a previous study (Singh *et al*., 2001).

Other white blood cells; neutrophils, lymphocytes, monocytes, eosinophils and basophils, play different roles in the body's immune response in tuberculosis; however, neutrophils and monocytes are regarded as essential components of white blood cells that respond to mycobacterial infection (Morris *et al*., 1989). Neutrophil counts of patients with tuberculosis were significantly higher ($p<0.05$) compared to controls. This can be attributed to neutrophilia which is indicative of inflammatory response. The lymphocyte counts were higher in tuberculosis subjects than controls. As reported by earlier authors (Jadoon *et al*., 2004; Ursavas *et al*., 2010), lymphocytosis is due to chronic inflammatory response, especially in active tuberculosis.

The reduction of circulatory cells is a common complication of tuberculosis infection which results in the development of anaemia (Onubogu *et al.,* 2010). There was significantly lower ($p < 0.05$) haemoglobin and PCV values in tuberculosis subjects when compared with apparently healthy controls. Male tuberculosis subjects had significantly lower ($p<0.05$) PCV than female subjects. This is in agreement with Eyshi *et al*. (2009) who observed the occurrence of anaemia among tuberculosis subjects in their study. This may be due to the effect of cytokines which are known to affect red cell production in the marrow. IL-1 and TNF-alpha inhibit the production of erythropoietin. A similar study by Shareef and Amin, (2012) and Akpan *et al*. (2012), observed a higher packed cell volume and attributed this to the consumption of diets containing vegetables and seafood in that locality. But in studies conducted in Kano (Nwankwo *et al*., 2005) *and* Benin City (Ajayi *et al*., 2005) had a contrasting view. Anaemia in tuberculosis subjects could be due to the invasion of *Mycobacterium tuberculosis*, which leads to activation of T-lymphocytes and macrophages, which exacts effect on the production of cytokines like IFN-gamma, tumour necrosis factor-alpha (TNF- α), IL-1, and IL-6 whose products causes a diversion of iron into the reticuloendothelial system resulting in decreased iron concentration in the red blood cells thereby reducing iron availability for haemoglobin synthesis (Means, 2003; Nemeth *et al*., 2004).

There was no significant difference in the mean cell volume, mean cell haemoglobin but significantly lower MCHC in tuberculosis subjects when compared with controls. This finding in variance with that of Ajayi *et al*. (2005) who reported a significant difference in haematological indices in tuberculosis subjects in Benin City. There were significant differences ($p < 0.05$) in white blood cell count, packed cell volume and platelets among tuberculosis patients. These findings are in agreement with those of Affusim *et al.* (2012).

Platelets cells play important roles as effector cells in inflammation and immunological response. They act to regulate the immune system by their intrinsic ability for stimulating cytokine and chemokine release by cells (Trzeciak-Ryczek *et al*., 2013). There was a significantly higher platelet count in tuberculosis patients than normal control subjects. This may be attributed to the reactive thrombocytosis that is found in clinical situations, including pulmonary tuberculosis (Unsal *et al*., 2005). There is a direct relationship existing between platelets and white blood cells in response to tuberculosis infection which may result in an increased number of circulating platelets. Bacillus-activated macrophages and lymphocytes produce such cytokines as IL-6 or TNF-α, which affect the maturation of megakaryocytes and platelet release. Therefore, reactive thrombocytosis is observed in tuberculosis, in which increased platelet count and size are associated with inflammation intensity (Feng *et al*., 2011; Unsal, 2005). Platelets stop bleeding by forming clots and scabs, which further prevents thrombocytopenia.

The values of red cell parameters (MCV and MCH) were not statistically significant except MCHC between the various groups when compared. Iron deficiency has been attributed to lower MCHC values. The tumour necrosis factor–α (TNF-α) and other cytokines released by activated monocytes suppress the erythropoietin production leading to anaemia. Tuberculosis imparts the haemopoietic system leading to a decrease in erythropoiesis (Kumar *et al*., 2013).

Platelet-lymphocytes ratio is a non-specific

marker of the systemic inflammatory response, which is calculated as a ratio of platelet counts to lymphocyte counts. Lymphocytes and platelets are haematology parameters that are related to immune surveillance, with both playing an important role in cytokine-dependent immune response. During chronic inflammatory processes, the megakaryocytes proliferate increasingly, and lymphocytes counts tend to decrease due to cell apoptosis. Hence platelet-to- lymphocyte ratio (PLR) can be affected in serve inflammatory diseases. Neutrophil lymphocyte ratio is a routinely available marker of the systemic response, which is derived from the absolute neutrophil and lymphocyte count. The neutrophil and lymphocyte ratio (in absolute or relative percentage values) is an easily measured parameter that is inexpensive, simple, rapid and reliable in the evaluation of the extent of systemic inflammation (Abakay *et al*., 2015).

Conclusion

Conclusively, the study shows a high prevalence rate of 29.5% for tuberculosis in the studied population. Females accounted for 42.0% of all the positive cases while male subjects accounted for a 19.2% prevalence rate in the study population and the highest rate of infection was in the age group 31- 45 years. Haematological abnormalities were observed in tuberculosis subjects. The study revealed that NLR and PLR might serve as novel biomarkers of systemic inflammation in tuberculosis

Conflict of interest-

The authors declare that there are no conflicts of interest.

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