Hematological Changes in Newly Diagnosed Pulmonary Tuberculosis Patients on Standard Anti-TB Treatment Regimen and their Influence on Smear Conversion

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ABSTRACT

Pulmonary tuberculosis (PTB) mortality remains high despite the availability of effective anti-TB therapy. Disease and treatment-associated hematological derangements at diagnosis and during therapy might contribute to this high TB mortality rate. This paper aims to determine hematological changes in newly diagnosed pulmonary tuberculosis patients on standard anti-TB treatment regimen and their influence on smear conversion. The study adopted a longitudinal design in which 55 newly diagnosed HIV negative PTB patients were followed up to the fifth month of anti-TB therapy. Blood samples (5ml) were collected for diagnosis during the second and fifth months and analyzed using automated HumaCount 5D hematology analyzer. Data was analyzed using Kruskal-wallis test with Dunn's multiple comparisons test in Graph-Pad prism version 6.0. Throughout therapy, there was a statistically significant time-dependent decrease in median total white blood cell counts from 6.82x10^3/ uL at diagnosis to 5.87x10^3/ uL at the fifth month (P=0.0358). This decrease in total WBC count was majorly driven by significant decrease in the neutrophil numbers from 4.31x10^3/ uL at diagnosis to 2.97x10^3/ uL at the fifth month. The proportion of patients who had anemia at the fifth month of treatment increased compared to the second month post intensive phase. There was also a significant decrease in MCV, MCH and MCHC at the second month compared to diagnosis. Moreover, the median platelet count, MPV, PDW and PCT% decreased from 314x10^3/ uL, 8.9 fL, 10.4 fL and 0.273 at diagnosis to 232x10^3/ uL, 10.15 fL, 12.5 fL and 0.235 at the fifth month, respectively. High WBC count, high platelet count, low lymphocyte count and low HGB at the end of month two of therapy were shown to increase the likelihood of sputum non-conversion (OR-2.42 (95% Ci 2.07-2.82)), (OR-1.5 (95% Ci 1.01-2.22)), (OR-1 (95% Ci 1-1)) and (OR-2.58 (95% Ci 1.68-3.95)), respectively (P<0.05). A significant number of PTB patients presented with anemia, thrombocytosis and leukocytosis. The proportion of patients with anemia increased over time. The study recommends screening for hematological abnormalities in patients for tailored patient interventions for better clinical outcomes.

Keywords: Anemia, Leukocytosis, Thrombocytosis

I. INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by the bacillus *Mycobacterium tuberculosis.* TB is a major cause of illness and death worldwide according to the World Health Organization (WHO, 2021) (Global Tuberculosis Report, 2021). *M. tuberculosis* is spread through expectoration, sneezing or by any other activity that generates droplet nuclei by individuals who are infected. The primary organs affected by the bacteria are the lungs, culminating into pulmonary tuberculosis. However, the bacterium can affect other parts of the body via blood or lymphatic drainage leading to milliary or extra-pulmonary tuberculosis. According to the 2021 annual global TB report, it was estimated that 10.6 million people fell ill with TB representing a 4.5% increase from 10.1 million people that fell ill with TB in 2020 (Global Tuberculosis Report, 2021). For the same year, 6.4 million newly diagnosed TB cases were reported globally. This was an increase in the number of new TB cases compared to 5.8 million cases that were reported in 2020 (Global Tuberculosis Report, 2021). In 2022, Kenya recorded 90, 500 cases of drug sensitive TB representing a 16% increase from 77, 854 cases that were reported in 2021 (MoH annual TB report, 2022).

Treatment of drug susceptible pulmonary TB consists of a 2 months intensive phase followed by a 4 month continuation phase according to WHO guidelines. This involves four drugs during the first two months (Rifampicin (R), Isoniazid (H), Pyrazinamide (Z) and Ethambutol (E) followed by two drugs (Rifampicin (R) and Isoniazid (H)) during the last four months of treatment (WHO guidelines, 2017) During treatment, patients are monitored for optic neuritis, peripheral neuropathy, acute hepatotoxicity and sputum conversion. Sputum smear monitoring at the end of month 2 of treatment provides information on patients' response to treatment (MoH Integrated Guideline, 2021) Subsequent monitoring of sputum smear conversion at month three, five and six is critical for evaluating overall

response to therapy by patients. However, monitoring for sputum smear conversion alone often overlooks other anomalies that occur in pulmonary TB patients on care. Furthermore, smear microscopy monitoring as used in most low income countries, has been proven to be less sensitive and specific as opposed culture conversion (Ramarokoto *et al*., 2002).

There is growing evidence that significant hematological changes occur in pulmonary TB patients on standard anti-TB therapy. Several studies around the world have reported various abnormalities in red blood cells, white blood cells and platelets in pulmonary TB patients undergoing treatment. For example, Obeagu *et al* in Nigeria reported significant changes in absolute WBC count, differential WBC count, RBC count, HGB, HCT%, MCV, MCH, MCHC and platelets count in pulmonary TB patients at baseline, month 2, month 4, and month 6 of treatment (Obeagu *et al*., 2019). Yadav et al. (2022) in India reported a prevalence of severe anemia, moderate anemia and mild anemia in 14%, 63% and 15% of pulmonary TB patients after completion of treatment compared to 10%, 57% and 13% before commencement of treatment, respectively. Prevalence of microcytic anemia among patients increased from 7% before treatment to 9% after completion on anti-TB therapy (Yadav *et al*., 2022).

Similarly, a study by Reta and colleagues in Ethiopia further confirmed occurrence of hematological derangements among pulmonary TB patients undergoing treatment. In their study, 45.3%, 14.2% and 23.6% of patients had low hemoglobin concentrations before initiation of treatment, after intensive phase and after completion of treatment, respectively. Thrombocytosis was reported among 51.4%, 42.6% and 15.5% of the pulmonary TB patients before initiation of treatment, after intensive phase and after completion of treatment, respectively (Reta *et al*., 2023). Additionally, a significant association between lymphopenia and delayed sputum smear conversion at month 2 of treatment was demonstrated by Nagu and colleagues in Tanzania (Nagu *et al*., 2014). According to the Kenya integrated guideline for PTB management (2021), patients are monitored with respect to sputum conversion, hepatotoxicity, peripheral neuropathy and optic neuritis. There is lack of emphasis on hematological aspects of patients newly diagnosed or those undergoing anti-tubercular treatment. Therefore, the incorporation of hematological profile monitoring into pulmonary TB management could offer predictive insights on response to treatment for pulmonary TB patients on treatment. The objective of this study was to evaluate hematological changes in hematological profiles of newly diagnosed pulmonary TB patients at baseline, month 2 and 5 of treatment. The study also determined the predictive effect of hematological changes on sputum smear conversion at month 2 of treatment.

1.1 Statement of the Problem

TB is a leading cause of mortality and morbidity from a single infectious agent worldwide as reported by WHO annually. For instance, according to the 2023 global TB report by WHO, 1.3 million people died due to TB. In the same year, 7.5 million people were newly diagnosed with TB worldwide. Kakamega County is among counties with the highest death rates among TB patients estimated at 10.8%. This is higher than the national target of <5% for TB related deaths in the country. This high mortality rates remain persistently high despite the availability of effective anti-tubercular treatment. The reasons for this persistently high mortality remain largely unknown. Additionally, there are no reliable markers that can be used to monitor patients on treatment. Therefore, this makes prognostication for the disease difficult hindering effective monitoring of patients on care (Harris and Kumar, 2018).

For patients on care, pharmaco-therapeutic monitoring relies on sputum smear conversion monitoring and occasional assessment for hepatotoxicity. Furthermore, due to associated drug toxicity, patients are also monitored for optic neuritis and peripheral neuropathy. However, no attention is paid to the potential hematological changes that occur in pulmonary TB patients due to *M. tuberculosis* infection or anti-tubercular therapy. The influence of hematological changes in patients hematological profiles on sputum smear conversion and clinical outcomes is not fully understand. Therefore, this study evaluated hematological profiles of pulmonary tuberculosis patients before enrollment on anti-tubercular therapy, at month two post intensive phase of treatment and at month five of treatment. The study also assessed the influence of hematological changes in hematological profiles of patients after the two months intensive phase of treatment on sputum conversion.

1.2 Research Objectives

i. To determine the hematological profiles of treatment naïve pulmonary TB patients.
ii. To determine the hematological changes in hematological profiles of pulmonary TB patients on treatment and their influence on sputum smear conversion.

III. METHODOLOGY

3.1 Study Site

The study was conducted in Kakamega County at the County Teaching and Referral Hospital. The County is part of the larger Western Kenya covering 3, 033KM^2 with a population of 1, 867, 579 according to the national census report of 2029, Kenya. The County referral hospital is the main referral facility within the County for Sub-County and faith-based hospitals within Kakamega. The hospital is located 379.8 KM West of Nairobi, 48 KM North of Kisumu along the Kisumu-Webuye road.

3.2 Study Design and Study Population

A prospective longitudinal study design was used for this study. The study was conducted from April 2023 to March 2024. A total of 55 newly diagnosed HIV negative pulmonary tuberculosis patients were recruited into the study at Kakamega County Teaching and Referral Hospital. PTB patients were bacteriologically diagnosed either through GeneXpert MTB/RIF or sputum smear fluorescence microscopy.

3.3 Sample Size Determination and Sampling

G power sample calculation software was used to determine the sample size where a power of 0.80, large effects size of 0.50 and alpha of 0.05 were used. Fifty one participants were obtained for PTB patients that were to be recruited and followed up. However, 55 PTB patients were recruited to cover for any attrition. All the 55 participants were conveniently sampled voluntarily after a thorough explanation of study objectives to them.

Inclusion Criteria

Newly diagnosed PTB patients who were HIV negative were eligible for recruitment. Furthermore, only those who consented in writing were recruited.

Exclusion Criteria

PTB patients who had chronic illness such as cancer, renal diseases or heart conditions. PTB patients who had other infectious diseases like HIV were excluded. Furthermore, patents with a known history of hematological disorders including thalassemia, hemophilia and sickle cell anemia were not recruited.

Laboratory Procedures

Blood Sample Collection

Blood samples were collected at diagnosis, post-intensive phase and at month 5 of anti-tubercular therapy. The evacuated system of blood collection was used to collect 5mL of blood from PTB patients via venipuncture. The blood was collected into ethylene-diamine tetra acetic acid vacutainer tubes to avoid coagulation of blood. The tubes were inverted gently 6-8 times for proper whole blood-EDTA mixing. The filled tubes were then labelled with participant serial number, laboratory tests, time and date of sample collection. Samples were then transported to the hematology laboratory via a designated cool box.

Complete Blood Count Analysis

To perform complete blood count analysis, the HumaCount 5D automated hematology analyzer (Human Gesellschaft för Biochemica und Diagnostica GmbH, Germany) was used. WBC count below 4.0x10^3/ µL and above 10.0x10^3/ µL was considered as leukopenia and leukocytosis, respectively. Neutropenia was defined as a count below 2.0x10^3/ µL and neutrophilia as a count above 7.0x10^3/ µL. Lymphocytopenia, monocytopenia and eosinopenia was considered as counts below 0.80x10^3/ µL, 0.12x10^3/ µL and 0.02x10^3/ µL, respectively. Counts above 4.0x10^3/ µL, 1.20x10^3/ µL and 0.50x10^3/ µL for lymphocytes, monocytes and eosinophils were termed as lymphocytosis, monocytosis and eosinophilia, respectively. Erythropenia and erythrocytosis was defined by counts below 3.50x10^6/ µL and above 5.50x10^6/ µL, respectively. Mild anemia was defined by hemoglobin values between 11.0-12.9 g/dL whereas moderate anemia ranged between 8.0-10.9 g/dL. Hemoglobin values below 8.0 g/dL were considered as severe anemia. Counts below 150x10^3/ µL and above 450x10^3/ µL for platelets were considered as thrombocytopenia and thrombocytosis, respectively.

PTB Diagnosis and Follow-Up

Presumptive cases of pulmonary tuberculosis were handled at the chest clinic where patients collected sputum samples upon interrogation by the clinicians. After thorough history taking, patients were given wide-mouthed sputum mugs or 50ml falcon tubes for sputum collection. Patients were directed to a designated sputum collection area for collection of spot samples. Before sputum collection, patients rinsed their mouths with sufficient water to get rid of

any food debris. Patients were instructed to inhale 2-3 times before coughing deeply to ensure quality sputa as opposed to saliva. Sputum samples were also collected at month 2 post-intensive phase and at month 5 of anti-tubercular treatment for sputum smear conversion monitoring. For PTB diagnosis, GeneXpert MTB/Rif was used according to the Kenya TB diagnostic algorithm. (Cepheid, 904 Caribbean drive Sunnyvale, CA 94089-1189 U.S.A). For smear follow-up at month 2 and 5, fluorescent microscopy was the only technique that was used.

3.4 Quality Assurance

Use of standard operating procedures in areas that involved patient handling, sample handling, sample collection and sample processing were strictly adhered to at all times. HumaCount 5D quality controls (low, medium and high) were used every day before sample analysis to perform internal quality control on the hematology analyzer. Continuous quality monitoring was done using automated HumaCount 5D Levey-Jennings charts corroborated with manual Levey-Jennings charts.

3.5 Statistical Analysis

Data was cleaned, entered into Microsoft excel and exported into GraphPad Prism version 6.0. Shapiro-Wilk normality test was performed to check for normal distribution of data. Since the data was not normally distributed, Kruskal-Wallis test was used to determine statistically significant differences between the hematological profiles of patients at diagnosis, at month 2 and month 5 of anti-tubercular therapy. A post-hoc analysis using Dunn's Multiple Comparisons test was performed to check for statistically significant mean rank differences in hematological values between diagnosis, month 2 and month 5 of anti-tubercular therapy. Multivariate logistic regression was used to check for the predictive effect of various hematological changes on sputum smear conversion at month 2 of treatment. A *P* value of <0.05 was considered statistically significant.

IV. FINDINGS & DISCUSSION

4.1 Significant Changes occur in Absolute WBC and Differential WBC Counts for PTB Patients on Treatment

Counts for absolute WBCs and differential WBC cells were obtained through automated analysis at diagnosis before initiation of treatment and at month 2 and 5 after initiation of treatment. The median WBC count for the patients was significantly lower at month 5 of treatment ($5.87x10^3$/ uL) compared to month 2 of treatment ($6.58x10^3$/ uL) and diagnosis ($6.82x10^3$/ uL) (P=0.0358). Similarly, the median neutrophil count at month 5 of treatment ($2.97x10^3$/ uL) was significantly lower compared to values that were recorded at month 2 of treatment ($3.28x10^3$/ uL) and diagnosis ($4.31x10^3$/ uL) for pulmonary tuberculosis patients (P=0.0021) Table 1. Differential median lymphocyte count was significantly higher at month 2 of treatment compared to diagnosis ($2.11x10^3$/ uL vs $1.74x10^3$/ uL) with a slight drop in the count being recorded at month 5 of treatment ($1.94x10^3$/ uL) (P=0.0397). Quite notably, our results showed no statistically significant differences in median monocyte, eosinophil and basophil counts at diagnosis vs month 2 vs month 5 (P=0.1807, P=0.1279 and P=0.2217, respectively) Table 1. A post hoc analysis using Dunn's multiple comparison's test revealed statistically significant differences in mean rank differences for WBC and neutrophil counts at diagnosis compared to month 5 of anti-tubercular therapy. Furthermore, post-hoc analysis revealed no statistically significant differences in mean rank differences for lymphocytes, monocytes, eosinophils and basophils at diagnosis vs month 2 vs month 5 Figure 1.

Table 1

Comparison of WBC, RBC, and Platelet Counts at Baseline, Month 2 and Month 5 of Treatment for PTB Patients

Parameter	**Baseline Median (min-max)**	**Month 2 Median (min-max)**	**Month 5 Median (min-max)**	***P*= Value**
WBC count (10^3/uL)	6.82 (3.22-13.12)^{a}	6.58 (3.26-21.14)^{b}	5.87 (2.72-10.30)^{a}	**0.0358**
Neutrophil (10^3/uL)	4.31 (1.78-11.61)^{a}	3.28 (0.73-18.90)^{b}	2.97 (1.21-7.78)^{a}	**0.0021**
Lymphocyte (10^3/uL)	1.74 (0.61-4.77)^{a}	2.11 (0.69-5.25)^{b}	1.94 (0.76-4.67)^{c}	**0.0397**
Monocyte (10^3/uL)	0.38 (0.08-1.89)^{a}	0.42 (0.08-1.65)^{b}	0.34 (0.10-0.97)^{c}	0.1807
Eosinophil (10^3/uL)	0.08 (0.0-1.07)^{a}	0.12 (0.0-0.90)^{b}	0.095 (0.010-0.95)^{c}	0.1279
Basophil (10^3/uL)	0.05 (0.01-0.15)^{a}	0.04 (0.01-0.17)^{b}	0.04 (0.02-0.12)^{c}	0.2217
RBC count (10^6/uL)	4.79 (2.88-6.33)^{a}	4.58 (2.42-5.69)^{b}	4.51 (2.79-5.68)^{c}	0.0937
HGB (g/dL)	12.80 (7.3-18.30)^{a}	11.60 (6.6-16.70)^{b}	13.0 (9.0-15.90)^{c}	0.0702
HCT%	37.90 (22.20-53.10)^{a}	34.70 (19.90-49.80)^{b}	37.80 (26.6-47.10)^{c}	0.0932
MCV (fL)	80.20 (55.70-93.80)^{a}	78.60 (56.70-92.60)$^{b}{}_{a}$	83.75 (57.30-95.30)$^{a}{}_{a}$	**0.0009**
MCH (pg)	27.0 (17.60-32.40)^{a}	26.50 (18.70-31.40)$^{b}{}_{a}$	28.40 (18.40-32.30)$^{a}{}_{a}$	**0.0005**
MCHC (g/dL)	33.80 (30.60-35.70)^{a}	33.50 (30.50-35.40)$^{b}{}_{a}$	33.95 (31.30-35.10)$^{a}{}_{a}$	**0.0066**
RDW-CV%	12.80 (11.20-18.70)^{a}	13.00 (11.20-18.90)^{b}	12.60 (11.0-17.80)^{c}	0.1485
RDW-SD	42.30 (36.30-70.70)^{a}	41.90 (33.90-57.90)^{b}	43.65 (36.8-54.0)^{c}	0.0809
Platelet count (10^3/uL)	314.0 (185.0-868.0)^{a}	312.0 (129.0-730.0)$^{b}{}_{a}$	232.0 (71.0-683.0)$^{a}{}_{a}$	**<0.0001**
MPV (fL)	8.90 (6.60-11.70)^{a}	9.2 (6.6-11.1)$^{b}{}_{a}$	10.15 (8.0-11.7)$^{a}{}_{a}$	**<0.0001**
PDW (fL)	10.40 (7.10-18.0)^{a}	10.7 (7.0-16.0)$^{b}{}_{a}$	12.5 (6.5-16.3)$^{a}{}_{a}$	**0.0023**
PCT%	0.273 (0.161-0.643)^{a}	0.269 (0.143-0.649)$^{b}{}_{a}$	0.235 (0.061-0.677)$^{a}{}_{a}$	**0.0025**

Data was analyzed using Kruskal-Wallis test and presented as median (min-max) with a *P* value of <0.05. Significant values are bolded. Similar superscripts and subscripts denote column proportions that are significant.

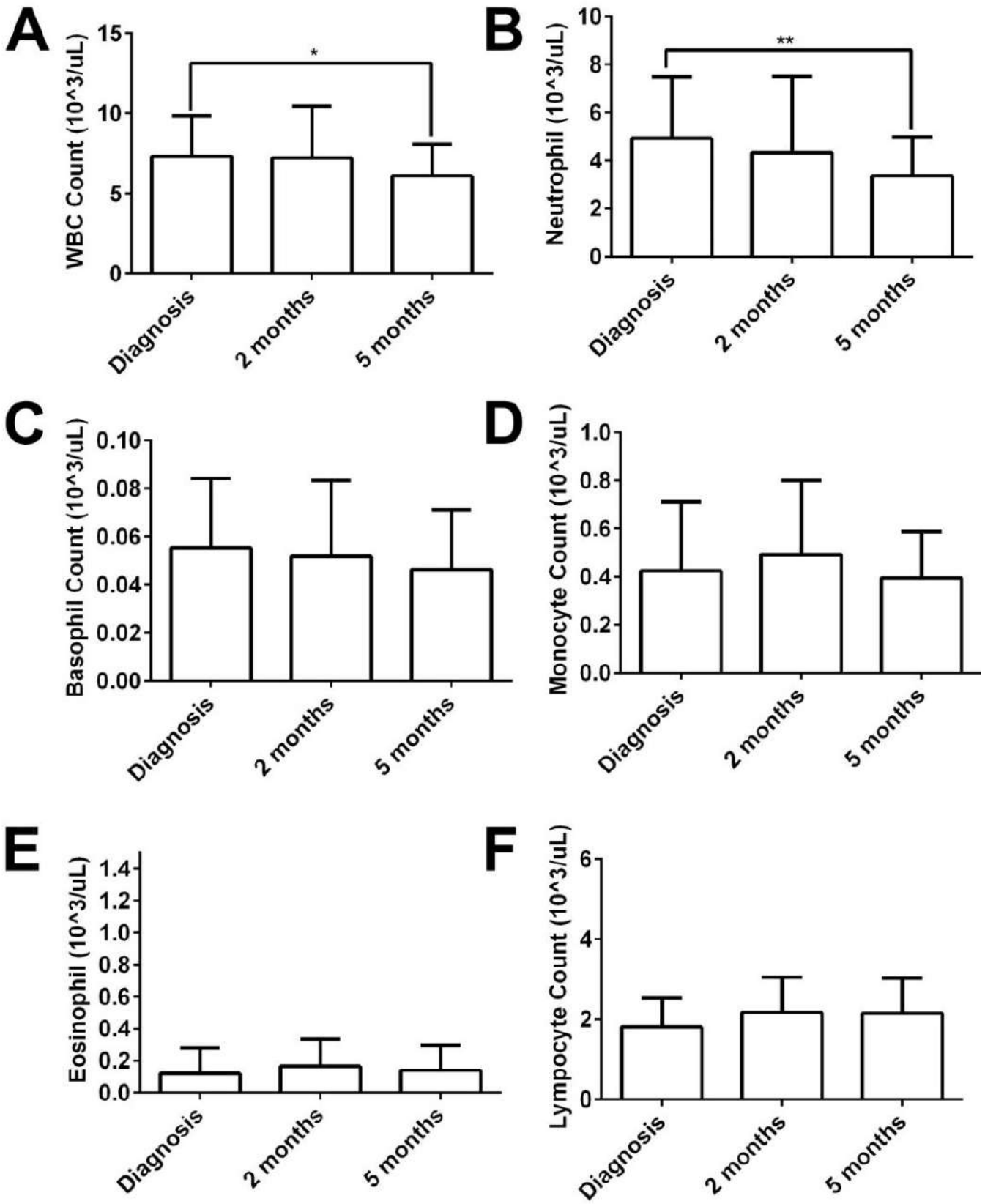

Figure 1

Time Dependent Changes in White Blood Cell Parameters from Diagnosis to the Fifth Month of Treatment

A and B -Denote statistically significant differences in WBC and neutrophil counts between mean rank differences at diagnosis versus month 5 of treatment, respectively. C, D, E and F-Denote no statistically significant

differences in basophil, monocyte, eosinophil and lymphocyte counts between mean rank differences at diagnosis, month 2 and month 5 of treatment, respectively. Data was analyzed using Dunn's Multiple Comparisons tests and presented as means with a *P* value of <0.05.

4.2 Significant Time-Dependent Changes occur in RBC Parameters and Indices in Pulmonary TB Patients at Diagnosis, Month 2 and Month 5 of Anti-Tubercular Therapy

As shown in Table 1, our results showed no statistically significant difference in median RBC count at diagnosis versus month 2 versus month 5 of anti-tubercular therapy ($4.79x10^6$/ uL vs $4.58x10^6$/ uL vs $4.51x10^6$/ uL, *P*=0.0937). Similarly, median HGB and HCT% values did not show statistically significant differences at diagnosis versus month 2 of treatment versus month 5 of treatment (*P*=0.0702 and *P*=0.0932, respectively). The median MCV value at month 5 of treatment (83.75 fL) for the patients was significantly higher compared to values recorded at diagnosis (80.20 fL) and month 2 of treatment (78.60 fL) (*P*=0.0009). Similarly, the median MCH and MCHC values at month 5 of treatment (28.40 pg and 33.95 g/dL, respectively) were significantly higher compared to median values at diagnosis (27.0 pg and 33.80 g/dL, respectively) and month 2 of anti-tubercular therapy (26.50 pg and 33.50 g/dL, respectively) (*P*=0.0005 and *P*=0.0066, respectively) Table 1. Interestingly, there was no statistically significant difference in median values for RDW-CV% and RDW-SD at diagnosis versus month 2 of treatment versus month 5 of treatment (*P*=0.1485 and *P*=0.0809, respectively). A post hoc analysis using Dunn's multiple comparisons test revealed statistically significant differences in mean rank differences in MCV, MCH and MCHC at diagnosis versus month 5 and month 2 versus month 5 of anti-tubercular therapy. On the contrary, post-hoc analysis in mean rank differences for RBC count, HGB, HCT%, RDW-CV% and RDW-SD showed no statistically significant difference at diagnosis versus month 2 versus month 5 of treatment Figure 2.

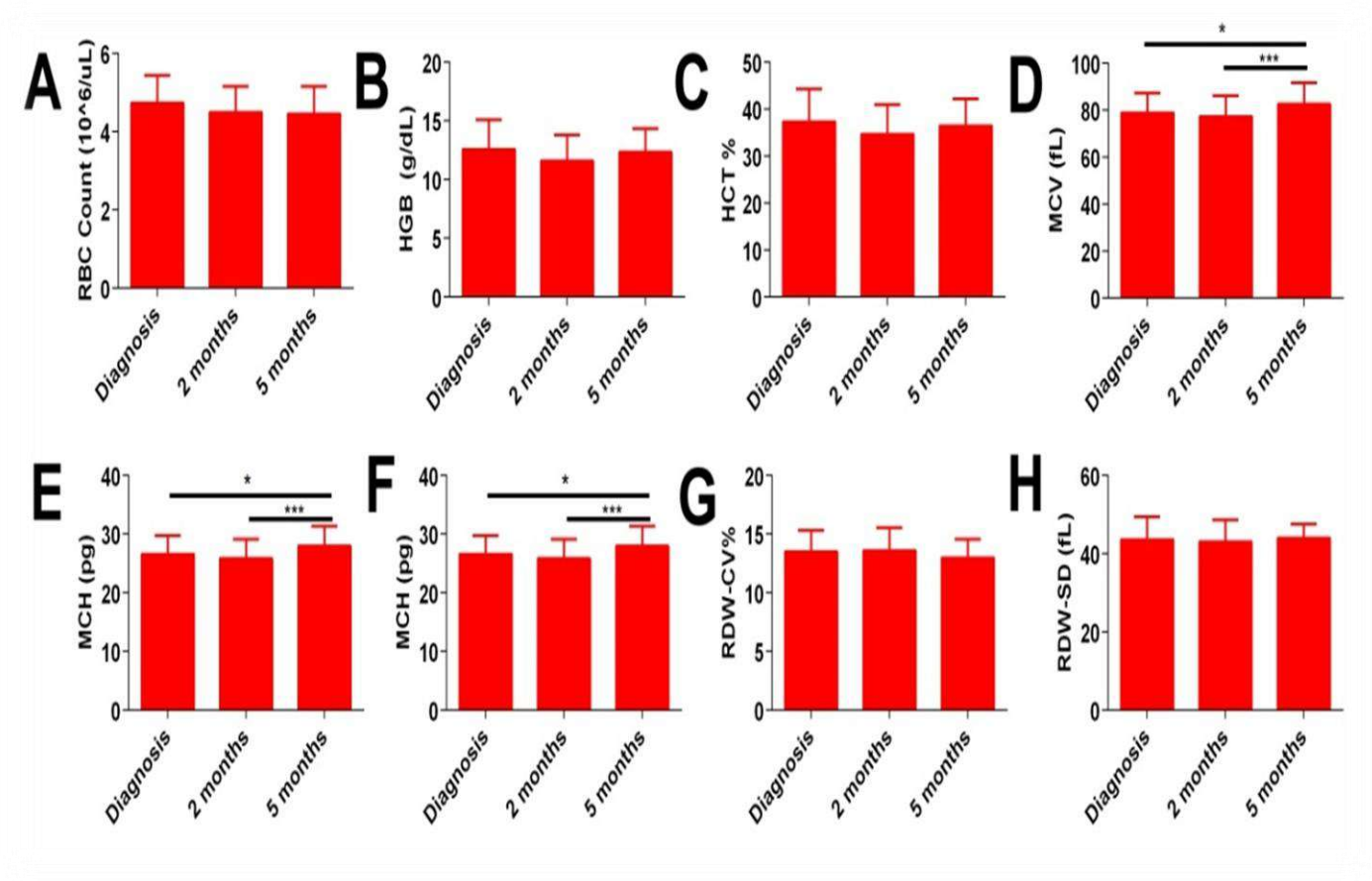

Figure 2

Time Dependent Changes in Red Blood Cell Parameters from Diagnosis to the Fifth Month of Treatment.

A, B, C, G and H-Denote no statistically significant differences in RBC counts, HGB, HCT%, RDW-CV% and RDW-SD between mean rank differences at diagnosis, month 2 and month 5 of treatment, respectively. D, E, and F denote statistically significant differences in MCV, MCH and MCHC between mean rank differences at diagnosis vs 5 months and month 2 vs month 5 of treatment, respectively. Data was analyzed using Dunn's Multiple Comparisons tests and presented as means with a *P* value of <0.05.

4.3 Significant Changes occur in Platelet Count and Associated Indices in Pulmonary TB Patients on Treatment

The median platelet count was significantly lower at month 5 of treatment (232.0x10^3/ uL) compared to median values that were recorded at month 2 of treatment (312.0x10^3/ uL) and at diagnosis (314.0x10^3/ uL) (P=0.0001) Table 1. Decrease in platelet counts was also reflected in the median plateletcrit (PCT %) values for the patients. The median PCT % value at month 5 of treatment (0.235%) was significantly lower than the median value at month 2 of treatment (0.269 %) and at diagnosis (0.273 %) (P=0.0025). The median MPV and PDW values for the PTB patients were significantly higher at month 5 of treatment (10.15 fL and 12.5 fL, respectively) compared to median values at month 2 of treatment (9.2 fL and 10.7 fL, respectively) and at diagnosis (8.90 fL and 10.40 fL, respectively) (P=0.0001 and P=0.0023, respectively) Table 1. A post-hoc analysis using Dunn's multiple comparison's test revealed statistically significant differences in mean rank differences for platelet count, MPV, PDW and PCT % at diagnosis versus 5 months of treatment and month 2 versus month 5 of treatment Figure 2.

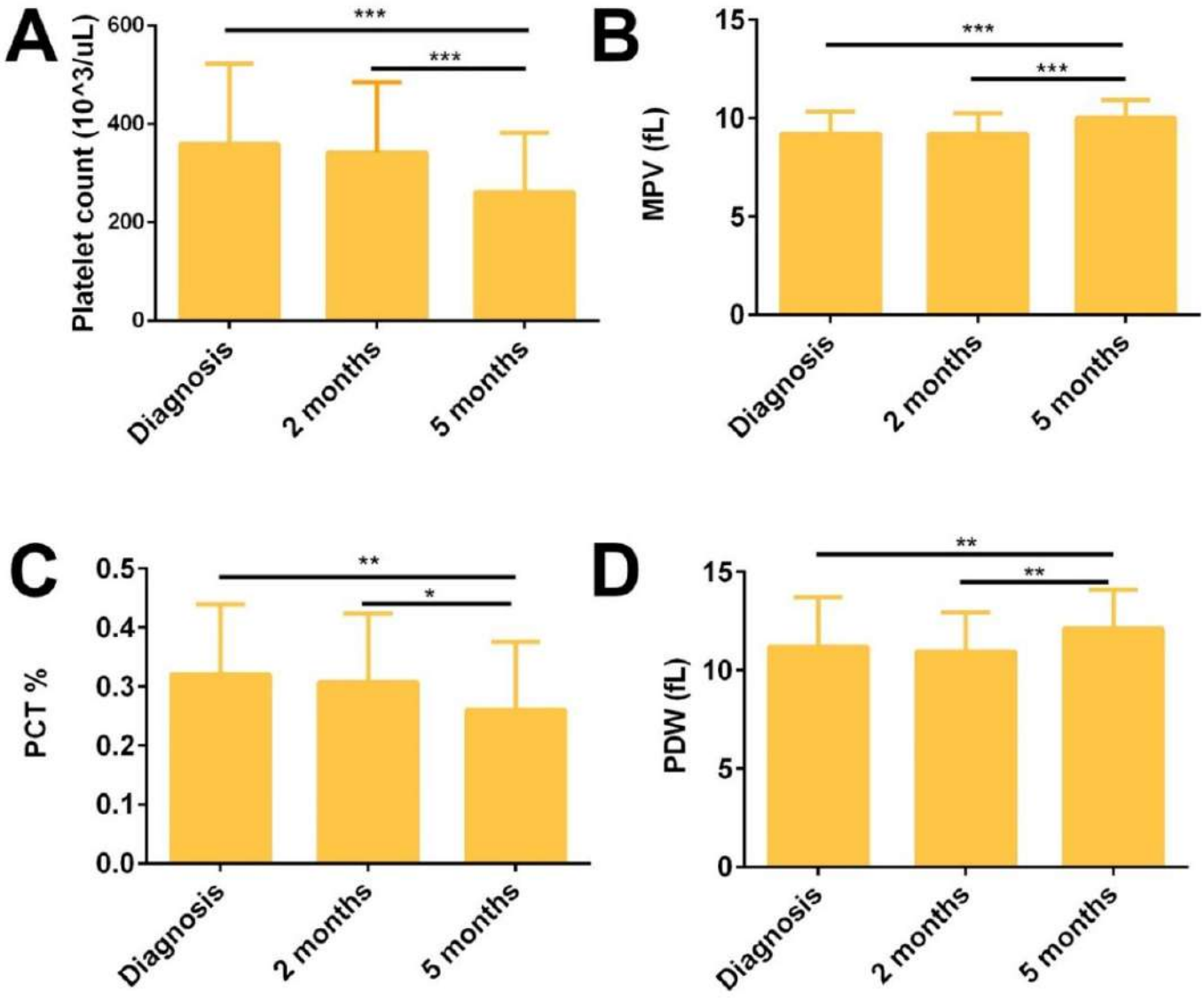

Figure 3

Time Dependent Changes in Red Blood Cell Parameters from Diagnosis to the Fifth Month of Treatment.

A, B, C, and D-Denote statistically significant differences in platelet counts, MPV, PCT% and PDW between mean rank differences at diagnosis, month 2 and month 5 of treatment, respectively. Data was analyzed using Dunn's Multiple Comparisons tests and presented as means with a P value of <0.05.

4.4 Hematological Parameters have Significant Associations with Sputum Smear Conversion Post-Intensive Phase of Anti-Tubercular Therapy

Having a high WBC count at the end of month two increased the odds of having a positive sputum smear post-intensive phase 2.4 times for pulmonary tuberculosis patients (OR=2.42, 95% CI=2.07-2.82), *P*<0.001 in comparison to the normal range Table 2. Similarly, having a high platelet count at the end of month two was associated with 1.5 times likelihood of sputum non conversion after the intensive phase of treatment (OR=1.5, 95% CI=1.01-2.22), *P*=0.043. Quite interestingly, having high counts of neutrophils, monocytes, eosinophils, basophils, RBCs and HGB were associated with a lower likelihood of sputum non conversion at the end of month two in comparison to the normal ranges ((OR=0.44, 95% CI=0.4-0.48, *P*=0.000), (OR=0.41, 95% CI=0.36-0.48, *P*=0.000), (OR=0.53, 95% CI=0.37-0.75, *P*=0.000), (OR=0.44, 95% CI=0.4-0.48, *P*=0.000), (OR=0.68, 95% CI=0.5-0.91, *P*=0.009), and (OR=0.62, 95% CI=0.44-0.88, *P*=0.008), respectively Table 2. Similarly, having a low WBC count, monocyte, RBC, MCV (fL), MCH (pg), RDW-SD (fL), and PDW (fL) were associated with lower odds of sputum non conversion post-intensive phase of treatment. On the other hand, patients with a low lymphocyte count and HGB were 1 and 2.58 times likely to have a positive sputum smear at the end of month two anti-tubercular therapy (OR=1.00, 95% CI=1.00-1.00, *P*<0.001), and (OR=2.58, 95% CI=1.68-3.95, *P*<0.001), respectively Table 2.

Table 2

The Predictive Effect of Hematological Parameters at the Second Month of Treatment on Sputum Smear Conversion Post-Intensive Phase

Parameter	Level	OR (95% ci)	*P* value
WBC (10^3/uL)	High	2.42(2.07-2.82)	**0.000**
	Low	0.58(0.38-0.88)	**0.011**
Neutrophil count	High	0.44(0.4-0.48)	**0.000**
	Low	0.69(0.44-1.09)	**0.112**
Lymphocyte count	High	1.19(1.08-1.31)	**0.001**
	Low	1(1-1)	**0.000**
Monocyte count	High	0.41(0.36-0.48)	**0.000**
	Low	0.44(0.4-0.48)	**0.000**
Eosinophil count	High	0.53(0.37-0.75)	**0.000**
Basophil count	High	0.44(0.4-0.48)	**0.000**
RBC (10^6/uL)	High	0.68(0.5-0.91)	**0.009**
	Low	0.65(0.44-0.97)	**0.033**
HGB (g/dL)	High	0.62(0.44-0.88)	**0.008**
	Low	2.58(1.68-3.95)	**0.000**
HCT%	Low	1(0.67-1.49)	0.999
MCV (fL)	Low	0.63(0.45-0.88)	**0.006**
MCH (pg)	Low	0.65(0.47-0.91)	**0.013**
MCHC (g/dL)	Low	0.99(0.81-1.22)	0.929
RDW-CV%	High	0.93(0.73-1.17)	0.528
RDW-SD (fL)	Low	0.59(0.41-0.84)	**0.004**
PLT (10^3/uL)	High	1.5(1.01-2.22)	**0.043**
PDW (fL)	Low	1.62(1.16-2.27)	**0.004**
PCT %	High	1.09(0.84-1.42)	0.515

Data is presented as odds ratio (95% confidence interval) values. Multivariate logistic regression was used to determine the predictive effect of hematological parameters at month two of treatment on sputum smear conversion post-intensive phase of treatment. A *P* value of 0.05 was used and significant values bolded.

4.5 Discussion

Following diagnosis, patients with drug susceptible pulmonary TB are put on a six month treatment regimen comprising of rifampicin, isoniazid, ethambutol and pyrazinamide for the first two months. This is followed by a two drug regimen comprising of rifampicin and isoniazid for the remaining four months (WHO Guidelines, 2017). The current study recruited fifty five newly diagnosed drug susceptible pulmonary TB patients who were longitudinally followed for the six months they were on treatment. Our study revealed significant changes in hematological profiles at month 2 and 5 of treatment compared to baseline hematological values. There was significant reduction in absolute

median WBC counts at month five compared to month two of treatment and at baseline. This finding is comparable to findings by Kassa *et al* (2016) in Ethiopia who reported significant reductions in WBC count at week two of treatment compared to baseline values. Similar findings were also documented by Bonsome, (2017) in Nigeria and Yadav *et al.*, (2022) in India. However, on differential WBC counts, our study reported significant reductions in neutrophil counts at month two and five of treatment compared to baseline values. This finding agrees with Obeagu *et al.* (2019) in Nigeria but contradicts that reported by Kassa *et al.* (2016) who reported an increase in neutrophil count post-intensive phase of treatment. Differences in neutrophil counts as reported could have been due to variations in bacillary loads in patients that were recruited by the two studies.

The decrease in WBC counts as measured at the three time points could have been due to reduction in bacillary loads occasioned by commencement of anti-tubercular therapy. This was evidenced by high sputum conversion rates at the end of month two of anti-tubercular therapy. Consequently, a decrease in bacillary load leads to a reduction in chemokines and inflammatory cytokines known to recruit more cells of the immune system into circulation. Therefore, response to treatment with significant reductions in granulocyte populations in pulmonary TB patients is largely responsible for variations seen in absolute WBC counts at baseline, month two and month five of treatment in this study. Our study reported no statistically significant changes in RBC counts, HGB and HCT% at baseline compared to month two and five of anti-tubercular therapy. However, there was an increase in the number of patients who had anemia at month five compared to month two of treatment. This finding is comparable to that documented by an Ethiopian study that was conducted by Reta and colleagues in 2023.

The median MCV, MCH, and MCHC in our study slightly dropped at month two of treatment with a slight increase being recorded at month five of anti-TB therapy. Obeagu *et al.* (2019) in Nigeria reported a significant increase in MCHC alone over the course of treatment with no statistically significant changes in MCV and MCH. Bonsome, 2017 reported contrasting findings in MCV, MCH and MCHC values to our study. The Nigerian study reported reductions in MCV, MCH, and MCHC values at week eight of treatment compared to week four and baseline values. Increase in the number of patients with low hemoglobin values could have further been compounded by anti-TB drugs. Several studies have associated anti-TB drugs to drug induced hematological syndromes such as megaloblastic anemia, red blood cell aplasia and sideroblastic anemia. However, anti-TB commencement in our study showed a significant reduction in bacillary loads as evidenced by sputum smear conversion rates at month two and five of treatment. Decrease in bacillary loads is associated with a reduction in the inflammatory milieu created by host immune cells. This could possibly explain the slight improvement in MCV, MCH and MCHC at month five compared to month two of treatment.

The current study documented significant reductions in platelet counts at month five of treatment compared to month two and baseline values. This finding agrees with studies by Bonsome, 2017, Yadav *et al.*, 2022 and Reta *et al.*, 2023 in Nigeria, India, and Ethiopia, respectively. The reduction in elevated platelet counts could have been due to reductions in acute phase inflammatory cytokines. Interleukin (IL)-6 has been implicated by previous studies as a key promoter of megakaryopoiesis (Kirwan *et al.*, 2021). Therefore, clearance of *Mycobacterium tuberculosis* by anti-TB drugs possibly reduces the inflammatory response in PTB patients thereby decreasing levels of IL-6 liberated by cells of the immune system. On the other hand, some studies have documented anti-tubercular induced destruction of platelets as one of the mechanism that leads to reduced platelet counts in PTB patients (Visentin & Liu, 2007; Aster & Bougie, 2007; Yakar *et al.*, 2013).

The current study also evaluated the predictive effect of hematological profiles on sputum smear conversion at month two of treatment. Having a high Absolute WBC count at the end of month two of treatment was significantly associated with sputum non conversion post-intensive phase of treatment. Persistently high absolute WBC counts even after anti-tubercular therapy for two months possibly would indicate active immunological status, correlating with residual *Mycobacterium tuberculosis* bacilli. Additionally, increase in the numbers of macrophages and dendritic cells has been demonstrated to aid *Mycobacterium tuberculosis* survivability. *Mycobacterium tuberculosis* has been shown to express several virulence factors that counter phagocytic roles of macrophages through inhibition of autophagy, inhibition of intracellular trafficking, neutralization of toxic components such as reactive oxygen species (de Martino *et al.*, 2019).

On the other hand, dendritic cells are prone to manipulation by *Mycobacterium tuberculosis* through their dendritic cell specific intracellular adhesion molecule 3-grabbing non-integrin receptor (DC-SIGN), also called CD209. The receptor has been demonstrated to couple with lipoarabinomannan mannose of *Mycobacterium tuberculosis* allowing the bacilli penetrate into the cell (Balboa *et al.*, 2010; Wu *et al.*, 2011). This penetration disrupts dendritic cell activity favoring continued *Mycobacterium tuberculosis* survival. Low lymphocyte and low hemoglobin values were equally associated with a likelihood of a positive sputum smear at month two of anti-tubercular therapy. Low lymphocyte counts have been shown to occur in patients with severe forms of TB, possibly delaying sputum conversion post intensive phase of treatment (Nagu *et al.*, 2014). Additionally, lymphocytes play key effector roles in elimination of pathogens, especially *Mycobacterium tuberculosis.* Therefore, decrease in their numbers would

somewhat impair rapid elimination of the pathogen by the immune system. Mechanisms by which anemia is associated with delayed sputum conversion are not yet fully understood. A Tanzanian study postulated iron deficiency as one of the mechanisms for delayed sputum conversion (Nagu *et al.,* 2014).

In our study, the inflammatory response during the acute phase of the infection could be one the mechanism by which anemia associates with sputum non conversion at month two of anti-tubercular therapy. During the acute phase, several inflammatory cytokines are liberated by immune cells in a bid to combat *Mycobacterium tuberculosis.* Most of these cytokines have been shown to have an inhibitory effect on erythropoietin, the chief hormone responsible for erythropoiesis stimulation. High numbers of platelets beyond the normal range according to the current study, were shown to increase the odds of a positive sputum smear post-intensive phase of treatment. Increases in platelet numbers in PTB patients has been linked to several undesirable outcomes. Platelets have been linked to upregulation of monocyte matrix metalloproteinase-1 production a tissue collagenase responsible for lung cavitation in PTB patients. Increase in tissue destruction occasioned by the collagenase coupled with an interleukin 1β, interleukin 10 high and interleukin 12 low milieu has been associated with an increase in *Mycobacterium tuberculosis* growth and survivability (Fox *et al.,* 2018).

V. CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

Significant time dependent changes in the values of WBC, RBC, and platelets occur in PTB patients from diagnosis to completion of treatment. The proportion of patients with anemia increases over time. Additionally, there are significant associations between high WBC count, high platelet count, low HGB and low lymphocyte count with delayed sputum conversion post-intensive phase of treatment. Therefore, patients undergoing treatment should be screened for any hematological abnormalities for tailored adjunctive therapies.

5.2 Recommendations

This study recommends screening of newly diagnosed pulmonary TB patients for hematological abnormalities before enrollment into care for tailoring specific treatment approaches. Similarly, pulmonary TB patients undergoing treatment should be monitored to detect any hematological anomalies that can exacerbated by treatment regimens used for managing pulmonary TB.

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