International Journal of Community Medicine and Public Health
Mwilitsa E et al. Int J Community Med Public Health. 2024 Jun;11(6):2225-2233
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20241480

Original Research Article

Hematological profiles of newly diagnosed pulmonary tuberculosis patients in Western Kenya

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Received: 09 April 2024
Revised: 20 May 2024
Accepted: 21 May 2024

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) mortality remains high despite current availability of effective anti-TB drugs. This could be due to pathophysiological derangements that are not fully understood and managed during anti-TB therapy. The objective of this study was to evaluate the hematological changes in newly diagnosed PTB patients.
Methods: 55 newly diagnosed PTB patients and 55 healthy controls were included in this cross-sectional non-randomized study. Complete hematological profiles were determined using an automatic analyzer. Peripheral blood films were used to evaluate cellular morphology. Data was analyzed using chi-square and Mann-Whitney tests (SPSS version 29.0).
Results: Males constituted 80% (44) of the newly diagnosed PTB patients and 81.8% of the blood donor controls. Compared with the control group, the PTB patients group exhibited significantly lower median red blood cell (RBC) count (4.79×10^6 µl vs 5.2×10^6 µl, p=0.001), hemoglobin levels (12.8 g/dl vs 14.3 g/dl, p=0.0001), hematocrit (37.9% vs 42.05%, p=0.0001), mean platelet volume (8.9 fl vs 10.5 fl, p=0.0001) and platelet distribution width (10.4 fl vs 13.0 fl, p=0.0001). The median platelet count for the PTB group was significantly higher relative to controls (314.0×10^3/µl vs 237.0×10^3/µl, p=0.0001). Similarly, the PTB group had a significantly higher PCT% compared to controls (0.27% vs 0.25%, p=0.002). Morphological analysis of peripheral blood films revealed normocytic normochromic anemia and microcytic hypochromic anemia in 54.5% (n=30) and 34.6% (n=19) of PTB patients, respectively.
Conclusions: Newly diagnosed PTB patients in Western Kenya present with leukocytosis, elevated platelet count and anemia, suggesting the need for appropriate management and routine monitoring of hematological profiles.

Keywords: PTB, Hematological profiles, Normocytic normochromic anemia, Hematocrit, Hemoglobin

INTRODUCTION

Pulmonary tuberculosis (PTB), is caused by *Mycobacterium tuberculosis*, and accounts for a large portion of the global health burden with majority of the developing nations bearing the consequences. Pulmonary TB is ranked as the 13th major cause of death worldwide and as the second leading infectious killer disease.[1] In 2020, 85% of the cumulative PTB deaths were recorded in the WHO South-East Asia and African regions.[1] Kenya, is among the high TB burdened countries in Africa, with an estimated incidence of 140, 000 and mortality rate of 7.1% in 2020.[2] In 2021, Kenya recorded a total of 77, 854 cases of all forms of TB with TB/HIV co-infection rate of 23.3%.[2] Of these, Kakamega County alone accounted for a total of 1, 899 cases with a TB/HIV co-infection rate of 30.8% and a mortality rate of 10.8%, which is above the national target of <5% for TB related deaths.[2] The potential factors contributing to this exaggerated PTB mortality rate in Kakamega County are largely unknown.

Mycobacterium tuberculosis bacterium is mainly acquired through exposure to aerosols from infected persons[3]. Upon exposure, the bacterium gets into the lungs where it induces release of several chemokines from resident macrophages, leading to recruitment of various immune cells, including neutrophils, monocytes, natural killer cells and lymphocytes, particularly $CD4^+$ T helper cells.[4] Following recruitment, inflammatory cytokines are released in large amounts including tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and interleukin 6 (IL-6), with both local and systemic pathological effects.[5,6] These inflammatory cytokines have been demonstrated to inhibit key hematopoietic processes such as erythropoiesis, resulting in anemia, with associated clinical sequelae that might influence PTB outcomes.[6] Interleukin-6 has been associated with promotion of megakaryopoiesis in PTB patients resulting in an increase of platelet counts.[7] Kirwan et al demonstrated that specific platelet gene transcripts were up-regulated in PTB patients, suggesting the potential pathological role played by elevated platelets in PTB infection.[7] Similarly, Fox et al evaluated the regulatory role of platelets in pulmonary inflammation and the resulting tissue damage in newly diagnosed patients.[8] The study demonstrated an up regulation of inflammatory mediators associated with platelets in PTB patients compared to controls.[8] Furthermore, hepcidin synthesis is induced during the inflammatory response to PTB.[9] Hepcidin, a key regulator of iron absorption and metabolism, is associated with decreased levels of serum iron. Consequently, this leads to reduced utilization of iron for blood formation with resultant bone marrow impairment.[9]

Therefore, hematopoietic disturbance might be a key, but often overlooked systemic factor in the pathogenesis and outcomes of PTB. In Kenya, PTB patients undergoing treatment are monitored for optic neuritis, peripheral neuropathy, hepatotoxicity and sputum conversion.[10] However, little or no attention is paid to the hematological profiles of PTB patients newly diagnosed or undergoing treatment. Therefore, this study, aimed at evaluating the hematological parameters of newly diagnosed PTB patients at Kakamega county teaching and referral hospital (KCTRH), western Kenya. The study also sought to determine the cellular morphological changes that occur in newly diagnosed PTB patients. We herein report that newly diagnosed pulmonary TB patients in western Kenya exhibit various hematological derangements.

METHODS

Study site

The study was conducted in Kakamega County located in the Western part of Kenya. The County covers an area of 3,033.8 km.[2] The County is the second most populous with a total population of 1, 867, 579 people with males and females being 897, 133 and 970, 406 respectively as per the national census report of 2019, Kenya. The County has 1 referral hospital, 12 sub-county hospitals, 47 health centers, 123 dispensaries and 44 clinics both public and private. The study was conducted at KCTRH located 379.8 km west of Nairobi, the capital city of Kenya, 48 km North of Kisumu, along the Kisumu-Webuye Road.

Study design and study population

A cross section design was used for this study. A total of 110 study participants (55 newly diagnosed PTB and 55 controls), were recruited into the study at KCTRH from April to November 2023. The PTB group were HIV negative patients who were newly diagnosed with PTB either through GeneXpert MTB/RIF or sputum smear microscopy. The control group was persons who qualified as blood donors at the hospital's blood donation center.

Sample size determination and sampling

The sample size was determined by the G power sample calculation software with a medium effect size of 0.50, a power of 0.80 and an alpha of 0.05. This gave a minimum sample size of 51 participants per group. The participation was voluntary and all the 110 participants who accepted to participate in the study were conveniently sampled after a thorough explanation of study objectives.

Inclusion criteria

All newly diagnosed HIV negative cases of PTB who consented were eligible for recruitment into the study. Blood donors who satisfied the Kenya tissue and transplant authority donor screening questionnaire were recruited after testing for HIV, hepatitis A, hepatitis B, hepatitis C, and syphilis.

Exclusion criteria

Patients who were suffering from any form of chronic illness such as cancer, heart disease and renal disease were excluded. Additionally, patients with a known history of hematological disorders such as sickle cell anemia, hemophilia or thalassemia were excluded. Blood donors that tested positive for HIV, hepatitis A, B and C, and syphilis were excluded from the control group. Additionally, donors who were lactating, pregnant or had tattoos were not recruited.

Laboratory procedures

Blood sample collection

Upon receiving consent, 5 ml of venous whole blood samples was collected by venipuncture using the evacuated system into EDTA-containing vacutainer tubes. Collected blood samples were labeled with participant serial number, tests to be performed, time and date of collection. Samples were then transported to the

laboratory department in designated cool boxes for analysis.

Complete blood count analysis

The HumaCount 5D automated hematology analyzer (Human Gesellschaft für Biochemica und Diagnostica GmbH, Germany) was used to perform complete blood counts on participant specimen. Leukopenia was considered as a white blood cell (WBC) count below 4.0×10^3/µl whereas leukocytosis as a count above 10.0×10^3/µl. A neutrophil count below 2.0×10^3/µl was categorized as neutropenia while a count above 7.0×10^3/µl was categorized as neutrophilia. Lymphocyte, monocyte and eosinophil counts below 0.80×10^3/µl, 0.12×10^3/µl and 0.02×10^3/µl were considered as lymphocytopenia, monocytopenia and eosinopenia. Conversely, counts above 4.0×10^3/µl, 1.20×10^3/µl and 0.50×10^3/µl were termed as lymphocytosis, monocytosis and eosinophilia. For RBCs, counts below 3.50×10^6/µl and above 5.50×10^6/µl were termed as erythropenia and erythrocytosis, respectively. The cut-off values, were mild anemia were 11.0-12.9 g/dl with those of moderate anemia being 8.0-10.9/dl. Severe forms of anemia were based on hemoglobin values that were less than 8.0 g/dl. For platelets, counts below 150×10^3/µl and above 450×10^3/µl were considered as thrombocytopenia and thrombocytosis, respectively.

Peripheral blood film examination

Peripheral blood films were prepared using venous blood collected in di-potassium EDTA-containing tubes. Prepared films were fixed using absolute methanol, stained with Leishman staining technique which involved flooding the smears with undiluted Leishman stain for 2 minutes followed by a dilution using buffered water (pH 6.8). The diluted stain was allowed to stand for 10 minutes after which slides were gently rinsed in tap water, air dried and observed using x100 immersion oil objective lens.

PTB diagnosis

All presumptive cases of PTB from the outpatient department were referred to and handled at the hospital's chest clinic department. After detailed history taking, patients were given wide-mouthed unbreakable leak proof sputum mugs or 50 ml falcon tubes for sputum collection. In most cases, patients collected a spot sample for PTB diagnosis. To ensure quality of collected sputum samples, patients were instructed to rinse their mouths thoroughly with clean water. They were further instructed to inhale deeply 2-3 times before coughing deeply to produce sputum as opposed to saliva. Collected sputa from presumptive cases of pulmonary TB were analyzed using GeneXpert Ultra/Rif (Danaher Corporation, U.S.A) and fluorescent microscopy for the diagnosis of pulmonary TB.

Quality assurance

Approved standard operating procedures were used at all times in areas that involved patient handling, sample collection, sample handling and sample processing. Analyzer specific quality controls for hematology parameters (low, medium and high) were used to perform internal quality control on the automated analyzer. Continuous quality monitoring was done using automated and manual Levey-Jennings charts.

Statistical analysis

Data was cleaned, entered into Microsoft excel and exported into SPSS version 29.0. Descriptive statistics were used to analyze socio-demographic data. The Kolmogorov-Smirnov test was performed to check for normal distribution of data. Since the data was not normally distributed, non-parametric Mann-Whitney U test was used to determine statistically significant differences between the hematological profiles of the PTB group and control group. Chi-square test was used to analyze categorical data to establish any statistically significant differences among the proportions of study participants with various hematological abnormalities.

Ethical approval

Ethical approval was obtained from the Masinde Muliro university ethical review committee, number MMUST/IERC/120/2023. A permit to conduct the research was obtained from the national commission for science, technology and innovation, number NACOSTI/P/23/23989. The ethical review committee of Kakamega County also approved the study, approval number ERC/190-03/2023. Written informed consent was obtained from all participants from the language of their choice (Luhya, Swahili or English), after explaining the study objectives thoroughly to them. Confidentiality of participant data was maintained through use of lockable cabinets, password-restricted access computers and use of participant serial numbers.

RESULTS

Participant characteristics

The participants' characteristics are summarized in Table 1. The study recruited 55 participants for both the control and PTB groups. In the PTB group, males accounted for 80% of the participants while females accounted for 20%. In the control group, males accounted for 81.8% of the participants whereas females accounted for 18.2 percentages.

The two groups were thus evenly matched on gender and age ($p=0.059$ and $p=0.121$, respectively). The mean weight was significantly higher in the control group compared to the PTB group ($p=0.046$) shown in the Table 1.

Newly diagnosed PTB patients exhibit altered WBC counts

Total WBCs were counted automatically and compared between PTB patients and controls. Our results revealed elevated total WBC counts among PTB patients relative to controls (p=0.0001). In comparison to the control group, PTB patients had significantly higher neutrophil counts (p=0.0001), monocyte counts (p=0.0001) and basophil counts (p=0.002; Table 2). The WBC parameters were further sub-categorized either as normal, high or low and the group proportions compared by Chi square test. As shown in Table 3, 14.5%, (n=8) of the PTB patients had higher than normal WBC counts (leukocytosis) compared to 0.0% (n=0) in the controls (p=0.001). A significantly higher proportion (18.2%, n=10) of the PTB patients had neutrophilia whereas a higher proportion of 34.5% (n=19) for controls exhibited neutropenia compared to 5.5% (n=3) for the PTB patients (p=0.0001). The proportions of PTB patients and controls in the lymphocyte and monocyte subcategories were comparable (p=0.361 and p=0.219, respectively; Table 3). Altogether these findings suggest that PTB is associated with WBC derangements at diagnosis.

Newly diagnosed PTB patients present with altered RBC parameters

The RBC count for the PTB group was significantly lower than that of the control group (p=0.001). Compared to the controls, the PTB patients also had significantly lower HGB levels (p=0.0001) and HCT (%) values, (p=0.0001). Moreover, the MCHC (g/dl) of the PTB group was significantly lower than that of the control group (p=0.010). Conversely, the PTB patients had higher RDW-CV% and RDW-SD (fl) values when compared to those of the control group (p=0.0001). There was no statistically significant difference observed between the MCV (fl) and MCH (pg) values of the PTB and control groups (p=0.152 and p=0.067, respectively; in the Table 2).

Sub-categorization of the participant's RBC parameters as normal, low or higher revealed a higher proportion of PTB patients with abnormal HCT, MCH and RDW-CV%. As presented in Table 3, a higher proportion of the PTB patients had lower than normal HCT% (p=0.001). Similarly, a higher proportion of the PTB patients had lower than normal MCH compared to controls (p=0.032). Furthermore, a higher proportion of PTB patients, had a significantly higher than normal RDW-CV (%) values when compared to the control group (p=0.032). HGB levels were used to group patients into normal, mild anemia, moderate anemia and severe anemia categories. As shown in Table 3, the proportion of PTB patients in the moderate anemia category (HGB of 8.0-10.9 gm/dl) was significantly higher in comparison to the controls (p=0.0001). Consistent with that finding, a significantly higher proportion of controls were found to have normal HGB levels (p=0.0001).

Newly diagnosed PTB patients altered platelet parameters

The platelet count of the PTB group was significantly higher than that of the control group (p=0.0001). The MPV and PDW values in the PTB group were significantly lower when compared to those of the control group (p=0.0001, respectively Table 2). Further, comparison of the proportion of participants with platelet abnormalities, showed that the PTB cases had higher than normal platelet counts compared to only 10.9% in the control group (p<0.0001). However, there was no statistically significant difference in PDW between the two groups when analyzed for participants that had low, normal or high PDW values (p=0.069, Table 3).

Blood cell morphological characteristics

To further elucidate the hematological abnormalities associated with PTB, peripheral blood films were observed and analyzed microscopically for WBC, RBC and platelet morphological changes. As shown in Table 4, a higher proportion (34.5%) of PTB patients had WBCs morphological abnormalities compared to only 1.8% in the control group (p=0.015). Of the PTB-associated WBC morphological abnormalities, 12.8% were classified as leukocytosis with neutrophilia and lymphopenia, 10.9% were neutrophilia with a left shift, 5.4% were neutrophilia with coarse brown granules and full lobulation, 3.6% lymphocytosis and 1.8% as slight eosinophilic leukocytosis with monocytosis. Notably also, a significantly higher proportion 45.5% of PTB patients compared to only 9.1% controls exhibited various RBC morphological abnormalities (p=0.005). The most prevalent red cell morphological abnormality was microcytic hypochromic anemia, which was observed in 38.2% of PTB patients (Table 4).

In addition, microscopic analysis of blood films showed that a higher proportion of PTB patients had higher platelet counts compared to controls (p=0.007, Table 4), which is consistent with the results of the automated analysis.

Table 1: Demographic characteristics of study participants.

Characteristics	Controls, N (%)	PTB patients, N (%)	P value
Gender			
Male	45 (81.8)	44 (80.0)	0.059
Female	10 (18.2)	11 (20.0)	
Weight (mean ± SD)	69.9±12.1	57.8±12.1	0.046*

Continued.

Characteristics	Controls, N (%)	PTB patients, N (%)	P value
Age (mean ± SD)	32.8±10.5	38.0±13.1	0.121
16-30	25 (45.5)	14 (25.5)	
31-44	22 (40.0)	24 (43.6)	
45-58	7 (12.7)	12 (21.8)	0.121
59-71	1 (1.8)	4 (7.3)	
>71	0 (0.0)	1 (1.8)	

Chi-square test was used to test for differences between the groups, with statistical significance set at p<0.05. *denotes statistically significant differences.

Table 2: Hematological parameters of control and cases.

Parameters	Controls median (minimum-maximum)	PTB patient's median (minimum-maximum)	P value
WBC (10^3/µl)	4.6 (2.8-8.2)	6.82 (3.2-13.1)	<0.0001*
Neutrophils	2.41 (1.2-5.0)	4.31 (1.8-11.6)	<0.0001*
Lymphocytes	1.91 (1.14-4.3)	1.74 (0.61-4.8)	0.193
Monocytes	0.23 (0.05-7.0)	0.38 (0.08-1.89)	<0.0001*
Eosinophils	0.12 (0.01-7.0)	0.08 (0.0-1.07)	0.040*
Basophils	0.03 (0.01-7.0)	0.05 (0.01-0.15)	0.002*
RBC (10^6/µl)	5.2 (3.78-6.5)	4.79 (2.88-6.33)	0.001*
HGB (g/dl)	14.3 (10.4-19.1)	12.8 (7.3-18.3)	<0.0001*
HCT%	42.05 (31.3-54.8)	37.9 (22.2-53.1)	<0.0001*
MCV (fl)	81.9 (68.1-96.0)	80.2 (55.7-93.7)	0.152
MCH (pg)	27.9 (22.8-33.5)	27.0 (17.6-32.4)	0.067
MCHC (g/dl)	34.1 (32.9-35.1)	33.8 (30.6-35.7)	0.01*
RDW-CV%	11.9 (10.8-13.7)	12.8 (11.2-18.7)	<0.0001*
RDW-SD (fl)	40.4 (36.5-44.8)	42.3 (36.3-70.7)	0.001*
PLT (10^3/µl)	237.0 (123.0-367.0)	314.0 (185.0-868.0)	<0.0001*
MPV (fl)	10.5 (8.5-12.6)	8.9 (6.60-11.70)	<0.0001*
PDW (fl)	13.0 (9.5-19.5)	10.4 (7.1-18.0)	<0.0001*
PCT %	0.25 (0.14-0.38)	0.27 (0.16-0.64)	0.002*

Mann-Whitney U test used to compare medians between 2 study groups, with statistical significance difference set at p<0.05. *denotes statistically significant differences. HCT-Hematocrit, MCV-Mean corpuscular volume-MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration, RDW-CV-Red blood cell distribution width-coefficient variation, PLT-Platelets, PDW-Platelet distribution width, MPV-Mean platelet volume PCT-Plateletcrit, fl-Femtoliters, pg-picograms, µl-Microliter.

Table 3: Proportion of participants with WBC, RBC and platelet abnormalities.

Parameters	Category	Controls (%)	PTB patients (%)	P value
Total WBC	Leukopenia	10 (18.2)[a]	2 (3.6)[b]	0.001*
	Normal	45 (81.8)[a]	45 (81.8)[a]	
	Leukocytosis	0 (0.0)[a]	8 (14.5)[b]	
Neutrophils	Neutropenia	19 (34.5)[a]	3 (5.5)[b]	<0.0001*
	Normal	36 (65.5)[a]	42 (76.4)[a]	
	Neutrophilia	0 (0.0)[a]	10 (18.2)[b]	
Lymphocytes	Lymphocytopenia	0 (0.0)[a]	2 (3.6)[a]	0.361
	Normal	54 (98.2)[a]	52 (94.5)[a]	
	Lymphocytosis	1 (1.8)[a]	1 (1.8)[a]	
Monocytes	Monocytopenia	7 (12.7)[a]	2 (3.6)[a]	0.219
	Normal	46 (83.6)[a]	51 (92.7)[a]	
	Monocytosis	2 (3.6)[a]	2 (3.6)[a]	
RBC	Erythrocytopenia	0 (0.0)[a]	3 (5.5)[a]	0.078
	Normal	43 (78.2)[a]	46 (93.6)[a]	
	Erythrocytosis	12 (21.8)[a]	6 (10.9)[a]	
HGB (g/dl)	Normal (>12.9)	47 (83.9)[a]	27 (49.1)[b]	<0.0001*
	Mild (11.0-12.9)	8 (14.3)[a]	13 (23.6)[a]	
	Moderate(8.0-10.9)	1 (1.8)[a]	13 (23.6)[b]	
	Severe (<8.0)	0 (0.0)[a]	2 (3.6)[a]	

Continued.

Parameters	Category	Controls, n (%)	PTB patients, n (%)	P value
HCT (%)	Low	7 (12.5)[a]	25 (45.5)[b]	0.001*
	Normal	47 (85.5)[a]	30 (54.5)[b]	
	High	1 (1.8)[a]	0 (0.0)[a]	
MCV (fl)	Low	21 (38.2)[a]	27 (49.1)[a]	0.249
	Normal	34 (61.8)[a]	28 (50.9)[a]	
	High	0 (0.0)[a]	0 (0.0)[a]	
MCH (pg)	Low	16 (29.1)[a]	27 (49.1)[b]	0.032*
	Normal	39 (70.9)[a]	28 (50.9)[b]	
	High	0 (0.0)[a]	0 (0.0)[a]	
MCHC(g/dl)	Low	0 (0.0)[a]	3 (5.5)[a]	0.079
	Normal	0 (0.0)[a]	0 (0.0)[a]	
	High	55 (100.0)[a]	52 (94.5)[a]	
RDW-CV (%)	Low	2 (3.6)[a]	0 (0.0)[a]	0.029*
	Normal	53 (96.4)[a]	50 (90.9)[a]	
	High	0 (0.0)[a]	5 (9.1)[b]	
Platelets	Thrombocytopenia	0 (0.0)[a]	0 (0.0)[a]	<0.0001*
	Normal	49 (89.1)[a]	27 (49.1)[b]	
	Thrombocytosis	6 (10.9)[a]	28 (50.9)[b]	
PDW (fl)	Low	0 (0.0)[a]	5 (9.1)[a]	0.069
	Normal	52 (94.5)[a]	48 (87.3)[a]	
	High	3 (5.5)[a]	2 (3.6)[a]	

Chi-square test was used to compare proportions of participants in each hematological sub-categories with statistical significance difference set at p<0.05. *denote significant group difference. Abbreviations: WBC-White blood cells, RBC- Red blood cells, HGB-Hemoglobin, HCT-Hematocrit, MCV-Mean corpuscular volume MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration, RDW-CV-Red blood cell distribution width-coefficient Variation, PDW-Platelet distribution width, fl-Femoliters, pg-Picograms, µl- Microliters. Values with similar superscripts denote no significant difference whereas different superscripts denote statistical significant differences.

Table 4: Proportion of study participants with various morphological characteristics.

Blood cell morphology/ distribution	Control (%)	PTB patients (%)	P value
WBCs			
Normal morphology	54 (98.2)[a]	36 (65.5)[b]	
Leukocytosis with neutrophilia and lymphopenia	0 (0.0)[a]	9 (12.8)[b]	
Mild leukopenia	1 (1.8)[a]	0 (0.0)[a]	
Neutrophilia with a left shift	0 (0.0)[a]	6 (10.9)[b]	0.015*
Neutrophilia with coarse brown granules with full lobulation	0 (0.0)[a]	3 (5.4)[a]	
Slight eosinophilic leukocytosis with monocytosis	0 (0.0)[a]	1 (1.8)[a]	
Lymphocytosis	0 (0.0)[a]	2 (3.6)[a]	
RBCs			
Normocytic normochromic	50 (90.9)[a]	30 (54.5)[b]	0.005*
Microcytic hypochromic red blood cells.	2 (3.6)[a]	21 (38.2)[b]	
Moderate erythrocytopenia, normocytic normochromic cells.	0 (0.0)[a]	1 (1.8)[a]	
Polycythemia, normocytic normochromic	2 (3.6)[a]	1 (1.8)[a]	
Mild microcytosis, hypochromic red cells, elliptocytes, target cells	1 (1.8)[a]	2 (3.6)[a]	
Platelets			
Adequate number of platelets	55 (100.0)[a]	44 (80.0)[b]	0.007*
Thrombocytosis	0 (0.0)[a]	11 (20.0)[b]	

Chi-square test was used to compare differences between the two study groups, significance level was set at a p=0.05 and * denotes significant differences. Dissimilar superscripts denote column proportions that are statistically different.

DISCUSSION

Accumulating evidence suggests that PTB is associated with various hematological derangements ranging from anemia to thrombocytosis.[4,6,7,9] In the present study we investigated the hematological derangements among PTB patients at diagnosis in a tertiary hospital in western

Kenya. Notably, males were overrepresented in our PTB study group. Alamlih et al in a study conducted in Qatar reported a male predominance at 80.2%.[11] Similarly, Batool et al in Pakistan reported a male overrepresentation of 71.6% among the 500 PTB cases studied.[12] In India, Shah et al reported a proportion of males at 64% among studied subjects.[13] These previous findings altogether agree with our documented finding on male predominance in this study. However, our study is in contrast to a study by Abdelkareem et al in Egypt, where the number of female PTB participants were higher compared to males.[14] One possible reason for the overrepresentation of males among our PTB study participants could be due to the generally higher number of male patients diagnosed of PTB compared to females at the study facility.

Majority of the participants in this study were aged between 31 and 44. This is in line with current national epidemiological trends, a clear indication that PTB affects adults in their productive age. The lower mean body weight of PTB patients compared to controls in our study is consistent with a study that was conducted in India.[15] This is likely due to the unintentional weight loss that generally occurs in PTB patients due to inflammatory mediators produced during the acute phase of the infection by *M. tuberculosis*.[16]

Under normal conditions, the human body mounts concerted immune responses, which includes expansion of different leucocytes to limit or clear bacterial infections. Consistent with this physiological response, we show that the median total WBC counts in PTB patients was increased by approximately 1.5-fold compared to the controls. This leukocytosis was mainly attributable to a significant increase in the number of polymorphonuclear neutrophils, monocytes, eosinophils and basophils, but not the lymphocytes. Lymphocytes mainly play a crucial role in adaptive immunity, which develops after the initial innate immune responses. In tuberculosis, these adaptive lymphocyte responses begin when *M. tuberculosis* spreads to the lymph nodes.[17] Therefore, the increase in the number of the leucocytes other than the lymphocytes might be explained by the fact that the PTB patients in our study were all newly diagnosed, and probably in the acute phase in which polymorphonuclear responses predominates. Previously, Ongwae et al documented leukocytosis among 15.9% of the PTB cases in Kisii, Kenya, a finding comparable to 14.5% of leukocytosis among PTB cases in our study.[18] However, a much higher proportion of PTB patients with leukocytosis than in our study was reported by Batool et al in Pakistan at 46.2%. Similarly, Shah et al in India reported leukocytosis in 53% of PTB patients while Kahase et al in Ethiopia reported leukocytosis in 27.5% of patients.[12,13,19] This variation might be due to differences in our studied PTB patient population in terms of age, immune status, disease stage and geographical locations. Altogether, these previous data and our results indicate that leukocytosis commonly occurs albeit in variable proportions in PTB patients in different parts of the world. Of note in our study was the finding of neutropenia in up to 34.5% of apparently healthy blood donor controls recruited. Neutropenia can occur due to viral infections, bacterial infections such as typhoid fever, brucellosis and overwhelming septicemia, splenomegaly, megaloblastic anemia and bone marrow failure. Other causes include drugs such as those used in cancer treatment.[20] Therefore, it is possible that a substantive number of blood donors in the study area had neutropenia-associated condition(s), which could not be detected by the current routine standard screening for transfusion transmissible infections and other healthy indicators at the blood donation facility. This raises an interesting question of whether; total and differential WBC counts should be included in addition to HGB measurement as part of routine hematological screening of potential blood donors. Such additional screening might not only enhance the safety of blood transfusion, but will also be beneficial in advising the potential donors to seek further diagnosis and management of the possible underlying conditions associated with neutropenia. The PTB patients were from the same catchment area as controls, but surprisingly none exhibited neutropenia. This can be attributed to ongoing PTB-driven marked leukocytosis.

In addition, previous investigations have reported strong associations between PTB and RBC abnormalities, including anemia in different countries. For instance, a study in Pakistan by Shafee et al reported anemia in 54% of the studied PTB patients.[21] Shafee et al further reported thrombocytosis in 12% of males and 10% of females studied.[21] Additional studies conducted in Nigeria also documented significant reductions in RBC counts, WBC counts, HCT and platelet counts at 22.74%, 27.94%, 30.88% and 20.59% among PTB patients compared to controls, respectively.[22] We extend these previous findings since we also demonstrated significantly lower RBC count, HCT, HGB, and MCHC in PTB patients compared to controls. In Malawi, 77 % of HIV-negative PTB patients were found to be anemic.[23] Batool et al reported a higher proportion (82.6%) of PTB patients with anemia in Pakistan.[12] In Nigeria, Erhabor et al reported anemia in 88.7% of PTB patients whereas Mukherjee et al reported anemia prevalence of 72.7% among PTB patients in India.[24,25] Abay et al reported anemia prevalence of 46% among PTB patients in Ethiopia.[26] In the present study, 50.8% of PTB cases had anemia, a finding comparable to that documented in Ethiopia by Abay et al but lower to that reported in Malawi, Pakistan, India and Nigeria.[12,23-26] Moreover, studies conducted in Ethiopia reported anemia in 25% of cases studied, a proportion which is substantially lower than that documented in this study.[19]

Morphological analysis of peripheral blood films revealed that 54.5% of the PTB patients had normocytic normochromic anemia while 38.2% were of the microcytic hypochromic type. This corroborates the

findings of Ongwae et al which revealed that 55% of PTB study participants in Kenya had normocytic normochromic anemia.[18] In contrast, lower proportions of normocytic normochromic anemia were reported in previous studies conducted in India and Egypt. In India, Shah et al reported normocytic normochromic anemia in 40% of PTB patients while Abdelkareem et al in Egypt reported 37% for the same.[13,14] The proportion of microcytic hypochromic anemia in this study was significantly lower when compared to proportions reported by Abdelkareem et al in Egypt at 55% and Shafee et al in Pakistan at 52%.[14,21] Conversely, Ongwae et al reported a proportion that was substantially lower for microcytic hypochromic at 20% in Kenya.[18] Consistently, Molay et al reported a lower proportion in India at 29.67% among PTB patients.[27]

Prior investigations have demonstrated that inflammatory mediators including, interleukin-1, interleukin-6, interferon gamma and tumor necrosis factor alpha that are released during the acute phase inhibit production of erythropoietin.[5,6] Consequently, decreased erythropoietin suppresses erythropoiesis, leading to reduced RBC levels.[6] Therefore, it is plausible that the observed increased levels of circulating inflammatory cells particularly neutrophils might have created an inflammatory milieu that probably inhibited erythropoiesis resulting in the high prevalence of anemia among the PTB patients in our study. However, the exact mechanisms through which PTB causes suppressed RBC counts and anemia remains to be elucidated in future studies, so as to inform targeted therapeutic strategies. Nevertheless, the high prevalence of anemia recorded among newly diagnosed PTB patients in this study and several other studies warrant screening, management and routine monitoring of anemia as part of standard care for PTB in western Kenya.[9,11-15] This is because anemia is considered major risk factor for TB-related severity and mortality.[28] Rohini et al showed that thrombocytopenia is the predominant platelet anomaly among PTB patients in India.[15] This is in sharp contrast to our observation where none of the PTB presented with thrombocytopenia. This might be due to differences in patient populations and staging of disease at diagnosis. We show that thrombocytosis was prevalent in 50.9% of PTB patients at diagnosis. Higher prevalence rates of thrombocytosis among PTB patients have previously been reported in India and Ethiopia.[13,19,29] Taken together, these findings indicate that thrombocytosis is a common PTB-associated hematological abnormality. Notably, pro-inflammatory interterleukin-6, which is increased during PTB is a key promoter of megakaryopoiesis.[19] Recent studies have also implicated platelets as key mediators of inflammatory responses in PTB and might play key role in pathogenesis and outcomes, which needs to be further investigated.[8]

Limitations

The cross-sectional nature of the design did not allow observation of the changes in hematological parameters in PTB patients over time. However, it has provided a snap shot of the patients. A validated questionnaire screening tool and clinical evaluation was used to rule out TB infection among blood donor controls, without any laboratory or radiological confirmatory tests.

CONCLUSION

In conclusion, leukocytosis, decreased RBC count, low hemoglobin levels, anemia, high RBC distribution width-coefficient of variation, high red blood distribution width-standard deviation and thrombocytosis are prevalent among newly diagnosed PTB patients in western Kenya. The impact of these hematological derangements on TB treatment outcome should be investigated in future studies.

ACKNOWLEDGEMENTS

Authors would like to thank the participants who volunteered to participate in the study. Also, technical support by the Kakamega County teaching and referral hospital staff especially at the chest clinic and laboratory department. We further appreciate Kakamega County research directorate for permitting our team to conduct the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Mwilitsa E, Kimoloi S, Raballah E. Hematological profiles of newly diagnosed pulmonary tuberculosis patients in Western Kenya. Int J Community Med Public Health 2024;11:2225-33.