Microbes, Infection and Chemotherapy

ORIGINAL RESEARCH

https://doi.org/10.54034/mic.e2286

Haematological Alterations and CD4+ Count in Patients with *Mycobacterium tuberculosis* and HIV coinfection in Adamawa, Nigeria

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Abstract

Objective: Tuberculosis remains a significant public health concern as a communicable disease. This study investigated CD4+ counts and hematological parameters in patients infected with *Mycobacterium tuberculosis*. **Methods**: *M. tuberculosis* detection was performed using GeneXpert MTB/RIF, while CD4+ counts and hematological parameters were analyzed using BD FACS Count and SYSMEX, respectively. **Results**: *Mycobacterium tuberculosis* strain L-form, *M. tuberculosis* strain H83, and *M. tuberculosis* strain bj were identified in all hospitals surveyed. The mean CD4+ count was significantly lower in M. tuberculosis-positive patients (521.4 ± 2.3) compared to the healthy control group (936 ± 0.43) ($p < 0.05$). Conversely, infected patients exhibited significantly higher mean leukocyte (18.3 ± 1.03 × 10^9/L, $p = 0.001$) and erythrocyte sedimentation rate (98.6 ± 21.00 mm/hr, $p = 0.002$) compared to controls (4.2 ± 06.3 × 10^9/L and 8.7 ± 2.10 mm/hr, respectively). Mean hemoglobin levels were lower in infected patients (8.6 ± 2.30) than in controls (15.3 ± 0.01), whereas the mean platelet count was significantly higher in infected patients (602.1 ± 2.70, $p = 0.001$) compared to the control group (312 ± 2.02). **Conclusion**: A baseline assessment of immunohematological parameters should be conducted in all *M. tuberculosis*-positive patients before initiating anti-TB therapy. Additionally, public health education on reducing exposure to TB risk factors is essential.

Key word: CD4+ count, erythrocyte sedimentation rate, GeneXpert, Haemoglobin, hematological, *Mycobacterium tuberculosis*.

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Submitted: november 27, 2024
Reviewed : january 10, 2025
Approved : february 21, 2025

How to cite: Doughari JH, Kadala I, Hamza F. Haematological Alterations and CD4+ Count in Patients with Mycobacterium tuberculosis and HIV coinfection in Adamawa, Nigeria. Microbes Infect Chemother. 2025; 5: e2286

Introduction

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), is an obligate aerobic pathogenic bacterium belonging to the *Mycobacteriaceae* family. MTB primarily infects the mammalian respiratory system, particularly the lungs (1). Tuberculosis remains a major global health concern, causing approximately 1.5 million deaths annually (2). It ranks as the second leading cause of death from infectious diseases worldwide, following human immunodeficiency virus (HIV) infection, with 9.0 million new TB cases and 1.5 million TB-related deaths reported in 2017 (3,4). The increasing prevalence of multidrug-resistant tuberculosis (MDR-TB) further complicates treatment options. Notably, Nigeria ranks third globally in TB burden (5).

Human cellular immunity against TB infection largely depends on CD4+ T-lymphocytes (6). Additionally, TB infection is associated with hematological abnormalities, including reduced hemoglobin levels and increased lymphocyte, monocyte, and platelet counts, as well as an elevated erythrocyte sedimentation rate (ESR) (7). Anemia is considered a major contributor to elevated ESR, while peripheral leukocytosis and neutrophilia are among the most common hematological manifestations of pulmonary tuberculosis (PTB) (8,9). Nwankwo et al. (10) also reported mild anemia, leukocytosis, neutrophilia with toxic granulation, thrombocytosis, and monocytosis in PTB cases.

Paddock et al. (11) highlighted that MTB infection can suppress the immune system by depleting CD4+ cells, leading to severe exacerbation of primary tuberculosis, increased extrapulmonary dissemination, and a higher bacterial burden. MTB-positive patients may exhibit altered CD4+ counts, white blood cell (WBC) counts, lymphocyte counts, and ESR values. Kaufmann (12) reported that CD4+ T lymphocytes coordinate the immune response by stimulating other immune cells, such as macrophages, B lymphocytes, and CD8+ T lymphocytes, to combat infection. Therefore, assessing CD4+ counts and hematological profiles in MTB-positive patients is crucial for detecting underlying alterations associated with infection, ensuring appropriate management alongside TB treatment.

Despite the significance of these hematological changes, they are often overlooked in TB disease management. Routine assessments of CD4+ counts and hematological parameters are not commonly performed, potentially leading to prolonged treatment duration or therapeutic failure. Given the diagnostic and therapeutic challenges posed by MDR-TB and the misdiagnosis of HIV-TB co-infections using conventional diagnostic techniques, evaluating CD4+ counts and hematological profiles in infected patients could provide valuable diagnostic and therapeutic insights.

Limited data on CD4+ levels in TB patients in Adamawa, Nigeria, underscores the need for further research to explore the correlation between CD4+ levels and TB infection. Additionally, a better understanding of how TB influences hematological parameters—including hemoglobin, leukocyte count, and platelet levels—is essential. Furthermore, the impact of TB treatment on hematological parameters remains largely unexplored. This study aims to assess hematological alterations and CD4+ counts in patients with Mycobacterium tuberculosis infection in Adamawa, Nigeria.

Methods

Study Area

The study was conducted in six selected health facilities in Adamawa State: General Hospitals in Ganye, Numan, Michika, and Mubi, as well as the State Specialist Hospital Yola and Modibbo Adama University Teaching Hospital Yola. These facilities serve as referral centers for local government areas and nearby communities. Adamawa State is one of Nigeria's 36 states, covering a total area of 39,742.12 square kilometers with a projected population of 3,106,585. It is located between latitudes 8°N and 11°N and longitudes 11.5°E and 13.5°E. The state experiences two distinct seasons: a dry season (November to March) and a rainy season (April to October), with an average rainfall ranging from 79 mm in the northern regions to 197 mm in the southern regions (13).

Study Period

The study was conducted over a twelve-month period, from September 2021 to August 2022.

Sample Size

A total of 300 blood samples (50 per health facility) were collected from both TB-suspected patients and healthy controls. The sample size was determined using Taro Yamane's formula for a finite population (14).

Sample Collection

A random sampling method was employed to ensure equal selection probability, thereby increasing validity, reliability, accuracy, and generalizability. Blood samples were aseptically obtained by venipuncture from patients attending the selected health facilities. A 3 mL blood sample was collected into an EDTA tube (ethylene diamine tetra-acetic acid) and transported in a cold box to the MTB referral laboratory at Modibbo Adama University Teaching Hospital, Yola, Adamawa State, Nigeria, for further analysis. To minimize bias, trained personnel, blinded to the study objectives, collected the samples following standardized procedures.

Inclusion and Exclusion Criteria

Only blood samples that tested positive for both HIV and MTB using GeneXpert analysis were included in the study. Samples negative for HIV and MTB were excluded. As controls, volunteers who tested negative for both HIV and MTB were randomly selected using specimen codes, ensuring a one-to-one match for each case in each zone.

Identification of *Mycobacterium tuberculosis* complex using GeneXpert MTB/RIF

For MTB complex identification, blood samples were processed using the GeneXpert MTB/RIF machine as described by Bodmer and Strohle (15).

Sequencing of multidrug resistant *Mycobacterium tuberculosis* (MDR-MTB) strains

DNA extraction from MDR-MTB blood samples was performed using the AccuPrep Genomic DNA extraction kit (Bioneer, Germany) following the manufacturer's protocol. The extracted DNA was stored at 4°C until further use (16). Sequencing was conducted using an automated ABI 3100 DNA sequencer and the BigDye Terminator v3.1 Cycle Sequencing Kit (Bioneer) with the following primers:

- Forward primer: rpoBS (5'-GCA GAC GTT GAT CAA CAT CC-3')
- Reverse primer: rpoBR (5'-GAG CCG ATC AGA CCG ATG T-3')

The sequencing reaction mixture contained 9.5 µL of distilled water, 10.0 µL of DNA template, 10.0 µL of primers, and 8.0 µL of BigDye master mix. PCR cycling conditions included an initial denaturation at 96°C for 20 seconds, followed by 30 cycles at 50°C for 20 seconds, and a final extension at 60°C for 4 minutes (17).

CD4+ count determination

CD4+ counts were determined using the BD FACSCount flow cytometry system (BD Biosciences, Germany) and BD FACSCount CD4+/CD3+ reagent kits, following the manufacturer's instructions (16,17). The automated system analyzed samples and displayed results within five minutes, which were printed on thermal paper.

Whole blood count

Whole blood samples in EDTA tubes were vortexed for homogenization and analyzed using the SYSMEX XN-330 5-Part Hematology Analyzer, following the manufacturer's

Table 1

NCBI Nucleotide BLAST of multidrug resistant Mycobacterium tuberculosis strains isolated from the samples

Sample	Sequence	Maximum score	Total score	Query cover	e-value	Percentage identity	Accession	Isolate
GHMc	AJ/GS CTCGGCTAGGCATTGCATAGGAGCTTCCGACCGCTCCGACCGACGGTGGATGCCTGCTCGAGCGTCGTGAA CGGATCGATGTGTACTGAGATCCCCTATCCGGATGGGGATAACGTCTTTCGGGTCAGACGCCTTCTTGGTGC GGCCGGATGGCTTGGTCAGCGGGGAGGACCATGAAGGGCCATCGGTGACGACCCGCAGCCAGGATCCTG AGGAGCTACGAGACAAAGGCCGCGAAGCAACCCTGCCCAGGCGACACATAGGAAGCTGCTACCCACAGC GGTTAGGCTGGGTACGAAGGATGGACAATCGGGGGGACAACCTGGAACCGGTCGGCGATCGGTGCCTGC GCTTTGCCGCGGGGTGACCGGAAACTCGAGTTTGGTCATAACCCTCGACAAAGCATTGGGAACACTCAATC TGATACGGTCAGGGTATCCACACTATCTCGCCACCGCCGCTCCGACCACCGCTGACGCGGGTGATGAGCTC TTGACTCATCATCCACATGTCGAGGGCTGCTATCCCGCTGATGCGCACACCAACTCTATGGATATCGGACAC CGCTCCTCACTATGTGCACATCATCACCCACCCAACATCGTCTCTCCCGGACCTGATGATCTCTCAAACAAG ATTTATCGCTGCCGTCGGGTCCTCCGAACATAGACCACGCTAC	412	7307	94%	1.00E-110	98.79%	CP044345.1	*M. tuberculosis* strain L
GHMb	BJ/GS GTCTCGGCTAGGCATTGGCATAGAGCTTCCGACCGCTCCGACCGACGGTTGGATGCCTGCTCGACGTCTGA CCGGATCGATGTGTACTGAGATCCCCTATCCGGATGGGGATAACGTCTTTCGGGTCAGACGCCTTCTTGGTG GGGCCGGATGGCTTGGTCAGCGGGGAGGACCATGAAGGGCCATCGTAACGACCCGCGCCAGGATCCTGC GAGCTACGAGACAAAGCCGCGAAGCAACCCTGCCCAGGCGACACATAGGTGAGGCTGCTACCCACAGCC GTTAGGCTGGGGTACGAAGGATGGACAATCGGGGGGACGGGCTGGAACCGGATCGGCGATCAGGCCTGC TGCTTTGCCGAAGGGTGACCAGAAACTCGAGTTGCCATAACCCCTCGACAAAGCATCTGGAACCTCAATGC CTCCGGTCCAGGCCACCACACTATCTCGCCACCGAAACTCCGGACCACCAGACCTGGACGCGGGTGATGAC CTCCTTGAATTATCATCCGCCTGTCGAGGGCTGCTATCCCGGCTGAGGCGCACACCAACCTATGGGGAATTC GGATGCCCAGCTCTTCCTATGGGCACATCCATCACCCCCCCAACCATCTCTCTCCCCGTGACCTGATGATCTA TCATACATCCGGAATTATCCGCATCCGGTCGAGGTTCTCCGACACATAGCACGCCCTTAAAATTCCCTAGGC TTCATCAATCCGCGTGGCCGGCAGCAG	329	659	92%	2.00E-85	98.22	Y17220.1	*M. tuberculosis* strain bj
SHY	CJ/GC CGTTGACGTCTCGGCTAGGCATTGCATAGAGCTTCCGACCGCTCCGACCGACGGTTGGATGCCTGCCTCGAC CCGTCGTGAACCGGATCGATGTGTACTGAGATCCCCTATCCGGATGGGATAACGTCTTTCAGGTCAGACGCC TTCTTGGTGGGGGCCAGATGGCTTGGTCAGCGGGGAGGACCATGGAGGGCCATCGAACGACCCGCAGCCA GGATCCTGCAGAGCCGGACAAAGCCACGAGCAACCCTGCCCAGGCGACACATAGGGAGCTGCTACCCAC CCGGTTAGGCTGGGTCCGAAGGTGGACAATCGGGGGGACGGGCTGGGGCCGGATCGGCGATCAGGCCTG AGCTTTGCCGAAGGGTGCCCAGACAACTCGAGTTTGGCATCACCCCTCGACAAGCATCTGGGAACCTCAAT CCCTCACGGTCCGGTACCACACTATGCCGCAACCGAAACTCCGGACTGAGCTGACGCGGGTGATGTGCTCC TGATCATCATCACATGTCGAGGGGCTGGTATCCCGGCTGAGGCGCACACAACCTATGGGGATATCGGGAC CCCAGCTCTTCCTATGGCAATCCAA	331	3919	91%	5.00E-86	98.26	CP019611.1	*M. tuberculosis* strain H83
MAUTHY	EJ/GS CGTCTCGGCTAGGCATTGTCATAGAGCTTCCGACCGCTCCGACCGACGGTTGGATGCCTGCCTCGGGAGCCC CTCGCTGAACCGGATCGATGTGTACTGAGATCCCCTATCCGGATGGGGATAACGTCTTTCGGGTCAGACGC TTCTTGGTGCGGGCCGGATGGCTTGGTCAGCGGGGGGAGGACCATGGAAGGGCCTCGTGACGACCCGGCA CCAGGATCCTGCAGATGCTACGAGACAAAGCCGCGAAGCAACCCTGCCCAGGCGACACATAGAACTCGCT GGTACCCACAGCCGGTTAGGCTGGGGTACGAAGTGATGGACCAATCGGGGGGATAACCTTGAACCGGATC GGCATCAGGGGCCTGCATGCTTTGCGAACGGGTGATGAGACAGGCGAGTTGGCATCACACCCGCGACAAA GCCTCTGGGACCACTCATTGCTCTCCGGCACAGGTACCACACTATCTCGCCCCGGAAACTTCGGACCACCAC CCTAAACGGTGTGATGAGCTCCTTTAACTTATCATCCACATCTCGAGGGGGCTGCTATCCCGGCTGATCGGC ACACCAACCTATGGGATCTTGGGATGCCCGCTCCTTCAACTGTGGCACATCATGCACCCCCCCAAACATCCA GTCTACCCGACCTGATGATCACTCAACAAGAAATTATCCCCTTACCGATAGGGGATCTCCGCACAAAAAGA CAGCTCACTTTAAGATTCCCCGAGGCCGTCATCAAACGGGTG	329	3896	98%	2.00E-85	98.95%	CP019611.1	*M. tuberculosis* strain H83
GHG	EY/GC CTCGGCTAGGCATTGCATAGGAGCTTCCGACCGCTCCGACCGACGGTGGATGCCTGCTCGAGCGTCGTGAA CGGATCGATGTGTACTGAGATCCCCTATCCGGATGGGGATAACGTCTTTCGGGTCAGACGCCTTCTTGGTGC GGCCGGATGGCTTGGTCAGCGGGGAGGACCATGAAGGGCCATCGGTGACGACCCGCAGCCAGGATCCTG AGGAGCTACGAGACAAAGGCCGCGAAGCAACCCTGCCCAGGCGACACATAGGAAGCTGCTACCCACAGC GGTTAGGCTGGGTACGAAGGATGGACAATCGGGGGGACAACCTGGAACCGGTCGGCGATCGGTGCCTGC GCTTTGCCGCGGGGTGACCGGAAACTCGAGTTTGGTCATAACCCTCGACAAAGCATTGGGAACACTCAATC TGATACGGTCAGGGTATCCACACTATCTCGCCACCGCCGCTCCGACCACCGCTGACGCGGGTGATGAGCTC TTGACTCATCATCCACATGTCGAGGGCTGCTATCCCGCTGATGCGCACACCAACTCTATGGATATCGGACAC CGCTCCTCACTATGTGCACATCATCACCCACCCAACATCGTCTCTCCCGGACCTGATGATCTCTCAAACAAG ATTTATCGCTGCCGTCGGGTCCTCCGAACATAGACCACGCTAC	412	7307	94%	1.00E-110	98.79%	CP044345.1	*M. tuberculosis* strain L
GHN	FJ/GN GTCTCGGCTAGGCATTGGCATAGAGCTTCCGACCGCTCCGACCGACGGTTGGATGCCTGCTCGACGTCTGA CCGGATCGATGTGTACTGAGATCCCCTATCCGGATGGGGATAACGTCTTTCGGGTCAGACGCCTTCTTGGTG GGGCCGGATGGCTTGGTCAGCGGGGAGGACCATGAAGGGCCATCGTAACGACCCGCGCCAGGATCCTGC GAGCTACGAGACAAAGCCGCGAAGCAACCCTGCCCAGGCGACACATAGGTGAGGCTGCTACCCACAGCC GTTAGGCTGGGGTACGAAGGATGGACAATCGGGGGGACGGGCTGGAACCGGATCGGCGATCAGGCCTGC TGCTTTGCCGAAGGGTGACCAGAAACTCGAGTTGCCATAACCCCTCGACAAAGCATCTGGAACCTCAATGC CTCCGGTCCAGGCCACCACACTATCTCGCCACCGAAACTCCGGACCACCAGACCTGGACGCGGGTGATGAC CTCCTTGAATTATCATCCGCCTGTCGAGGGCTGCTATCCCGGCTGAGGCGCACACCAACCTATGGGGAATTC GGATGCCCAGCTCTTCCTATGGGCACATCCATCACCCCCCCAACCATCTCTCTCCCCGTGACCTGATGATCTA TCATACATCCGGAATTATCCGCATCCGGTCGAGGTTCTCCGACACATAGCACGCCCTTAAAATTCCCTAGGC TTCATCAATCCGCGTGGCCGGCAGCAG	315	5545	95%	5.00E-81	99.72	CP044345.1	*M. tuberculosis* strain L

Key: GHMc – General Hospital Michika; GHMb - General Hospital Mubi; SHY –Specialist Hospital Yola; MAUTH – Modibbo Adama University Teaching Hospital; GHG – General Hospital Ganye; GHN – General Hospital Numan.

guidelines (18).

Erythrocyte Sedimentation Rate determination (ESR)

Anticoagulated blood was transferred into Westergren pipettes and allowed to stand in a pipette stand for one hour. ESR was determined by measuring the plasma column height in millimeters per hour (mm/h) (18).

Data Analysis

Statistical analyses were performed using SPSS version 22.0 (IBM, USA). Data were expressed as mean ± standard deviation (SD). T-tests and ANOVA were used under the assumption that data were normally distributed, observations were independent, and variances were equal across groups.

Ethical approval

Ethical approval was obtained from the Adamawa State Health Services Management Board Research Ethical Committee (ASDHBREC No. 2020/269). Informed consent was obtained from participants, and confidentiality was maintained through anonymized unique codes. Data was stored on password-protected devices with restricted access.

Results

Sequencing of multidrug resistant *Mycobacterium tuberculosis* (MDR-MTB) strains

Among the 300 samples analyzed, 45 (15.67%) were positive for either HIV (32; 10.67%) or *Mycobacterium tuberculosis* (13; 4.33%), while 255 (84.33%) were negative for both infections. Of the 45 positive cases, 9 (20.00%) were co-infected with HIV and multidrug-resistant tuberculosis (MDR-TB). Further screening of these 9 co-infected cases using GeneXpert confirmed that 6 (66.67%) were MDR-MTB.

Sequencing of the six MDR-TB isolates from the HIV and MDR-TB co-infected patients identified three MDR-TB L strains (50.00%), two H8 strains (33.33%), and one Beijing bj strain (16.67%) from the studied healthcare facilities. The nucleotide BLAST analysis revealed sequence identity percentages ranging from 95.79% to 99.72%, based on the NCBI database (https://www.ncbi.nlm.nih.gov) (Table 1)

Additionally, three MDR-TB isolates (AJ/GS, BJ/GS, and EJ/GS) from samples obtained at Michika and Mubi General Hospitals showed the highest prevalence rates (23.5% and 17.6%, respectively). The Specialist Hospital and Modibbo Adama Teaching Hospital exhibited identical prevalence rates (11.8%), while the lowest prevalence rate (5.9%) was recorded at Ganye and Numan General Hospitals.

Relationship Between Pulmonary Tuberculosis, CD4+ Count, Leukocyte Parameters, and Erythrocyte Sedimentation Rate (ESR) in Infected Patients

Analysis of pulmonary tuberculosis in relation to CD4+ count, quantitative and qualitative leukocyte parameters, and erythrocyte sedimentation rate (ESR) in HIV and MDR-TB positive patients revealed significant hematological alterations.

Patients co-infected with M. tuberculosis and HIV had a significantly lower mean CD4+ count (521.4 ± 2.3) compared to the apparently healthy control group (936 ± 0.43) ($p < 0.05$). Conversely, infected patients showed significantly higher mean leukocyte counts (18.3 ± 1.03 × 10^9/L, $p = 0.001$) and erythrocyte sedimentation rates (98.6 ± 21.00 mm/hr, $p = 0.002$) compared to the control group (4.2 ± 6.3 × 10^9/L and 8.7 ± 2.10 mm/hr, respectively).

Moreover, the mean hemoglobin (Hb) concentration was lower in infected patients (8.6 ± 2.30) compared to controls (15.3 ± 0.01). The mean platelet count was significantly higher, while neutrophil count was lower in infected patients compared to the apparently healthy control group.

Table 2

Mean values of the relationship of Haematological parameters with HIV and MDR MTB positive, with apparently health controls

Haematological parameters	MTB positive mean ± SD	P-value	MTB negative mean ± SD	P-value	Control ± SD	P-value
Hb (g/dL)	8.6 ± 2.30	0.003	13.3 ± 2.53	0.003	15.3 ± 0.01	0.003
PCV (%)	42 ± 3.06	0.004	40.4 ± 07.01	0.002	53.4 ± 0.02	0.003
Platelets ($x10^{9/L}$)	602.1 ± 2.70	0.001	310.3 ± 16.47	0.001	312 ± 2.02	0.004
Neutrophils (%)	43.4 ± 2.30	0.003	65.02 ± 2.32	0.001	62.3 ± 0.41	0.002
Lymphocytes (%)	37.26 ± 3.22	0.004	44.6 ± 2.42	0.003	46.2 ± 1.07	0.003
Monocytes (%)	12.1 ± 3.81	0.001	6.6 ± 1.40	0.005	4.3 ± 2.01	0.004
Eosinophils (%)	2.7 ± 1.75	0.002	3.1 ± 1.04	0.003	2.4 ± 1.01	0.003

Key: WBC – White Blood cell Count, RBC – Red Blood Cell count, PCV – Packed Cell Volume, Hb – Haemoglobin, ESR – Erythrocyte sedimentation rate, mm – millimeter, hr - hour

Discussion

In this study, multidrug-resistant tuberculosis (MDR-TB) strains, including *Mycobacterium tuberculosis* strain L-form, strain H83, and strain bj (Beijing), were identified in all investigated hospitals. The prevalence of these strains varied, with *M. tuberculosis* strain L-form and strain H83 being more frequent in the General Hospitals of Michika and Mubi, while their prevalence was lower in the Specialist Hospital, Modibbo Adama University Teaching Hospital in Yola, and the General Hospitals in Ganye and Numan. Similar bacterial strains have been previously reported in Gombe State, Nigeria (19).

M. tuberculosis strain L-form is a cell wall-deficient variant that exhibits resistance to antibiotics. First isolated in 1935 by Emmy Klieneberger-Nobel, it was named "L-forms" after the Lister Institute in London. This strain has been reported in Malawi, Cape Town (South Africa), Chad, and the United States (17). The absence of a cell wall is a key factor in the development of antibiotic resistance (17), as L-form transition can be induced by antibiotics targeting cell wall synthesis (18). Additionally, L-forms can survive in unfavorable conditions for extended periods, increasing their resilience compared to wild-type bacteria (18).

Resistance to rifampicin and isoniazid in *M. tuberculosis* strain L-form may result from the absence of specific drug targets due to cell wall alterations or loss. The production of capsule-like structures and biofilm matrix may further contribute to drug resistance and bacterial survival. The absence of a cell wall also facilitates the secretion of large amounts of proteins that would otherwise accumulate in the periplasmic space, enhancing antibiotic resistance (20). The mechanisms underlying drug resistance in *M. tuberculosis* L-forms likely involve both target loss and the formation of protective structures that hinder drug penetration.

M. tuberculosis strain bj (Beijing) is one of the most successful genotypes of *M. tuberculosis*, responsible for over a quarter of the global tuberculosis epidemic (17). This strain is known for its high adaptability and capacity to develop drug resistance through rapid genetic mutations (17). The Beijing strain is predominant in East Asia but has also emerged in Malawi, Cape Town, the U.S., and Asia, often associated with disease outbreaks and antibiotic resistance. It is believed to have originated around 30,000 years ago in Southeast Asia, coinciding with early human migration (21). The hypervirulent Beijing subtype has gained global attention due to its increasing prevalence in recent decades (22), its tendency to cause outbreaks, and its strong association with antibiotic resistance (21). Resistance to rifampicin and isoniazid has been particularly linked to the Beijing strain in regions such as Cuba, South Africa, and countries of the former Soviet Union (23).

M. tuberculosis strain H83 belongs to a clade predominantly composed of *Mycobacterium foliorum*. First isolated from the phyllosphere of grasses in Germany, this strain has demonstrated resistance to rifampicin and isoniazid (23). Although the study was conducted on a small sample size within a limited geographical area, the widespread occurrence of L-form, strain H83, and Beijing strain among HIV-positive individuals has significant public health implications.

M. tuberculosis interacts with host immune responses through various surface molecules, modulating immune regulation and bacterial survival (11). The protective immune response against TB primarily relies on cell-mediated immunity, particularly CD4+ and CD8+ T-cells. A normal CD4+ count ranges from 500 to 1,500 cells per microliter of blood. In this study, the mean CD4+ count among MTB-positive patients (337.0 ±1.4) was significantly lower than that of the healthy control group (753.6 ±1.28). This finding is consistent with a study conducted in Pune, India, which also reported significantly lower CD4+ counts in pulmonary TB (PTB) patients (24).

The reduced mean CD4+ count among TB patients observed in this study aligns with findings from other studies (25). CD4+ depletion alters the cytokine environment, affecting pulmonary trafficking of T-effector cells. Infected cells trigger local inflammatory responses, attracting immune cells to the infection site. These cellular aggregates, known as granulomas, are pathological hallmarks of TB (26). CD4+ T-cell deficiency results in reduced immune cell recruitment to the lungs during *M. tuberculosis* infection, underscoring the crucial role of CD4+ T-cells in sustaining pulmonary immune responses. CD4+ T-helper cells and their cytokines are essential for maintaining the functionality of T-effector cells, which produce IFN-γ, TNF-α, IL-17, IL-22, and perforin in infected tissues.

IFN-γ, primarily produced by CD4+ T-cells, is essential for enhancing CD8+ T-cell effector functions during *M. tuberculosis* infection. The diminished pulmonary recruitment of IFN-γ-producing CD4+ and CD8+ T-cells following CD4+ depletion suggests a novel role for CD4+ T-cells in TB immunity (27). Previous studies have shown that tobacco smokers tend to have higher CD4+ counts compared to non-smokers, whereas alcohol consumption is associated with decreased CD4+ levels due to its immunosuppressive effects (28, 29). While direct comparisons of hematological changes and CD4+ counts among TB patients across different regions are limited, research from diverse geographical locations, including India, Africa, and Asia, suggests similar trends. Studies have reported significant hematological changes in TB patients, including anemia, leukocytosis, and altered CD4+ counts (30).

TB is a contributing factor to wasting syndrome, a condition commonly associated with malnutrition in infected patients. Malnutrition often results in subnormal CD4+ counts, while pulmonary TB (PTB) is typically associated with elevated white blood cell (WBC) counts. Increased WBC levels in TB patients are a well-established immune response, driven by heightened polymorphonuclear leukocytes and macrophages, which play a crucial role in combating bacterial infections. Infection-induced inflammation further stimulates WBC proliferation, reflecting the host's immune response to *M. tuberculosis* (25).

Complete blood count determination

Anemia is defined as hemoglobin (Hb) levels below 12.5 g/dL in women and 13.5 g/dL in men (31). It is a common hematologic complication among tuberculosis (TB) patients and a significant risk factor for mortality (32). One study reported a mean hemoglobin level of 10.6 g/dL among TB patients (33), while previous studies in sub-Saharan Africa, including Malawi, have also documented a high prevalence of anemia in this population (34). Other investigations have found hemoglobin levels at TB diagnosis ranging from 10.7 g/dL to 11.2 g/dL (35). Despite its epidemiologic relevance, the causes of anemia in TB patients are not systematically investigated in Adamawa State. Excessive production of pro-inflammatory cytokines such as IL-6, TNF-α, and IFN-γ contributes to anemia by reducing erythropoietin production, suppressing the bone marrow response to erythropoietin, and altering iron metabolism, which may impair erythropoiesis (36).

Anemia in TB patients can have multiple causes, including iron deficiency and chronic inflammation. It is often attributed to nutritional deficiencies, malabsorption syndromes, failure of iron utilization, and bone marrow suppression. The findings of this study reinforce the notion that anemia is highly prevalent in active TB cases and that TB-associated anemia is primarily driven by inflammation. A possible explanation for this increased inflammation is that, as TB infection progresses, chronic inflammation leads to suppressed hemoglobin synthesis, consistent with the anemia of chronic disease (37). The burden of M. tuberculosis infection has been linked to inflammatory status (38). Similar studies have reported lower hemoglobin levels among TB patients (11, 39, 40). However, Milman and Pederson (41) found that hemoglobin levels were higher among smokers and that alcohol consumption had no significant effect on hemoglobin levels.

The mean platelet count in MTB-positive patients (523.1 ±123.5 × 10 /L) was significantly higher than that of the apparently healthy control group (238 ±124.52 × 10 /L), consistent with findings from studies in Iraq and Pakistan (42, 43). This difference may be attributed to reactive thrombocytosis, which occurs in various clinical conditions, including infectious diseases such as pulmonary tuberculosis. This response is likely due to the increased production of thrombopoietic factors like IL-6, which is released by inflamed cells during the immune response (44). In PTB patients, IL-6 secretion stimulates platelet production (39). A study conducted at the All India Institute of Medical Sciences in New Delhi found that thrombocytosis was more common among PTB patients (6). Similarly, research from São Paulo State University, Brazil, indicated elevated platelet counts in PTB patients (45). This may be due to strong pro-inflammatory cytokine activity (IFN-γ and TNF-α) at the onset of TB infection, which stimulates the expression of acute-phase proteins and promotes thrombocytosis (45).

Physiological immune responses to infections often involve increased neutrophil counts and decreased lymphocyte counts. Elevated total WBC and neutrophil levels indicate an inflammatory response, particularly in bacterial infections. Lymphocytopenia has also been proposed as a diagnostic marker for bacterial infections (41). This study found that the mean monocyte count in MTB-positive patients (10.4 ±8.19%) was significantly higher ($p = 0.002$) than in the healthy control group (5.9 ±4.12%). The interaction between M. tuberculosis and the host immune system determines the disease outcome (44). Monocytes and macrophages, derived from hematopoietic stem cells in the bone marrow, circulate in the bloodstream and play a crucial role in innate immunity by linking it to the adaptive immune response through antigen presentation. During MTB infection, monocytes and macrophages phagocytize the bacteria and are recruited to form granulomas, the hallmark of TB pathology (46).

Determination of Erythrocyte Sedimentation Rate determination (ESR)

The relative increase in monocytes and decrease in the lymphocyte-to-monocyte ratio may reflect the host immune response efficiency against infection. Studies have shown that an elevated monocyte percentage in peripheral blood is associated with a higher risk of tuberculosis (41). In this study, the mean ESR in infected patients was significantly elevated (100.8 ±28.31 mm/hour) compared to the healthy control group (6.5 ±14.3 mm/hour). ESR, a routine clinical marker for diagnosing TB, is a non-specific measure of inflammation. However, several non-inflammatory conditions, such as anemia, kidney failure, obesity, aging, and female sex, can also elevate ESR. Additionally, ESR levels increase in women during menstruation and pregnancy due to increased fibrinogen and globulin levels and decreased albumin levels (40).

Conclusion

The findings of this study provide baseline data on the association between CD4+ counts in HIV patients and hematological abnormalities in TB patients. While further comparative studies are necessary, research from Nigeria and other regions suggests that hematological changes and CD4+ counts serve as critical indicators of TB disease severity and treatment outcomes.

Author Contribution Statement

Doughari, J.H.: study design, analysis and interpretation of data, manuscript writing, collection of data, critical revision. **Hamza F.**: study design, analysis and interpretation of data, collection of data. **Kadala I.**: study design, manuscript writing, critical revision.

Funding

This work was supported by the National Research Fund (NRF) Intervention Batch No. 80, Federal Republic of Nigeria.

Ethics statement

The authors declare that the present study was conducted under the strictest ethical conditions after getting approval from Adamawa Sate Health Services Management Board Research Ethical Committee (ASDHBREC No.2020/269).

Conflict of interest

There's no conflict of interest.

Availability of data

The datasets generated and/or analyzed during current study are available from the corresponding author on reasonable request.

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