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Leucocyte Adherence and Migration Indices in Newly Diagnosed Pulmonary Tuberculosis Patients and at 4-Months of Chemotherapy

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Abstract

Background: Pulmonary tuberculosis (PTB) caused by *Mycobacterium tuberculosis* (Mtb) remains a major public health problem in Nigeria. Leucocytes control the onset, progression of Mtb infection, and response to treatment of PTB patients; however, adhesion and migration of leucocytes are not extensively studied in Mtb patients. Leucocyte adherence and migration in response to antigenic stimulation are key steps in immune surveillance, recruitment of immune cells to infected sites, and granuloma dynamics. This study assessed and compared leucocyte adherence and migration indices in newly diagnosed pulmonary tuberculosis patients, 4-months drug-treated tuberculosis patients, and apparently healthy controls.

Methods: A case-control, cross-sectional study design was used. Venous blood (5 ml) was collected from newly diagnosed TB patients (ND-TB, n=20), 4-months drug-treated TB patients (DT-TB, n=20), and apparently healthy controls (HC, n=20). Leucocytes were isolated using dextran sedimentation method. Leucocyte adherence was assessed by a glass slide adherence method, while leucocyte migration was assessed using a capillary tube-based method under unstimulated and BCG-stimulated conditions. Data were analysed using one-way ANOVA, and post-hoc comparisons were performed using Tukey's test at $p < 0.05$.

Results: Leucocyte migration indices (LMI) differed significantly across groups ($F = 7.343$, $p = 0.001$). ND-TB participants demonstrated a significantly higher mean migration index compared with DT-TB (mean difference = 76.505, $p = 0.005$) and compared with HC (mean difference = 77.893, $p = 0.004$). No significant difference was observed between DT-TB and HC (mean difference = 1.389, $p = 0.998$). Leucocyte adherence indices did not differ significantly across all groups ($F = 0.199$, $p = 0.820$).

Conclusion: Leucocyte migration but not leucocyte adherence is defected during Mtb infection and this improves with treatment. These findings suggest that leucocyte migration index may serve as a low-cost functional immune marker in pulmonary TB management.

Keywords: Leucocyte migration, Leucocyte adherence, Pulmonary tuberculosis, Chemotherapy, BCG.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), predominantly affects the lungs and other organs and has returned to being the leading cause of mortality from a single infectious agent, with 80% of cases and deaths occurring in low- and middle-income countries

[1]. Nigeria is one of the 10 high-burden countries in Africa, with approximately 467,000 new TB cases reported annually, ranking first in Africa and sixth globally, accounting for about 4.6% of the global TB burden [2, 3].

Both innate and adaptive immunity are involved in the host immune response to Mtb in the pulmonary airway [4]. After Mtb enters the alveolar space via inhalation, it is phagocytosed by alveolar resident macrophages and dendritic cells as an innate immune response to contain and eradicate the bacilli [5]. The central feature of the immune defense is the recruitment of immune cells to the site of infection, which originates from their adhesion to the vascular endothelium and progresses by transendothelial migration into infected tissues [6]. These cellular functions are critical for granuloma development, immunological surveillance, and bacilli containment [7].

While an increased number of leucocytes implies an inflammatory condition, in tuberculosis the body's immune system responds by producing more leucocytes [8]. Leucocyte adherence and migration are essential first steps in this immune mobilisation. Deficiencies in leucocyte migration or adherence are proposed to contribute to the advancement of drug-sensitive tuberculosis to multi-drug-resistant TB (MDR-TB), where bacterial persistence may be connected to immune evasion tactics [9].

The standard drug regimen for drug-sensitive TB includes isoniazid, rifampicin, pyrazinamide, and ethambutol, which targets bacterial replication and also leads to bacterial clearance in many cases [10]. Drug treatment does not simply eradicate bacteria; it also influences the immune milieu [11]. A study reported therapy-associated alterations in T-cell changes, with decreased frequencies of tmTNFR1 and tmTNFR2 CD4 T cells after six months of treatment [12], demonstrating that standard anti-TB treatment reshapes cellular immune markers in both drug-sensitive and drug-resistant TB cases [13].

While numerous studies have described T-cell subsets and cytokine profiles in TB [14, 15], there are few functional assays of leucocyte behaviour, such as adherence and migratory capacity, especially in African populations. Additionally, these few studies did not compare these parameters among newly diagnosed, drug-treated TB, and apparently healthy controls. Determining differences in leucocyte behaviour among these groups will provide important information on immunopathogenesis in tuberculosis, early indicators of immunological defects, and immunotherapeutic approaches.

This study therefore assessed and compared leucocyte adherence and migration indices in newly diagnosed (ND-TB), drug-treated (DT-TB) patients, and apparently healthy individuals, with the aim of improving understanding of host immune responses in tuberculosis.

Materials and Methods

Study Design and Setting

This is a case-control cross-sectional study conducted at the Outpatient Clinic of Government Chest Hospital, Jericho, Ibadan North Local Government Area, Oyo State, Nigeria. Study

participants were recruited from the Directly Observed Treatment Scheme (DOTS) at this facility.

Sample Size Determination

Sample size was calculated using the one-way ANOVA design since the study compared mean values of continuous indices across three independent groups, applying an effect size (Cohen's f) of 0.40, significance level of 0.05, and 80% statistical power. This yielded approximately 20 participants per group, giving a total of 60 participants across the three groups [16].

Ethical Consideration

Ethical approval was obtained from the Oyo State Research Ethics Review Committee (NHREC/OYOSHRIEC/10/11/22) before commencement of the study. Informed consent was obtained from all participants prior to sample collection.

Study Population and Sampling

A total of sixty (60) consenting adults (>18 years) were recruited. Newly diagnosed and drug-treated TB patients were consecutively enrolled.

Inclusion criteria for cases: Adults aged 18–70 years; newly diagnosed with pulmonary TB confirmed by clinical symptoms and GeneXpert MTB/RIF; confirmed pulmonary TB patients receiving standard first-line treatment at 4 months post-treatment; no co-morbidities or pregnancy; and provision of informed consent.

Inclusion criteria for controls: Adults aged 18–70 years; age and sex-matched apparently healthy individuals with no active infections; no history of HIV, hypertension, diabetes mellitus, obesity, or stroke; and provision of informed consent.

Exclusion criteria: Failure to meet inclusion criteria or refusal to provide informed consent.

TB Patients' Treatment: Patients at the DOTS clinic were treated with a combination of Rifampicin (150 mg), Isoniazid (75 mg), Pyrazinamide (400 mg), and Ethambutol (275 mg) for the first 2 months, followed by Isoniazid and Rifampicin combination for the subsequent four months.

Sample Collection

Five (5) ml of venous blood was obtained from each participant into EDTA-containing sample bottles. Samples were transported in labelled, temperature-maintained containers and processed within 2 hours to maintain cell viability.

Leucocyte Isolation

Leucocytes were isolated following the procedure of Edem and Arinola [17]. Five ml of fresh venous blood was added to an equal volume of 3% dextran solution, mixed gently, and incubated at 37°C for 60 minutes in an upright position. The upper plasma layer containing leucocytes was carefully aspirated and centrifuged at 1000 rpm for 5 minutes. The pellet was treated with RBC lysis buffer, washed twice with phosphate-buffered saline (PBS), and re-

suspended in 400 µL of PBS. The suspension was divided into two tubes labeled unstimulated and stimulated. To the stimulated tube, 20 µL of BCG vaccine was added and incubated at 37°C for 30 minutes. The cell count used for assays was 4×10^6 cells/µL of PBS.

Leucocyte Adherence Assay

The leucocyte adherence assay was performed following the procedure of Bullen and Losowsky [18]. Ten µL of the leucocyte suspension was diluted 1:100 with PBS and dispensed onto a sterile glass slide. Leucocytes were counted under a microscope at X10 objective magnification as the pre-adherence (baseline) count. The slide was washed at 120° angle with sterile PBS to remove non-adherent cells, allowed to drain for 1 minute, and re-examined. Remaining adherent leucocytes were counted. The same procedure was repeated for stimulated cells.

The percentage leucocyte adherence index was calculated as:

% Adherence Index = [(% adherence of stimulated leucocytes − % adherence of unstimulated leucocytes) / % adherence of unstimulated leucocytes] × 100

Leucocyte Migration Assay

The leucocyte migration assay was carried out using a modified method of Edem and Arinola [17]. The remaining leucocyte suspension was drawn into a sterile plain capillary tube and sealed with plasticine. The capillary tube was centrifuged at 1000 rpm for 10 minutes to pack leucocytes, then incubated at 37°C overnight in a humid box. The distance migrated by leucocytes from the packed cell column was measured using a metre rule.

The percentage leucocyte migration index was calculated as:

% Migration Index = [Distance of migration in antigen (mm) / Distance of migration in medium alone (mm)] × 100

Data Analysis

Data were entered into Microsoft Excel and exported to SPSS version 27.0. Data were assessed for Gaussian distribution before analysis. One-way ANOVA was used to assess differences in continuous variables across groups. Post-hoc comparisons were performed using Tukey's test. Statistical significance was set at $p < 0.05$.

Results

Leucocyte adherence and migration indices in newly diagnosed pulmonary tuberculosis patients and at 4 months of chemotherapy

Study participants

Sixty participants were included in the analysis, with 20 each in the newly diagnosed pulmonary tuberculosis (ND-TB) group, the 4-month drug-treated tuberculosis (DT-TB) group and the healthy control (HC) group. Leucocyte migration and adherence indices were determined for every participant using BCG-stimulated leucocytes, with the unstimulated sample as the reference. Data are presented as mean ± standard deviation (SD) unless stated otherwise.

Leucocyte migration index

The leucocyte migration index (LMI) differed significantly across the three groups (one-way ANOVA, $F = 7.343$, $p = 0.001$; Table 1, Fig. 1). The ND-TB group had the highest mean LMI (145.58 ± 106.35), while the DT-TB group (69.08 ± 54.53) and the HC group (67.69 ± 44.17) had similar and much lower values.

Tukey post hoc testing showed that LMI in ND-TB patients was significantly higher than in DT-TB patients (mean difference 76.51, $p = 0.005$) and in healthy controls (mean difference 77.89, $p = 0.004$). LMI did not differ between DT-TB patients and healthy controls (mean difference 1.39, $p = 0.998$; Table 2).

Leucocyte adherence index

The leucocyte adherence index (LAI) did not differ significantly among the groups ($F = 0.199$, $p = 0.820$; Table 1, Fig. 2). Mean LAI was 72.96 ± 34.98 in ND-TB, 81.84 ± 50.60 in DT-TB and 78.85 ± 48.85 in HC. LAI was numerically lowest in the ND-TB group, but none of the pairwise comparisons reached statistical significance (Table 2).

Table 1. Leucocyte migration and adherence indices in the study groups (mean ± SD)

Index	ND-TB (n = 20)	DT-TB (n = 20)	HC (n = 20)	F	p
LMI	145.58 ± 106.35	69.08 ± 54.53	67.69 ± 44.17	7.343	0.001
LAI	72.96 ± 34.98	81.84 ± 50.60	78.85 ± 48.85	0.199	0.820

ND-TB, newly diagnosed tuberculosis; DT-TB, drug-treated tuberculosis (4 months); HC, healthy controls; LMI, leucocyte migration index; LAI, leucocyte adherence index. F and p values are from one-way ANOVA.

Table 2. Tukey post hoc comparisons of leucocyte migration and adherence indices

Index	Comparison	Mean difference	p value
LMI	ND-TB vs DT-TB	76.51	0.005
LMI	ND-TB vs HC	77.89	0.004
LMI	DT-TB vs HC	1.39	0.998
LAI	ND-TB vs DT-TB	8.89	0.810
LAI	ND-TB vs HC	5.90	0.911
LAI	DT-TB vs HC	2.99	0.976

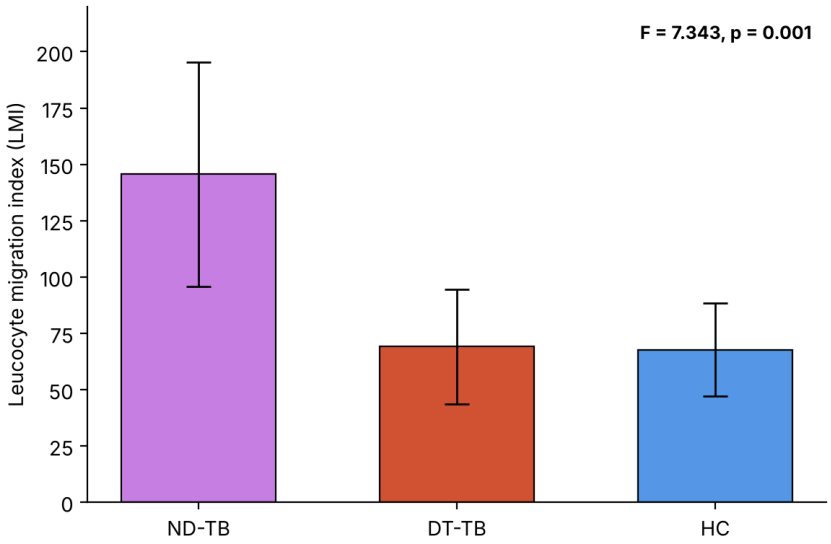

Fig. 1 Mean leucocyte migration index in ND-TB, DT-TB and HC. Bars show means and error bars show 95% confidence intervals (one-way ANOVA, F = 7.343, p = 0.001).

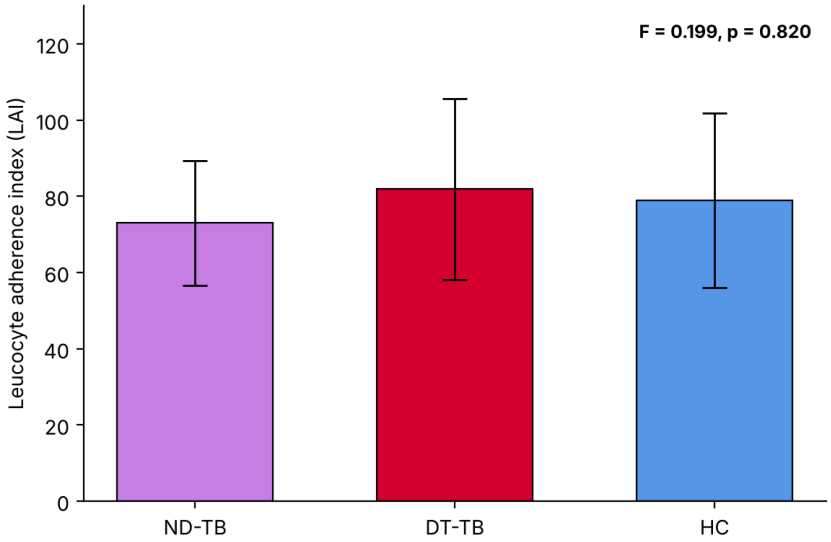

Fig. 2 Mean leucocyte adherence index in ND-TB, DT-TB and HC. Bars show means and error bars show 95% confidence intervals (one-way ANOVA, F = 0.199, p = 0.820).

Discussion

This study evaluated leucocyte migration and adherence responses in newly diagnosed pulmonary tuberculosis patients, patients who had received four months of anti-tuberculosis chemotherapy, and apparently healthy controls. The principal finding was that leucocyte migration index (LMI) was significantly higher in ND-TB patients than in both DT-TB patients and healthy controls, whereas leucocyte adherence index (LAI) did not differ significantly among the study groups. These findings suggest that leucocyte migration is more sensitive than leucocyte adherence in reflecting immunological alterations associated with active pulmonary tuberculosis and treatment-induced immune modulation.

Leucocyte migration is a critical component of host defence against Mycobacterium tuberculosis because recruitment of immune cells to infected tissues is essential for granuloma formation, maintenance of immune surveillance, and containment of bacilli [7]. In the leucocyte migration assay, increased migration index reflects diminished levels of lymphocyte migration inhibitory factors produced by antigen-sensitised T lymphocytes. Therefore, the elevated migration index recorded among ND-TB patients reflected reduced production of LMIF to BCG antigen during active tuberculosis. BCG is a mycobacteria-derived vaccine, therefore the reduced LMIF production due to the BCG antigen might be n evasion strategy of Mtb to reduce BCG efficacy.

The finding is consistent with previous observations that active pulmonary TB is associated with disturbances in leucocyte trafficking and immune-cell function. Earlier studies reported defective monocyte chemotaxis and altered leucocyte migratory responses in active TB patients [17], and altered expression of adhesion molecules and chemokine receptors [19]. Furthermore, active TB is characterised by increased production of chemokines such as CXCL10 and other interferon-inducible mediators that drive leucocyte recruitment to infected tissues [20, 21]. This persistent leucocyte stimulation might lead to immune-cell exhaustion, low LMIF production,

and impaired regulation of migration-related pathways, resulting in the elevated migration indices observed in the present study.

Another observation in the present study is the absence of a significant difference in LMI between DT-TB patients and healthy controls. This finding suggests that four months of anti-tuberculosis chemotherapy may partially restore normal leucocyte migratory responses. Effective treatment reduces mycobacterial burden and decreases chronic immune stimulation, leading to modulation of cytokine and chemokine networks involved in leucocyte trafficking. Previous studies have shown that anti-TB therapy is accompanied by reductions in inflammatory mediators, including CXCL10/IP-10, TNF-α, IL-6, and C-reactive protein, as well as alterations in T-cell and monocyte phenotypes [12, 20, 22]. The similarity in migration indices between DT-TB patients and healthy controls in this study therefore supports the concept of treatment-associated immune reconstitution and normalisation of leucocyte function.

In contrast to the migration findings, leucocyte adherence indices did not differ significantly among ND-TB patients, DT-TB patients, and healthy controls. Although adhesion molecules play essential roles in leucocyte recruitment through endothelial attachment and transendothelial migration [6, 23], the results suggest that overall adherence capacity may remain relatively preserved during both active disease and treatment. Alternatively, adhesion-related abnormalities in TB may be more assessed at the molecular level using changes in the expression of adhesion molecule rather than through gross functional adherence measurements.

Previous studies have demonstrated elevated circulating levels of soluble ICAM-1, VCAM-1, and E-selectin in pulmonary TB patients, indicating endothelial activation and dysregulation of adhesion pathways [24]. Experimental evidence also highlights the importance of adhesion molecules such as CD157 in facilitating effective neutrophil and monocyte migration during mycobacterial infection [25]. However, despite these molecular alterations, the present study did not demonstrate measurable differences in overall leucocyte adherence among the study groups. This finding suggests that leucocyte adherence may be less affected than migration during active pulmonary tuberculosis or may require more sensitive methodologies for detection.

Taken together, the findings indicate that leucocyte migration, rather than leucocyte adherence, reflects disease-associated immune alterations in pulmonary tuberculosis. The significantly elevated migration index observed in newly diagnosed patients and its apparent normalisation following chemotherapy suggest that migration-based functional assays may provide useful information regarding disease activity and treatment response. These observations support the growing recognition that functional immune assays can complement conventional immunological markers in understanding host-pathogen interactions during tuberculosis and may have value in monitoring immune recovery during treatment.

Conclusion

Leucocytes from newly diagnosed pulmonary TB patients exhibited the least BCG-stimulated adherence and the highest migration responses compared with drug-treated patients and healthy

controls. Tuberculosis chemotherapy changed leucocyte migration and adherence indices to levels closer to healthy controls, suggesting partial normalisation of leucocyte functions during treatment. These findings support the concept that leucocyte trafficking dynamics are altered in active pulmonary TB and are modulated during chemotherapy, consistent with contemporary evidence linking inflammatory recruitment pathways to both host protection and lung damage. Leucocyte migration but not leucocyte adherence reflects PTB-related leucocyte activation and treatment-associated immune modulation in these Nigerian PTB patients.

Ethics Approval and Consent to Participate

Ethical approval (NHREC/OYOSHRIEC/10/11/22) and informed consent were obtained prior to study commencement.

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