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Immunological Biomarkers and Epidemiological Evaluation of Lymphatic Filariasis Elimination Efforts in Osun State, Nigeria

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Abstract

Lymphatic filariasis (LF) remains a neglected tropical disease affecting millions globally, despite extensive elimination efforts. While the interruption of transmission is a key goal, the long-term immunological effects on individuals with existing complications, like elephantiasis, remain poorly understood. This study assessed the current transmission status of LF and the hematological profiles of infected individuals across selected Local Government Areas (LGAs) in Osun State, Nigeria. A total of 7,388 individuals from 12 LGAs were screened for circulating filarial antigen (CFA) using Abbott Filarial Test Strips (FTS). Additionally, hematological parameters, including white blood cells (WBCs), lymphocytes, monocytes, neutrophils, and eosinophils, were evaluated in five previously diagnosed LF patients to determine immune cell modulation associated with chronic infection. The pre-transmission assessment survey (pre-TAS) yielded a 0% antigenemia rate across all 12 LGAs, which falls within the WHO threshold (< 2%) for the cessation of mass drug administration (MDA). This indicates probable interruption of LF transmission in Osun State. Data from the community survey also revealed moderate compliance with preventive measures, with 41.09% of participants reporting sleeping under long-lasting insecticide-treated nets (LLINs), and 56.09% reporting participation MDA. Hematological assessments revealed significant variations in immune cell profiles among the LF patients. While some exhibited a reduction in WBCs, lymphocytes, suggestive of immune suppression, others displayed elevated counts consistent with active immune response or recovery. Persistent monocytosis and eosinophil reduction were common, indicating ongoing immune modulation despite parasite clearance. It is concluded that Osun State has entered the post-transmission phase of LF elimination, reflecting the effectiveness of sustained MDA and vector control interventions. Nonetheless, continued immune monitoring, post-MDA surveillance, and improved LLIN usage are essential to prevent reinfection and ensure lasting elimination.

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Keywords: Circulating filarial antigen, immune cells, lymphatic filariasis, mass drug administration, post-elimination surveillance

INTRODUCTION

The Lymphatic filariasis (LF) is a debilitating neglected tropical disease caused by parasitic worms, primarily *Wuchereria bancrofti*, which is responsible for about 90% of cases, while *Brugia malayi* and *Brugia timori* account for the remaining infections. The parasites are transmitted through mosquito vectors and can lead to progressive disability and physical deformity, most notably elephantiasis [1]. Historical accounts suggest that symptoms resembling LF have been recognized since antiquity, but a major scientific breakthrough came in the 19th century with the identifying of microfilariae in body fluids and the discovery of mosquitoes as their vectors [2]. Sir Ronald Ross later demonstrated that adult worms inhabit the lymphatic system, where they produce microfilariae that circulate in the bloodstream, which marked the beginning of medical entomology [3]. Today, LF remains a pressing global health challenge, impacting over 120 million people across 72 countries, with the greatest burden in Africa and Asia [4].

The complex life cycle and pathogenesis of the parasite are intrinsically linked to the clinical manifestations of LF. The life cycle begins when a mosquito ingests microfilariae during a blood meal from an infected individual. Within the vector, the parasites develop into infective third-stage (L3) larvae. These larvae are subsequently transferred to a new human host and migrate to the lymphatic vessels, where they mature into adult worms capable of surviving for several years [5]. The presence of these adult worms disrupts normal lymphatic function, triggering inflammation and progressive damage to the lymphatic system [6]. Clinically, LF manifests in three distinct stages: asymptomatic, acute, and chronic. Although many infected individuals remain asymptomatic, they often suffer from subclinical lymphatic damage. Acute episodes are characterized by painful inflammatory attacks, localized swelling, and chills [3]. Chronic disease results in irreversible conditions, including lymphedema, elephantiasis, and hydrocele, which contribute substantially to morbidity, social stigma, and economic hardship [2].

The complexity of LF is further compounded by the parasite's sophisticated modulation of the host immune responses, which enables long-term survival [6]. This dynamic interaction requires the parasite to undergo metabolic switches to adapt to drastically different environments, from the mosquito to the human host [7]. In humans, they evade both innate and adaptive immune mechanisms. The innate immune system, involving natural killer (NK) cells, eosinophils, and macrophages, is often subverted. Research has shown that infective larvae can rapidly activate NK cells, followed by apoptosis, thereby weakening early host defenses [8]. Similarly, immune mediators, such as IL-5 and eosinophils, typically involved in parasite clearance, may sometimes signal accelerated larval growth [9]. Adaptive immune response also plays a crucial role in controlling filarial infections. Antigen presentation by macrophage activates CD4+ T cells and promotes cytokine secretion (IL-3, IL-4, IL-5, IL-9) leading to mast cell activation eosinophil recruitment, and B-cell-mediated antibody production [10, 11]. Specific antibody profiles have diagnostic and functional significance. For example, high levels of IgG4 are often indicative of a *W. bancrofti* infection, while IgG1 may confer protection against *B. malayi* [12]. Effector mechanisms, including antibody-dependent cellular cytotoxicity and complement-mediated lysis, further contribute to parasite clearance [13, 14].

Verifying current transmission status of LF is essential for guiding government decisions, including whether to discontinue mass drug administration (MDA) in the local government areas studied or to recommend additional intervention rounds by the Government/NGOs. Additionally, evaluating hematological profiles of LF patients provides insights into the disease's impact on immune function and support future morbidity management and elimination strategies, including the development of targeted anthelmintic therapies. Therefore, this study aimed to determine the prevalence of lymphatic filariasis across 12 LGAs of Osun State, Nigeria, and assess the hematological profiles and immune cells mobilization in patients presenting with elephantiasis-related complications.

MATERIALS AND METHODS

The Ethics Statement

This study was conducted in compliance with institutional and state ethical standards. The ethical clearance was obtained from the Ethical Review Committee of Adeleke University, Ede with the

reference number given as AUERC/2025/IND/BCH/02, dated 18 September 2024 and Osun State Health Research Ethics Committee (OSHREC), Ministry of Health, Osun State, (referenced as OSHREC/PRS/25/079 and dated 26 August 2024). All participants were fully informed of the study's objectives, procedures, potential risks, and their rights. A written informed consent form was filled out by each participant before inclusion in the study. Confidentiality and anonymity were strictly upheld, and all participants retained the right to withdraw at any time without consequences.

Design and Sample Area

Pre-Transmission Assessments were carried out in 12 Local Government Areas, LGAs (Aiyedade, Boluwaduro, Ede South, Ife North, Ife South, Ifedayo, Ilesha East, Isokan, Iwo, Ola Oluwa, Orolu, and Oshogbo) of Osun State, Nigeria from 17th–25th September 2024, in collaboration with the Department of Public Health, Ministry of Health, Osun State, Nigeria with support of United Nations Children's Fund (UNICEF), Amen Health & Empowerment Foundation (AHF). A total of seven thousand three hundred and eighty-eight (7388) individuals across the 12 LGAs, and a sample size of not less than 600 participants in each local government, according to the WHO standard (Table 1).

Table 1. Represents the state, study sites, geographical mapping and survey type.

State	LGA	Survey site	Survey type	Latitude ^{o}N	Longitude ^{o}E
Osun	Orolu	Eleesi	SS	7.86363	4.48197
	Orolu	Ooye	SC	7.85121	4.46404
	Oshogbo	Irepodun	SS	7.72572	4.56826
	Oshogbo	Iludun	SC	7.78286	4.58184
	Boluwaduro	Igbajo	SS	7.90593	4.81562
	Boluwaduro	Iresi	SC	7.93683	4.84542
	Ifedayo	Oke-Ila	SS	7.95073	4.98840
	Ifedayo	Ora	SC	8.03626	5.04864
	Iwo	Agberire	SS	7.59408	4.14288
	Iwo	Ogburo	SC	7.60305	4.15748
	Ola-Oluwa	Ikire-Ile	SS	7.71179	4.23652
	Ola-Oluwa	Telemu	SC	7.66829	4.25593
	Aiyedade	Akiriboto	SS	7.46304	4.32435
	Aiyedade	Odeomu	SC	7.53956	4.39584
	Isokan	Ayepe	SS	7.33131	4.27427
	Isokan	Adesina	SC	7.22428	4.10327
	Orolu	Eleesi	SS	7.86363	4.48197
	Orolu	Ooye	SC	7.85121	4.46404
	Oshogbo	Irepodun	SS	7.72572	4.56826
	Oshogbo	Iludun	SC	7.78286	4.58184
	Boluwaduro	Igbajo	SS	7.90593	4.81562
	Boluwaduro	Iresi	SC	7.93683	4.84542
	Ifedayo	Oke-Ila	SS	7.95073	4.98840
	Ifedayo	Ora	SC	8.03626	5.04864
	Ife North	Akinlalu	SS	7.46448	4.41881
	Ife North	Mooro	SC	7.53102	4.46272
	Ife South	Ifetedo	SS	7.48034	4.69696
	Ife South	Olode	SC	7.25882	4.62841
	Ede South	Akoda	SS	7.67899	4.46189
	Ede South	Obada	SC	7.71047	4.44514
	Ilesha East	Ilemo	SS	7.62597	4.75844
	Ilesha East	Irojo	SC	7.60820	4.75153

Note: SS: sentinel sites, SC: sport check sites.

Sample Collection and Analysis

Blood samples (75µl) from the consenting participants were collected via finger-prick and then emptied by drops using the calibrated capillary tubes onto the sample pad of the Abbott Filarial Test Strip (FTS), an immunochromatographic test (ICT) strip used for detecting circulating filarial antigen. The test result was read after 10 minutes. In a positive test, the control and test lines appear to indicate the presence of lymphatic filarial antigen with a positive result (presence of filarial antigen) indicated by the appearance of both control and test lines, while the appearance of only the control line indicated a negative result. In addition, blood samples were collected from elephantiasis patients, in Ethylene-diamine-tetra-acetic acid-containing anticoagulant tubes (50uL) for hematological immune assays. The samples were diluted with a cell pack solution in the White Blood Cell (WBC) counting chamber. A fixed volume of Stromatolyser-W solution (1 volume of Stromatolyser-WH to 2 volumes of cell pack) was added to obtain a final dilution of 1:500. The cells were subsequently counted by the Direct Counted Method (DCM). Hemoglobin released during RBC lysis was converted to the red methemoglobin and read photometrically at 555 nm.

Statistical Analysis

Data were analyzed by one-way ANOVA (Analysis of Variance) followed by Tukey–Kramer Post Hoc tests using the Graph Pad Instat (GPIS) package version 5. Results were considered significant at $p < 0.05$. Values were expressed as Mean ± SEM ($n = 600$/LGA) for field analysis of LF prevalence in 12 LGAs. Data analysis of blood samples collected from elephantiasis patients and controls was performed using two-way ANOVA at $p < 0.05$ level of significance. Values were expressed as Mean ± SEM ($n = 6$). The results were compared using the Bonferroni comparison test.

RESULTS

Participants' Use of Long-Lasting Insecticidal Nets (LLINs) and Participation in Mass Drug Administration (MDA)

The assessment revealed that 41.09% of the surveyed population reported using long-lasting insecticidal nets (LLINs), while 56.09% had participated in the Mass Drug Administration (MDA) for LF. The MDA coverage varied across LGAs, with the lowest participation observed in Ilesha East (31.66%) and the highest in Ola-Oluwa (76.70%) Figure 1.

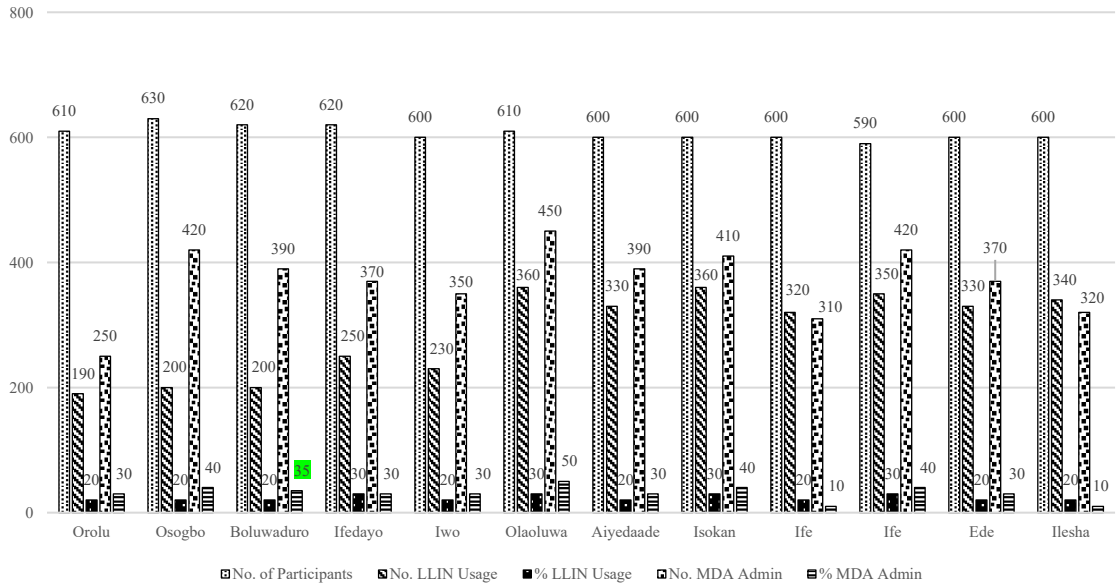


Figure 1. Respondents reporting use of LLINs and participation in MDA for LF.

Note: (*) indicates a significant difference in the number of participants compared with the percentage of LLIN usage.
(#) indicates a significant difference in the number of participants compared with the percentage of MDA participation.
(a) indicates no significant difference between LLIN Usage and MDA participation.
Statistical significance level was set at $p < 0.05$.

Lymphatic Filariasis Pre-Transmission Assessment Survey (Pre-TAS) Results for 12 LGAs of Osun State

The Abbott Filarial Test Strip (FTS) results revealed a 0% prevalence of circulating filarial antigen (CFA) across all 7,388 individuals sampled in the 12 LGAs Figure 2.

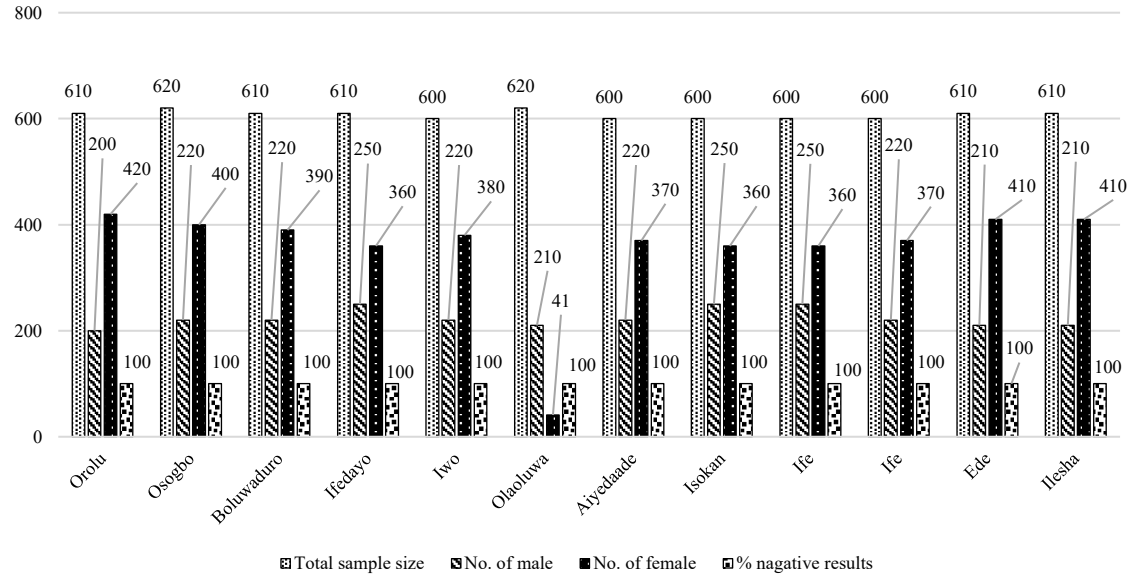
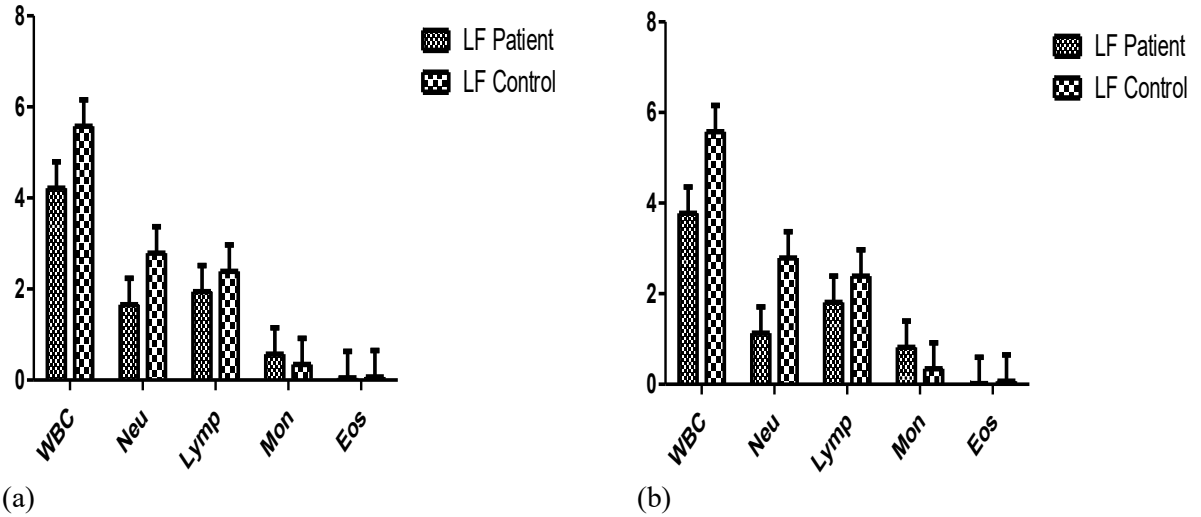


Figure 2. Lymphatic filariasis pre-transmission assessment survey (pre-TAS) in 12 LGAs of Osun State.
Note: (*) indicates a significant difference between the total sample size and the number of male participants.
(#) indicates a significant difference between the total sample size and the number of female participants.
(a) indicates no significant difference between the numbers of female and male participants.
Statistical significance level was set at $p < 0.05$.

Hematological Assessment of Immune Cell Expression in the Blood of LF Patients and Group Control Samples

Hematological evaluation revealed statistically significant variations ($p < 0.05$) in white blood cell (WBC), lymphocyte, monocyte, and eosinophil counts in LF patients compared to controls. LF Patients A and B exhibited a marked decrease in white blood cells (WBCs), lymphocytes, neutrophils, and eosinophils, but an increase in monocyte levels. LF Patients C and D demonstrated a significantly increased counts of WBCs, lymphocytes, neutrophils, and monocytes. Patient E showed elevated levels of WBCs, lymphocytes, and monocytes, but a reduced neutrophil and eosinophil counts Figure 3.



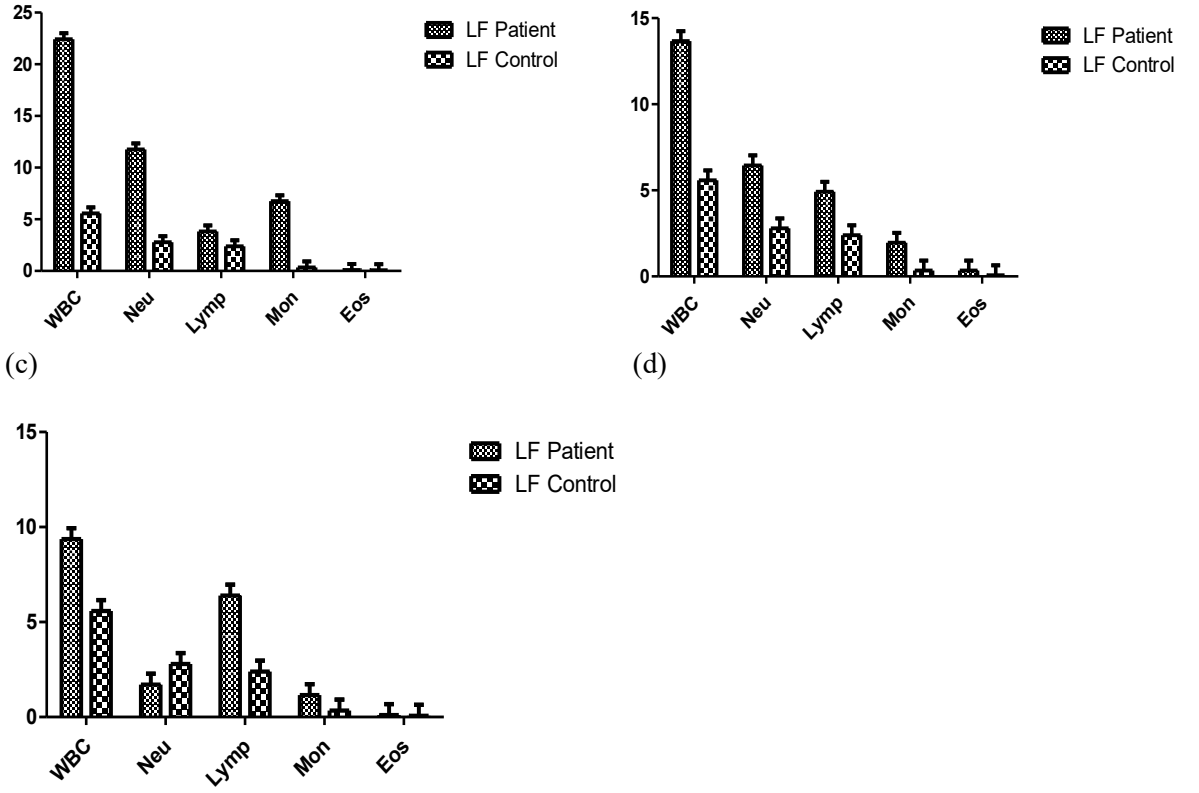


Figure 3. Hematological assessment of immune cell concentrations in LF patients and controls.
Note: A, B, C, D, and E represent the results of hematological assays in blood samples of Patients A, B, C, D, and E compared to healthy control samples.
White blood cells (WBC), Neutrocytes (Neu), Lymphocytes (Lymp), Monocytes (Mon) and Eosinophils (Eos) were the Immune Cells analyzed.
(*) indicates a significant difference between the immune cells of LF patients and the control group.
Statistical significance level was set at $p < 0.05$.

DISCUSSION

Lymphatic filariasis (LF) has been defined by the complex interplay between the parasite and the host immune system, which results in a spectrum of clinical outcomes ranging from asymptomatic infection to severe lymphatic pathology, such as lymphedema and elephantiasis, among other symptoms [15]. The World Health Organization (WHO) recommends a minimum of 65% therapeutic coverage to effectively interrupt LF transmission [4]. This study revealed that 41.09% of the surveyed population reported using long-lasting insecticidal nets (LLINs), while 56.09% had participated in the Mass Drug Administration (MDA) programs for LF. The observed variation in MDA participation across Local Government Areas (LGAs), ranging from 31.66% in Ilesha East to 76.70% in Ola-Oluwa, indicates moderate community compliance with preventive measures. Participation in MDA was higher than LLIN usage, suggesting that vector control practices are not as successfully adopted by the population as the medication campaigns. In areas, like Ola-Oluwa and Isokan, the high MDA rates likely contributed to effective suppression of parasite transmission. Conversely, low LLIN usage, especially in Orolu (28.71%) and Ife North (36.90%), presents a potential reinfection risk if MDA is discontinued prematurely. Richards et al. [16]. highlighted the synergistic effect of MDA and vector control through LLINs in reducing the LF burden. Therefore, while drug uptake is encouraging, LLIN usage must be scaled up to sustain interruption of transmission.

The Abbott Filarial Test Strip (FTS) results showed a 0% prevalence of circulating filarial antigen (CFA) across all 7,388 individuals sampled across the 12 LGAs. This finding was well below the WHO threshold (<2%) for stopping MDA, indicating that LF transmission has been successfully interrupted

in the surveyed areas. This outcome aligns with the WHO's global LF elimination framework and mirrors successful outcomes reported in other endemic countries [4]. Biritwum et al. [17] reported a 0% CFA rate in Ghana after multiple MDA rounds, while Dorkenoo et al. [18]. documented a similar outcome in Togo. Collectively, these findings reaffirm that consistent MDA campaigns and vector control can achieve the interruption of LF transmission. Nevertheless, achieving 0% prevalence does not imply the disease is eradicated. The WHO emphasizes the importance of post-MDA surveillance, continued for several years, to ensure no resurgence, particularly in communities with low LLIN usage or those at risk of reinfection due to migration from endemic regions [4].

Hematological analyses revealed statistically significant differences ($p < 0.05$) in immune cell counts between LF patients and healthy controls, particularly in WBCs, lymphocytes, monocytes, and eosinophils. Basophils were not found in all patient samples. Patients A and B exhibited marked decreases in WBCs, lymphocytes, neutrophils, and eosinophils, but an increase in monocyte levels. This pattern suggests parasite-induced immunosuppression, reflecting chronic antigen exposure and immune exhaustion, well-documented hallmarks of long-term filarial infection [9]. The reduction in eosinophils and neutrophils further indicates impaired adaptive and innate immune responses, while the elevated monocyte count may reflect compensatory activation of phagocytic and inflammatory pathways during chronic infection [10]. This persistent monocyte activity appeared to be associated with prolonged exposure to filarial antigens and may also contribute to the fibrotic and inflammatory tissue changes seen in LF-affected individuals.

In contrast, Patients C and D demonstrated elevated WBC, lymphocyte, neutrophil, and monocyte counts, suggesting an active immune response, possibly associated with antigenic stimulation, parasite death, or superimposed bacterial infection. The increased neutrophil levels imply ongoing inflammation, secondary infection, or acute tissue damage [19], while simultaneous lymphocytosis and monocytosis reflect an engaged adaptive and innate immune system. Such immune reactivation has been observed in individuals transitioning from immune tolerance to active inflammatory disease in LF [20]. Whereas patient E demonstrated increased levels of WBCs, lymphocytes, and monocytes, but decreased neutrophils and eosinophils. This mixed immune profile suggests an immune imbalance, possibly driven by chronic immune regulation. The reduced granulocyte levels (neutrophils and eosinophils) may be attributed to the regulatory T cells (Treg) activity suppressing Th2-mediated responses, a mechanism frequently described in chronic LF [21]. The suppression of eosinophils could reflect altered cytokine profiles, particularly reduced IL-5 levels, which is vital for eosinophil activation and survival [22]. This pattern likely represents an adaptive immune deviation in which Treg-mediated pathways dominate to limit excessive tissue-damage while maintaining low-grade immune surveillance during chronic infection.

Overall, the hematological patterns observed in this study demonstrate the heterogeneity of immune responses in LF patients, ranging from profound immunosuppression to hyper-inflammatory states. The prevalence of monocytosis across most LF patients underscores the continued involvement of innate immunity, even in immunosuppressed states. These findings are consistent with reports of Babu & Nutman [9] and Mukherjee et al. [10], who described LF as a disease of immune dysregulation characterized by T-cell hypo-responsiveness, eosinophil dysfunction, and monocyte-driven chronic inflammation.

CONCLUSION

This study assessed the prevalence of lymphatic filariasis in 12 LGAs (Orolu, Osogbo, Boluwaduro, Ifedayo, Iwo, Ola-Oluwa, Aiyedade, Isokan, Ife North, Ife South, Ede South and Ilesa East) of Osun State, Nigeria, and examined hematological profiles of immune cells, including white blood cell (WBC), lymphocyte, monocyte, and eosinophil counts in individuals with clinical manifestations of lymphatic filariasis. The results provide compelling evidence that active transmission of LF in the surveyed LGAs has been successfully interrupted, as demonstrated by the 0% prevalence rate recorded across all sampled individuals using the Abbott Filarial Test Strip. This indicates that Osun State, Nigeria has attained the post-transmission phase of LF elimination, demonstrating the success of mass drug administration (MDA), vector control, and community-based health interventions.

Nonetheless, the hematological analyses of previously diagnosed LF patients revealed lingering immune alterations, characterized by variable levels of WBCs, lymphocytes, neutrophils, monocytes, eosinophils, and the absence of basophils. These findings indicate ongoing immune modulation even after parasite clearance, reinforcing the necessity of continuous care for chronic cases through morbidity management and disability prevention (MMDP) programs, as recommended by the WHO. Although the findings show that Osun State is progressing toward complete LF elimination, post-MDA surveillance and strengthened community participation, particularly in improving LLIN usage and maintaining high MDA coverage, remain critical to sustain these gains and prevent resurgence.

Authors' Contributions

Conceptualization, project administration, methodology, data analysis, writing original draft, supervision, and editing, N.S.C.; project supervision, review, and editing, B.S.O., M.A.E., A.A.O.; project administration, supervision, and data collection, B.A.L., A.T.A., A.M.O., A.T.A.; conceptualization, writing review, data collection and data analysis, K.D.O., O.M.O.; methodology, project administration, project review and editing, O.O., O.D.A., O.A.O., O.A.B.; partly funding acquisition, B.A.L.

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Institutional Review Board Statement

All procedures were conducted in accordance with the ethical guidelines of the Ethics Committee. This study received ethical clearance from two independent review bodies to ensure full compliance with national and institutional guidelines for research involving human participants. First, the research protocol was reviewed and approved by the Adeleke University Ethical Review Committee, with the approval reference number AUERC/2025/IND/BCH/02, dated 18th September 2024. Additionally, ethical approval was obtained from the Osun State Health Research Ethics Committee (OSHREC) under the Ministry of Health, Osun State, Nigeria with the reference number OSHREC/PRS/25/079, dated 26th August 2024.

Informed Consent Statement

All participants involved were duly informed and filled out participation forms.

Data Availability Statement

The data generated or analyzed during this study are attached to this manuscript as a document.

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Conflicts of Interest

The authors declare no conflicts of interest.

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