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Neutrophils as a cellular biomarkerof inflammation among adult patients with pulmonary tuberculosis in Zaria, Nigeria

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ABSTRACT

Background: Tuberculosis (TB) remains a significant cause of morbidity and mortality worldwide. Complications of the disease is associated with the host's inflammatory response. The study aimed to determine percentage neutrophil as a cellular biomarker of inflammation among adult patients with pulmonary tuberculosis in Zaria. **Methods**: This was a cross sectional study. Blood samples were taken from 30 treatment-naïve (TN), 30 treatment-experienced (TE) and 30 healthy controls (HC) for percentage neutrophils count. **Results**: The mean values of percentage neutrophils count for TN, TE and HC were 65.8 ± 7.3, 46.0 ± 11.8 and 55.8 ± 8.3, respectively. There was a decrease in neutrophils counts of TE patients compared with HC, an increased in neutrophils counts of TN compared with HC and a significant difference between TE and TN (P<0.0001).**Conclusion** Percentage Neutrophils should be use as a cellular biomarker for the monitoring of treatment response among adult patients with pulmonary Tuberculosis.

Introduction

Tuberculosis (TB) caused by Mycobacterium tuberculosis (Mtb) is an infectious bacterial disease which represents one of the leading causes of death by infectious diseases worldwide. Studies report that approximately one-third of the world's population is infected with the organism; out of which 8 million develop symptoms and approximately 2 million die from the infection annually [1]. Nigeria is the 4th among the 22 high TB-burden countries in the world and ranks number one in Africa with no fewer than 460,000 cases of TB and estimated prevalence of 616 cases annually. It is a major target in the global control of the disease [2]. The emergence of multidrug-resistant (MDR), extensive drug-resistant (XDR) strains and spread of human-immunodeficiency virus (HIV) among TB patients has added new formidable dimensions to the problem of TB [3].

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Tuberculosis infection starts by inhalation of aerosol containing Mtb into the pulmonary alveoli which is accompanied by the binding of the phagocytic receptors that aid organism entrance into resident alveolar macrophages, dendritic cells, and monocytes recruited from blood stream. However, Tuberculosis infection in general has been traditionally linked to failed immunity. Successful immune mechanisms operating during the onset of Mtb infection and during active disease differ. In particular, an inflammatory response is prerequisite for efficient control of Mtb at initial stages of the infection, but may become deleterious at chronic stage of disease [4]. Recent work has also implicated excessive inflammation in increased TB susceptibility [5]. However, it is usually very difficult to dissect whether severe inflammation is a cause or a result of disease severity; whether it develops due to intrinsic host hyper-reactivity to the pathogen or whether it mirrors high pathogen load (i.e. deficient Mtb control).

Tuberculosis biomarkers for monitoring disease outcome especially inflammation in Mtb infection are urgently needed because management of Tuberculosis (TB) infection remains a challenge. Mtb as a disease has been with humans for 70,000 years with evolutionary trade off that often compromises host survival [6]. This unique potential of Mtb has been attributed to dysregulated immunity to the infection. Understanding of natural immunity in 90 to 95% of infected individuals who eliminate the disease is extremely limited. This immune population constitutes approximately a quarter of the world population, and as such, it is necessary that the mechanisms underlying host resistance are elucidated [7].

As it is, poor use of existing medications, lack of effective vaccines and inadequacy of newly developed medications, require, identification of inflammatory or immunomodulatory markers in Mtb infection. Currently,one of the treatment indicators that measure progress of successful treatment in Mtb infection includes sputum smear conversion rate at the end of two months or intensive phase of treatment which provides an early assessment to the effectiveness of treatment [8]. This study, therefore, aimed at determining percentage neutrophils as a cellular biomarker of inflammation among adult patients with pulmonary Mtb infection with a view of identifying immune markers associated with inflammation and poor TB outcome.

Materials and Methods:

Study Area

This study was conducted in National TB and Leprosy Training Center (NTBLTC), Zaria, Kaduna State. Kaduna State is located in the North-West geopolitical zone of Nigeria with Zaria as a major city in the State after Kaduna. Geographically Zaria has 11o04’N 7o42’E as coordinates, a total land area of 300km2and a population of 408,198, by 2006 census [9]. NTBLTC being the largest referral centre in northern Nigeria with several hundred patients receiving TB/HIV treatment in the facility made it possible to accommodate patients of the above mentioned inclusion criteria and so made the research feasible

Study Population

The study participants included adult patients diagnosed clinically with pulmonary TB attending the centre who are drug-susceptible positive for AFB by gene-Xpert. These included the following case definition/classification of cases based on National TB guidelines for TB 2016 [10].

Inclusion Criteria

The participants were drug susceptible Adult patients with Mtb gene-Xpert positivity; above 18 and below 60yrs attending NTBLTC Clinic with informed consent.

Exclusion Criteria

This study excludes the following categories of patients with TB:

I. TB patients with co-morbidities like HIV, HCV, HBSAg positivity, Allergies or Asthma;

II. TB positive patient below 18 and above 60years;

III. TB patients on immunosuppressive or anti-inflammatory drugs; and

IV. None Tuberculosis patients

Controls

Inclusion Criteria

The control group for this research included apparently healthy adults without active TB, comparatively similar for age and sex with the study participants.

Exclusion Criteria

The control group for this research excluded the following:

I. Apparently healthy, age and sex matched adults aged below 18 and above 60 years without active TB;

II. Apparently healthy, age and sex matched adults with known history of infections like HCV and HBV, allergies or asthma.

Study Design

This is an analytical cross-sectional study. The study measured associations between the percentage Neutrophils among TB Treatment naïve and experienced patients who are comparatively similar and its possible outcome in comparison with healthy controls at the NTBLTC.A structured Questionnaire was drawn up to facilitate the analysis.

Minimum Sample Size Determination

The sample size was determined using the Fischers expression and prevalence of 73% for QFT-GIT test positive for the study on Pro and anti-inflammatory cytokine among patients with TB [11]. This was calculated using the formula as shown;

$$n = \frac{Z^2pq}{d^2}$$

Where;

Z = Standard normal deviate at 90%confidence interval (1.64)

p = Proportion of QFT-GIT test positive for TB = 73% (0.73)

q = Complementary probability (1 – p) = 1– 0.73=0.27

d = Tolerance limit, the minimum is 0.1

Therefore, $n = \frac{(1.64)2 \times 0.73 \times 0.27}{(0.1)^2} = 52$

These together give a minimum sample size of 52 participants and, at 10% attrition it gives 57 which was approximated to 60 PTB positive participants. However, a total of ninety (90) participants were enrolled and the ratio of the study group to control group was 2:1 for PTB participants to non-TB apparently healthy control (HC) respectively. This study grouped the PTB positive participants into Treatment naïve (TN) and treatment experienced (TE) based on the status of their anti-TB treatment which includes 30 treatments naïve, 30 treatment experienced and (30) non-TB apparently healthy controls.

Ethical Considerations

Approval from the Health Research Ethical Committees of NTBLTC Saye and written informed consent from the participants/controls of the participants were sought and obtained prior to the commencement of samples collection. Confidentiality and anonymity of the participants were protected by informing them their rights to withdraw at any time, respect for dignity and fidelity, and use of coding systems in maintaining data security.

Sampling Technique

The study adopted a purposive sampling technique with participants recruited as they present to the facility. National Tuberculosis and leprosy training centre [NTBLTC] Saye Zaria, is one of the referral centers for many health institutions in Kaduna State and northwest geopolitical zone of Nigeria. Here, control of tuberculosis (TB) continues to be important because of the ease of spread due to its transmission by airborne droplets; and the increasing mortality and morbidity from the disease constitute what make TB patients readily available. The total duration of study was seven months.

Study instruments

The patients/controls were interviewed with the aid of questionnaires. The questionnaires were designed to obtain personal bio-data of the study participant which included sex, age and occupation etc and anthropometric measurements such as weight, height and BMI, history of known risk factors, nutritional status and any other relevant information.

Blood Sample Collection

A four milliliter (4mL) blood sample was collected from each participant in K2EDTA liquid BD Vacutainer tube as described [12]. Neutrophil count was estimated manually through microscopic examination of thin blood film stained by Leishman staining technique [13].

Data management/Statistical analyses

The data were collated and validated using Epi info® questionnaire database. It was then analyzed using GraphPadprism 6 statistical software package. Qualitative variables were expressed as frequencies and percentages while quantitative variables were presented as mean (±SD) and median (and IQR) which was used appropriately. For quantitative data that followed the Gaussian distribution, One-way ANOVA was used to compare statistical differences among the variables. For data that were not normally distributed, Kruskal-Wallis was used to determine difference. The

significance level was set at p≤0.05 with 90% confidence interval.

RESULTS

Socio demographic characteristics of study participants

A total of ninety (90) participants were recruited for the study, comprising thirty (30) treatment naive [TN] patients with Pulmonary TB (PTB), thirty (30) treatment experienced [TE] patients with Pulmonary TB (PTB) and thirty healthy controls [HC]. The socio demographic characteristics of the study population are shown in Table 1.

Age and body mass index (BMI) of study participants

The means (±standard deviation [SD]) of ages for TB treatment-naïve (TN), experienced (TE) and their apparently healthy control (HC) counterparts were 32.9±11.3 years, 35.3±11.5years and 31.6±8.2 years, respectively. There was no significant difference in age between any of the three groups (p=0.3909) (Table 2).The means(±SD) values of the BMI were 25.1±2.7kg/m2, 23.4±3.1 kg/m2and 27.7±5.0kg/m2 for the TN, TE and HC groups, respectively. There was significant difference in BMI (p=0.0001) between the test and control groups (Table 2).

Percentage Neutrophil counts among Study participants

The means (±SD) of percentage neutrophil counts for TN, TE and HC groups were 65.8 ± 7.3%, 46.0 ± 11.8% and 55.8 ± 8.3%, respectively. There was a significant decrease in neutrophil counts of TE patients compared with HC, a significant increase in neutrophil counts of TN patients compared with HC and significant difference between TE and TN (P<0.0001)(Figure 1).

Table 1. Socio-demographic characteristics of study participants according to the categories

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Characteristics	**Frequency (%)**		
	TE	**TN**	**HC**
Sex			
Male	15(16.7)	20 (22.2)	28 (31.1)
Female	15 (16.7)	10(11.1)	2 (2.2)
Educational Status			
Primary	0 (0.0)	1 (1.1)	2(2.2)
Secondary	9 (10.0)	10 (11.1)	19 (21.1)
Undergraduate	8(8.9)	7(7.8)	0 (0.0)
Graduate	8 (8.9)	4(4.4)	5(5.6)
Islamiyya/Qur'anic	0 (0.0)	1(1.1)	0 (0.0)
No formal education	5 (5.6)	7 (7.8)	4 (4.4)
Occupation			
Civil Servant	8 (8.9)	5 (5.6)	2 (2.2)
Driver	2 (2.2)	10 (11.1)	8 (8.9)
Housewife	3 (3.3)	1 (1.1)	0 (0.0)
Unemployed	12 (13.3)	5 (5.6)	10 (11.1)
Others	5 (5.6)	9 (10.0)	10 (11.1)

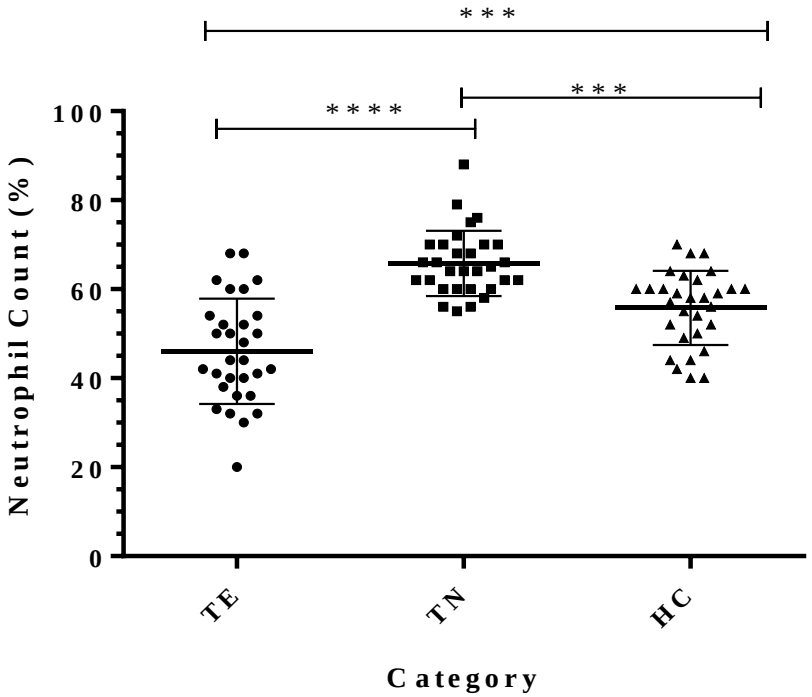
TE: Treatment Experienced; TN: Treatment Naïve; HC: Healthy control

Table 2. Age and BMI of study participants

	TE(n=30)	TN(n=30)	HC(n=30)	p-value
Age, yrs	35.3 ± 11.5	32.9 ± 11.3	31.6 ± 8.2	0.3909
BMI (kg/m^2)	25.1 ± 2.7	23.4 ± 3.1	27.7 ± 5.0	0.0001*

TE: Treatment Experienced; TN: Treatment Naïve; HC: Healthy control, * significant

Figure 1: Neutrophil counts among treatment-experienced, treatment-naïve and healthy control study participant.



Discussion

The high death rate and progressive spread of tuberculosis emphasizes the need to address the complexities associated with the disease and its treatment. Complications associated with the disease are attributed to the process of inflammation which is known to be caused by a group of cytokines and inflammatory immune cells of host [18]. Hence this study investigated the percentage of peripheral blood neutrophils which is known to have pro-inflammatory effects, in patients with TB (Treatment naïve and experienced) in comparison with those without TB.

This study found the mean value and standard deviations of ages for Mtb infected patients to be 35.0 ± 11.5 years which is consistent with the documented global epidemiology of tuberculosis disease [17]. This signifies that adults in their most productive years are the category of individuals that are predominantly infected with Mtb, probably because they are relatively involved in occupations that increase their exposure to higher risk of contracting the bacilli.

Findings from this study found the mean value and standard deviation of BMI increase significantly among TB treatment naïve, treatment experience and apparently healthy controls respectively and this agrees with findings of Reinout [16]. Tuberculosis often leads to severe weight loss (wasting), probably through the production of leptin by adipocytes which binds to specific receptors in the hypothalamus, from which it suppresses appetite and food intake and be one of the mechanisms underlying weight loss hence substantially lower BMI among TB treatment naïve compared to treatment experience and healthy controls. There was substantially higher BMI among TB treatment experience compare to treatment naïve which is theoretically attributed to reduce in leptin concentration.

A significant increase was revealed in neutrophil counts of treatment naive patients compared with healthy control. This is because neutrophils are the first defensive cells recruited to tissue following infection, where their role has been thought to involve eliminating invading pathogens via mechanisms such as the generation of reactive oxygen species, neutrophil traps and the release of preformed oxidants and proteolytic enzymes from granules. There was a significant decrease in Neutrophil counts of TE patients compared with HC and this might be attributed to the role of of anti-TB drugs which agrees with the report of Eyuel [14] which states that anti-TB drug-induced hematological disorders can span almost the entire spectrum of hematology, affecting red cells, white cells, platelets, and the coagulation system. There is also a significant decrease in percentage Neutrophil counts among TE participants compared with the treatment naïve participants. This neutropenia is a rare complication of anti-tuberculosis therapy and is usually due to a single agent, most frequently isoniazid but it can occur with other anti-tuberculous antibiotics such as rifampicin ethambutol and streptomycin [15]. Neutropenia in an immunocompetent patient coupled with sepsis carries a significant cause of morbidity and mortality associated with Tuberculosis and this calls for support with granulocyte-colony stimulating factor (G-CSF) to enable safe and successful completion of therapy.

As it is Tuberculosis treatment monitoring is paramount to clinical decision-making and the host biomarkers appear to play a significant role. Neutrophil is a key players in the cellular and molecular processes leading to the manifestation of clinical tuberculosis and the significant differences of the percentage neutrophils before and during treatment could serve as a useful biomarker for the management of patients with active tuberculosis.

Limitation of the Study

This study was unable to include drug resistant TB patients due to their unavailability and the time frame of the program.

Conclusion and Recommendations:

Significant differences in percentage neutrophils among three different participants suggest it use as surrogate cellular biomarker of inflammation for monitoring the therapeutic response and management of patients with pulmonary tuberculosis under medication, More studies to address the immunological imbalance created due to Mtb infection as well as the number fold (cut off value) of the studied parameters to be identified as surrogate biomarkers of inflammation for the management of patients with active TB to avoid risk of relapse.

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Conflict of interest

No conflict of interest

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