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Assessment of interleukin 6 (IL-6) as a marker of inflammation among adult patients with pulmonary tuberculosis in Zaria, Nigeria

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Abstract

Tuberculosis (TB) remains a significant cause of morbidity and mortality worldwide. Complications of the disease are associated with the host's inflammatory response. The study aimed to determine the plasma level of interleukin-6 as a biomarker of inflammation among adult patients with pulmonary tuberculosis in Zaria.

Method This was a cross-sectional study. Blood samples were taken from 30 treatment-naïve (TN), 30 treatment-experienced (TE), and 30 healthy controls (HC).

Results The means and standard deviations of interleukin-6 plasma levels for tuberculosis treatment naive, treatment experience and apparently healthy control are 64.4 ± 19.4, 57.9 ± 21.4, and 49.9 ± 7.7 pg/L, respectively. This study found upregulated plasma levels of interleukin-6 among treatment naive compared to treatment experience but the statistically not significant and significantly upregulated level of interleukin 6 among treatment naïve compared to apparently healthy control ($p = 0.006$). There was a downregulated level of interleukin-6 among HC compared to TN and TE but statistically not significant.

Conclusion The role of interleukin-6 as a surrogate biomarker for the management of patients with pulmonary tuberculosis is promising but requires further study.

Keywords Interleukin, Tuberculosis, Biomarker, Inflammation

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 an 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 the spread of the human immunodeficiency virus (HIV) among TB patients has added new formidable dimensions to the problem of TB [3].

Tuberculosis infection starts by inhalation of aerosol containing *Mtb* into the pulmonary alveoli which are accompanied by the binding of the phagocytic receptors that aid organism entrance into resident alveolar

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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 a prerequisite for efficient control of *Mtb* at the initial stages of the infection but may become deleterious at the chronic stage of the 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 an 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 the identification of inflammatory or immunomodulatory markers in *Mtb* infection. Currently, one of the treatment indicators that measure the progress of successful treatment in *Mtb* infection includes the sputum smear conversion rate at the end of 2 months or intensive phase of treatment which provides an early assessment of the effectiveness of treatment [8]. This study, therefore, this research aims at determining the plasma level of IL-6 as a possible biomarker of inflammation among adult patients with pulmonary *Mtb* infection with a view to identifying immune markers associated with inflammation and poor TB outcome.

Materials and methods

Study area

This study was conducted in the 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 11° 04′ N 7° 42′ E as coordinates, a total land area of 300 km^2, and a population of 408,198, by the 2006 census [9]. NTBLTC being the largest referral center 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 center 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 [10].

Inclusion criteria

The participants were drug susceptible adult patients with *Mtb* gene-Xpert positivity and were above 18 and below 60 years 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 patients below 18 and above 60 years;
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 in 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 a known history of infections like HCV and HBV, allergies, or asthma.

Study design

This is a cross-sectional study. The study measured associations between interleukin-6 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 0.05.

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

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 90 participants were enrolled and the ratio of the study group to the 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 treatments 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 sample collection. The confidentiality and anonymity of the participants were protected by informing them of their rights to withdraw at any time, respect for dignity and fidelity, and use of coding systems in maintaining data security.

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.

Sampling technique

The study adopted a purposive sampling technique with participants recruited as they presented 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 the 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 the study was 7 months.

Study instruments

The patients/controls were interviewed with the aid of questionnaires. The questionnaires were designed to obtain the personal bio-data of the study participant which included sex, age, 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 4-mL blood sample was collected from each participant in a K2EDTA liquid BD Vacutainer tube as described [12]. The blood samples were assayed for IL-6 using the ELISA technique as described by the manufacturers.

Data management/statistical analyses

The data were collated and validated using the Epi info® questionnaire database. It was then analyzed using the *GraphPad prism* 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 the difference. The significance level was set at $p \leq 0.05$ with 90% confidence interval.

Results

Socio demographic characteristics of study participants

A total of 90 participants were recruited for the study, comprising 30 treatment naive (TN) patients with pulmonary TB (PTB), 30 treatment experienced (TE) patients with pulmonary TB (PTB), and 30 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 ± 11.5 years, and 31.6 ± 8.2 years,

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

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

respectively. There was no significant difference in age between any of the three groups ($p=0.3909$) (Table 2). The mean (± SD) values of the BMI were 25.1 ± 2.7 kg/m^2, 23.4 ± 3.1 kg/m^2, and 27.7 ± 5.0 kg/m^2 for the TN, TE, and HC groups, respectively. There was a significant difference in BMI ($p=0.0001$) between the test and control groups (Table 2).

Plasma levels of interleukin-6(IL-6) among study participants

The means and standard deviations of interleukin-6 plasma level for TB treatment naïve and experienced patients as well as apparently healthy controls were 64.4 ± 19.4, 57.9 ± 21.4, and 49.9 ± 7.7 pg/ml, respectively. There was a significant difference between TN and HC ($p=0.006$), and no significant differences between TE and TN and between TE and HC by one-way ANOVA (Fig. 1).

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

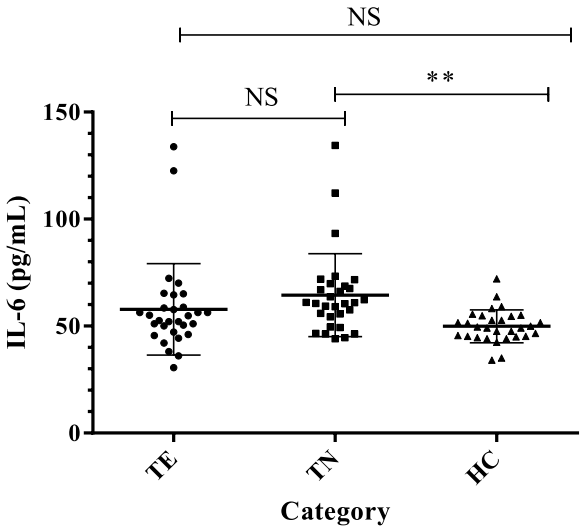

Fig. 1 Interleukin-6 levels among treatment-experienced, treatment-naïve, and healthy control study participants. *Significant, NS not significant

Discussion

The high death rate and progressive spread of tuberculosis emphasize 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 the host [13]. Hence, this study investigated the plasma level of IL-6 known to have pro-inflammatory effects.

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 [14]. This signifies that *adults* in their most productive years are the category of individuals who are predominantly infected with *Mtb*, probably because they are relatively involved in occupations that increase their exposure to a 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 the findings of [15]. 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 is 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 compared to treatment

naïve which is theoretically attributed to a reduction in leptin concentration.

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 the findings of [16] which state that leptin concentration is proportional to BMI. Tuberculosis often leads to severe weight loss (wasting), probably through the production of inflammatory mediators which cause depletion in leptin concentration that leads to suppressed appetite and food intake and is 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 compared to treatment naïve which is theoretically attributed to the state of immunocompetency characterized by reduced TB-induced catabolism hence leptin level improvement in wellness and appetite.

Findings from this study revealed a significant increase in IL-6 among treatment naïve (TN) compared to treatment experienced (TE) participants with TB as demonstrated by a relative increase in the expression of plasma levels of IL-6 in the treatment naïve compared to treatment experienced (64.4 pg/mL ± 19.4 versus 57.9 ± 21.4 pg/mL). This indicates that IL-6 signaling is increased in active tuberculosis in this study and agrees with the findings of [17] which state that there is an increased in the IL-6 signals in patients with active TB and decreased in IL-6 signals in treatment experienced patients. This is in consonance with the theoretical speculation that after TB antigen stimulation, a good marker would be significantly up-regulated in patients with infection but would not be significantly changed in healthy people. Therefore, this may be possible because mycobacteria, including *M. tuberculosis*, may induce the augmented elaboration of IL-6 to support their own growth or mobilize other immune mechanism to eliminate the *Mycobacteria* [18]. Interleukin-6 among treatment naïve plays a crucial role in restricting Mycobacterial replication by inhibiting type 1 interferon hence decreasing disease progression. Probably the remarkable decrease observed in the plasma concentration of IL 6 of the patients on treatment (intensive phase of treatment) might coincide with the period of sputum conversion for most of the patients. Nevertheless, this present study also revealed a slight increase in plasma level of IL-6 among treatment experienced (TE) compared with healthy control (HC) but statistically not significant. This shows that there is an up-regulated expression of IL-6 in treatment experienced compared with healthy control. Evidence to support this interpretation has been reported in a study demonstrating that pyrazinamide treatment can affect cytokine production in J774 cells and bone marrow-derived mouse macrophages and dendritic cells by increasing activation of J774 cells, macrophages, and dendritic cells causing the release of co-stimulatory molecules and pro-inflammatory cytokines and nitric oxide [19]. Studies have shown that an increase in IL-6 among treatment experienced especially on follow up is a potential indication of pretreatment failure [20]. These together signify that IL-6 is a key player in the cellular and molecular processes leading to the manifestation of clinical tuberculosis and the plasma concentration of IL-6 before and during treatment and could serve as a useful biomarker for monitoring the progression of TB in patients with active tuberculosis on treatment.

As it is tuberculosis treatment monitoring is paramount to clinical decision-making and the host biomarkers appear to play a significant role. Interleukin is a key player in the cellular and molecular processes leading to the manifestation of clinical tuberculosis and the significant differences in its levels before and during treatment could serve as a useful biomarker for the management of patients with active tuberculosis as such it could serve as immunological tools for monitoring patients' response to TB treatment in order to minimize the increased risk of relapse following completion of treatment for pulmonary TB.

Conclusion and recommendations

This study shows up-regulated IL-6 among treatment naïve compared to treatment experience patients and up-regulated IL-6 among TE compared with HC. These differences in the levels of IL-6 before and during treatment could serve as a useful biomarker for the management of patients with active tuberculosis. 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.

Abbreviations

TB	Tuberculosis
TN	Treatment naive
TE	Treatment experience
HC	Healthy control
NTBLTC	National Tuberculosis and leprocy training center
WHO	World health organization
MDR	Multi drugs resistance tuberculosis
XDR	Extensive drug resistance
HIV	Human immune deficiency virus
HCV	Hepatitis C virus
HBSArg	Hepatitis B surface antigen
BMI	Body mass index
AFB	Acid fast bacilli

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Authors' contributions
Concept, design, and preparation of the research article (Mairiga Sa'ad); manuscript editing and reviewing (Abdullah A. Abba and Bolanle Olufunke Priscilla Musa); statistical analysis of the research article (Abdurrahman El-fulaty Ahmad); and data acquisition and sample storage (Musa Muhammed).

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Availability of data and materials
The data that support the findings of this research article will be rendered available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
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 sample collection.

Consent for publication
I, Mairiga Saad, the author of this manuscript, qualify and warrant that nobody who qualifies for authorship has been excluded. I agree to its submission to the *Egyptian Journal of Bronchology* and, if accepted, to its publication. This article is not under consideration for publication by any journal.

Competing interests
The authors declare that they have no competing interests.

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