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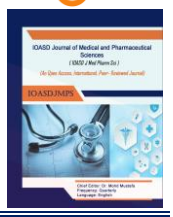

Evaluation of Immunoglobulin Levels in Pulmonary Tuberculosis Patients on Anti-Tuberculosis Therapy in Owerri, Imo State, Nigeria

Ukamaka Edward[1*], Festus Chidi Emengaha[2], and Eberechi Nwanguma[1]
[1]Department of Medical Laboratory Science, Faculty of Health Science, Imo State University Owerri, Nigeria.
[2]Department of Medical Biochemistry Faculty of Basic Medical Science Imo State University Owerri, Nigeria.

Corresponding Author: Ukamaka Edward
Department of Medical Laboratory Science, Faculty of Health Science, Imo State University Owerri, Nigeria.

ABSTRACT

Pulmonary tuberculosis (PTB) is a persistent infectious illness characterised by prolonged immunological activation. Cell-mediated immunity is fundamental to tuberculosis control; but, humoral immune responses, indicated by variations in serum immunoglobulin levels, can significantly influence disease development and treatment efficacy. Anti-tuberculosis therapy (ATT) may affect immunoglobulin profiles; however, data from Nigerian populations are still scarce.This study assessed and compared the mean serum concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin E (IgE) in pulmonary tuberculosis patients on anti-tuberculosis treatment with seemingly healthy control participants. A cross-sectional comparison study was executed with 300 volunteers, consisting of 150 pulmonary tuberculosis patients undergoing anti-tuberculosis therapy and 150 seemingly healthy controls. Blood samples were taken from veins, and standard immunoassay methods were used to find the levels of IgG, IgA, IgM, and IgE in the serum. The data were presented as mean ± standard deviation (SD). Statistical comparisons among groups were conducted, with $p < 0.05$ regarded as statistically significant. The mean serum IgG levels in PTB patients on therapy were significantly elevated compared to controls (1473.24 ± 124.07 mg/dL vs 1356.17 ± 40.91 mg/dL; $p = 0.001$). There was a statistically significant difference in serum IgA levels between the two groups. The PTB patients had lower levels than the controls (212.19 ± 21.32 mg/dL vs 231.47 ± 11.66 mg/dL; $p = 0.001$). The IgM levels in PTB patients were somewhat elevated compared to controls (126.30 ± 7.01 mg/dL vs 125.30 ± 5.33 mg/dL), although this difference lacked statistical significance ($p = 0.06$). In PTB patients receiving therapy, mean serum IgE levels were substantially higher than in controls (97.42 ± 25.58 IU/mL vs 83.33 ± 5.76 IU/mL; $p = 0.001$). Patients with pulmonary TB undergoing anti-tuberculosis therapy demonstrate notable changes in serum immunoglobulin profiles, marked by increased IgG and IgE levels and decreased IgA levels in comparison to healthy controls. These results underscore the role of humoral immune responses in tuberculosis and indicate that immunoglobulin evaluation may be a valuable tool for monitoring immunological status throughout treatment.

Key words: Pulmonary tuberculosis, immunoglobulins, IgG, IgA, IgM, IgE, and anti-tuberculosis therapy are all important terms.

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INTRODUCTION

Pulmonary tuberculosis (PTB) is a long-term infectious disease caused by Mycobacterium tuberculosis. It is still one of the biggest public health problems in the world. Tuberculosis remains one of the top causes of illness and death in the world, even though it may be avoided and treated. This is especially true in low- and middle-income countries [1]. Global estimates say that millions of new cases of tuberculosis happen every year, and that nations in sub-Saharan Africa and South Asia bear an unfair amount of the burden. Nigeria is routinely ranked among the countries with the highest rates of tuberculosis in the world. This is because the disease spreads quickly, takes a long time to diagnose, and there are holes in the control of the disease. The disease has serious effects on public health, society, and

the economy, such as decreased productivity, higher healthcare costs, and long-term incapacity [2].

There are a number of reasons why tuberculosis is still a problem in Nigeria. These include poverty, overcrowding, malnutrition, poor access to healthcare, and other infectious diseases that are also present. Additionally, issues like late case discovery, poor treatment adherence, the rise of drug-resistant strains, and problems connected to the immune system make it harder to control tuberculosis. The use of standardised diagnostic methods and anti-tuberculosis therapy (ATT) has made treatment outcomes better, but tuberculosis is still a complicated illness with different clinical and immunological signs [3]. Consequently, comprehending host immune responses during infection and treatment is vital for enhancing disease surveillance and control [4].

The pathogenesis of tuberculosis is complex and mostly relies on the interplay between M. tuberculosis and the human immune system. After inhaling infected droplets, the bacilli get to the alveoli, where alveolar macrophages eat them. The result of an infection depends on how well the host's immune system can keep the pathogen in check or get rid of it. Historically, cell-mediated immunity has been considered the fundamental element of defence against tuberculosis. T lymphocytes, especially $CD4^+$ T cells, are very important because they activate macrophages through cytokine signalling, which makes it easier for M. tuberculosis to kill cells inside the body. The development of granulomas, a characteristic feature of tuberculosis, signifies a host defence mechanism designed to restrict the infection [5].

Cell-mediated immunity is essential, however increasing data indicates that humoral immunity also plays a significant role in the host response to M. tuberculosis. For a long time, people thought that antibodies didn't do much against tuberculosis since the bacteria live inside cells. Recent research has shown that immunoglobulins can affect the course of a disease, the removal of microorganisms, and the regulation of the immune system in different ways [6]. These encompass the opsonisation of mycobacteria, control of macrophage and dendritic cell activity, regulation of inflammatory responses, and augmentation of antigen presentation. Consequently, interest in the function of immunoglobulins in tuberculosis aetiology and therapy surveillance has intensified [7].

Immunoglobulins are glycoproteins that are specific to antigens and are made by plasma cells after B lymphocytes are activated. They are important parts of the adaptive immune system, and they are divided into distinct isotypes based on their structure and function. Immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) are the three main types of immunoglobulins that are important for infectious disorders [8]. IgM is usually the first antibody made when the body comes into contact with an antigen for the first time. It is a sign of an early or acute immune response. IgG is the most common immunoglobulin in serum. It is linked to long-term immunity, opsonisation, complement activation, and antibody-dependent cellular cytotoxicity. IgA is very important for mucosal immunity, and it is especially vital for keeping pathogens out of the respiratory and gastrointestinal systems [9].

In the case of pulmonary tuberculosis, immunoglobulins may show how active the disease is and how well the host's immune system is working. IgA is particularly noteworthy because of its function in mucosal defence in the respiratory system, the primary site of M. tuberculosis infection. High amounts of IgA may be a way for the body to protect itself from bacteria sticking to and invading mucosal surfaces. IgG, however, may signify persistent antigenic stimulation and continuous immunological activation, which are hallmark characteristics of tuberculosis. Changes in IgM levels may help us understand how far along the infection is or how well treatment is working [10].

Numerous investigations have indicated modified immunoglobulin profiles in persons with active tuberculosis, frequently marked by increased IgG and IgA levels relative to healthy counterparts [11]. These increases are believed to be caused by ongoing exposure to antigens and long-term immunological activation. Anti-tuberculosis therapy not only decreases the mycobacterial load but also modulates host immunological responses over time. As the bacterial load diminishes with efficient medication, immunological indicators, such as immunoglobulin levels, may exhibit alterations, indicating immune recovery or the resolution of inflammation. Keeping an eye on how immunoglobulin levels change during treatment may give you useful further information about how well the treatment is working and how the condition is becoming worse [12].

The treatment for tuberculosis entails giving various antimicrobial drugs for a long time, usually in two phases: an intense phase and a continuing phase. The main purpose of ATT is to get rid of microorganisms, but the treatment also slowly changes how the immune system works as the antigenic stimulus decreases. Learning how immunoglobulin levels change during different stages of treatment may help us understand how to make the immune system normal again and how well the treatment works. This kind of information could be very helpful in places where there aren't many resources and where advanced diagnostic and monitoring technologies aren't available [13].

Even though more and more people are realising how important humoral immunity is in tuberculosis, there isn't much information on immunoglobulin patterns in Nigerian patients getting treatment for tuberculosis. The majority of current

investigations have concentrated on cell-mediated immune responses or have been executed outside southeastern Nigeria. Owerri, the capital of Imo State, is an important city with active tuberculosis treatment programs. However, there isn't much local data on how patients respond to treatment in terms of their immune systems. It is important to collect data that is relevant to each region since genetic, environmental, dietary, and epidemiological factors that are unique to that population can affect immune responses [14].

In this context, assessing serum immunoglobulin levels in pulmonary tuberculosis patients undergoing treatment in Owerri is of significant clinical and public health importance. Comparing these immunoglobulin profiles with those of ostensibly healthy persons may facilitate the understanding of the effects of tuberculosis and its treatment on humoral immunity. This knowledge could help us learn more about how diseases work, help us find possible immunological markers for keeping an eye on treatment, and in the end, improve how we treat tuberculosis [15].

Consequently, this study sought to assess serum immunoglobulin levels—namely IgG, IgA, and IgM—in pulmonary tuberculosis patients on anti-tuberculosis treatment in Owerri, Imo State, and to juxtapose these results with those from ostensibly healthy controls. The results of this study are anticipated to enhance the existing information regarding humoral immune responses in tuberculosis and establish foundational data for subsequent immunological and clinical investigations in the region.

MATERIALS AND METHODS

Study Area

This study was conducted at the Federal Teaching Hospital (FTH), Owerri, Imo State, Nigeria. Owerri, the capital of Imo State, is located in southeastern Nigeria within the Igbo ethnic region. It is the largest urban center in the state and comprises three Local Government Areas: Owerri Municipal, Owerri North, and Owerri West. The Federal Teaching Hospital is situated in Owerri Municipal. Geographically, Owerri lies at latitude 5.485°N and longitude 7.035°E, approximately 150 m above sea level, covering an estimated land area of about 100 km², with a population of approximately 1,401,873 as of 2016. The city is bordered by the Otamiri River to the east and the Nworie River to the south. Owerri experiences a humid tropical climate with distinct wet (April–October) and dry (November–March) seasons, conditions that may influence the epidemiology of communicable diseases, including tuberculosis.

Ethical approval

The ethical clearance (FTH/OW/HREC/VOL1/157) was obtained from the Ethics Committee of Federal Teaching Hospital, Owerri. Informed consent was obtained from all study participants.

Study Design and Population

This was a hospital-based case–control study conducted between October and December 2024. The study population consisted of adults aged 20–60 years attending the Directly Observed Treatment, Short-course (DOTS) Clinic of the Federal Teaching Hospital, Owerri.

Two main groups were enrolled:
Cases: One hundred and fifty (100) confirmed pulmonary tuberculosis (PTB) patients on anti-tuberculosis therapy.

Controls: One hundred and fifty (100) apparently healthy individuals, age- and sex-matched with the cases, with no clinical or laboratory evidence of tuberculosis or other chronic illnesses.

Inclusion Criteria

- Confirmed diagnosis of pulmonary tuberculosis by sputum smear microscopy, GeneXpert, or chest radiography.
- Patients aged 18 years and above.
- Patients who had been on anti-tuberculosis therapy for at least two months.
- Willingness to provide informed consent.

Exclusion Criteria

- Patients with extrapulmonary tuberculosis.
- Individuals co-infected with HIV, hepatitis B, or hepatitis C.
- Patients with known autoimmune diseases or chronic inflammatory conditions.
- Pregnant women.

Sample Collection and Processing

Sputum Collection and Analysis

Sputum samples were collected from suspected PTB patients under standard biosafety conditions. Participants were instructed to rinse their mouths with clean water before expectorating sputum into sterile, labeled containers in a well-ventilated area. Samples with a minimum volume of 1 mL were disinfected externally with 5% sodium hypochlorite and transported to the laboratory within 48 hours. Only samples confirmed positive for *Mycobacterium tuberculosis* by GeneXpert MTB/RIF assay were included for further analysis.

Blood Collection and Serum Preparation

Five milliliters (5 mL) of venous blood were collected aseptically from each participant via venipuncture into plain tubes. Samples were allowed to clot and centrifuged at 3000 rpm for 5 minutes to separate serum. The sera were aliquoted into labeled containers and stored at –20°C until analysis.

Laboratory Analysis

Detection of *Mycobacterium tuberculosis*

The GeneXpert MTB/RIF assay (Cepheid, USA) was used for the qualitative detection of *Mycobacterium tuberculosis* complex DNA and rifampicin resistance. The assay is based on real-time polymerase chain reaction (PCR) and molecular beacon technology targeting the *rpoB* gene, providing automated results with minimal operator intervention.

Determination of Serum Immunoglobulins

IgG and IgM: Serum IgG and IgM concentrations were determined using immunoturbidimetric methods, using Biosino Biotechnology reagents. Turbidity was measured spectrophotometrically at 700 nm for IgG and 340 nm for IgM, with concentrations extrapolated from calibration curves.

IgA: Serum IgA was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, USA). Optical density was read at 450 nm, and concentrations were determined using a four-parameter logistic standard curve.

IgE: Serum IgE levels were measured using a sandwich ELISA method (Elabscience, USA). Absorbance was read at 450 nm, and concentrations were derived from standard curves generated using curve-fitting software.

All assays were performed in duplicate, strictly following manufacturers' instructions. Quality control procedures were observed throughout.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0. Results were expressed as mean ± standard deviation (SD). The independent Student's *t*-test was used to compare means between two groups. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Table 1: Mean Values of Serum IgG, IgA, IgM and IgE in Pulmonary TB Patients on antitherapy versus Controls (Mean ± SD)

Parameter	Pulmonary TB Patients on therapy (n=150)	Control Subjects (n=150)	p-value
IgG (mg/dL)	1473.24 ± 124.07	1356.17 ± 40.91	0.001*
IgA (mg/dL)	212.19 ± 21.32	231.47 ± 11.66	0.001*
IgM (mg/dL)	126.3 ± 7.01	125.30 ± 5.33	0.06
IgE (IU/mL)	97..42 ± 25.58	83.33 ± 5.76	0.001*

KEY:
n: sample size
***: Statistically significant ($p < 0.05$)**

ANALYSIS

The average levels of IgG and IgA in the serum were much greater in PTB patients who were getting treatment than in controls ($p < 0.05$). IgM levels were somewhat higher in PTB patients, although the difference was not statistically significant ($p > 0.05$).

DISCUSSION

This study assessed serum immunoglobulin levels in pulmonary tuberculosis (PTB) patients on anti-tuberculosis therapy in Owerri, Imo State, to clarify alterations in humoral immune responses throughout treatment. The results revealed substantial modifications in immunoglobulin profiles, notably increased IgG and IgA levels in PTB patients relative to seemingly healthy controls, highlighting the considerable influence of Mycobacterium tuberculosis infection on antibody-mediated immunity. These changes show how the pathogen and the host immune system interact in a dynamic way. They also show how important humoral responses are in addition to cell-mediated immunity in the development and control of tuberculosis [16].

The markedly increased IgG levels found in PTB patients align with the prolonged antigenic stimulation typical of M. tuberculosis infection. IgG is the main immunoglobulin in serum and is very important for opsonisation, complement activation, and antibody-dependent cellular cytotoxicity. All of these things help phagocytosis and getting rid of mycobacteria [17]. The sustained elevation of IgG levels during anti-tuberculosis medication may signify ongoing immune identification of leftover mycobacterial antigens or slowly replicating bacilli. This finding is consistent with earlier studies indicating that IgG responses may persist at elevated levels for extended durations owing to the chronic characteristics of tuberculosis and the extended timeframe necessary for complete microbiological eradication [18]. Furthermore, increased IgG levels may indicate immunological memory and ongoing host efforts to avert illness reactivation.

The substantial rise in IgA levels in PTB patients underscores the critical role of mucosal immunity in respiratory infections. IgA is the main immunoglobulin in mucosal secretions. It protects the

respiratory system by stopping pathogens from sticking to surfaces, neutralising poisons, and stopping microbes from invading epithelial surfaces. In the setting of pulmonary tuberculosis, increased IgA levels may signify an adaptive immune response designed to restrict mycobacterial colonisation and dissemination inside the airways. Previous investigations have also found that IgA levels were higher in people with active PTB, which suggests that it could be a measure of continuous mucosal immune engagement and disease activity [19]. The elevated IgA response noted in this study may indicate prolonged activation of the mucosal immune system by inhaled mycobacterial antigens.

Conversely, IgM levels exhibited no statistically significant difference between PTB patients and ostensibly healthy controls. IgM is usually the first immunoglobulin made when an acute infection starts, and it is commonly linked to recent exposure to an antigen. The greater stability of IgM levels in this study may be due to the patients already receiving anti-tuberculosis therapy and likely being beyond the acute phase of illness at the time of sampling. As treatment advances and the initial immune response diminishes, IgM levels may decrease, while class-switched antibodies, including IgG and IgA, become more prevalent [20]. This finding corroborates the hypothesis that IgM may possess restricted efficacy as a biomarker for disease activity in patients receiving standard anti-tuberculosis therapy.

Additionally, the noted decrease in immunoglobulin levels when patients transitioned from the intense phase to the continuation phase of therapy indicates immunological recovery and a less antigenic burden. Effective anti-tuberculosis therapy results in a diminished mycobacterial load, subsequently leading to reduced immune system activation and antibody synthesis. This pattern suggests a gradual normalisation of humoral immune responses following effective treatment and corroborates recent findings indicating decreasing immunoglobulin levels in individuals successfully responding to medication [21]. The fluctuating immunoglobulin concentrations noted in this study underscore their potential utility as supplementary immunological indicators for assessing therapy response and illness resolution [22].

This study's results further support the notion that humoral immunity, specifically IgG and IgA responses, plays an active role in pulmonary tuberculosis and is influenced by anti-tuberculosis therapy. Consequently, monitoring serum immunoglobulin levels may yield supplementary insights to traditional clinical, microbiological, and radiological evaluations in assessing disease activity and therapy efficacy. Nevertheless, additional longitudinal studies with more substantial sample numbers are necessary to more precisely delineate the predictive significance of immunoglobulin profile and to elucidate its function in standard tuberculosis care [23].

CONCLUSION

Patients with pulmonary tuberculosis on anti-tuberculosis therapy in Owerri demonstrate notable changes in serum immunoglobulin profiles, namely increased levels of IgG and IgA. These alterations show that the humoral immune system is still active because of the Mycobacterium TB infection and constant exposure to antigens, even while treatment is going on. The higher IgG levels show how important chronic immune engagement and immunological memory are, whereas the higher IgA levels show how important mucosal immunity is in the respiratory tract during pulmonary tuberculosis. The lack of a substantial alteration in IgM levels indicates that the majority of patients had progressed past the acute phase of infection at the time of evaluation, aligning with their treatment status. Moreover, the observed pattern of decreasing immunoglobulin levels as patients transition from the intensive to the continuation phase of therapy suggests immunological recovery and a diminished mycobacterial burden with good treatment.

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