

**ASSESSMENT OF THE UTILITY OF SEROLOGICAL DIAGNOSIS AMONG
PATIENTS WITH PULMONARY TUBERCULOSIS IN ZARIA, NIGERIA**

BY

SANMI EMIADE

**DEPARTMENT OF MEDICINE
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

NOVEMBER, 2016

**ASSESSMENT OF THE UTILITY OF SEROLOGICAL DIAGNOSIS AMONG
PATIENTS WITH PULMONARY TUBERCULOSIS IN ZARIA, NIGERIA**

BY

**Sanmi EMIADE, B. Tech (MINNA) 2010
MSC/MED/43456/2012-2013**

**A DISSERTATION SUBMITTED TO THE
SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD
OF A
MASTER OF SCIENCE DEGREE IN IMMUNOLOGY**

**DEPARTMENT OF MEDICINE,
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

NOVEMBER, 2016

DECLARATION

I declare that the work in this thesis entitled ‘ASSESSMENT OF THE UTILITY OF SEROLOGICAL DIAGNOSIS AMONG PATIENTS WITH PULMONARY TUBERCULOSIS IN ZARIA, NIGERIA’ has been performed by me in the Department of Medicine. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

SanmiEmiade

Name of Student

Signature

Date

CERTIFICATION

This dissertation entitled ‘ASSESSMENT OF THE UTILITY OF SEROLOGICAL DIAGNOSIS AMONG PATIENTS WITH PULMONARY TUBERCULOSIS IN ZARIA, NIGERIA’ by Sanmi EMIADÉ meets the regulations governing the award of the degree of Master of Science in Immunology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Dr. B.O.P. Musa

Chairman,
Supervisory Committee

Signature

Date

Dr. M.A. Adeiza

Member,
Supervisory Committee

Signature

Date

Prof. B. G. Sani

Head of Department

Signature

Date

Prof. K. Bala

Dean,
School of Postgraduate Studies

Signature

Date

DEDICATION

This dissertation is dedicated to God Almighty, to my parents Mr. and Mrs. Emiade, and to my sisters, Bose and Yemi.

ACKNOWLEDGMENT

To God Almighty be the glory, great things he has done. I acknowledge God's great provisions, protections and support throughout the duration of this course.

I must appreciate constructive suggestions, criticisms and encouragement of my supervisors, Dr. B.O.P. Musa and Dr. M.A. Adeiza.

I must also appreciate the Department of Medicine and the Management of Ahmadu Bello University, Zaria for giving me the permission and approval to conduct the study.

I would like to express my sincere gratitude to my dear parents Mr. and Mrs. Emiade, Shola, Ruth and to my entire family. I would not forget to extend my sincere appreciation to my friends and classmate; you will always be remembered for your support:

I also thank Dr. Clement Adesigbin, Dr. Leshack Emmanuel, Dr. Zakari, Dr. Ishaku Ahmadu and the entire staff of the TB reference laboratory, National Tuberculosis and Leprosy Training Centre Saye.

I wish to express my heartfelt thanks to; Dr. B.Y. Jamoh; Dr. Shamsudeen Aliyu of the Department of Medical Microbiology, Ishaya Gaius, Mrs. F. Baba and members of PGF, ABU, Zaria, Dr. Nasir Adabara of the Department of Microbiology, FUTMINNA and Dr. Iyanda, Dr. Jiya, Dr. Toli, Dr. Aina, Dr. Al Gwozani and Matron Ozigi of the TB Annex, ABUTH, Zaria.

I must also appreciate the cooperation of Mrs. L. Okonkwo, Mr. Joseph, Ahmad AE, Dr. Akase, Dr. Popoola, Mr. Oduma, Mrs. Ariba and Bolaji Yemi of the Immunology unit ABU Zaria and all those who participated in this study.

ABSTRACT

Tuberculosis (TB) remains the leading single microbial illness globally, with one-third of the world's population infected with *Mycobacterium tuberculosis*.

Pulmonary tuberculosis is the most common of all forms of TB and accounts for 70% of all cases. In Nigeria, pulmonary tuberculosis is diagnosed by the sputum smear microscopy after staining by Zehl-Neelsen method. The problems associated with the diagnosis of TB using smear and culture techniques have necessitated the exploration of the utility of serodiagnosis to support clinical suspicion of TB. Anti- *M. tuberculosis* IgG antibodies may aid in the diagnosis of active *M. tuberculosis* disease. The aim of the study was to test the diagnostic utility of the specific IgG serological assays for the diagnosis of pulmonary TB. It was studied whether anti-*M. tuberculosis* IgG antibodies are elevated in active *M. tuberculosis* disease. A diagnostic utility test of 184 participants in which 92 were smear positive pulmonary tuberculosis cases and 92 were apparently healthy controls was conducted at the National Tuberculosis and Leprosy Training Centre Saye and the Ahmadu Bello University Teaching Hospital, Zaria, Kaduna. All samples were tested with the anti- *M. tuberculosis* IgG antibody assay. Of 92 patients with a positive sputum smear microscopy test, 17 (18.5%) were positive for *M. tuberculosis* antibodies. Eleven of the 92 (11.9%) controls were positive for *M. tuberculosis* antibodies. The sensitivity of IgG ELISA tests was 18% and the specificity was 90%, while the positive and negative predictive values were 66% and 53% respectively.

Although anti- *M. tuberculosis* antibodies can be detected in patients with active *M. tuberculosis* disease, its detection may not be useful as an ideal adjunctive diagnostic

test. The serological test evaluated showed low sensitivity and relatively high specificity and suggests serological tests are not useful in the diagnostic algorithm of pulmonary TB.

TABLE OF CONTENTS

	Page
Title page- - - - - - - - - -	-iii
Declaration- - - - - - - - - -	-iv
Certification- - - - - - - - - -	-v
Dedication - - - - - - - - - -	-vi
Acknowledgements- - - - - - - - - -	-vii
Abstract- - - - - - - - - -	-viii
Table of Contents - - - - - - - - - -	-ix
List of Tables- - - - - - - - - -	-xiv
List of Figures- - - - - - - - - -	-xv
List of Appendices- - - - - - - - - -	-xvi
List of Abbreviation - - - - - - - - -	-xvii
1.0 INTRODUCTION- - - - - - - - - -	-1
1.1 Background to the Study- - - - - - - - -	-1
1.2 Statement of Research Problem- - - - - - - -	-3
1.3 Justification for the Study - - - - - - - -	-4

1.4 Aim of the study	-	-	-	-	-	-	-	-	-5
1.5 Objectives of the study	-	-	-	-	-	-	-	-	-5
2.0 LITERATURE REVIEW									
2.1 Description of <i>Mycobacterium tuberculosis</i>	-	-	-	-	-	-	-	-	-6
2.1.1 <i>Mycobacterium tuberculosis</i> complex	-	-	-	-	-	-	-	-	-8
2.1.2 Cell wall structure	-	-	-	-	-	-	-	-	-8
2.1.3 Acid fastness	-	-	-	-	-	-	-	-	-9
2.1.4 Culture character	-	-	-	-	-	-	-	-	-10
2.1.5 Permeability barriers	-	-	-	-	-	-	-	-	-10
2.1.6 Nutritional and environmental requirements	-	-	-	-	-	-	-	-	-10
2.1.7 Generation time	-	-	-	-	-	-	-	-	-11
2.1.8 Metabolic and biochemical marker	-	-	-	-	-	-	-	-	-11
2.1.9 Resistance to physical and chemical challenges	-	-	-	-	-	-	-	-	-11
2.1.10 Virulence mechanisms and virulence factors	-	-	-	-	-	-	-	-	-12
2.2 Pathogenesis of tuberculosis	-	-	-	-	-	-	-	-	-14
2.2.1 The initial lesion	-	-	-	-	-	-	-	-	-14
2.2.2 Formation of granuloma	-	-	-	-	-	-	-	-	-15
2.4 Epidemiology of Tuberculosis	-	-	-	-	-	-	-	-	-16
2.4.1 Prevalence of tuberculosis	-	-	-	-	-	-	-	-	-16
2.4.2 Transmission	-	-	-	-	-	-	-	-	-17
2.4.3 Reservoirs	-	-	-	-	-	-	-	-	-17
2.4.4 Habitat	-	-	-	-	-	-	-	-	-17

2.4.5 Predisposing factors for TB infection-	-	-	-	-	-	-	-	-18
2.5 Cells involved in immune response-	-	-	-	-	-	-	-	-18
2.5.1 Macrophages-	-	-	-	-	-	-	-	-18
2.5.2 Dendritic cells -	-	-	-	-	-	-	-	-19
2.5.3 Natural killer cells-	-	-	-	-	-	-	-	-19
2.5.4 Neutrophils-	-	-	-	-	-	-	-	-19
2.5.5 CD4+ T cells-	-	-	-	-	-	-	-	-20
2.5.6 CD8+ T cells-	-	-	-	-	-	-	-	-20
2.6 Host Immune Mechanisms in TB-	-	-	-	-	-	-	-	-20
2.6.2 Specific immune response-	-	-	-	-	-	-	-	-20
2.6.2 Immunoglobulins-	-	-	-	-	-	-	-	-21
2.7 Host Genetics and Susceptibility-	-	-	-	-	-	-	-	-23
2.7.1 Influence of HIV on the clinical presentation of TB	-	-	-	-	-	-	-	-24
2.8Serological Tests- -	-	-	-	-	-	-	-	-25
2.8.1 Enzyme Linked Immunosorbent Assay (ELISA)	-	-	-	-	-	-	-	-25
2.8.2 Tuberculin skin test-	-	-	-	-	-	-	-	-28
2.8.3Interferon-gamma determination-	-	-	-	-	-	-	-	-29
2.8.4Quantiferon-TB Gold assay-	-	-	-	-	-	-	-	-29
3.0 MATERIALS AND METHODS-	-	-	-	-	-	-	-	-30
3.1Study Area-	-	-	-	-	-	-	-	-30
3.2 Study design-	-	-	-	-	-	-	-	-30

3.3 Ethical approval-	-	-	-	-	-	-	-	-30
3.4 Study Population-	-	-	-	-	-	-	-	-30
3.4.1 Inclusion Criteria-	-	-	-	-	-	-	-	-31
3.4.2 Exclusion Criteria-	-	-	-	-	-	-	-	-31
3.4.3 Controls-	-	-	-	-	-	-	-	-31
3.5 Sampling Technique-	-	-	-	-	-	-	-	-32
3.6 Determination of Sample size-	-	-	-	-	-	-	-	-32
3.7. Data Collection Method-	-	-	-	-	-	-	-	-33
3.7.1 Administration of questionnaire-	-	-	-	-	-	-	-	-33
3.7.2 Collection of Sample-	-	-	-	-	-	-	-	-33
3.8 Sample analysis-	-	-	-	-	-	-	-	-34
3.8.1 Sputum Sample Analysis-	-	-	-	-	-	-	-	-34
3.8.2 Microscopic Examination of Slides	-	-	-	-	-	-	-	-34
3.9 Statistical Analysis-	-	-	-	-	-	-	-	-37
4.0 RESULTS-	-	-	-	-	-	-	-	-38
4.1 Socio-Demographic Characteristics of the Study Participants-	-	-	-	-	-	-	-	-38
4.2 Clinical Symptoms of Participants with Pulmonary TB	-	-	-	-	-	-	-	-38
4.3 TB IgG concentration among participants with Pulmonary TB and Controls	-	-	-	-	-	-	-	-38

4.4 Relationship between TB IgG concentration by Sex-	-	-	-42
4.5 Sputum AFB bacillary density and TB IgG in participants with Pulmonary TB			
-42			
5.0 DISCUSSION-	-	-	-46
6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATION-	-		-51
6.1 CONCLUSIONS-	-	-	-51
6.3 LIMITATION-	-	-	-52
6.4 RECOMMENDATION--	-	-	-52
REFERENCES-	-	-	-53
APPENDICES-	-	-	-60

LIST OF TABLES

Table	Page
3.1 Interpretations of Ziehl-Neelsen acid-fast staining- - - -	-35
4.1 Socio-demographic Characteristics of study participants- - -	-39
4.2 Frequency distribution of Clinical symptoms in 92 participants with Pulmonary TB-	
- - - - - - - - -	-40
4.3 TB IgG concentrations among participants with Pulmonary TB and Controls-	
- - - - - - - - -	-41
4.4 TB IgG concentration by Sex of participants with Pulmonary TB- -	-43
4.5 Sputum AFB bacillary Density and TB IgG Positivity in 92 participants with	
PulmonaryTB-- - - - - - - -	-44

LIST OF FIGURES

Figure	Page
2.1 Structure of <i>M. tuberculosis</i> - - - - - - -	-7
4.1 Relationship between bacillary density and IgG concentration of patients with pulmonary TB- - - - - - - - -	-45

LIST OF APPENDICES

Appendix	Page
I: Informed Consent form - - - - -	-60
II: Questionnaire - - - - -	-61
III: Ethical Clearance (ABUTH- Zaria)- - - - -	-64

LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
APC	Antigen Presenting Cell
CD	Cluster of Differentiation
DC	Dendritic Cells
ELISA	Enzyme Linked Immunosorbent Assay
HBHA	Heparin-Binding Haemagglutinin
HIV	Human Immunodeficiency Virus
IBL	Immunobiology
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LAM	Lipoarabinomannan
MHC	Major Histocompatibility Complex
NK	Natural Killer Cells
NTBLTC	National Tuberculosis and Leprosy Training Centre
NTBLTCP	National TB and Leprosy Control Programme
NTM	Non-Tubercular Mycobacteria
TNF	Tumour Necrosis Factor
TB	Tuberculosis
TMB	Tetramethylbenzidine
WHO	World Health Organization
Z-N	ZiehlNeelsen

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and remains a major global public health problem responsible for over 1.5 million deaths in 2014 (WHO, 2015). Recent analysis of the global burden of TB revealed that Nigeria ranked third among the 22 high burden countries that account for 80% of the global TB epidemic with the highest TB burden in the world (WHO, 2015). An efficient TB control program requires early and accurate diagnosis and treatment of patients with active pulmonary TB.

A major obstacle to early treatment of TB is the lack of rapid accurate diagnostic methods that can be applied in low-income areas. Diagnosis of active mycobacterial infections in resource-constrained settings is primarily based on clinical suspicion, radiological findings, and identification of acid-fast bacilli (AFB) in sputum by smear microscopy. Microbiological culture requires specialized laboratories which are costly and time-consuming.

TB most often affects the lungs, is curable, preventable and is spread from person to person as aerosol. When people with pulmonary TB cough they propel the TB bacilli into the air and common symptoms of active pulmonary TB are cough productive of sputum, low-grade fever, hemoptysis, chest pain, weight loss and night sweats (Demkow *et al.*, 2007).

According to the global report on TB by the World Health Organization (WHO) in 2014, 1.5 million people died from TB, including 400,000 among people who were HIV-positive (WHO, 2015). The WHO estimates that 2 billion people are infected worldwide,

and in 2014, there were an estimated 9.6 million incident cases of TB with 88% of these occurring in HIV-negative patients and 12% in HIV-positive patients(WHO, 2015). While the host's immune system typically prevents the organism from spreading beyond the primary site of infection, 5 to 10% of these latent *M. tuberculosis* infections progress to active disease. Once the disease becomes active, it is contagious and lethal with a mortality rate of greater than 50% in untreated individuals (Welch *et al.*, 2012). Therefore, early diagnosis of active TB is an essential step in the successful treatment through rapid isolation of infected individuals and the early initiation of anti-TB therapy.

An issue of global concern presently is the emergence of drug-resistant TB, which is on the increase in many countries of the world (WHO, 2015).The WHO recommended strategy for global TB control is a short-course, clinically administered treatment (Rojas *et al.*, 2010; WHO, 2015).

A few studies have explored the role of serology in the early diagnosis of TB in Asia (Maers, 2013). Serological tests detecting circulating antibodies against specific mycobacterial antigens in serum could be an attractive approach for the diagnosis of TB due to its quick to perform ability and relatively simple technology. However, serological tests have been hampered by low sensitivity and cross-reactivity with other mycobacteria, and have relatively limited usefulness in the diagnosis of TB in endemic countries (Steingart *et al.*, 2007). Additionally, there is great variability in specific antibody expression in different individuals (Samanich *et al.*, 2001) and antigen composition of the bacilli recognized by antibodies also varies with stages of infection and disease progression. Recent systematic reviews of commercial serological tests showed that, commercial tests vary widely in performance (Bukhary, 2007; Steingart *et al.*, 2007; Singh

etal., 2012). Anti- *M. tuberculosis* IgG antibodies have been shown to increase in patients with active disease (Hoff, 2007; Mizusawa, 2008). While the function of anti-*M. tuberculosis* antibodies in providing protective immunity is still under investigation, it has been proposed that they may be utilized as a diagnostic marker of active disease (Anderson *etal.*, 2008; Abebe and Bjune, 2009).

However, serological methods may have low discriminatory power differentiating latent from active TB infections in endemic regions (Steingart *etal.*, 2007). Several serologic tests have been proposed among which measuring IgM, IgG and IgA against combined antigens of *M. tuberculosis* by ELISA have received further attention (Ahmad, 2011).

ELISA based serological methods do not require sophisticated instrumentation, are simple, and the reagents that it employed are relatively inexpensive, so that the ELISA can be performed in every laboratory. The use of the ELISA method with highly specific antigens could be the best option for TB serodiagnosis (Demkow *etal.*, 2007). Serological tests may be especially useful for a rapid diagnosis of TB in low-income countries, which shoulder over 80% of the global burden of TB cases.

1.2 Statement of the Research Problem

Current TB diagnostics rely mostly on identification of *M. tuberculosis* clinical isolates by acid-fast bacilli (AFB) staining or culture. Although AFB smear staining allows rapid detection of mycobacteria in clinical specimens, it has relatively low sensitivity, with a greater failure rate in children and immunocompromised groups, such as the elderly and patients with AIDS. The culture method is more sensitive than AFB staining, but it takes several weeks to obtain results and requires laboratory facilities that may be unavailable in resource-limited settings.

Despite several diagnostic approaches, TB diagnosis is still difficult, as a result of the low sensitivity, specificity and diagnostic accuracy that characterizes the diagnostic tools.

The WHO stated the development of good diagnostic tools as one of the approaches at stopping the spread of TB; this may requires the use of serology as an adjunctive diagnostic tool that can be useful in the diagnostic algorithm of TB (WHO, 2011).

1.3 Justification for the Study

Development of a serodiagnostic method for TB diagnosis has garnered considerable interest, as these assays are both simple and cost-effective; however, efforts have been hampered by inconsistent results (Steingart *et al.*, 2011). Notably, while serodiagnosis of TB by combining immunoglobulin classes was suggested as a useful point-of-care test in South Africa (Baumann *et al.*, 2014), poor performance of the commercial serological tests for diagnosing pulmonary TB was reported in India (Singh *et al.*, 2012), and to date, no commercial serodiagnostic tests have demonstrated acceptable sensitivity and specificity for routine laboratory use. In previously developed tests, these have varied, depending on the recombinant capture antigens or cocktail of antigen tested, assay format, population variation, stage of infection (Zhang *et al.*, 2013; She and Litwin, 2015) and changes in antibody levels following successful anti-TB treatment (Huret *et al.*, 2015). Anti- *M. tuberculosis* IgG antibodies have been shown to increase in patients with active disease. While the function of anti-*M. tuberculosis* antibodies in providing protective immunity is still under investigation, it has been proposed that they may be utilized as a diagnostic marker of active disease (Welch *et al.*, 2012).

To control TB, it is still necessary to find a diagnostic method that is both more rapid to carry out and more sensitive than traditional methods (smear and culture) but which is

simpler and less expensive than the new molecular diagnostic tests that are based on the amplification of nucleic acids. Using the ELISA technique, several studies have been performed with these antigens, and an extensive variability in immunoglobulin G (IgG) or IgM titers was seen (Hossain and Norazmi, 2013). It may therefore, be pertinent to measure the IgG response in pulmonary TB.

It has also been seen that patients with TB were usually positive for IgG antibody than IgM. It has been seen that IgG holds the great promise in diagnosing active disease in both children as well as adults (Bhatia *et al.*, 2003; Maers, 2013).

Finally, there is a dearth of knowledge on the use of diagnostic emerging serological assays of pulmonary tuberculosis in Zaria.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study is to assess the utility of IgG antibody in the diagnosis of patients with pulmonary TB.

1.4.2 Specific Objectives

1. To measure the antigen specific immunoglobulin G antibody levels in patients with pulmonary TB and controls attending clinic in Zaria.
2. To determine the sensitivity and specificity of the TB IgG ELISA assay compared with sputum AFB microscopy.
3. To determine the association between immunoglobulin G antibody with bacillary density in pulmonary TB patients and controls.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is the causative organism of TB, a disease usually characterized by cough productive of sputum, hemoptysis, chest pain, weight loss and night sweats (Thomas et al., 2011). It is an acid fast bacillus, which can form acid-stable complexes when certain arylmethane dyes are added (Uhíaetal., 2011). All species of mycobacteria have rope-like structures of peptidoglycan that are arranged in such a way to give them properties of an acid fast bacillus (Uhíaetal., 2011). Mycobacteria are abundant in soil and water, but *M. tuberculosis* is mainly identified as a pathogen that lives in the host. Some species in the *M. tuberculosis* complex have adapted their genetic structure specifically to infect human populations. *M. tuberculosis* can be isolated in laboratories and stored at -80°C to be studied extensively, and the most commonly used strain of *M. tuberculosis* is the H37Rv strain(Uhíaetal., 2011). *M. tuberculosis* has very simple growth requirements and is able to grow slowly in harsh conditions. Their acid-fast property is the strongest when there is glycerol around. However, glutamate, and not glucose, is actually the main source of nutrient for initiating growth of *M. tuberculosis*(Thomas et al., 2011).



Figure 2.1: Structure of *M. tuberculosis*

Source: Levinson (2006)

M. tuberculosis has a tough cell wall that prevents passage of nutrients into and excreted from the cell, therefore giving it the characteristic of slow growth rate (Figure 2.1). The cell wall of the pathogen looks like a Gram-positive cell wall. The cell envelope contains a polypeptide layer, a peptidoglycan layer, and free lipids. The cell wall also contains lipid complexes including acyl glycolipids and other complex such as free lipids and sulfolipids and porins in the membrane to facilitate transport beneath the cell wall (Thomas *etal.*, 2011).

2.1.1 *Mycobacterium tuberculosis* complex:

The *M. tuberculosis* complex is comprised of *M. tuberculosis*, *M. bovis*, *M. caprae*, *M. pinnipedii*, *M. africanum*, *M. canettii* and *M. microti*. The mycobacteria grouped in the complex are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences but differ widely in terms of their host tropisms, phenotypes, and pathogenicity (Levinson, 2006).

2.1.2 Cell wall structure:

As the most distinctive anatomical feature of the bacillus, the cell envelope is composed of the plasma membrane, a cell wall, and an outer capsule like layer (Levinson, 2006). The mycobacterial cell wall, however, is unique among prokaryotes. The wall is constituted by an inner peptidoglycan layer, which seems to be responsible for the shape-forming property and the structural integrity of the bacterium. The structure of this stratum is different from that of common bacteria as it presents some particular chemical residues and an unusually high number of crosslinks (Thomas, *etal.*, 2011).

The conformations of these mycolic acids differ from species to species, a property that allows the recognition of many species of mycobacteria by gas-liquid, high-performance

liquid or thin layer chromatography. The mycolic acids specific to *M. tuberculosis* are alpha, keto and methoxymycolates containing 76 to 82, 84 to 89, and 83 to 90 carbons respectively. The outer layer of the cell wall presents an array of free glycolipids such as lipomannan (LM) and lipoarabinomannan (LAM) which are anchored to the plasma membrane and extend to the exterior of the cell wall. LAMs are specific for different species and constitute more than half of the dry weight of the *Mycobacteria* and act as carbon and energy reserves. The waxy coat confers the distinctive characteristics of the genus: acid fastness, extreme hydrophobicity, resistance to injury, including that of many antibiotics, unique immunological properties and probably also contributes to the slow growth rate of some species by restricting the uptake of nutrients. The capsule-like layer contains proteins, polysaccharides and minor amounts of inner lipids which might be protective and bioactive (Riley *et al.*, 2006).

2.1.3 Acid fastness:

Acid-fastness describes the resistance of certain microorganisms to decolourization with acid-alcohol solutions after staining with carbolfuchsin (Kaplan *et al.*, 2003). This feature is of utmost practical importance in identifying the tubercle bacillus, particularly in pathological specimens. Indeed, there is a widely differing gap between the increasing degree of acid fastness displayed by microorganisms in the genera *Corynebacterium*, *Nocardia*, and *Mycobacterium*, and the increasing length of mycolic acid chains in their walls. However, the property is completely dependent on the integrity of the bacillus (Kaplan *et al.*, 2003).

2.1.4 Cultural character:

M. tuberculosis grows only in enriched medium containing egg, asparagine, potatoes, serum and meat extracts. Virulent strains often grow as twisted rope like colonies which are called serpentine cords (Levinson, 2006). In contrast, non-virulent *Mycobacteria* and tubercle bacilli attenuated by prolonged cultures usually develop smooth colonies on solid media. The recognition of that peculiarity of cord formation provides a reliable clue to the experienced microbiologist for the presumptive distinction of *M. tuberculosis*. These distinct features of the virulent bacilli have been attributed to the trehalose 6, 6'-dimycolate which is the cord factor (Chesnutt *et al.*, 2007).

2.1.5 Permeability barriers:

The tightly packed mycolic acids provide the bacillus with an efficient protection and a special impermeability along with a thicker layer of carbohydrate and protein outside the lipid layer. The impermeability is at least one of the determinants for two *M. tuberculosis* characteristics: its slow growth and its intrinsic drug resistance (Sohaskey, 2005).

2.1.6 Nutritional and environmental requirements:

M. tuberculosis macromolecular structure and metabolic proficiencies result in high adaptation to the specific environment. Trace elements as Magnesium and iron are essential for life. In nature, the bacillus grows most successfully in tissues with high oxygen partial tension, such as the lungs, particularly the well-aerated upper lobes. In the laboratory, an atmosphere of 5 to 10 % carbon dioxide favors culture growth, at least during the early stage of incubation (Levinson, 2006).

2.1.7 Generation time:

Under favorable laboratory conditions, *M. tuberculosis* divides every 12 to 24 hours (Uthia *et al.*, 2011). This slow growth rate might be partially determined by the cell wall impermeability that limits nutrient uptake. Ribonucleic acid (RNA) synthesis is a major factor associated with the long generation time of the tubercle bacillus (Sohaskey, 2005). The low multiplication could be responsible for the typically sub-acute to chronic development of the disease and the long time required in reaching visible growth in vitro.

2.1.8 Metabolic and biochemical markers:

In addition to some susceptibility tests, the analysis of niacin accumulation, nitrate reductase and urease activity allows the distinction of *M. tuberculosis* complex and species differentiation within the complex (Levinson, 2006). The main *M. tuberculosis* antioxidant enzyme is a heat labile catalase-peroxidase with both catalase and peroxidase activities. The catalase is not only self-protective but can also be self-destructive as it activates the antituberculous prodrug isoniazid (INH) (Sohaskey, 2005).

2.1.9 Resistance to physical and chemical challenges:

Though tubercle bacilli do not form spores, there is a remarkable capacity to endure unfavorable conditions (Pfyffer, 2007). The bacillus is able to limit the access to the bacterial targets of hydrophilic antiseptics and antibiotics. The bacillus also withstands very low temperatures; its viability may be increasingly well-preserved for a long term between 2 to -70°C. When ultrafrozen, the viability of the bacilli remains almost intact as well as the taxonomic, serologic, immunologic, and pathogenic properties. The bacilli are however, very sensitive to heat, sunlight and ultraviolet (UV) irradiation (Pfyffer, 2007)

2.1.10 Virulence Mechanisms and Virulence Factors:

M. tuberculosis does not possess the classic bacterial virulence factors such as toxins, capsules and fimbriae. However, a number of structural and physiological properties of the bacterium are beginning to be recognized for their contribution to bacterial virulence and the pathology of TB. *M. tuberculosis* has special mechanisms for cell entry; the bacillus can bind directly to mannose receptors on macrophages via the cell wall-associated mannosylated glycolipid, LAM, or indirectly via certain complement receptors or F_c receptors (Chesnut *et al.*, 2007).

Since *M. tuberculosis* has slow generation time, the immune system may not readily recognize the bacteria or may not be appropriately triggered to eliminate them.

The first step in understanding the mechanisms of recognition of pathogenic bacteria is a solid knowledge of the structure of the cell wall of the microorganism, which is the first structure to come in contact and to be recognized by the cells of the immune system. It has been demonstrated that dectin and toll-like receptor 4 (TLR4) play a role in induction of IL-10 by TB (Thomas *et al.*, 2011). The cytokine IL-10, which is generally secreted by Th2 cells, affects macrophages by suppressing cytokine production, and thereby down-regulating Th1 cell activity and proliferation, and one study suggests that IL-10 plays a central role in enhancing the survival of *M. tuberculosis* within macrophages (Russell *et al.*, 2010). Thomas *et al.* (2011) showed that both cytolytic T lymphocytes and IFN- γ -activated macrophages are necessary for conferring protective immunity against *M. tuberculosis*. These immunological pathways may therefore be important in the development of an efficacious vaccine to tuberculosis. The Th1 pathway of the immune response including cytotoxic T lymphocytes activation is best stimulated by

antigens that have been synthesized and degraded inside the cell and presented on the cell surface with MHC Class I. IFN- γ is an important cytokine for immune protection, but, in excess, it is also a key mediator of immunopathology. Massive activation of macrophages within tubercles by IFN- γ results in concentrated release of lytic enzymes. These enzymes destroy neighbouring healthy cells and create circular regions of necrotic tissue (Ahmad, 2011).

M. tuberculosis persists within macrophages through a variety of immune evasion strategies:

(1)*Entry into macrophages via multiple receptors*: Entry of Mycobacteria into phagocytic cells can occur through binding to multiple receptors, all leading to the delivery of the bacilli into macrophage phagosomes.

(2)*Phagosome*: To persist in the host, *M. tuberculosis* arrests the maturation of bacilli-containing phagosomes into phagolysosomes (Robbins *et al.*, 2005). It could interfere with phagolysosomal fusion by retention of host protein on the phagosome behaving as self-antigens.

(3) *Modulation of antigen presentation*: *M. tuberculosis* avoids elimination by the immune system through the inhibition of antigen processing and presentation by macrophages and dendritic cells.

(4) *Evasion of toxic effects of reactive nitrogen intermediates*: *M. tuberculosis* expresses genes that counteract the bactericidal or bacteriostatic effects of reactive nitrogen intermediate and nitric oxide.

2.2 Pathogenesis of TB:

2.2.1 The initial lesion:

On inhalation, most tubercle bacilli are trapped in the mucosa of the upper respiratory tract, trachea and bronchi, especially when inhaled in masses, and are eradicated by the mucocilliary defense mechanisms. Tiny particles or droplet nuclei smaller than 5 μm perform as a gas and overcome this barrier and reach the inferior respiratory tract, especially inside the alveoli, where they are readily phagocytosed by alveolar macrophages(Cooper, 2009).

Levinson (2006) noted that after *M. tuberculosis* enters the lung, one of four potential fates might occur:

- i. The initial response can be effective in the killing and elimination of the bacilli, and these individuals do not come down with TB at any time point in the future.
- ii. The bacilli can grow and proliferate immediately after infection, to cause clinical disease (primary TB).
- iii. The bacilli may turn out to be inactive and never cause disease at all, resulting in a latent infection that is manifested only as positive tuberculin skin test (latent TB).
- iv. The latent bacilli can eventually begin to grow with consequential disease (Reactivation TB).

The development of clinical TB will occur in 5-10 % of infected persons at some point in their lives. This is different for people infected with HIV where 50% (WHO, 2004) develop TB.

Dissemination of TB outside the lungs (extrapulmonary TB) is more common in children and HIV infected individuals (Thomaset *al*, 2011) leading to the appearance of a number of unusual findings with typical patterns:

(1) Skeletal TB involves mainly the thoracic and lumbar vertebrae also known as Pott's disease.

(2) Genital tract TB involves the fallopian tube, prostate and epididymis.

(3) Urinary tract TB.

(4) TB of the central nervous system

(5) Cardiac TB and

(6) Scrofula (lymphadenitis TB) (Raviglioneet *al*., 2005).

2.2.2 Formation of granuloma:

The granulomatous response is the hallmark of chronic *M. tuberculosis* infection, which is an exertion by the host immune system to arrest multiplication and further dissemination of bacteria to other organs. It is proposed that stimulated alveolar macrophages in the airways invade the lung epithelium ensuing internalization of inhaled bacteria (Ulrichset *al*., 2006).

Algoodet *al*, (2005) noted that the granuloma serves major purposes as a local environment in which immune cells can interact to kill bacteria, a focus of inflammatory cells. While the disruption of the granuloma structure or function appears to be detrimental to the control of bacterial replication and the control of immunopathology in the lung, and also drives the destruction of the extracellular matrix that is necessary for the spread of *M. tuberculosis*, understanding of this process remains quite vague (Russell *et al*., 2010). TB immunopathology is currently considered to be a form of

caseous necrosis and it is divided into fundamental processes. At least 3 mechanisms must take place to cause the immunopathology that result in caseous necrosis. First, *M. tuberculosis* infection is directly toxic to macrophages and causes cell necrosis (Divangahi *et al.*, 2010). Second, several lines of evidence demonstrate that activated T-cells drive pathology (Cooper, 2009). For example, in advanced HIV infection during which patients present with TB in the context of a low CD4⁺ cell count, caseating granulomas are not observed (Cooper, 2009), but as the immune system reconstitutes during anti-retroviral therapy, pathology develops (Meintjes, *et al.*, 2008). Likewise, in the rabbit model, pre-sensitization with serial injections of heat killed *M. bovis* drive a delayed type hypersensitivity response in the skin accelerates pathology and results in cavitation in the lungs (Nedelcheva *et al.*, 2008). Third, the matrix metallo-protease activity must be a final effector causing destruction of the extracellular matrix. Considering these processes separately permits evaluation of where therapies can be most effectively targeted to limit immune-mediated tissue damage.

2.4 Epidemiology of Tuberculosis

2.4.1 Prevalence of tuberculosis:

The WHO ranked Nigeria third among the world's 22 high burden TB countries in 2015, with more than 570,000 new cases and 170,000 deaths (WHO, 2015). TB hinders socioeconomic development as 75% of people with TB are within the economically productive age group of 15-54 years.

There were an estimated 13 million prevalent cases (range, 11 million–14 million) of TB in 2014 (WHO, 2015), equivalent to 174 cases per 100 000 population (WHO, 2015). By the end of 2015, it is estimated that the prevalence rate will have fallen 42%

globally. Since 1990, some of the high burden countries are missing the target (WHO, 2015), whereas, two regions met the target before 2015 (the Region of the Americas and the Western Pacific Region) and the South-East Asia Region reached the target (according to the best estimate) in 2015, TB prevalence is falling in all of the other three regions. Among the 22 high burden countries, nine are assessed to have met the target of a 50% reduction from the 1990 levels (WHO, 2015).

2.4.2 Transmission:

M. tuberculosis is an obligate, aerobic, intracellular pathogen, which has a predilection for the lung tissue rich in oxygen (Chesnutt *et al.*, 2007). TB occurs almost exclusively from inhalation of droplet nuclei containing *M. tuberculosis*, which disperse primarily through cough, sneezing and other forced respiratory maneuvers by a person with smear positive Pulmonary TB. Normally, recurrent exposure to a TB patient is necessary for infection to take place (Chesnutt *et al.*, 2007).

2.4.3 Reservoirs:

Incorporated within the *M. tuberculosis* complex and generically called the tubercle bacilli, the various etiologic agents of TB have distinct hosts and reservoirs. *M. tuberculosis*, and the regional variants or subtypes *M. africanum* and *M. canettii* are primarily pathogenic in humans. *M. bovis* and *M. microti* are the causative agents of TB in animals, and can be transmitted to humans. Some particular strains isolated from goats have been named *M. caprae* and *M. pinnipedi* (Levinson, 2006).

2.4.4 Habitat:

Only a few mycobacteria became successful pathogens of higher vertebrates, preferentially inhabiting the intracellular environment of mononuclear phagocytes. The

host-dependent mycobacteria that cannot replicate in the environment are *M. leprae*, *M. lepraemurium*, *M. avium* subsp. *paratuberculosis*, and the members of the *M. tuberculosis* complex. Bacteria within the *M. tuberculosis* complex are able to reproduce *in vitro*, in contrast to *M. leprae* and *M. lepraemurium*, which are uncultivable and require the intracellular environment for survival and propagation. The major pathogens *M. tuberculosis* and *M. leprae* are not capable of replicating on inanimate matter whereas non-tubercular mycobacterium (NTM) are free living mycobacteria and are usually found in watery habitats such as lake, rivers and wet soil (Levinson, 2006).

2.4.5 Predisposing factors for TB infection include:

Factors which predispose to TB infection include close contact with large populations, i.e., schools, nursing homes, dormitories and prisons. Poor nutrition, *i/v* drug use, alcoholism and HIV infection are also predisposing factors for *M. tuberculosis* infection (Crompton, 2000). Only 3-4% of infected individuals will develop active disease upon initial infection and 5-10% within one year (Crompton, 2000).

2.5 Cells involved in immune response:

2.5.1 Macrophages:

Macrophages play a central effector role in the immune response to *M. tuberculosis* infection. Once infected by the bacillus, macrophages present antigens on both class I and II major histocompatibility complex (MHC) to T cells, which in turn secrete IFN- α resulting in activation of the macrophages to kill the bacteria (Ahmad, 2011). Tumor necrosis factor (TNF) is an important pro-inflammatory cytokine secreted by activated monocytes/ macrophages (Bekker *et al.*, 2001). Additionally, the pro-inflammatory cytokines IL-1 and IL-6 secreted during inflammation play an important role in

recruitment of cells to the site of infection. A major effector mechanism is the induction of nitric oxide (NO) and related reactive nitrogen intermediates (RNIs) by macrophages (Giacomini *et al.*, 2001) and phagolysosome synthesis (Robins *et al.*, 2005).

2.5.2 Dendritic cells (DC):

It is now established that DCs are also involved in an effector role against *M. tuberculosis* infection and are essential for the generation of acquired immunity after conveying of antigens to draining lymph nodes, where recognition by T cells can be exploited (Flynn, 2004). The proficient antigen-capturing causes immature DCs to be transformed into mature T cell stimulating DCs, which migrate into draining lymph nodes. In these compartments, the stimulatory capacity of mature DCs ultimately leads to effector T cell differentiation and memory T cell expansion, which in turn, confer protection against *M. tuberculosis* in the lungs (Kaufmann *et al.*, 2005).

2.5.3 Natural killer cells:

Natural killer cells play a very important role in the development of the innate immune response associated with the progress of cytotoxicity to mark cells and the first cell populations to produce IFN- α during the immune response (Junqueira-Kipnis *et al.*, 2003).

2.5.4 Neutrophils:

Even though macrophages are considered the main targets for infection by *M. tuberculosis*, neutrophils are among the earliest cells recruited into infection sites and inflammatory signals are triggered along with the formation of neutrophil extracellular traps (Urban *et al.*, 2006).

2.5.5 CD4+ T cells:

Effector CD4+Th1 cells play a dominant role in TB infection (Kaufmann *et al.*, 2005) *M. tuberculosis* resides primarily in a vacuole within the macrophage resulting in MHCII presentation of mycobacterial antigens to CD4+ T cells. Upon activation, CD4+ T cells secrete IFN- α and TNF, which in turn induce anti mycobacterial mechanisms in macrophages (Flynn *et al.*, 2001). Other roles played by CD4+ T cells include induction of apoptosis suggested to be important in controlling *M. tuberculosis* infection, conditioning of antigen presenting cells, help for B cells and CD8+ T cells, and production of other cytokines (Scanga *et al.*, 2000).

2.5.6 CD8+ T cells:

Stenger *et al.*, (1998) demonstrated that mycobacterial antigens derived from infected cells can be presented by MHC I to CD8+ T cells in humans. CD8+ T cells also recognize various antigens from *M. tuberculosis* that are not presented by classical Major Histocompatibility Complex I (MHC I) molecules. In humans, CD8+ T cells can kill intracellular mycobacteria via the release of the antimicrobial peptide granulysin.

Selvaraj *et al.*, (2001) showed that plasma lysozyme and other enzymes may play an important role in the first line defense of innate immunity to *M. tuberculosis*. The role of CD-1 restricted CD8+ T cells and non-MHC restricted T cells have been implicated but not completely understood.

2.6 Host Immune Mechanism in TB

2.6.1 Specific Immune Response:

In general, humoral immunity does not contribute to induction of the protective immunity against *M. tuberculosis*. However, antibodies specific for heparin-binding

haemagglutinin(HBHA) and arabinomannan are reported to contribute to reducing the bacterial load in the lung (Raja, 2004). Antibodies specific for antigens presented on the surface of *M. tuberculosis* could affect the prevention of invading *M. tuberculosis* into the host cells.

It is well documented that mycobacteria activates the complement system. Several studies suggest that anti-LAM antibodies may have an important protective role. Jolleret *al*, (2010) noted that mycobacteria coated with IgG-specific antibodies were more effectively processed and presented by dendritic cells for stimulation of CD4+ and CD8+ T cell responses. Mice treated with monoclonal antibodies IgG3 specific for arabinomannan and then challenged with *M. tuberculosis* localized the pathogen within granuloma centres suggesting that the monoclonal antibody conferred protection by enhancing a cellular immune response. It has been observed that during active *M. tuberculosis* disease, a humoral response occurs in the host, which can be measured using anti- *M. tuberculosis* antibodies.

2.6.2 Immunoglobulin

An immunoglobulin (Ig) also known as an antibody (Ab) is a large protein produced by B-cells that is used by the immune system to identify and neutralized foreign objects such as bacteria and viruses. Immunoglobulins are glycoproteins typically made of basic structural units each with two large heavy chains and two small light chains. There are several different types of antibody heavy chains, and several different kinds of antibodies, which are grouped into different isotypes based on which heavy chain they possess (Tolaretal, 2008). Five different immunoglobulin isotypes or classes are known which perform different roles and help direct the appropriate immune response for each

different type of foreign object they encounter. They include IgA, IgD, IgE, IgG and IgM. They differ in their biological properties, functional locations and ability to deal with different antigens.

Immunoglobulins are used in flow cytometry to differentiate cell types by the protein they express. Different types of cell express different combinations of cluster of differentiation molecules on their surface and produce different intracellular and secreted proteins. Proteins can also be detected and quantified with antibodies using ELISA and ELISPOT techniques.

IgG is increased in patients with pulmonary tuberculosis (Jain *et al*, 2011; Fahey and Mekeley, 2007). IgM is decrease in patients with pulmonary TB (Daniel and Baum, 2008).

M. tuberculosis is a classic example of a pathogen for which the protective response relies on cell-mediated-immunity. In the mouse model, within 1 week of infection with virulent *M. tuberculosis*, the number of activated CD4⁺ and CD8⁺ T cells in the lung draining lymph nodes increases (Hossain and Norazmi, 2013). Between 2 and 4 week post-infection, both CD4⁺ and CD8⁺ T cells migrate to the lungs and demonstrate an effector/memory phenotype (CD44^{hi}CD45^{lo}CD62L⁻); approximately 50 per cent of these cells are CD69⁺ (Hossain and Norazmi, 2013). This indicates that activated T cells migrate to the site of infection and interact with APCs. The tuberculous granulomas contain both CD4⁺ and CD8⁺ T cells (Robbins *et al.*, 2005) that contains the infection within the granuloma and prevent reactivation.

M. tuberculosis is equipped with numerous immune evasion strategies, including modulation of antigen presentation to avoid elimination by T cells. Protein secreted by *M.*

tuberculosis such as superoxide dismutase and catalase are antagonistic to reactive oxygen intermediates (Raja, 2004). Mycobacterial components such as sulphonamides, LAM and phenolic- glycolipid I (PGL-I) are potent oxygen radical scavengers (Robbins *et al.*, 2005). *M. tuberculosis*-infected macrophages appear to be diminished in their ability to present antigens to CD4⁺T cells, which leads to persistent infection (Russell *et al.*, 2010). Another mechanism by which antigen presenting cells (APCs) contribute to defective T cell proliferation and function is by the production of cytokines, including TGF- β , IL-10 or IL-6 which are involved in killing of the mycobacteria. In addition, it has also been reported that virulent mycobacteria were able to escape from fused phagosomes and multiply (Sultan *et al.*, 2012).

2.7 Host Genetics and Susceptibility:

Several aspects of TB epidemiology are not explained by the germ theory, and suggest that there are individual differences in susceptibility as not everyone exposed to *M tuberculosis* becomes infected. TB is known to be more common in those who additionally had a family history of TB, suggesting the greater importance of familial susceptibility.

Asians, Africans and African Americans have less innate resistance than Whites. Mutations causing Mendelian susceptibility to Mycobacterial diseases (MSMD) have been found due to different genes (Ottenhoff *et al.*, 2005). The polymorphic human leukocyte antigens (HLA) were the first proteins to be examined for associations with TB susceptibility. HLA-B13 has a protective effect while individuals with DR8 have a greater probability of developing TB (Ahmad, 2011).

2.7.1 Influence of HIV on the Clinical Presentation of TB

Infection with HIV greatly increases the risk of developing TB and accelerates its progress. Progression to AIDS is also accelerated in those co-infected and HIV has a significant effect on the risk of relapse of tuberculosis. WHO estimates that tuberculosis causes up to 40% of AIDS deaths in sub-Saharan Africa and Southeast Asia. In sub-Saharan Africa, HIV is the most important cause of the increasing incidence of tuberculosis. Up to 70% of those with tuberculosis are also HIV positive (WHO, 2015).

As HIV infection progresses, CD4 lymphocytes decline by about 50–80 cells/mm³/year, and the immune system becomes less able to prevent the growth and local spread of *M. tuberculosis*.

Pulmonary TB remains, especially in adults, the commonest form of TB, but its presentation depends on the degree of immunosuppression. The clinical pictures, sputum-smear results and chest X-rays are often different in the early stage of HIV infection (CD4 >350 cells/mm³) and the late stage (CD4 <200 cells/mm³). The clinical presentation of TB cases in early HIV infection is similar to that of individuals without HIV infection, resembling post-primary PTB, that is, with positive sputum smears (defined as two or more initial smear examinations that are positive for acid-fast bacilli (AFB), or one plus consistent radiographic abnormalities) and often with cavities in the chest X-ray. In contrast, the clinical presentation in late HIV cases resembles primary PTB: the sputum smear is often negative and radiological infiltrates are present instead of cavities (WHO, 2015).

The interaction between HIV and TB is summarized in the following:

TB is a most important opportunistic disease which is infectious not only to HIV-infected persons but also to non-infected persons, it causes severe illness and increases progression to AIDS and TB kills – it is the number one killer in HIV (WHO, 2013).

HIV is the main risk factor for progression from latent TB infection to active disease. It increases TB incidence, leading to hot spots of TB transmission. HIV increases morbidity and mortality rates in TB patients because of HIV-related diseases and also increases adverse drug reactions to TB treatment and HIV increases risk of recurrent TB.

2.8 Serological Tests:

2.8.1. Enzyme Linked Immunosorbent Assay (ELISA):

In TB patients, the serological response to *Mycobacterial* antigens has been principally evaluated using standard ELISA with in-house procedures and practices which certainly differ between laboratories (Welch *et al.*, 2012). Few commercial tests based on the detection of specific antigens, such as the 36kDa protein, and A-60, have been developed and have been in use, chiefly in developing countries (Walid *et al.*, 2010). There is still a need to improve the sensitivity or specificity of commercial serological tests. The sensitivity and specificity of specific antigen enzyme linked immunosorbent assay (ELISA) have been shown to be highly variable in previous studies in different settings, notwithstanding the small sample size (Julian *et al.*, 2000; Butt *et al.*, 2004).

In Nigeria, Kagu *et al* (2006) used the smart check recombinant TB assay, and obtained sensitivity and specificity of 96.7% and 82.1% respectively, Garbati *et al.*, (2009) Maduguri, reported sensitivity and specificity 94% and 74% respectively for the Myco G. Ekundayo *et al.*, (2012), using the Clinotech TB screentest obtained 24.1% and 78.8% as sensitivity and specificity respectively. Using a sample size of 100 patients with

pulmonary TB; they calculated predictive values 78% and 89%. Enwuruet *al.*, (2004), in Lagos, Nigeria, obtained sensitivity and specificity of 76% and 100% respectively for Myco G EIA. Adjei *et al* (2003), with 92 participants in Ghana, obtained sensitivity of 80% and specificity of 98.2% while positive and negative predictive values were obtained as 96.6 and 88.9 respectively. They suggested that the serodiagnostic test kit may be a useful tool for the diagnosis of TB and can provide simple and satisfactory results, particularly in cases where TB is clinically difficult to diagnose by usual clinical and laboratory tests. Kassa-kelembo *et al* (2006), with 99 participants in Central African Republic, obtained sensitivity of 20.6% and specificity of 90.3% with the SDHO test, they concluded that despite acceptable specificity, the test lacks the sensitivity to replace sputum smear microscopy in their environment. With the MycoG enzyme immunoassay, Singh *et al* (2008), in India obtained sensitivity of 24.4% and specificity of 76.6%, they concluded that the commercial serological test evaluated showed poor sensitivity and specificity, and suggests no utility for the detection of pulmonary TB. The same kits were also evaluated on 94 pulmonary TB cases in Pakistan by Butt *et al*, (2004) showing sensitivities of 67% and 46% and specificity of 98% and 93% for MycoM and MycoG. Imaz *et al*, (2004) from Argentina also evaluated Pathozyme Myco M, MycoA and Myco G EIA on only 58 patients with pulmonary TB in a hospital setting and demonstrated high specificities of 93.3%, 97.8% and 100% respectively. Their respective sensitivity rates were 29.4%, 76.5% and 82.3% for 17 smear positive pulmonary TB cases, and 31.7%, 34.2% and 48.8% for 41 smear negative pulmonary TB cases (Singh *et al.*, 2006). The WHO published a policy statement regarding commercial serodiagnostic tests for diagnosis of TB. Based on a bivariate meta-analysis of commercially available tests,

including 67 studies, the authors of the WHO statement concluded that *M. tuberculosis* antibody tests should not be used for the diagnosis of pulmonary and extrapulmonary *M. tuberculosis* infections (WHO, 2011). In their summary statement, they stated specifically that the Anda-TB IgG (the most commonly evaluated test in their study) had a pooled sensitivity of 76% in smear-positive patients and 59% in smear-negative patients. In studies involving smear-positive patients, anda-TB IgG yielded a pooled sensitivity of 76% (95% CI 63–87) and a pooled specificity of 92% (95% CI 74–98). In studies involving smear-negative patients, the pooled sensitivity of anda- TB IgG decreased to 59% (95% CI 10–96); the 95% CI was very wide, reflecting the imprecision of the sensitivity estimate. Pooled specificity was 91% (95% CI 79–96). Compared with ELISA (pooled sensitivity 60% [95% CI 6–65]; pooled specificity 98% [95% CI 96–99]), immunochromatographic assays yielded lower pooled sensitivity (53%, 95% CI 42–64) and comparable pooled specificity (98%, 95% CI 94–99). The probability that the pooled sensitivity of ELISA tests exceeds that of immunochromatographic assays was estimated at 0.88% of TB in HIV-infected patients. The only study identified involving HIV-infected patients compared the performance of the SDHO MTB test (SDHO Laboratories) head-to-head with smear microscopy in 55 individuals suspected of having pulmonary TB residing in the Central African Republic (Steingart *et al.*, 2011). TB was confirmed by culture. Compared with smear microscopy (sensitivity 68% [95% CI 49–83]); SDHO yielded a sensitivity of only 16% (95% CI 5–34). Specificity of SDHO was 90% (95% CI 74–98); lower than the specificity of smear microscopy (100% [95% CI 89–100]) (WHO, 2011). Community specific cohort studies of patients were not available. The sensitivity is higher in smear positive than smear-negative samples; specificity is

higher in healthy volunteers than in non-TB suspects where TB was part of the differential diagnosis (Steingart *et al.*, 2007).

2.8.2 Tuberculin skin test:

Tuberculin skin test can be done by two methods

(1) Mantoux test and (2) Heaf test.

2.8.2.1 *Mantoux test* (MT) has been used to identify patients actively infected with TB, to measure the prevalence of infection in a community, and to select susceptible or high-risk patients for BCG vaccination. The test has been in existence for more than 100 years and has remained more or less unchanged for the last 60 years (Hoff, 2007).

MT works by intradermally injecting 0.1 mL of 5 tuberculin unit (TU PPD) on the forearm. On examination, after 48-72 hours, a positive reaction is indicated by erythema and induration of > 10 mm in size. Erythema (redness) alone is not taken as a positive reaction. All persons with prior infection with tubercle bacilli will mount an immune response to bacilli proteins (Hoff *et al.*, 2007). As the active ingredient used in the skin test contains a whole series of proteins that are shared with the BCG vaccine and other mycobacteria common in the environment, the skin test is often falsely positive. It is currently estimated that almost one third of people positive to MT do not actually have TB infection. The sensitivity of the skin test is estimated to be around just 70 % in known active TB cases; so the test misses up to 30 % of people who are infected. This sensitivity decreases to as low as 30 % in immunocompromised people.

2.8.2.2 *Heaf test*: This test is done with multiple puncture apparatus. Although it is not widely used, it is used in epidemiological survey, and as test for immunity before BCG vaccination (Levinson, 2006).

2.8.3. Interferon-gamma (IFN- γ) determination:

One of the most significant developments in the diagnosis of TB in the last hundred years seems to be the assays based on IFN- γ determination based on the principle that T cells of sensitized individuals produce IFN- γ when they re-encounter the antigens of *M. tuberculosis* (Tufariello *et al.*, 2003). Recent evaluations showed that IFN- γ assays have advantages over tuberculin skin testing (Lalvani *et al.*, 2001).

IFN- γ assay that are commercially available include: the enzyme-linked immunospot (ELISPOT), T SPOT-TB assay, the novel **Quantiferon-TB**, and its improved variety.

2.8.4 Quantiferon-TB Gold assay

Quantiferon-TB test measures the release of interferon-gamma in whole blood from human subjects infected with *M. tuberculosis* and *M. bovis* respectively, in response to stimulation by PPD. The IFN- γ secreted by T-cells into the plasma is measured by ELISA to indicate the likelihood of TB infection (Hossain and Norazmi, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Zaria, Kaduna state, Nigeria. It lies at latitude 11° 07' 51" N and longitude 7° 43' 43" E. Zaria is an urban town and it is located in the Guinean savanna zone of northern Nigeria (NPC, 2006).

3.2 Study Design

This was a diagnostic utility study. The duration of the study was from October 2014-April, 2015 (6 months)

3.3 Ethical Approval

Ethical approval was obtained from the Health Research and Ethics Committee of the Ahmadu Bello University Teaching Hospital where the participants were drawn from. After proper education on the procedure and significance of the investigation, informed consent was obtained from the consenting individuals.

3.4 Study Population

The study population comprised patients with pulmonary TB attending the National Tuberculosis and Leprosy Training Centre Saye and the TB annex Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

The National Tuberculosis and Leprosy Training Centre (NTBLTC), Zaria located in Saye village, is about four kilometres from Zaria town, along the old Zaria – Kaduna road. The Centre was established in January, 1991 as a human resource development centre for the National TBL Control Programme (NTBLTCP).

The NTBLTC comprises the training department as well as 140 beds capacity hospital wing as a practical area for the trainees. The Hospital has the following facilities

- Two General out Patient Departments (GOPD) with ten consulting rooms, pharmacy and monitoring and evaluation unit.
- Five wards with 140 beds capacity
- Eye Clinic
- Laboratories: 1 multipurpose, 1 TB culture and DST under construction
- Physiotherapy Department
- Operating Theatre

The TB annex of ABUTH is located in Zaria city, opposite the city gate. It has ten bed spaces for in-patients. Most of the patients attending the TB annex are out-patients.

3.4.1 Inclusion Criteria

Newly diagnosed patients with sputum smear positive Pulmonary TB, who are eighteen years of age and over.

3.4.2 Exclusion Criteria

1. Patients on anti-tuberculosis drug.
2. Patients less than eighteen years of age.
3. Patients with HIV infection.
4. Pregnant women

3.4.3 Controls

The control group comprised apparently healthy individuals, who did not have any clinical symptoms of TB like cough productive of sputum, fever, and night sweat.

3.5 Sampling Technique

The study involved a consecutive sampling method which was employed to recruit the participants as they presented.

3.6 Determination of Sample Size

The sample size was determined according to Araoye, (2004) using the formula:

$$n = (Z^2 p q) / d^2$$

Where

n = minimum sample size,

Z = standard normal deviate at 95% confidence interval i.e. 1.96.

p = proportion of IgG antibodies among patients with PulmonaryTB based on a previous study, according to Garbattietal (2009) p = 0.94 (94%)

q = 1 – p, i.e. complementary probability and

d = tolerance limit, the minimum is 0.05.

Therefore, $n = (1.96)^2 \times 0.94 \times 0.06 / (0.05)^2 = 84$

Assuming the non-response rate is 10%;therefore, nine (8) more participants were added to the calculated minimum sample size to make up a total of **92** participants.

3.7 Data Collection Method

3.7.1 Administration of Questionnaire

At the point of recruitment, a structured questionnaire was used to obtain each participant's data. Information collected included age, sex, educational status, occupational status, physical examination, weight/height, test result of sputum microscopy for AFB and Quantitative IgG antibody assay.

Patients with confirmed smear positive pulmonary TB were recruited while patients who decline their enrolment were not involved in the study.

3.7.2 Collection of Sample

3.7.2.1 Collection of Sputum Sample

The sample collection was done with the assistance of medical personnel. The patients were given sterile universal bottles in which they produced three samples (one spot, one early morning and one spot) (WHO, 2011).

3.7.2.2 Collection of Blood Sample

Peripheral blood samples (3ml) were collected from the ante-cubital vein and transferred into plain tubes in order to obtain the serum for specific anti-TB IgG assay. Samples were collected at the tuberculosis annex of the Ahmadu Bello University Teaching Hospital, Zaria and at the National for Tuberculosis and Leprosy Training Centre Saye Zaria, Kaduna.

3.8 Sample Analysis

3.8.1 Sputum Sample Analysis

Sputum samples were analyzed using the traditional microscopy;

Three consecutive sputum samples as detailed above were collected in leak proof universal plastic containers, stained using Ziehl-Neelsen's method and examined in accordance with standard methods (WHO, 2011). Three smears were prepared from each patient; heat fixed and stained using Ziehl-Neelsen technique as follows: Strong carbolfuchsin was flooded on the slides and steamed. It was then allowed to stain for 5 minutes followed by decolourization with 3% acid-alcohol and washing with water. Counterstaining was with 0.3% methylene blue for 1 minute, followed by washing with water and drying before examination for Acid Fast Bacilli.

3.8.2 Microscopic Examination of Slides

The stained smears were examined with the microscope under the oil immersion objective, scanning all fields at high power field for the presence of bright red slender rods, the presence of which signifies positive AFB and the absence, negative (Cheesebrough, 2008).

Interpretation: According to WHO & National tuberculosis control programme, interpretations of Ziehl-Neelsen acid-fast staining were as shown below:

Table 3.1 Interpretations of Ziehl-Neelsen acid-fast staining

AFB	RESULT	FIELDS TO BE EXAMINED
0/100 fields	Negative	100 fields
1-9/100 fields	Scanty	200 fields
10-99/100 fields	1+	100 fields
1-10/field	2+	20 fields
>10/field	3+	20 fields

With carbolfuchsin staining, tubercle bacilli look like fine red rods, slightly curved, more or less granular, isolated, in pairs or in groups, standing out clearly against the blue background.

3.8.3.1 Immunological test using ELISA:

Commercially available 16, 36 and 40kDa IgG- specific ELISA kits (IBL USA) were used in accordance with the manufacturer's instructions to determine levels of *M. tuberculosis* specific IgG in serum samples frozen at -70°C and were run for both cases and control. Standards supplied by the manufacturer were used to produce a standard curve. The optical density of each sample was read on a densitometer at 450nm and plotted against a standard curve with values. The serum samples were diluted 1: 100 in the sample diluents, the diluted samples and controls were then added (100 µl) to the wells and incubated at 37°C for 1 h. The wells were washed five times, then 100 µl of anti-human IgG-POD conjugate were distributed in each well and later on incubated for

30 min at 37°C, then the wells were washed again five times with the buffer solution and 100 ml tetramethylbenzidine (TMB) substrate was added in each well. The reaction was stopped by the addition of 100 ml H₂SO₄ after 15 min incubation at 37°C and then absorbance measured at 450 nm using MULTISCAN-ELISA plate reader.

Interpretation:

The optical densities values of different groups of case and control were used to interpret the result of ELISA.

Calibrator B with its concentration of 10 U/ml serves as cut-off standard. Analogous to the cut-off evaluation a range of +/-20% around the cut-off is defined as a grey zone. Thus results between 8 and 12 U/ml are reported as borderline.

3.9.4.1 Sensitivity and Specificity

The sensitivity and specificity of TB IgG ELISA was determined using the formula as stated below:

Formula for sensitivity and specificity=

Sensitivity =	True positive	× 100
	True positive + False negative	

Specificity =	True negative	× 100
	True negative + False positive	

3.8.3.4 Positive and Negative Predictive Values

The positive and negative predictive values of the performance of the TB IgG ELISA were calculated using the formulae below:

Formula for Predictive Values =

Positive predictive value =	True positive	× 100
	True positive + False positive	

Negative predictive value =	True negative	× 100
	True negative + False negative	

3.9 Statistical Analysis

The data collected were normally distributed and analyzed and values of the TB IgG concentrations in sera from all participants were compared using SPSS for windows version 20.0. Means were compared using independent t-test ($p < 0.005$). Chi-square was used for categorical data in comparing the results.

Descriptive statistics were presented in tables while quantitative variables were analyzed using the independent t-test.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-Demographic Characteristics of the Study Participants

A total of one hundred and eighty-four (184) participants were recruited for the study, comprising ninety-two (92) patients with Pulmonary TB (PTB) and ninety-two (92) controls. The socio demographic characteristics of the study population are shown (Table 4.1).

Male to female ratio was 4:1. The age distribution of participants is shown below; Most Pulmonary TB cases were within the 30-39 years.

The 92 participants with pulmonary TB comprised 89.7% males and 10.3% females (Table 4.1).

4.2 Clinical Symptoms of Participants with Pulmonary TB

The controls were symptom free during the study. The clinical symptom at presentation in the 92 participants with Pulmonary TB is shown below (Table 4.3) Cough, weight loss and fever were the commonest symptoms seen in 90 (98%), 87 (95%) and 85 (92%) of patients respectively.

4.3 TB IgG concentration among participants with Pulmonary TB and Controls

TB IgG concentration was measured using ELISA technique. The means and standard deviations of IgG concentration for participants with pulmonary TB was 5.71U/ml $\pm$ 5.47U/ml, while for controls, it was 13.77U/ml $\pm$ 11.31U/ml. This difference was statistically significant. $p=0.003$ (Table 4.4.1).

Table 4.1 Socio-demographic characteristics of study participants

	Category of participants (%)		Total
	PTB (n=92)	Controls (n=92)	
Age, years	n (%)	n (%)	
<20	11 (12.0)	2 (2.2)	13 (7.06)
20-29	26 (28.3)	39 (42.4)	65 (35.3)
30-39	30 (33.0)	31 (33.7)	61 (33.2)
40-49	15 (16.3)	16 (17.4)	31 (16.8)
50-59	8 (8.7)	4 (4.3)	12 (6.5)
60-69	2 (2.2)	0 (0.0)	2 (1.1)
Sex			
Male	76 (82.6)	89 (96.7)	165 (89.7)
Female	16 (17.3)	3 (3.3)	19 (10.3)
Marital status			
Single	33 (35.9)	42 (45.7)	75 (40.8)
Married	58 (63.0)	50 (54.3)	108 (58.7)
Divorced	1 (1.1)	0 (0.0)	1 (0.5)
Education			
Primary	9 (9.8)	12 (13.0)	21 (11.4)
Secondary	30 (33.0)	25 (27.2)	55 (29.9)
Tertiary	11 (12.0)	48 (52.2)	59 (32.1)
Qur'anic only	7 (7.6)	0 (0.0)	7 (3.8)
None	35 (38.0)	7 (7.6)	42 (22.8)
Ethnicity			
Hausa	79 (85.9)	68 (73.9)	147 (79.9)
Yoruba	5 (5.4)	8 (8.7)	13 (7.1)
Igbo	2 (2.2)	3 (3.3)	5 (2.7)
Others	6 (6.5)	13 (14.1)	19 (10.3)
Occupation			
Student	24 (26.1)	23 (25.0)	47 (25.5)
Civil servant	5 (5.4)	32 (34.8)	36 (19.6)
Business	21 (22.8)	14 (15.2)	35 (19.0)
Farmer	19 (20.7)	10 (10.9)	29 (15.8)
Housewife	7 (7.6)	0 (0.0)	7 (3.8)
Others	16 (17.4)	13 (14.1)	29 (15.8)

Table 4.2 Frequency distribution of Clinical symptoms in 92 participants with Pulmonary TB

Symptom	Frequency	Percentage
Cough	90	98.0
Weight Loss	87	95.0
Fever	85	92.0
Chest Pain	78	85.0
Fatigue	75	82.0
Night Sweat	69	75.0
Haemoptysis	74	80.4

Table 4.3 TB IgG concentrations among participants with Pulmonary TB and Controls

TB IgG Concentration (U/ml)		
(Pulmonary TB) (n=92)	(Controls) (n=92)	P value [#]
Mean ± SD	Mean ± SD	
13.77 ± 11.31	5.71 ± 5.47	0.003*

TB IgG- Tubercle Bacilli Immunoglobulin G

[#]determined by independent t-test.

*Significant difference between values for IgG concentration.

4.4 Relationship between TB IgG concentration and Sex

Among participants with Pulmonary TB, TB IgG concentration was higher among females. The mean and standard deviation of the TB IgG concentration for the 76 male participants was obtained as 12.8U/ml $\pm$ 8.2U/ml and for the 16 female participants, was 16.3U/ml $\pm$ 10.7U/ml (Table 4.5).

4.5 Sputum AFB bacillary density and TB IgG in participants with Pulmonary TB

Table 4.6 shows the sputum AFB bacillary density and TB IgG antibody positivity. Seventeen (18.5%) participants were positive by the TB IgG antibody. In TB IgG antibody negative participants, (1+) was seen in 29 (32%) of patients while (3+) was seen in 27 (29%) of the participants with Pulmonary TB. For TB IgG antibody positivity, a low sputum AFB bacillary density (1+) was seen in 6 (7%) of patients while a high density (3+) was also seen in 6 (7%) of participants with Pulmonary TB. The Pearson Chi-square was 0.132 with $p = 0.937$. This relationship was not statistically significant.

The relationship between sputum AFB bacillary density and TB IgG antibody concentration was determined. The median for (1+) was 3.52U/ml while for (3+), it was 4.33U/ml (Figure 4.1).

In the 92 participants with Pulmonary TB diagnosed with sputum AFB microscopy, the sensitivity and specificity of TB IgG antibody ELISA was 18% and 90% respectively.

While the positive and negative predictive values of TB IgG antibody ELISA was 66% and 53% respectively.

**Table 4.4 TB IgG concentration by Sex of participants with Pulmonary TB
(n=92)**

	Sex	Mean	Std. Deviation
IgG concentration (U/ml)	Male	12.8	8.2
	Female	16.3	10.7

TB IgG- Tubercle Bacilli Immunoglobulin G

[#] determined by independent t-test.

*Significant difference between values for IgG concentration.

Table 4.5 Sputum AFB bacillary Density and TB IgG Positivity in 92 participants with Pulmonary TB

Bacillary density	IgG Positivity		Total
	Positive	Negative	
	1+	6	29
AFB	2+	5	19
	3+	6	27
Total	17(18)	75(82)	92(100)

$\chi^2 = 0.132$; $p = 0.936$; $df = 2$

AFB= Acid-fast Bacilli; IgG= Immunoglobulin G

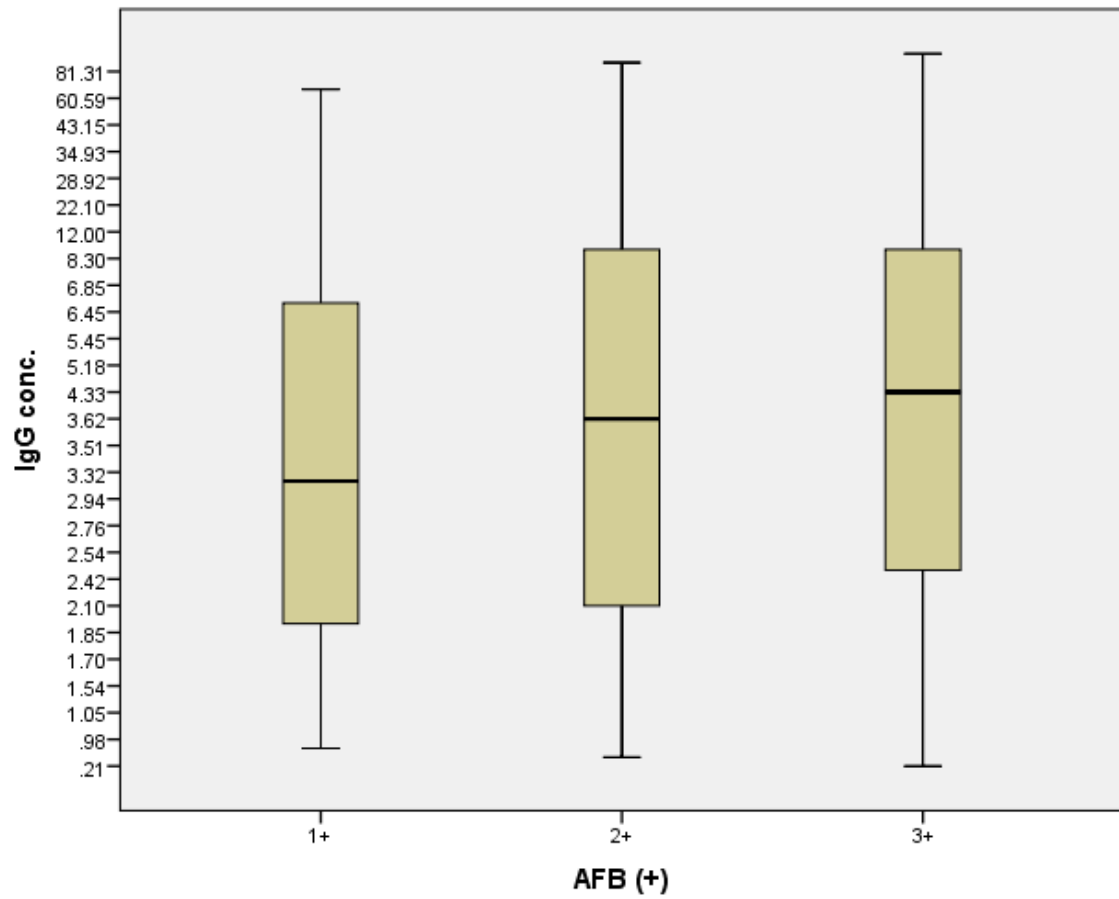


Figure 4.1 Relationship between bacillary density and IgG concentration of patients with pulmonary TB

CHAPTER FIVE

5.0 DISCUSSION

The use of sputum smear microscopy for the identification of acid fast bacilli remains the most important diagnostic method for the diagnosis of pulmonary tuberculosis in high endemic countries (Deunet *al.*, 2005).ELISA as a test for diagnosis of TB still presents substantial variability in sensitivities (16-100%) and specificities (71-100%) and hence has not acquired a defined role in clinical practice(Steingart*et al.*, 2009).

This study evaluated the performance of a cocktail 18, 36 and 40kDa TB antigen by IgG ELISA (IBL, USA) in 92 microscopically confirmed active pulmonary TB cases and 92 controls.

It has been observed that during active *M. tuberculosis* disease, a humoral response occurs in the host, which can be measured by quantifying anti-*M. tuberculosis* antibodies. IgG antibodies directed against several *M. tuberculosis* antigens have been proposed as potential markers of TB, of which the 18, 36, 40-kDa protein, as antigens are contained on the TB IgG assay (Anderson *et al.*, 2008). These proteins have been well characterized as immunodominant proteins present in *M. tuberculosis* culture filtrates, and although these protein antibodies offer good specificity, they suffer from low sensitivity when utilized alone (Silva *et al.*, 2003; Steingart, *et al.*, 2009;Welch *et al.*, 2012). Therefore, the cocktail of mycobacterial 18, 36, 40-kDa protein assay has a combination of antigens in an attempt to maximize sensitivity and specificity.

The present study has identified some weaknesses of the TB-IgG antibody assay in terms of sensitivity and specificity. This studyhas shown that a significant proportion of patient

with active pulmonary TB as demonstrated by AFB microscopy and other clinical parametres were found to be negative by the TB IgG assay, this is comparable to the work of Kassa-kelembo *et al.* (2006). The pro-zone effect that is usually associated with ELISA may be responsible for some of the false negative results. Since infections with non-tuberculous mycobacteria can coexist with *M. tuberculosis* infections, it is possible that the TB IgG assay was detecting antibodies which mimic or are concurrent to *M. tuberculosis* infection.

Other false-negative results may be due to immunosuppression, associated with nutritional deficiencies which may be due to loss of appetite that is associated with pulmonary TB (Welch *et al.*, 2012). This could potentially cause a false-negative result on an antibody detection based assay due to a decrease in IgG levels (Sultan *et al.*, 2012; Welch *et al.*, 2012). Some patients may however, have no history that would explain a negative antibody result. In this present study, patients with HIV co-infected with *M. tuberculosis* infection were excluded as it has been demonstrated in previous studies that HIV patients could potentially be negative by the TB IgG assay due to immunodeficiency (Anderson *et al.*, 2008; Welch *et al.*, 2012). Immunosuppression, immunodeficiency, or immunosenescence due to advanced age may also cause false negatives (Welch *et al.*, 2012).

Only 8% of the controls were positive for the TB IgG assay, this is similar to the findings by W.H.O. (2015). Cross-reactions with non-tuberculous mycobacteria in the TB IgG assay have not been previously investigated, except with the *Mycobacterium bovis* (BCG) (Anderson *et al.*, 2008), and may be responsible for positive TB IgG in the controls.

Additionally, the false positive results among the apparently healthy controls may be due to sub-clinical TB in individuals who are immunocompetent, since Zaria, the study area is a TB endemic region of Nigeria (Ogboi *et al.*, 2010). It may be inferred that environmental mycobacteria could be present in apparently healthy individuals but in the latent phase.

The use of purified and recombinant antigen(s) of various infectious agents improves the sensitivity and specificity of assays but such specific antigens are yet to be discovered for TB diagnosis (Steingart *et al.*, 2009). Serology is not known to be used in Nigeria for TB diagnosis.

In the study, TB IgG ELISA demonstrated barely 18% sensitivity with 90% specificity which agrees with those reported by Kassa-Kelembho *et al.*, (2006), Steingart *et al.*, (2007) and Singh *et al.*, (2007), while these rates are much lower than those reported in other studies (Imaz *et al.*, 2004; Bukhary, 2007; Garbat *et al.*, 2009). Moreover, as mentioned above these published studies used very small sample size and pulmonary TB patients were compared with non-TB patients. This has serious implications for any TB control programme as more than one third infectious pulmonary TB patients could be missed if serology is used as the sole criteria for administering anti-tubercular treatment. IgG ELISA demonstrated positive and negative predictive values of just 65% and 53% respectively. The diagnostic potential of a test in clinical practice also depends on its predictive values of the test. High positive predictive values of a test make it useful in strengthening the clinical suspicion of disease, while high negative predictive values of test makes the test useful in exclusion of disease in negative cases (Singh *et al.*, 2007),

indicating that this test has weak ability in confirmatory testing for TB infection, and has weak ability in ruling out TB in controls.

The comparison of mean value of serum TB IgG antibody assay by ELISA in pulmonary TB cases in this study was similar to another study in Saudi Arabia (Ahmed *et al.*, 2008) and also in the United States (Welch *et al.*, 2012) which shows that the difference in value of serum IgG between the combined control group and the tuberculosis cases was statistically significant, as a high percentage of serum samples from the patients with tuberculosis were found to be positive while a lower percentage of control samples were also positive. Bukhary (2007) reported high ELISA IgG in tuberculosis patient but there was sufficient discrimination between patients and control. Considering the healthy population as a control it became highly significant similar to western countries where the prevalence of tuberculosis is low and a clear distinction between case and control prevails (Hossain and Norazmi, 2013). It is difficult to obtain a specificity of 100% in developing countries particularly in tropical area where people are permanently in contact with various pathogens and develop cross reacting antibodies such as those of *Norcadia* and other mycobacteria resulting in poor test specificity. As such, every new serodiagnostic test should be validated with controls from countries where the test would apply (Hossain and Norazmi, 2013) and where it is possible to alter the cut off and obtain appropriate sensitivity, specificity and predictive value according to the prevalence of the disease in different areas (Kameswaran *et al.*, 2002).

On the basis of data showing that circulating *M. tuberculosis* specific antibodies can be formed not only in individual with TB diseases but also in the individual with latent *M.*

tuberculosis infection, it is suggested that the poor performance of sensitivities and specificities may at least reflect the high prevalence of latent TB in this region (Hoff *et al.*, 2007).

It was found in this study that there was an increase in TB IgG antibodies in patients with high sputum AFB bacillary density. This is similar to the findings of Turneer *et al.*, (1998) who pointed out the significant IgG elevation in highly smear positive patients (Turneer *et al.*, 1998). It has also been found that there is usually a decrease in IgG antibody concentration of pulmonary TB.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Pulmonary TB is the most common form of TB and is a major public health issue.

This diagnostic utility study with 184 participants of which 92 were positive for TB sputum smear microscopy while 92 were apparently healthy individuals revealed a higher TB IgG concentration in patients with pulmonary TB and lesser in controls and this was statistically significant. There is also a slight increase in the concentration of the TB IgG antibody with increase in bacillary density.

It was found that the TB IgG ELISA (IBL, USA) is poorly sensitive (18%) but with a relatively high specificity (90%). While the diagnostic efficacy of ELISA based serological diagnosis of TB is not efficient enough for the diagnosis of TB in a high prevalence country like Nigeria, and so it is not useful in the diagnostic algorithm TB.

6.2 Conclusion

Anti-18, 36 and 40kDa IgG ELISA results were not significantly positive and associated with low concentration TB IgG concentration in participants with sputum smear microscopically confirmed active pulmonary TB compared with the controls. The low diagnostic performance of the test makes it an unsuitable, though simple and rapid tool to validate clinical suspicion of active pulmonary TB.

As such serological tests may not be suitable for screening or diagnosing pulmonary tuberculosis in Nigeria because the extent of the antibody response to mycobacterial antigens may be associated with geographical variations (Singh *et al.*, 2007; Welch *et al.*, 2012).

Considering the various findings of present study it was concluded that the Immunodiagnostic methods for the laboratory diagnosis of pulmonary tuberculosis cannot be used as an adjunctive diagnostic tool but can help in prognosis of the disease in a low income and resource constraint country like Nigeria. It is relatively cost effective, yet large scale studies can be done in the evaluation of the test prior to application in the National level.

6.3 Limitation

The gold standard for the diagnosis of pulmonary TB (culture) was not used in the study.

6.4 Recommendations

1. Increasing the sensitivity and specificity of serological tests by selecting epitopes to enhance antibody responses such as combined antigens like TB 7.7, 38kda, ESAT 6 and CRP 10.
2. Anti-18, 36 and 40kDa may also be combined with other capture antigens that may perform better in the diagnostic algorithm pulmonary TB.
3. Combined antibody assay of other capture antigens to also be evaluated.

REFERENCES

- Abebe, F., and Bjune, G. (2009). The protective role of antibody responses during *Mycobacterium tuberculosis* infection. *Clinical and Experimental Immunology*, 157:235–243.
- Adjei, A., Amah, H., Duah, O., Adku, T. and Hesse, I. (2003). Evaluation of rapid serological chromatographic immunoassay for the diagnosis of pulmonary tuberculosis in Accra Ghana. *Japanese journal of infectious diseases*, 56:161-164
- Ahmad, S. (2011). Pathogenesis, immunology, and diagnosis of latent *Mycobacterium tuberculosis* infection. *Clinical and Developmental Immunology*, Article ID 814943, 176.
- Algood, H. M., Lin, P. L., and Flynn, J. L. (2005). Tumor necrosis factor and chemokine interactions in the formation and maintenance of granulomas in tuberculosis. *Clinical Infectious Diseases*, 41: 189-193.
- American Thoracic Society and the CDC (2000). Diagnostic standards and classification of tuberculosis in adults and children. *American Journal of Respiratory and Critical Care Medicine*, 161 (4): 1376– 1395.
- Anderson, B., Welch, R., and Litwin, C. (2008). Assessment of three commercially available serologic assays for detection of antibodies to *Mycobacterium tuberculosis* and identification of active tuberculosis. *Clinical and Vaccine Immunology*, 15:1644 –1649.
- Araoye, M. O. (2004). *Research methodology with statistics for health and social sciences*. Mathader Publishers, Ilorin, Nigeria. pp. 115 - 121.
- Azzurri, A. (2006). Serological markers of pulmonary tuberculosis and of response to anti-tuberculosis treatment in a patient population in Guinea. *International Journal of Immunopathology and Pharmacology*, 19:199 –208.
- Barry, C., Boshoff, H., Dartois, V., Dick, T., Ehrh, S., Flynn, J., ... Young D. (2009). The spectrum of latent tuberculosis: rethinking the biology and intervention strategies, *National Review of Microbiology* 7, 845-855
- Baumann, R., Kaempfer, S., Chegou, N., Oehlmann, W., Loxton, A.G., Kaufmann, S., van Helden, D., Black, F., Singh, M., and Walzl, G. (2014). Serologic diagnosis of tuberculosis by combining Ig classes against selected mycobacterial targets. *Journal of Infections*, 69:581-589.
- Bekker, L. G., Freeman, S., Murray, P. J., Ryffel, B., and Kaplan, G. (2001). TNF- α controls intracellular mycobacterial growth by both inducible nitric oxide synthase dependent and inducible nitric oxide synthase-independent pathways. *Journal of Immunology*, 166: 6728-6734.

- Bhatia, A., Kumar, S., and Harinath, B. (2003). Immunodiagnosis of tuberculosis: An Update. *Indian Journal of Clinical Biochemistry*, 18 (2) 1-5
- Bukhary, Z. (2007). Evaluation of anti-A60 antigen IgG enzyme-linked immunosorbent assay for serodiagnosis of pulmonary tuberculosis. *Annals of Thoracic Medicine*, 2: 47–51.
- Caruso, A., Serbina, N., Klein, E., Triebold, K., Bloom, B., and Flynn, L. (2001). Mice deficient in CD4 T cells have only transiently diminished levels of IFN- γ , yet succumb to tuberculosis, *Journal of Immunology*; 162: 5407-16.
- Chesnutt, M. S., and Prendergast T. J. (2007). Lung, Pulmonary Tuberculosis. *Current Medical Diagnosis and treatment*, 46th edition, McGraw Hill Medical, 222-315.
- Cheesebrough, M. (2009). District laboratory practices in tropical countries. 4th edition, Cambridge, 34-35
- Cooper, A. (2009). Cell-mediated immune responses in tuberculosis. *Annual Review of Immunology*, 27, 393-422.
- Davidow, A., Kanaujia, G., Shi, L., Kaviar, J., and Guo, X. (2005). Antibody profiles characteristic of Mycobacterium tuberculosis infection state. *Infections and Immunity* 73: 6846–6851.
- Del pezzo, M., De philippis, A., D'alessio, A., Rosiello, M., Tosone, G., and Gulletta E. (1990). A serological study of mycobacterial infections in AIDS and HIV-1 positive patients. *Journal Bacteriology and Virology Immunology*, 83:3-9.
- Delacourt, C., Gobin, J., and Guillard, J. (1993). Value of ELISA using antigen 60 for the diagnosis of tuberculosis in children. *Chest*, 104:393-98
- Demkow. U, Filewskai, M., Chromiec, B. B., Zielonka, T., Michalowsk, A., Kua, J., and Augustynowiczi, E. (2004). Diagnostic Value Of Different Serological Tests For Tuberculosis In Poland., *Journal Of Physiology And Pharmacology*, 55, 57-66.
- Deun, V. A., Salim, A. H., Aung, K. J. M., Hossain, M. A., Chambugonj, A., Hye, M. A., Kawria, A., and Declercq, E. 2005, .Performance of variations of carbolfuchsin staining of sputum smears for AFB under field conditions., *International Journal Tuberculosis and Lung Diseases*, 9 (10), 1127.1133.
- Divangahi, M., Desjardins, D., Nunes-Alves, C., Remold, H., and Behar, G. (2010). Eicosanoid pathways regulate adaptive immunity to *Mycobacterium tuberculosis*. *Nature Immunology*, 11, 751-758.
- Dye, B., and Williams, G. (2010). The population dynamics and control of tuberculosis.

- Science*, 328, 856-861.
- Flynn, J. L. (2004). Mutual attraction: does it benefit the host or the bug? *Nature Immunology*, 8,778-779.
- Garbati, M., Yusuph, H., Kagu, M., and Moses, A. (2009). Diagnosis of Pulmonary tuberculosis: utility of serology and Mantoux reaction in a resource-limited setting. *The Internet Journal of Infectious Diseases*,8:1-5
- Ghadiri, K., Izadi, B., Afsharian, M., Vaziri, S., Rezaei, M., and Sohbatollah, N. (2008). Diagnostic value of serological tests (IgA, IgG, IgM) against A-60 antigen in tuberculosis.*IranianJournalofClinicalInfectiousDiseases*, 3(4):205-208.
- Glatman-Freedman, A., and Casadevall, A. (1998). Serum therapy for tuberculosis revisited: reappraisal of the role of antibody-mediated immunity against *Mycobacterium tuberculosis*. *ClinicalMicrobiologyReview*. 11:514 –532.
- Greenaway C. (2005). Humoral response to *Mycobacterium tuberculosis* antigens in patients with tuberculosis in the Gambia. *InternationalJournalTubercleLungDiseases*. 9:1112–1119.
- Gruver, A.L., Hudson, L.L., and Sempowski, G.D. (2007). Immunosenescence of ageing. *JournalofPathology*,211:144 –156.
- Gupta, S., Shende, N., Bhatia, A., Kumar, S., and Harinath, B. (2005). IgGsubclass antibody response to mycobacterial serine protease at differentstages of pulmonary tuberculosis. *MedicalScienceMonitor*,11:585–588.
- Helke, J., Mankowski, Y., and Manabe, Y. (2006). Animal models of cavitation in pulmonary tuberculosis. *Tuberculosis*, 86, 337-348.
- Hoff, T. S., Abebe, M., Ravn, P., Range, N., Malenganisho W.,... Weldingh, K. (2007). Evaluation of *Mycobacterium tuberculosis*- Specific Antibody Responses inPopulations with Different Levels of Exposure from Tanzania, Ethiopia, Brazil, andDenmark.*clinical Infectious Disease* , 45: 575-582.
- Hossain, M. and Norazmi, M. (2013). Pattern Recognition Receptors and Cytokines in *Mycobacterium tuberculosis*Infection—The DoubleEdged Sword? Hindawi Publishing Corporation. *BioMedResearchInternational*, 20:171-174.
- Hur Y-G., Kim A., Kang Y.A., Kim A.S., Kim D.Y., Kim Y., Kim Y., Lee H. and Cho S.N. (2015). Evaluation of antigen-specific immunoglobulin G responses in pulmonary tuberculosis patients and contacts. *Journal of Clinical Microbiology* 53:904 –909.

- Imaz, M.S., Comini, M. A., Zerbini, E., Sequeira, M.D., and Latini, O. (2004) Evaluation of commercial enzyme-linked immunosorbent assay kits for detection of tuberculosis in Argentinean population. *Journal of Clinical Microbiology*, 42: 884–887.
- Jain, A.E., Faulkner, J.B., Carpenter, R.L., Patnode, R.A. (2011). Serum Protein and Ig levels in TB. *American Journal of Clinical Pathology*, 48:48-50
- Joller, N., Webera, S., Mullera A., Sporria, R., Selchowb, P., Sanderb P., and Hilibic, H. (2010). Antibodies protect against intracellular bacteria by Fc receptor-mediated lysosomal targeting. *National Academy of Science USA*, 107-204.
- Junqueira-Kipnis, A. P, Kipnis, A. and Jamieson A. (2003). NK cells respond to pulmonary infection with *Mycobacterium tuberculosis*, but play a minimal role in protection. *Journal of Immunology*, 171: 6039-45.
- Kassa-Kelembho, E., Kassa, E., Zandanga, G., Service, Y., Ignaleamoko, A. and Talarmin A.(2006). Poor Performance of a Novel Serological Test for Diagnosis of Pulmonary Tuberculosis in Bangui, Central African Republic *Clinical and Vaccine Immunology*, 13: 702–703.
- Lalvani, A., Nagvenkar, P. and Udwardia , Z. (2001). Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians., *Journal of Infectious Diseases*, 183, 469-77.
- Levinson, W. (2006). *Mycobacterium*. 9th edition, Lange Medical Books, Mc Graw hills, Medical publishing division, 161-165.
- Maes, R. (2013). Was the WHO Ban on TB Serodiagnosis Justified? *Science Journal of Medicine and Clinical Trials*, ISSN: 2276-7487.
- Manivannan, S., Narayan, V., and Ramanathan, V. (2012). Role of complement activation and antibody in the interaction between *Mycobacterium tuberculosis* and Human Macrophages. *Iranian Journal of Experimental Biology*, vol. 50: 542-550
- Meintjes, S., Lawn, F., Scano, G., Maartens M., French W., Worodria J. H.,... Colebunders, R. (2008). Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource limited settings. *Lancet Infectious Diseases*, 8, 516-523.
- National Populations Commissions (2006). Population Census. www.npc.gov.ng.

- Nedeltchev, T., Raghunand, M., Jassal, S., Lun, Q., Cheng, W., and Bishai, R. (2008). Pathway profiling in *Mycobacterium tuberculosis*: elucidation of cholesterol-derived catabolite and enzymes that catalyze its metabolism. *The Journal of Biological Chemistry*, 286, 43668-43678.
- Ongradi, J., Stercz, B., Kovesdi, V. and Vertes, L. (2009). Immunosenescence and vaccination of the elderly, I. Age-related immune impairment. *Acta Microbiology and Immunology Hungary*, 56:199 –210.
- Ottenhoff, T. H., Verreck, F. A., Hoeve, M. A. and Vosse, E. (2005). Control of human host immunity to mycobacteria. *Tuberculosis (Edinburgh)*, 85: 53-64.
- Pai, M., Riley, L. W. and Colford J. M. (2004). Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review., *Lancet Infectious Disease*., 4, 761-76.
- Pfyffer, G. E. (2007). *Mycobacterium*: General characteristics, Laboratory detection and staining procedure. *Manual of Clinical Microbiology*. 9TH edition, vol-1, pp-543-572.
- Raja, A., Acharyulu, G., Selvaraj, R., and Khudoos, A. (2001). Evaluation of antibody level to purified mycobacterial antigens for identification of tuberculous infection. *Biomedicine*, 21: 63-9.70.
- Ramos, J. (2005). The TetR family of transcriptional repressors. *Microbiology and Molecular Biology Reviews*, 69, 326-356.
- Raviglione, M. C. and Brien, R. J. O. (2005) .Tuberculosis. in *Harrisons Principles of Internal Medicine*, ed. Kasper, D. L., Branwald, E., Fauci, A. S., Hauser, S. L., Longo, D. L. and Jameson, J. L., 16th edition, McGraw Hill, 953-66.
- Robbins, S. L. and Cortan, R. S. (2005). Infectious Diseases. in *Pathologic basis of disease*, Kumar, V., Abbas, A. K., Fausto, N. 7th edition, Saunders, Elsevier, 381- 386.
- Russell, B., VanderVen, W., Lee R., Abramovitch, M., Kim, S., Homolka, S., Niemann, K., and Rohde, H. (2010). *Mycobacterium tuberculosis* wears what it eats. *Cell Host Microbe*, 8, 68-76.
- Samanich, K., Belisle, J., and Laal, S. (2001). Homogeneity of antibody responses in tuberculosis patients. *Infections and Immunology*, 69: 4600–4609.
- Scanga, C. A., Mohan V. P. and Yu, K. (2000). Depletion of CD4(+) T cells causes reactivation of murine persistent tuberculosis despite continued expression of

- interferon gamma and nitric oxide synthase 2. *Journal of Experimental Medicine*, 192, 347-58.
- Selvaraj P., Kannapiran M., Kurian S., and Narayanan, P. (2001). Effect of plasma lysozyme on live *Mycobacterium tuberculosis*. *Current Science*, 81:201-3.
- Senthil Kumar, K., Raja, A., Uma Devi, R., and Paranjape, S. (2000). Production and characterization of monoclonal antibodies to *Mycobacterium tuberculosis*. *Indian Journal of Medicine*, 112: 37-46.
- She, R.C. and Litwin, C. M. (2015). Performance of a tuberculosis serologic assay in various patient populations. *American Journal of Clinical Pathology*. 144:240-246.
- Shrestha, D., Bhattacharya, K., Lekhak, B. and Kumar, B. (2005). Evaluation of Different Staining Techniques (Ziehl-Neelsen Stain, Kinyoun Stain, Modified Cold Stain, Fluorochrome Stain) for the Diagnosis of Pulmonary Tuberculosis., *Journal of Nepal Health Research Council*, 39(2), 8-16.
- Singh S., Singh J., Kumar S., Gopinath K., Balooni V., Singh N. and Mani K. (2012). Poor performance of serological tests in the diagnosis of pulmonary tuberculosis: evidence from a contact tracing field study. *PLoS One* 7:e40213
- Sohaskey, C. D. (2005). Regulation of nitrate reductase activity in *Mycobacterium tuberculosis* by oxygen and nitric oxide. *Microbiology*, 151, 3803-10.
- Stenger, S., Niazi, K. R. and Modlin, R. L. (1998). Down-regulation of CD1 on antigen presenting cells by infection with *Mycobacterium tuberculosis*. *Journal of Immunology*, 161:3582-8.
- Steingart, K., Ramsay, A., and Pai, M. (2007) Commercial serological tests for the diagnosis of tuberculosis: do they work? *Future Microbiology*, 2: 355–359.
- Thomas, S., VanderVen, B., Sherman D., Russell D., and Sampson, N. (2011). Pathway profiling in *Mycobacterium tuberculosis*: elucidation of cholesterol-derived catabolite and enzymes that catalyze its metabolism. *The Journal of Biological Chemistry*, 286: 43668-43678.
- Todar, K. (2008) .*Mycobacterium tuberculosis* and Tuberculosis. In Todar's online Textbook of Bacteriology, 123-125.
- Tolar, P., Sohn, H.W., Pierce, S.K. (2008). Viewing the antigen-induced initiation of B-cell activation in living cells. *Immunology Review*, 221:1-4.
- Tripathi, D. G., Sriram, N., Naik, V. K., Smita, P., Seema, A., Shakila, G. and Desai, M. W. (2004). Efficacy of immunochromatographic technique for the Sero-diagnosis of tuberculosis., *Indian Journal of Medical Microbiology*, 22, 131-132.

- Tufariello, J. M., Chan, J. and Flynn, J. L. (2003). Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. *Lancet Infectious Diseases*, 3, 578-90.
- Tully, G., Kortsik, C. and Hohn H. (2005). Highly focused T cell responses in latent human pulmonary *Mycobacterium tuberculosis* infection., *Journal of Immunology*, 174: 2174-2184.
- Turneer, M., Van J. P. and Bruyn, J. D. (1988). Humoral immune response in human tuberculosis imminoglobulins G, A, and M Directed Against The Purified P-32 Antigen of *Mycobacterium bovis*, *bacilli calmetteGuerrin*., *Journal of Clinical Microbiology*, 26:1714-9.
- Uhía, I., Galán, B., Medrano, F. and García J. (2011). Characterization of the KstR-dependent promoter of the gene for the first step of the cholesterol degradative pathway in *Mycobacteriumsmegmatis*. *Microbiology*, 157: 2670
- Urban, C., Lourido, S., and Zychlinsky, A. (2006). How do microbes evade neutrophil killing? *Cell Microbiology*, 8: 1687-96.
- Vijaysekaran, D., Kumar, R.A., Gourishankar, N.C., Neduchekian, k. and Sethuraman, S. (2006). Mantoux and contact positivity in Tuberculosis., *Indian Journal of Paediatrics*, 73, 989-993.
- Walid, B., Hedi, H., Manel, M., Imen, B., Foued, B., Asma, F., and Jalel, B. (2010). Evaluation of the diagnostic value of measuring IgG, IgM, and IgA antibodies to mycobacterial A60 antigen in active tuberculosis. *Diagnostic Microbiology and Infectious Disease*, 68: 55–59
- Welch, R., Lawless, K., and Litwin, C. (2012). AntituberculosisIgG Antibodies as a Marker of Active *Mycobacterium tuberculosis* Disease. *ClinicalandVaccine Immunology*, p. 522–526
- World Health Organization (2004). Interim Policy on Collaborative TB/HIV Activities. <http://apps/who.int/tb/hivinterim policy/1826>
- World Health Organization (2011). Commercial serodiagnostic tests for diagnosis of tuberculosis: policy statement.<http://apps/who.int/tb/policystatement/1066>
- World Health Organization (2015). Global Tuberculosis Report. <http://www.who.int/tb/publications/tbglobalreport/2015/gtbr/11main>
- Zhang X., Su Z., Zhang X., Hu C., Yu J., Gao Q., and Wang H. (2013). Generation of

Mycobacterium tuberculosis-specific recombinant antigens and evaluation of the clinical value of antibody detection for serological diagnosis of pulmonary tuberculosis. *International Journal of Molecular Medicine*, 31:751–757.

APPENDIX I

INFORMED CONSENT

RESEARCHER'S DECLARATION

GSM NUMBER: - 08076877644

My name is SanmiEmiade, I am a postgraduate student of the Immunology Unit, Department of Medicine, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, where I am carrying out a study on the 'Assessment of the utility of serological diagnosis among patients with pulmonary tuberculosis in Zaria, Nigeria'. The study will involve you being asked some questions with respect to your illness. During the course of this exercise, you will be asked some questions, physically examined in the usual way for your routine clinic visits and blood sample will be taken from your veins for some tests. This will cause no harm.

The laboratory tests will include sputum smear tests and levels of a chemical substance (known as immunoglobulin G) which has been found to be protective against tuberculosis antigen. The tests will be done free-of-charge and will be interpreted in local dialects of the subjects. Please note that your responses and test results will be kept strictly confidential.

The overall findings of this study will help in the management of future patients with pulmonary tuberculosis.

I appreciate your help and participation in this study.

SUBJECTS' AFFIRMATION

The purpose and method of this study have been explained to me and I understand that refusal to participate will not affect my treatment in any way and that I may withdraw at any time I so wish, without giving any explanation. I am willing to participate in the study.

Signature/ thumbprint of the participant/ date -----

Signature of the interviewer/research assistant/ date -----

Signature of witness / date -----

APPENDIX II

Questionnaire

Assessment of the utility of serological diagnosis among patients with pulmonary tuberculosis in Zaria, Nigeria

QUESTIONNAIRE

SUBJECT [] CONTROL []

PROFORMA

Serial number -----

Date -----

Hospital number-----

1. BIODATA

- Age _____years
- Gender_____
- Occupation
 - Student []
 - Civil servant []
 - Business []
 - Others (specify) -----
- Marital status
 - Single []
 - Married []

- Divorced []
- Others (specify) -----

- Ethnicity

- Hausa []
- Yoruba []
- Igbo []
- Others (specify) -----

- Level of Education

- None
- Primary []
- Secondary []
- Tertiary []
- Others (specify) -----

2. CLINICAL INFORMATION	YES	NO
-------------------------	-----	----

- | | | |
|---------------------|-----|-----|
| a) Chest pain | [] | [] |
| b) Pleural effusion | [] | [] |
| c) Weight loss | [] | [] |
| d) Fatigue | [] | [] |
| e) Night sweat | [] | [] |
| f) Haemoptysis | [] | [] |
| g) Cough | [] | [] |
| h) Lymphadenopathy | [] | [] |

i) Others symptoms(specify) -----

3. Temperature _____ °C

4. Height-----

5. Weight-----

6. BMI-----

7. PHYSICAL EXAMINATION

General Examination	YES	NO
a) Wasting	[]	[]
b) Distress	[]	[]
c) Dyspnoea	[]	[]
d) Dehydration	[]	[]
e) Jaundice	[]	[]
f) Pallor	[]	[]
g) Lymphadenopathy	[]	[]
h) Cutaneous lesions	[]	[]
i) Pedal oedema	[]	[]
j) Others (specify) -----		

8. DIAGNOSIS AT RECRUITMENT -----

9. LABORATORY RESULTS

- Sputum smear_____
- Immunoglobulin G assay _____

APPENDIX III

Ethical Clearance (ABUTH - Zaria)



HEALTH RESEARCH ETHICS COMMITTEE

AHMADU BELLO UNIVERSITY TEACHING HOSPITAL

SHIKA - ZARIA, NIGERIA.

E-mail: abuth@yahoo.com Website: www.abuth.org

Chairman of Board: Chief. Shuaib Oyedokun Afolabi Fnil
Chief Medical Director: Prof. Lawal Khalid, MBBS, FMCS, FWACS, FRCS(ED) mni
Chairman, Medical Advisory Committee: Prof. Abdullahi Mohammed, MBBS, FWACP, FICS
Director of Administration: Barr. Ishak Bello, LL.B, BL., LL.M, PGDM, AHAN, FCAI

Our Ref: ABUTHZ/HREC/36/2014 Date: 23rd Sept. 2014

Your Ref: _____

ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE

Anti-A-60 Antigen Immunoglobulin G Antibody Assay in patients with Pulmonary Tuberculosis in Zaria.

ABUTH Ethics Committee assigned number: - ABUTHZ/HREC/L23/2014

Name of the principal Investigator: - Mr. Emiade Sanmi

Address of the Principal Investigator: - Immunology Unit, Dept of Medicine
F/Medicine ABU Zaria

Date of receipt of valid application: - 11/8/ 2014

Date of meeting when final determination
on ethical approval was made: - 3rd & 4th Sept, 2014

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and *given full approval by the Health Research Ethics Committee.*

Please note: this approval dates from 24th September, 2014– 24th September, 2015

No participant recruitment into this research may be conducted outside these dates.

All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: <http://www.nhrec.net>.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.


Prof. A. I. Mamman
Chairman, ABUTH HREC