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Immunological and Molecular Study among Patients with Pulmonary Tuberculosis

A Thesis
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Degree of Doctorate of Philosophy in Science / Medical Microbiology

By

Sarah Kassab Shandaway Hamzah

B.Sc. College of Science /Microbiology/University of Babylon (2008)
M.Sc. College of Science /Microbiology/University of Babylon (2011)

Supervisors

Professor	**Consultant physician**
Dr. Jawad Kadhim Tarrad	**Dr. Ahmed Asmer Mankhi**

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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الزمر: 9

صدق الله العظيم

Supervisors Certification

We certify that this thesis was prepared under our supervision at the college of Medicine, University of Babylon, as a partial fulfillment of the degree of Ph.D. of Science in Medical Microbiology.

Supervisor	**Supervisor**
Dr. Jawad Kadhim Tarrad	**Dr. Ahmed Asmer Mankhi**
Professor	**Consultant physician**
College of Medicine	**Directorate of General Health**
University of Babylon	**National Tuberculosis Institute**
/ /2022	**/ / 2022**

Recommendation of Head of the Department of Medical Microbiology in view of the available recommendations, I forward this thesis for debate by the examining committee.

Professor
Dr. Hayam Khalis Al-Masoudi
Head of Department of Medical Microbiology
College of Medicine
University of Babylon
/ /2022

Certification of Examining Committee

We, the members of examining committee, certify after reading this thesis **(Immunological and Molecular Study among Patients with Pulmonary Tuberculosis)** and after examining the student, **Sarah Kassab Shandaway Hamzah**, in its content, it was found adequate for Doctorate of Philosophy degree in Medical Microbiology with **Excellent** degree.

Professor
Dr. Alaa Jawad Hassan
College of Science
University of Babylon
(Chairman)

Professor
Dr. Huda Hadi AL-Hasnawy
College of Medicine
University of Babylon
(Member)

Professor
Dr. Ifad Kerim Abd Al-shibly
College of Medicine
University of Babylon
(Member)

Assistant Professor
Dr. Salih Abdul-Mahdi AL-Khalidi
College of Biotechnology
Al-Qasim Green University
(Member)

Assistant Professor
Dr.Mohammed A. Al-Aaskari
College of Biotecnology
University of Al-Qadisiyah
(Member)

Professor
Dr. Jawad Kadhim Tarrad
College of Medicine
University of Babylon
(Member and supervisor)

Consultant physician
Dr. Ahmed Asmer Mankhi
National Tuberculosis Institute
(Member and supervisor)

Approved by the college committee on graduate studies
Professor
Dr.Mohned A.N.Al-Shalah
The Dean of college of Medicine
University of Babylon

Dedication

To the symbols of giving and love……

My mother and My father……

who are present despite their absence……

I dedicate this research to their soul,

hoping that it will be goodness and charity

for them.

Sarah 2022

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Summary

Despite international control programs, tuberculosis remains a public health issue. Individuals with latent TB infection significantly increase the number of active tuberculosis cases and carry a lifelong risk of developing the disease. The present study aims to determine some of the immunological and genetic markers at two phases during the development of active TB disease and latent TB infection and to evaluate their role as predictive markers in latent TB infection and active TB patients. This study was designed as case-control, performed from February to September 2021 at the National Tuberculosis Institute / National Reference Laboratory for Tuberculosis in Baghdad.

Out of 140 sputum specimens were collected from patients suspected with TB, 60 patients were confirmed with active pulmonary tuberculosis based on positive results from staining of acid-fast bacilli, bacterial culture or GeneXpert assay. The demographic data for active pulmonary TB patients included age groups ranging from 16 to 68 years. The mean age was (36.16 ± 14.16) years, 21 (35.0 %) were females, and 39 (65.0 %) were males and there was a statistically significant difference in the incidence of active pulmonary tuberculosis among males and females (P = 0.02) and the he age groups (25-34years) showed higher frequencies of patient TB (33.3 %) than other age groups, with highly significant differences between all age groups (p=0.001). To assess the efficacy of the diagnostic tests using culture as the gold standard. Gene Xpert MTB/RIF assay gave high sensitivity (100%) and specificity (80.8%), respectively. Among 60 specimens with MTB detected by GeneXpert, the results showed that seven isolates (11.67%) of *Mycobacterium tuberculosis* were rifampicin resistance.

Diagnosis of subjects suspected of latent tuberculosis and apparently healthy controls were by QuantiFERON-TB Gold (QFT- Plus) assay. In total, 164 subjects with suspected latent TB were tested. Overall, sixty subjects (36.6%) displayed positive results in this test and were defined as latent TB infected, consisting of 37 males and 23 females ranging from (18 – 66) years, and 104 samples (63.4%) were

negative. Forty healthy persons without known TB exposure who were negative by this test included 22 males and 18 females ranging from 15 - 70 years. In this test, the positivity rates of the first antigen tube TB1 and the second antigen tubeTB2 were 27.4% and 30.5%, respectively. Also, there was no significant difference in positivity rate between TB1 and TB2 tubes(P=0.6) and the concordance rate between it was 84.8%, and the κ coefficient was 0.630. The median IFN-γ release of TB2 was insignificantly higher (p=0. 142) compared to TB1 in the QFT-Plus positive results.

To assess the specific contribution of CD8+ T-cells ,TB2−TB1 differential values were used as an indirect estimate of specific CD8+ stimulation with TB2 antigens. In the present study a greater TB2 antigen response was observed (TB2−TB1 difference >0.6 IU/mL) in 22 (36.7 %) individuals with latent TB from all QFT-Plus positive. Regarding the risk factors associated with QuantiFERON-TB Gold assay positive results, the most frequent age group was 45-54 years (30.0%), followed by the age groups ≥55 (23.3%) and were associated significantly with positive results for this test (OR: 3.8, P=0.022 and OR: 4.03, P=0.024), respectively. Males (61.7 %) were more frequent than females (38.3 %) in the Latent TB infection group and associated significantly with positive results for this test (OR: 2.19, P=0.018).

The results of the ELISA technique showed that there was a significant increase in the mean concentration of IL-18 in the serum of the active tuberculosis group compared to the latent and apparently healthy controls(P < 0.001), and there was also a significant increase in the mean concentration of IL-18 in the serum of the group of Latent tuberculosis compared to the apparently healthy controls (P<0.001). While there was no significant difference in the mean concentration of IL-17A in the serum of the active tuberculosis group compared to the apparently healthy controls (p=0.069). In contrast, there was a significant increase in the mean concentration of IL-17A in the serum of the latent TB group compared to the apparently healthy controls (p=0.002) and active TB group (p<0.001).ROC analysis was performed using IL-17A and IL-18 to estimate the capacity of these parameters to predict active TB and latent TB. IL-17A exhibited significant discriminatory power with high AUC values, sensitivity and specificity in discriminating latent TB from apparently healthy controls (sensitivity 98 % , specificity 95 % , AUC=0.993). IL-18 exhibited significant discriminatory power with high AUC values, sensitivity and specificity in discriminating between active TB vs. apparently healthy controls (sensitivity 92% , specificity 90 % , AUC= 0.952).

The molecular study included, detection of rs1946518 C>A of IL-18 gene and rs2275913 G > A of IL17A gene in active and latent TB by allele-specific polymerase chain reaction analysis and these results confirmed by Sanger sequencing method. To study the association between the above genetic variations and susceptibility to active and latent tuberculosis, Genotypic and allelic frequencies of the studied cohorts were calculated. In apparently healthy controls, the genotype distribution had no deviation from Hardy-Weinberg equilibrium (P>0.05).

For IL-18 -607C/A (rs1946518), the results demonstrated that the A allele of rs1946518 frequency was more common in TB patients than in apparently healthy controls (60% vs. 38.75%), and this allele confers a 2.37-fold increased risk of developing active tuberculosis (OR: 2.37, P=0.003) compared to wild type , and AA and CA mutant genotypes were associated with active TB risk (OR: 4.67 , p=0.007 and OR: 3.17, P=0.024) ; respectively. Also , TB patients had a higher frequency of the A allele of rs1946518 than LTBI group and that A- allele confers a 1.77-fold increased risk of TB (60% vs. 45.83% , OR: 1.77, P =0.028) compared to wild type allele. The mutant homozygous genotype AA of rs1946518 are significantly associated with TB risk (OR: 2.99, p =0.035) compared to LTBI group. For IL17A (rs2275913 G>A),the present study found no statistically significant relationship between genotypes or alleles frequency of IL-17A (rs2275913 G>A) in active TB patients, latent TB group compared to apparently healthy controls.

SNPs genotyping of *Sp110 gene* (rs7580900, rs7580912; rs9061) in the latent TB groups by Sanger Sequencing method. The results demonstrated that the mutant allele C of rs7580900 and rs7580912 frequency was more common in LTBI group than in apparently healthy controls (47% vs.36.25% ,44% vs. 27.5%), respectively and the mutant heterozygous genotype “TC” in rs7580912 (P=0.02, OR:2.88) and “TC” in rs7580900 (P=0.011, OR: 3.53) were associated with LTBI risk. In addition ,the mutant heterozygous genotype "GA" of rs9061 was more common in LTBI group compared apparently healthy controls (34% vs.15%) respectively, and this genotype was significantly associated with LTBI risk (P=0.03, OR: 3.14).

The current study concluded that early detection of rifampicin resistance may contribute to reducing the incidence of (MDR) strains among tuberculosis patients. Serum levels of IL-17A and IL-18 play differential roles in the two stages of tuberculosis infection and may be exploited to create new diagnoses .On the other hand, IL-18 gene polymorphisms(IL-18-607 C > A rs1946518) are associated with susceptibility to active TB and genetic variants of the SP110 (rs7580900,rs7580912,rs9061) are linked to susceptibility to latent TB and displayed the potential to predict of the risk of active and latent tuberculosis, respectively . Furthermore, Individuals with latent tuberculosis were diagnosed and those with a higher risk of progressing to active tuberculosis in future were identified based on immunological and molecular parameters, thus host biomarkers could improve the diagnosis of tuberculosis.

List of abbreviations

Abbreviation	**Meaning**
AS-PCR	Allele-specific polymerase chain reaction
ATB	Active Tuberculosis
AUC	Area under curve
BBA	Binding Buffer A
BSA	Bovine serum albumin
CFP-10	Culture filtrate protein-10 kDa
CT	Cycle threshold
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
CWD	Column wash solution
CXCL	Chemokine (C-X-C motif) ligand
DC	Dendritic cell
dNTP	Deoxynucleoside triphosphate
DST	Drug susceptibility testing
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ESAT-6	Early Secreted Antigenic Target 6 kDa
EtBr	Ethidium Bromide
FDA	Food and Drug Administration
FOXp3	forkhead box P3
gDNA	genomic DNA
GM-CSF	Granulocyte macrophage colony-stimulating factor
GWAS	Genome-wide association studies
AHC	Apparently Healthy control
HLAs	Human leukocyte antigens
HRP	Horseradish Peroxidase
HWE	Hardy Weinberg Equilibrium
IGRA	Interferon-release assays
ILCs	Innate lymphoid cells
iNOS	Inducible nitric oxide synthase
Ipr1	Intracellular pathogen resistance-1 gene in mouse
IUAT	International Union against Tuberculosis
KIR	Killer immunoglobulin-like receptor
LTBI	Latent TB infection
LTi	lymphoid tissue-inducer cells
MBL	Mannan-binding lectin
MDR-TB	Multidrug resistance tuberculosis
MHC	major histocompatibility complex
miRNAs	MicroRNA

MTC	*M. tuberculosis* complex
NFAT	Nuclear factor of activated T-cells
NF-kB	Nuclear factor kappa activated B cells
NOS	Nitric oxide synthase
NOS2A	nitric oxide synthase 2A
NTM	Non-tuberculous mycobacteria
OD	Optical Density
Omics	Includes: Genomic, Transcriptomic , Proteomic and Metabolomic
PAMPs	Pathogen-associated molecular pattern molecules
PBMCs	Peripheral blood mononuclear cells
PD-1	Programmed death 1receptor
PPD	Purified protein derivative
PRRs	Pattern Recognition Receptors
QFT- Plus	QuantiFERON -TB Gold
QFT-GIT	QuantiFERON-TB Gold In-Tube
RLB	RBC Lysis Buffer
RNI	Reactive nitrogen intermediates
ROC	Receiver operating characteristic
rpoB	RNA polymerase subunit B
RRDR	RIF-resistance determining region
RR-TB	Rifampicin Resistance tuberculosis
SCL11A1	Solute carrier family 11 member 1
SNPs	Single nucleotide polymorphism
SP110	Speckled 110 gene
Sst1	Super-susceptibility to TB 1 (sst1) locus
STAT3	Signal transducer and activator of transcription 3
TBE	Tris-Borate EDTA
VDR	Vitamin D receptor
VODI	Hepatic veno-occlusive disease with immunodeficiency
XDR-TB	Extensively drug resistance tuberculosis

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Chapter One

Introduction

and

Literatures review

1.1 Introduction

Tuberculosis (TB) is a public health problem despite the existence of international control programs. Active tuberculosis is primarily a lung disease caused by *Mycobacterium tuberculosis* , and it is one of the top ten causes of death worldwide (Gong *et al*., 2018). According to the Global Tuberculosis Report 2020 released by the World Health Organization (WHO), more than 10 million new TB cases and 1.4 million deaths (WHO, 2020) .Furthermore, multi-drug-resistant TB (MDR-TB) rates are increasing (Hayward *et al*.,2018).

Latent TB infection (LTBI) is defined as a state during which a persistent host immune response to stimulation by Mtb antigens is sustained without evidence of clinically manifested active TB (WHO,2018). One-fourth of the human population is estimated to have been exposed to *Mycobacterium tuberculosis* (Mtb) and carries the infection in its latent form and this latent infection presents a lifelong risk of developing active tuberculosis (TB) disease, and persons with latent TB infection (LTBI) are significant contributors to the pool of active TB cases. About 5-15 % of those people with latent TB infection would develop the disease in the near or distant future in a process known as "TB reactivation" (Pai and Behr, 2016; Gong and Wu, 2021). Hence, screening of TB-exposed individuals might help in the early detection of disease or infection for the better control of TB.

Cytokines are key regulators of immune responses and are imbalanced in tuberculosis (TB) which may reflect the status of the disease. Therefore, cytokines could be introduced as potential diagnostic and prognostic biomarkers for TB (Seyedhosseini,2019). Diagnosis of TB infection remains challenging with laboratory diagnostic tools. Immunological assays can bring light to this problem, QuantiFERON-TB Gold assay which measures the amount of IFN-γ released from sensitized human T cells after exposure to *M. tuberculosis* used to identify latent

TB infection (Zellweger *et al*., 2020). Moreover, published studies have reported on the diagnostic potential of IL-17A and IL-18 cytokines to distinguish individuals with latent or active TB from those who are uninfected (Kumar *et al*.,2019; He *et al*.,2020). In addition , a comparison of latent and active tuberculosis cases may provide some insight into factors that protect them from disease development, as well as new insights into the roles of cytokines at two critical stages of the *M. tuberculosis* infection.

Genetic polymorphisms among hosts have been shown to contribute to the outcome of Mtb infection. Polymorphisms can influence and modulate the immune responses, and it may be one possible reason for not all infected people with TB progress to active tuberculosis. Many studies have suggested that polymorphisms in immune response genes such as the IL-17A gene (rs2275913) and 1L-18 gene (rs1946518) are associated with TB susceptibility among different populations (Han *et al*.,2011; Shi and Zhang ,2015; Aravindan , 2019). Also, the SP110 gene (Speckled 110), which encodes an interferon-induced nuclear protein, has been shown to control innate host immunity to Mtb infection. Several SNPs were identified in the SP110 gene associated with susceptibility to LTBI and/or TB disease (Chang *et al*.,2018). Therefore, the identification of SNPs in these genes could be used as a marker to screen individuals who are at risk of latent and active TB Infections.

1.1.1 Aim of Study

The study was aimed to determine some of the immunological and molecular markers at two phases during the development of active TB disease and latent TB infection , and to evaluate their role as predictive markers in latent TB infection and active TB patients, through the following objectives:

1. Detection of *Mycobacterium tuberculosis* in active TB patients by phenotypic and genotypic methods.
2. Diagnosis of individuals with latent TB infection by using QuantiFERON-TB Gold assay.
3. Assessment the serum levels of cytokines linked to the immune response to *M.tuberculosis.*
4. Identification of the immunogenetics markers of interleukin -17A and interleukin-18 SNPs and study of the association between these genetic markers and susceptibility to active TB disease and latent TB infection.
5. Molecular detection of single nucleotide polymorphism of the Sp110 gene and susceptibility to latent TB infection.

1.2 Literatures Review

1.2.1 Active tuberculosis

Human tuberculosis is a contagious disease caused by *Mycobacterium tuberculosis*. TB is transmissible by a respiratory droplet nucleus of 1–5 μm in diameter, containing 1–10 TB bacilli. TB transmission is affected by the strength of the infectious source, TB bacilli's infectiousness, host immunoresistance, environmental stresses, and biosocial factors (Lee, 2016). Tuberculosis most commonly affects the lungs (pulmonary tuberculosis) but may affect any organ or tissue outside the lungs (extrapulmonary tuberculosis). Active tuberculosis (TB) has a more significant burden of TB bacilli than latent TB and acts as an infection source for contacts. Signs and symptoms of pulmonary tuberculosis include cough, fever, chills, night sweats, weight loss, haemoptysis (coughing up blood), and fatigue. Signs and symptoms of extrapulmonary tuberculosis depend on the site of the disease (Loddenkemper *et al.,*2016).

Once diagnosed, the current recommended post-exposure treatment for drug-susceptible TB is a 6- month regimen of four first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide; this serves to clear the infection and prevent the development of antibiotic resistance (Wu *et al*.,2016). Drug-resistant tuberculosis is a serious threat to global health. For surveillance and treatment, drug-resistant tuberculosis is classified as rifampicin-resistant tuberculosis, multidrug-resistant tuberculosis (MDR-TB), and extensively drug-resistant tuberculosis (XDR-TB). (WHO,2020). There is growing comprehension that focusing only on treatment or treatment outcomes will not eliminate TB. Rather, attention needs to be given to other aspects that drive high TB incidence, including poverty, overcrowding, malnutrition, continued transmission, and weak health systems (Naidoo *et al*.,2017).The most effective strategy implemented by governments to control TB in humans has been immunization with Bacille Calmette-Guérin (BCG) applied during childhood. This vaccine induces a robust Th1 response and promotes the development of mycobacterial-specific polyfunctional and cytotoxic T cells. Although it confers a variable and incomplete protection against pulmonary TB in humans, it is highly effective in preventing the severe forms of TB in childhood (Weiner and Kaufmann, 2014).

In 2014, the World Health Assembly unanimously approved the WHO End TB Strategy, a 20-year strategy devised to end the global tuberculosis epidemic (WHO , 2015). Early diagnosis of tuberculosis, including universal drug susceptibility testing and systematic screening of contacts and high-risk groups, is a vital part of the strategy. After more than a century since its original identification, TB remains a major global health challenge worldwide. Most researchers efforts directed at identifying host immune biomarkers of protection, disease progression, treatment response, and disease recurrence suggest that studying human genetic determinants of TB infection outcome holds promise for new vaccines and therapeutic options (Satta ,2018).

1.2.2 Latent tuberculosis infection

Latent tuberculosis infection (LTBI) is a subclinical mycobacterial infection defined based on the cellular immune response to mycobacterial antigens (Kiazyk and Ball , 2017). The tuberculin skin test (TST) and the interferon-gamma release assay (IGRA) are currently used to establish the diagnosis of LTB. Although about 90% to 95% of the people infected with *M. tuberculosis* do not develop the active disease and remain asymptomatic, around a small proportion, approximately 5–10% of the individuals infected with *Mycobacterium tuberculosis*, develop the disease during the first 2–5 years after infection (WHO.,2018). In the rest of them, the innate immune response will either fully eliminate the infection without leaving a trace of immunological response (resistance to TB infection) or lead to a state of persistent immune response to *M. tuberculosis* antigens without clinical evidence of active disease. This last outcome is indeed the basis to consider that one-fourth of the world population is infected with *M. tuberculosis* (WHO, 2018; Simmons *et al.*,2018).

The identification of individuals in contact with active TB cases during the first two years of exposure is essential for two reasons: first, it allows the implementation of public health policies of disease control by identifying individuals with an increased risk of developing active tuberculosis; in particular, those who acquired the infection recently. Second, it can lead to a better understanding of the immune response during infection, which could be instrumental in developing better therapeutic and prophylactic interventions (Behr *et al*.,2018). In persons with LTBI, several factors increase the risk of developing active TB and most of them are related to an impaired immune response, such as concurrent HIV infection, cancer, immunosuppressive therapy or renal transplant, and diabetes (Ayelign *et al*.,2019).

Some other factors, though, are related to specific components of host response, such as macrophages activation, maintenance of granuloma structure, CD4 T cells, CD8 T cells, interferon-gamma (IFN-γ), and tumour necrosis factor-alpha (TNF-α) production, all of them important for the control of the pathogen during LTBI and some studies using whole-blood transcriptomic profiling have been performed to identify signatures that could differentiate between LTBI and active tuberculosis and predict different outcomes of treatment (Thompson *et al*.,2017).

1.2.3 *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is an ancient master of the art of causing human disease (Tiwari *et al*., 2019). *M. tuberculosis*, then known as the tubercule bacillus (TB), was first described on 24 March 1882 by Robert Koch (now World TB Day), who subsequently received a Nobel Prize for this discovery in 1905; the bacterium is also known as Koch's bacillus. *M. tuberculosis* is acid-fast bacilli, an aerobic, non–spore-forming, non-motile bacillus with a high cell wall content of high-molecular-weight lipids. Growth is slow, the generation time is 15 to 20 hours, compared with much less than 1 hour for most common bacterial pathogens, and visible growth takes from 3 to 8 weeks on solid media. The organism tends to grow in parallel groups, producing the colony characteristic of serpentine cording (Bennett *et al*.,2019). Unlike most pathogens, *M. tuberculosis* has evolved within humans, which are both host and reservoir (Ehrt *et al*.,2018).

It has a complex, lipid-rich cell wall responsible for many of the bacteria characteristic such as acid fastness; slow growth; resistance to detergents, common antibacterial antibiotics, and the host immune response; antigenicity. Strains of *M. tuberculosis* resistant to antibiotics have become a worldwide problem because no plasmids were found in this organism, this resistance is attributed to one or more chromosomal mutations (Murray *et al*.,2016).

The organism's virulence depends on several antigenic compounds (toxic substances) of the cell wall. *M. tuberculosis* lacks the classic virulence factors that other organisms encode to enable the invasion of host tissues, such as pili, flagella, fimbriae, and classic toxins (Cambier *et al*.,2014). The unique composition of the cell wall is composed of mycolic acid, glycolipids such as diacyltrehaloses, polyacyltrehalose, lipomannan, lipoarabinomannan (LAM), mannose-capped-

LAM, sulfolipids, and trehalose-6,6’-dimycolate, all have been implicated in providing the pathogen with an advantage in the host (Ghazaei,2018).

Mycobacterial virulence is not depending solely based on bacteria strains and/or bacteria burden in respiratory secretion but is also involved in differential expression of *M. tuberculosis* genes at different stages of infections . Essential virulence factors mainly attributed to the genes encoding lipid pathways, proteins and regulators of signal transduction systems and cell surface proteins (Echeverria-Valencia *et al*., 2018).

1.2.4 Epidemiology

Globally, 1.7 billion individuals are Mtb infected. In 2018, 10 million individuals developed active TB disease and 1.5 million died as shown in figure (1.1). In addition, it is estimated that only 70% of new cases of active TB were reported in 2018, leaving 30% undiagnosed, untreated, and capable of transmitting Mtb or even drug-resistant Mtb (WHO,2019) .

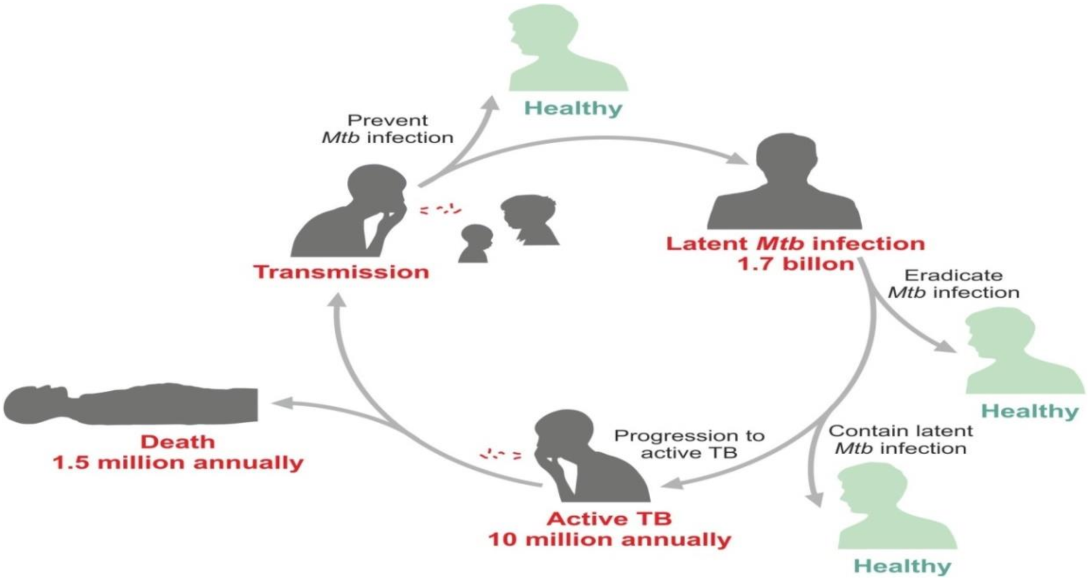


Figure (1.1): Epidemiologic data for tuberculosis (Kaufmann,2020)

However, in 2019, around 208,000 HIV-positive people died from tuberculosis. Furthermore WHO estimates that, from 2000 to 2019, more than 60 million lives were saved by diagnosing and treating tuberculosis. There were approximately half a million new cases of rifampicin-resistant tuberculosis (of which 78% had MDR-TB) worldwide and 12,350 cases of XDR-TB. The COVID-19 pandemic threatens to reverse the gains made in recent years. A modeling study by the WHO suggests that there could have been between 200,000 and 400,000 additional tuberculosis deaths in 2020 if, for three months, 25% to 50% fewer people were detected with and treated for tuberculosis (WHO, 2020).

Iraq is one of the countries in the World Health Organization Eastern Mediterranean Region (WHO-EMR) with a TB incidence rate of 42 per 100,000 and a TB case detection rate of 54% (WHO,2018). Overall, according to the WHO country profile, Iraq has a higher incidence rate of TB than all neighboring countries, that is, Syria, Jordan, Iran, Saudi Arabia, and Turkey. TB is an important public health concern in Iraq. Iraq is a middle burden country with TB, and is ranked 108th globally and 7th in the Eastern Mediterranean region among countries with TB burden size (Durib,2018).

Given this immense disease burden, prophylactic and post-exposure treatments are the logical steps toward eradicating TB. Treatment for individuals with RR-TB and MDR-TB is longer, more toxic, and more expensive and three times higher than that of an antibiotic-susceptible TB case (Oh *et al*.,2017). The extreme biological, social, and economic burden emphasizes the rising threat of TB and the need for researchers and funders to collaborate to develop higher efficacy antibiotics and a more effective TB vaccine (Shah *et al*.,2017).

1.2.5 Pathogenesis

Human tuberculosis pathogenesis is a complicated process that involves a complex interaction between the host immune system and bacterial elements. Several recently published reviews demonstrate the role of host immunity, which includes both innate and adaptive immunological responses, in mediating the pathogenesis of human tuberculosis caused by *Mycobacterium tuberculosis* (Liu *et al*., 2017). Macrophages, granulomas and IFN- γ produced by CD4+ T cells in primary TB representing the "central dogma" of protective immunity that produces systemic immune responses to protect entire body from disseminated infection (Queval *et al*., 2017). The pathogenesis of human tuberculosis and progression of the infection and disease outcomes in immunocompetent hosts following exposure to infectious droplet nuclei containing *M. tuberculosis* are described in figure (1.2).

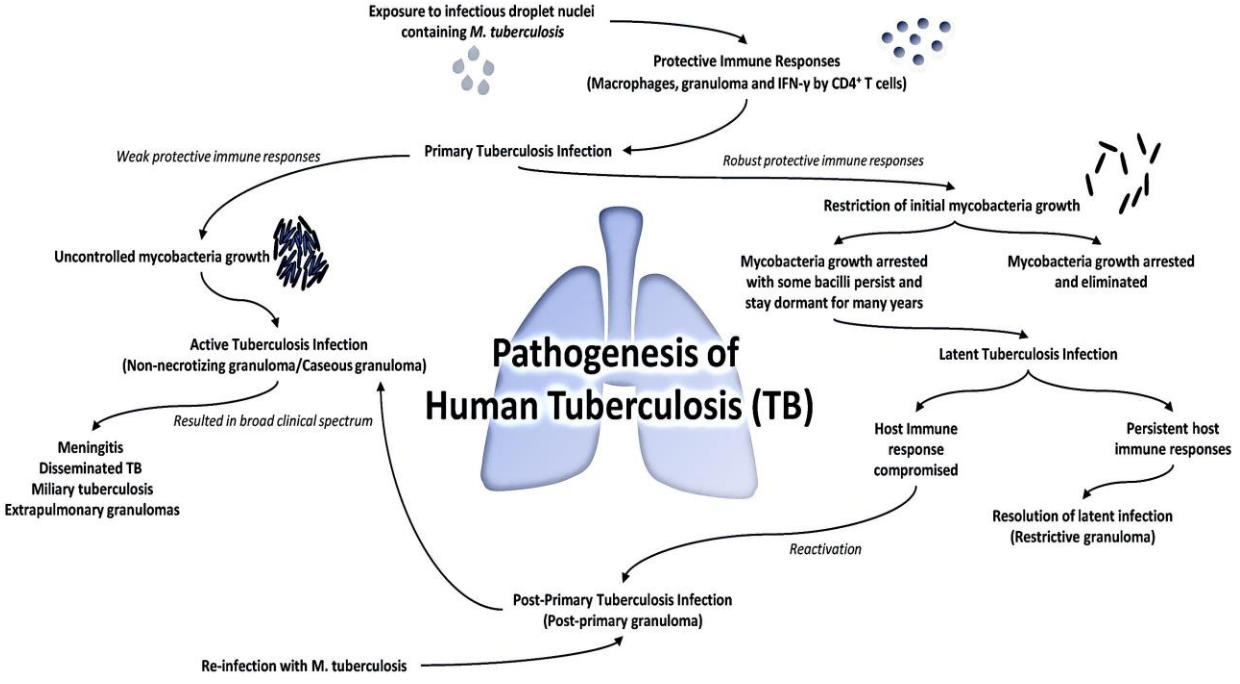


Figure (1.2): Pathogenesis of human tuberculosis (Kanabalan *et al*.,2021).

Pathogenesis begins with inhaling *M. tuberculosis* bacilli-droplet nuclei from active TB patients. These infectious droplets travel down the respiratory tract and into terminal alveoli, where they are phagocytosed by alveolar macrophages and other phagocytic immune cells. Alveolar macrophages provide a primary cellular niche for *M. tuberculosis* to replicate intracellularly during the initial stage of infection . *M. tuberculosis* replicates inside cells and spreads to lymph nodes and extrapulmonary tissues via lymphatics and blood. Adaptive immunity begins when *M. tuberculosis* spreads to lymph nodes (Guirado and Schlesinger, 2013).

Activation of macrophages, together with the elicitation of adaptive immune responses, result in phagosome-lysosome fusion and production of cytokines such as IFN-α, TNF-α, IFN-β, IL-1β, IL-6 and IL-12 that while consolidating the host bactericidal response, can also potentially cause significant lung inflammation and tissue destruction, a hallmark for active tuberculosis (Zumla *et al*., 2015). Active tuberculosis, developed through either reinfection with *M. tuberculosis* or reactivation from latent infection, is manifested by active replication of *M. tuberculosis* and host tissue damage caused by excessive host inflammatory responses, leading to necrosis and cavitation (Kiran *et al*., 2016).

The hallmark of pulmonary tuberculosis in humans is granuloma formation surrounded by the influx of macrophages and other cells (dendritic cells, neutrophils, fibroblast, T lymphocytes and B lymphocytes), which begins shortly after the infection. Histologically, granulomas are compact and organized immunological structures rich in a wide spectrum of transformed macrophages such as epithelioid cells, multinucleated giant cells and foamy macrophage cells, and the presence of other immune cells including dendritic cells, monocytes and neutrophils (Palucci and Delogu, 2018).

In latent infection, *M. tuberculosis* has been found to reside inside the macrophages in the hypoxic center in a metabolically altered state. In active disease, *M. tuberculosis* can replicate in peripheral oxygenated areas. Granulomas formation is a consequence of a complex and well-coordinated mechanism between both innate and adaptive immunity that sealed *M. tuberculosis* inside granulomas. Formation of granuloma is to restrict mycobacterial dissemination, and also to control and to limit tissue damage at the site of infection through inflammation (Ramakrishnan, 2012). However, not all granulomatous lesions are sterilized, and some lesions might promote the persistence of viable *M. tuberculosis* (non-replicating-persistence phase) in the granuloma (Ernst , 2012).

M. tuberculosis may stay dormant in the granuloma for decades without any active clinical signs and symptoms. This condition occurs in at least 90 % of the infected individuals, which then progresses into latent tuberculosis infection. The infected individual will likely be tested positive using IGRA and tuberculin skin test. However, dormant mycobacteria may actively replicate and disseminate to lungs or extrapulmonary tissues in immunocompromised populations. At this stage, the infected individual can transmit the disease actively due to the opening of granuloma in the bronchial lumen, where *M. tuberculosis* is expelled when coughing (de Martino *et al*., 2019).

1.2. 6 Pathogenicity

The pathogenicity of *M. tuberculosis* mainly lies in its capability to manipulate macrophages, granulomas and host cellular metabolism (Miggiano *et al*., 2020). Also, the *M. tuberculosis* genome undergoes evolutionary changes during its co-adaptation to humans to assure its transmissibility and evasion from host immune surveillance (Copin *et al*., 2016).

1.2.7 Diagnosis of *Mycobacterium tuberculosis*

1.2.7 .1 AFB Smear microscopy

Early laboratory diagnosis of tuberculosis still relies on the examination of stained smears. This technique is based on the fact that the cell wall of the *Mycobacterium spp*. genus is rich in complex lipids, and their hydrophobic cell wall resists penetration of aniline dyes such as crystal violet; however, under certain conditions, arylmethane stains are able to form stable complexes with the mycolic acids within mycobacterial cell walls (Caulfield and Wengenack, 2016). In the presence of heat, carbol fuchsin dye can be used as performed during Ziehl–Neelsen staining, which also utilizes methylene blue as a counterstain, but, when stained with carbol-fuchsin, these are not easily decolourized, even with alcohol-acid solutions (AFB) (David *et al*.,2018).

A significant limitation of smear microscopy is its low sensitivity (25–75% compared to culture) and the high number of bacilli required for positivity. Sensitivity and the positive predictive value (PPV) of smear microscopy are influenced by numerous factors such as the disease's prevalence and severity, the specimen's type and quality, the number of mycobacteria in the sample and the quality of the smear preparation, staining and reading process. Smear microscopy does not allow for mycobacterial species identification nor gives an indication of mycobacteria's viability in the sample (WHO,2010). The Ziehl-Neelsen stain detects the MTC but is unreliable in detecting nontuberculous mycobacteria (Aitken *et al*., 2019).

1.2.7.2 Cultivation of *M.tuberculosis*

Culture is the most reliable test for TB diagnosis; however, *Mycobacterium tuberculosis* grows slowly. It has a doubling time of 18 hours, in contrast to most bacteria, which can double in number in 1 hour or less. Sputum mycobacterial culture served as the "gold standard" diagnostic assay in TB diagnosis (Bourassa, 2018), and it also facilitates DST for definitive diagnosis of drug-resistant forms of tuberculosis unable to detect RIF/DR simultaneously . Detection of the number of TB cases is increased by 30–50% with culture techniques (Migliori *et al*. 2012). The Löwenstein–Jensen medium, more commonly known as the LJ medium, is the most popular means of culturing Mycobacteria, as recommended by the International Union against Tuberculosis (IUAT); also, several alternative media have been investigated (Chinedum *et al*.,2018).

1.2.7.3 Xpert MTB/RIF assay

The GeneXpert MTB/RIF assay (Cepheid, USA) is a probe-based, fully automated polymerase chain reaction (PCR) test that simultaneously detects *M. tuberculosis* complex and resistance to rifampin. Sample processing, PCR amplification, and detection occur in a single cartridge to minimize hands-on technical time and opportunities for contamination. Additionally, the assay's sample reagent has tuberculocidal activity, which decreases biosafety concerns during the test procedure (Alame-Emane *et al*.,2017).

GeneXpert MTB/RIF is approved by the US Food and Drug Administration (FDA) for direct testing on smear-positive or -negative sputum specimens in patients who have received fewer than three days of antituberculosis therapy and has a turnaround time of 2 hr. Additionally, one or two GeneXpert MTB/RIF tests can be used as an alternative to AFB smears to inform the decision to discontinue airborne isolation in patients with suspected pulmonary TB, thereby decreasing the length of time that a patient remains in isolation (Cowan *et al*.,2017).

GeneXpert MTB/RIF has been extensively studied and validated in various clinical settings (Rice *et al.*,2017). A meta-analysis that included data from low- and middle-income countries found that the pooled sensitivity and specificity for GeneXpert MTB/RIF as an initial test replacing smear microscopy were 89% and 99%, respectively (Steingart *et al*.,2014).

Implementing GeneXpert MTB/RIF can lead to considerable operational and logistical challenges, including infrastructure renovation, device training, and regular instrument troubleshooting and maintenance (Ardizzoni *et al*.,2015). Cost is probably the most significant limitation and has hindered widespread utilization of GeneXpert MTB/RIF. However, multiple studies have shown that GeneXpert MTB/RIF is a cost-effective tool for diagnosing TB and drug-resistant TB (Oxlade *et al*.,2016).

1.2.8 Diagnosis of Latent TB infection

The diagnosis of LTBI is indirect and relies on detecting an immune response against *M. tuberculosis* antigens, assuming that the immune response has developed after contact with MTB. Tuberculin skin test (TST) and interferon-gamma release assays (IGRAs) are the main diagnostic tests for LTBI; both are indirect tests based on the immune response to TB and do not directly assess the presence or viability of TB bacilli. Furthermore, no test is currently available which would allow the distinction between an immune reaction due to LTBI from that induced by active TB (Carranza *et al.*,2020). Thus, the difference between LTBI and active TB relies on clinical, bacteriological, and radiological findings. Indeed, the low tissue bacterial burden associated with LTBI works against any diagnostic strategy focused on identifying the bacteria or its components. There is no standard gold test for LTBI (WHO,2018).

1.2.8.1 The tuberculin skin test (TST)

The TST is a diagnostic method performed through intradermal injection of old tuberculin (OT) or purified protein derivative (PPD) as an antigen (Mosavari *et al*., 2021). It has been applied in the screening, diagnosis, and epidemiological studies of primary infection with *M. tuberculosis* for over 100 years and is the preliminary diagnostic test for LTBI. The TST is widely used to screen and detect TB because it is affordable, simple to perform, and requires minimal laboratory equipment. However, its disadvantages cannot be neglected, such as its requirement for a second visit after 48–72 h, inability to distinguish LTBI from ATB, false positives with Bacillus Calmette–Guérin (BCG) vaccination, cross-reactivity with nontuberculous mycobacteria (NTM), and false negatives in immunosuppression and deficiency (Pourakbari *et al*., 2019).

1.2.8.2 Interferon Gamma Release Assays (IGRAs)

IGRAs is a blood-based test relying on the in *vitro* measurement of gamma-interferon release by sensitized lymphocytes after stimulation with antigens from MTB. The test uses the same principle as the TST (evaluation of the release of cytokines by sensitized lymphocytes). Until recently, two IGRAs have been commercialized: the T-SPOT.TB assay and the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay. The QFT-GIT assay measures interferon-γ concentration to detect *M. tuberculosis* infection using a single mixture of synthetic peptides (ESAT-6, CFP-10, and TB7.7) and these two epitopes mainly stimulate CD4+ T cells to secrete IFN- γ when exposed to *M. tuberculosis* antigens (Pai *et al*.,2014).

Therefore, the newest generation of the QuantiFERON-TB Gold Plus (QFT-Plus), was recently developed and launched in 2015. In contrast to the QFT-GIT assay, QFT-Plus has two TB antigen tubes: TB antigen tube 1 (TB1) and the TB antigen tube 2 (TB2). TB1 contains long peptides from ESAT-6 and CFP-10, designed to stimulate an immune response from CD4+ T cells. In contrast, TB2 contains relatively short peptides that elicit immune responses from both CD4+ and CD8+ T cells. The TB7.7 present in the QFT-GIT has been removed in the QFT-Plus assay (Kim et al.,2020).

The mycobacterial antigens used in these assays are the early secretory antigenic target (ESAT-6) and the 10-kDa culture filtrate protein (CFP-10). ESAT-6 and CFP-10 antigens are encoded in the region of differentiation 1 (RD1) present in the *M. tuberculosis* genome and are absent in the Bacillus Calmette–Guerin vaccine (BCG) and most environmental mycobacteria .Therefore, IGRA results are affected by neither BCG vaccination nor exposure to environmental mycobacteria (Arend *et al*.,2000).

QuantiFERON-TB Gold Plus (QFT-Plus) has a higher specificity and the second positive feature of the test is the standardization and objectivity of the procedure, without the subjective bias due to the injection technique and the reading of the skin reaction. Furthermore, the test is performed with the negative and positive control, thus decreasing the risk of error. The test can be repeated without the risk of boosting the result. Nevertheless, apart from cost and technical requirements, the performance of the test requires attention to several conditions, among which are the transport conditions of the blood samples to the laboratory, the delay in processing, and the availability of a specialized laboratory (Pai *et al*.,2014).

The technical variations that may affect IGRA results include those related to blood sampling (time, volume), tube shaking, incubation or processing delay (cell viability in the blood may be affected), incubation duration, analytical errors, and manufacturing defects (Pai and Behr,2016).T-SPOT.TB, another commercially available assay, uses the M. tuberculosis antigens ESAT-6 and CFP-10. This assay is based on the ELISPOT technique, which quantifies the number of IFN-γ-producing T cells (spot-forming cells). It requires expensive readers and software and specialized trained personnel, which restricts its clinical application in developing countries (Sztajnbok *et al*.,2014).

1.2.9 Genomics and host biomarkers discovery

As a result of current challenges in diagnosing tuberculosis in particular, to differentiate between active tuberculosis (active TB) and latent tuberculosis infection. Therefore, the usage of host biomarkers responsive to pathological events or host immune responses to active tuberculosis , extrapulmonary disease, and latent tuberculosis is deemed a better choice. Omics methods measure and analyze a class of biological components, including DNA, RNA, protein, and metabolites (Hasin *et al*., 2017; Goff *et al*., 2020).The advancement of "omics" technologies such as genomics , transcriptomics , proteomics , metabolomics and deep sequencing technologies have enabled us to understand *Mycobacterium tuberculosis* complex and tuberculosis infection from multi-dimensional views and in an unbiased manner (Ahamad *et al*.,2022).

The drastic spike in host omics data accompanied by the discovery of unique signatures of gene variations in MTC, in particular, *M. tuberculosis* infection, has provided a valuable window into understanding the impact of genetic variation in diagnosing tuberculosis(Kanabalan *et al* .,2021). For example, Chang *et al*., (2018) analyzed the genetic variants of the gene encoding IFN-induced protein-SP110 in a large cohort of patients in Taiwan consisting of 278 healthy controls, 301 active tuberculosis and 68 latent tuberculosis infection. They revealed that several SNPs in the SP110 gene are significantly linked with susceptibility to LTB1. This suggests that genetic polymorphisms in SP110 may serve as genetic markers for human susceptibility to active tuberculosis and latent tuberculosis infection.

Challenges and shortcomings of currently available diagnostic tools in tuberculosis diagnosis, in particular, to differentiate between active tuberculosis (active TB) and latent tuberculosis infection (LTBI) and the potentials and considerations of adopting different "omics" approaches as alternatives in the quest for promising host biomarkers to improve tuberculosis diagnosis and to discern between active TB and LTBI are shown in figure (1.3).

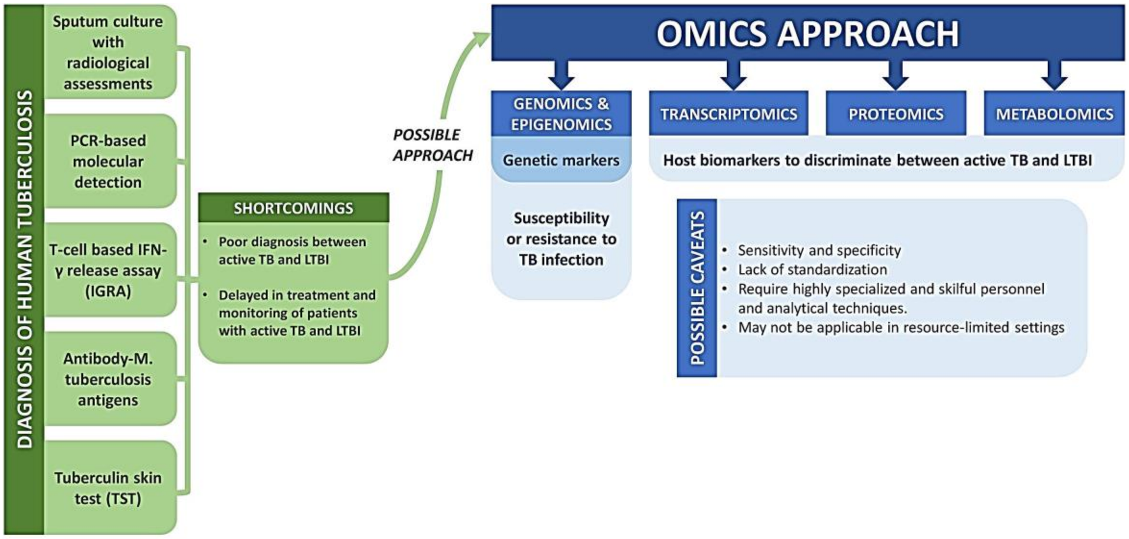


Figure (1.3): Challenges and shortcomings of currently available diagnostic tools in tuberculosis diagnosis (Kanabalan *et al* .,2021).

1.2.10 Host innate immune mechanisms against Mtb

The host's innate immune response to Mtb has recently attracted increasing attention. An initial trigger to prompt researchers to consider the role of innate immunity in TB was the conspicuous delay of adaptive immunity during Mtb infection. There is a considerable interval of 2–3 weeks before host T cell priming after being infected with Mtb. (Jasenosky *et al*.,2015) , a time span that could be beneficial for mycobacterial colonization. However, still > 90% of infected individuals can successfully control the infection (WHO,2018), hinting at essential roles for host innate mechanisms against Mtb before activation of adaptive immunity Hence, it could be valuable to revisit host innate immunity for clues to develop novel TB therapies (Chai *et al*.,2020).

The major innate immune cell types involved in Mtb infection include macrophages, DCs, neutrophils and NK cells . Engagement of *M. tuberculosis* PAMPs (carbohydrates, lipoproteins and glycolipids) with the PRRs in macrophages induces several cellular mechanisms encompassing oxidative burst, phagocytosis, apoptosis and autophagy that could contribute to the effective control and removal of *M. tuberculosis* and/or intensify the antigen-presenting and subsequent induction of adaptive immunity (Liu *et al*., 2017).

M. tuberculosis is capable to reprogrammed host macrophages to prevent its own elimination after primary tuberculosis infection (Miggiano *et al*., 2020). Once activated by cytokines, notably Interferon-γ (IFN-γ), mononuclear phagocytes increase their antibacterial capacities and pose a more serious threat to *Mtb* although they generally fail to eradicate it completely. The innate immune response mediated by professional phagocytes serves as a first barrier for *Mtb*. Recent evidence suggests that epigenetic changes induced by *Mtb* in professional phagocytes leads to trained immunity. Such trained immunity could play a role in early defense against repeated *Mtb* infections (Aaby and Benn., 2019; Netea *et al*.,2019). However, thus far compelling evidence for this notion is still incomplete.

Natural killer (NK) cells are innate lymphocytes with the capacity to secrete IFN-γ and perform cytolytic functions in order to mediate control of a variety of pathogens, including *M. tuberculosis*. Various components of the *M. tuberculosis* cell wall can bind directly to NKp44 found on NK cells (Esin *et al*.,2013) and NK cells can also recognize stress molecules upregulated on the surface of *M. tuberculosis* infected cells NK cells can mediate direct killing of *M. tuberculosis* infected macrophages, but can also restrict intracellular bacterial replication via secretion of IL-22 and IFN-γ to increase phagolysosomal fusion of *M. tuberculosis*-containing phagosomes (Sia and Rengarajan, 2019)

Innate lymphoid cells (ILCs) are subsets of lymphocytes enriched at mucosal surfaces, such as the intestine and lungs, and act early during Mtb infection. Different from T and B cells, ILCs lack rearranged antigen-specifc receptors and are thus not MHC restricted, but they mirror the functions of adaptive lymphocytes . According to their different transcription factors and effector functions, ILCs can be categorized into: ILC1s, ILC2s, ILC3s, and lymphoid tissue-inducer (LTi) cells. ILC1s, ILC2s, and ILC3s parallel the corresponding T-helper subsets including Th1, Th2, and Th17, respectively (Vacca *et al*.,2019). Cytokines produced by iLC-1 are of TH1 type, iLC-2 cytokines are of TH2 and iLC-3 cytokines are of TH17 type. The iLC-1 and iLC-3 probably contribute to resistance to *Mtb* and the iLC-2 to healing of lesions (Ardain *et al*.,2019).

1.2.11 Host adaptive immune mechanisms against Mtb

CD4 T cells have been proven central to acquired resistance against and containment of *Mtb* . The major protective immune response against *Mycobacterium tuberculosis* is T cell–mediated recruitment and activation of phagocytes (cell-mediated immunity) (Ottenhoff and Kaufmann,2012).

T cells provide defense against infections by two types of reactions: CD4+ T cells respond to class II MHC–associated peptide antigens derived from the intravesicular bacteria and CD4+ T cells activate phagocytes through the actions of CD40 ligand and IFN-γ, resulting in killing of microbes that are ingested by and survive within the phagosomes of phagocytes, and CD8+ T cells respond to class I–associated peptides derived from cytosolic antigens and CD8+cytotoxic T lymphocytes (CTLs) kill infected cells, eliminating microbes that escape the killing mechanisms of phagocytes. CD4+ T cells differentiate into Th1 effectors under the influence of IL-12, which is produced by macrophages and dendritic cells. The T cells express CD40 ligand and secrete IFN-γ and IL-2, and these three stimuli activate macrophages to produce several microbicidal substances, including nitric oxide, lysosomal enzymes, and reactive oxygen species (Abbas *et al*.,2016) .

Th1 cells are preferentially stimulated during *Mtb* infection and are of major importance for defense. They produce cytokines such as IFN-γ, interleukin-2 (IL-2) and tumor necrosis-α (TNF-α). Th2 cells are only weakly induced. They are often considered harmful in TB since they induce inappropriate effector mechanisms (O'Garra *et al*., 2013). Their major cytokines are IL-4, IL-5, IL-10, and IL-13. However, evidence has been provided that TH2 cytokines, at least in part, can contribute to tissue healing. Th17 cells induce rapid proinflammatory responses by secreting IL-17, IL-22 and IL-6. They are stimulated during *Mtb* infection and evidence has been published that they participate in protection against TB, notably at early stages of infection. The role of CD8 T cells in protection and containment – although less profound – is also widely accepted. CD8 T cells often produce cytokines of TH1 type and in addition express cytolytic activity (Ottenhoff and Kaufmann,2012).

Progression to active disease is due to the weakening of the immune response via several incompletely understood mechanisms. It is likely that myeloid-derived suppressor cells and regulatory T lymphocytes participate in the dampening of protective immunity. These cells produce inhibitory cytokines, including IL-4, IL-10, and transforming growth factor-β (TGF-β) (Barber *et al*., 2019).

On the other hand, diverse immune cells further aggregate to Mtb-infected sites and subsequently lead to the formation of granulomas to restrain the spread of the bacteria. These immune cells mainly include macrophages, DCs, neutrophils, natural killer (NK) cells, T cells, and B cells (Chai *et al*.,2020), as shown in figure (1.4).

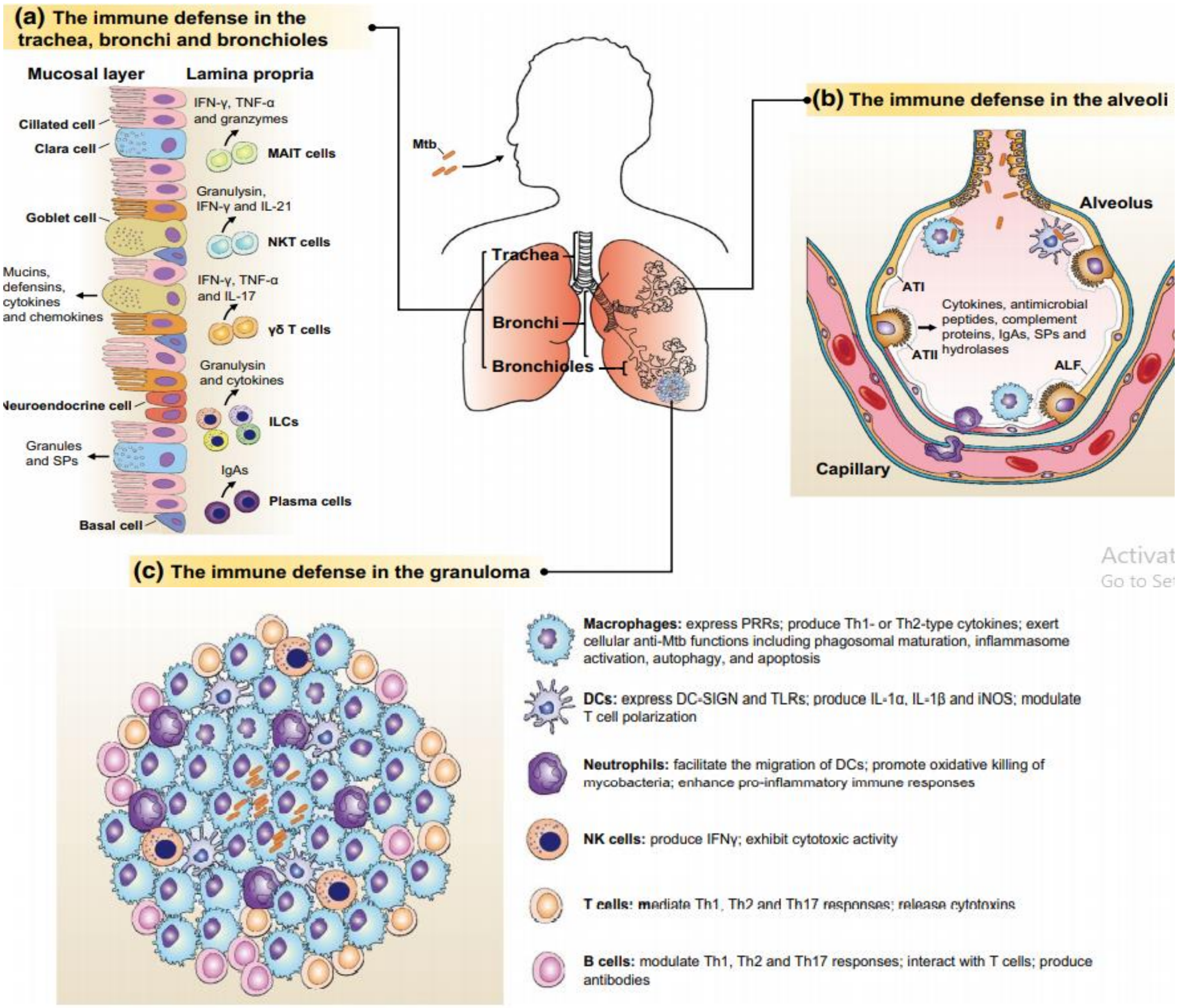


Figure (1.4): Integrated host defense system against Mtb (Chai *et al*.,2020).

1.2.12 Some studied cytokines and their roles in MTB infection

Cytokines can serve as a double-edged weapon, with the levels and timing of cytokine production acting as determinants of protection versus pathology (Dorhoi and Kaufmann,2016). After infection, the interplay of proinflammatory and anti-inflammatory signals is vital in establishing the granuloma and influencing its eventual trajectory. Skewing towards a robust proinflammatory cytokine milieu can promote bacterial dissemination, remodelling of the granuloma, and destruction of lung parenchyma. These processes typically underlie active TB and aid transmission (Cadena *et al*.,2017).

1.2.12.1 IL-17 and Th17 cells

The cellular immune response has a key role in tuberculosis infection. Th17 cells play a key role in immunity against *Mycobacterium tuberculosis* (Beringer et al., 2016). Th17 cells represent the third T helper lineage in addition to the well-known Th1 and Th2 cells. Th17 cells are the main IL-17-producing cells, and Th17 cells differentiate with the induction of IL-6, transforming growth factor-β (TGF-β) and IL-1β and are expanded by IL-23 via the STAT3 signaling pathway (Patel and Kuchroo, 2015). Although first characterized in autoimmune encephalitis, Th17 signals play a pivotal role in host defense against pulmonary diseases (Wang *et al* .,2020).

Moreover, The IL-17 cytokine family comprises six members, IL-17A-F, with IL-17A and IL-17F being the most studied. Production of IL-17 has conventionally attributed to T cells; however, other lymphocytes and innate immune cells can produce this cytokine . Among six members, IL-17A and IL-17F share a similar structure and have similar roles in the immune response against Mtb infection (Tateosian *et al*.,2017). The roles of other members of the IL-17 family are currently not known in Mtb infection. The immune protective role of IL-17F in Mtb infection is similar to IL-17A, which is 50% homologous to IL-17F in structure. The sequence variant of IL-17F is also correlated with susceptibility to TB (Shen and Chen.,2018).

The roles of Th17 cells are complicated in TB because Th17 cells are dysregulated in TB patients. Th17 cells might have a protective role in TB and are negatively regulated by Mtb virulent factor TlyA (Ahmed *et al*.,2017) .

Furthermore, IL-17A enhances autophagy in Mtb-infected monocytes and is essential for IFN-γ and TNF production in protective immunity against Mtb infection (Umemura *et al*.,2016). Early granuloma formation may depend on IL-17A and direct evidence of Th17 protection comes from the observation that IL17 deficient mice demonstrate compromised ability to control hypervirulent strain of HN878 (Gopal *et al*.,2014). Induction of chemokines including mainly CXCL8 and CXCL9, CXCL10 as well as CXCL11 by IL-17 leads to the recruitment of IFN-γ-producing T cells to the infection site and inhibits bacterial growth following Mtb infection (Lombard *et al*.,2016).

The majority of IL-17 was produced by Th17 cells (median 71%), followed by γδ T cells (6.4%) and MAIT cells (5.8%). TB patients had a significantly lower proportion of Th17 cells and CD4+CD161+Vα7.2+ cells producing both IL-17 and IFN-γ compared to LTBI subjects (Coulter *et al*.,2017).

Despite the protective outcome of IL-17 mentioned above, Th17 responses in TB are presumed to participate in pathology according to their activities, such as neutrophil recruitment and promoting inflammatory responses in infection sites (Lyadova and Panteleev, 2015), which will cause serious tissue damage in redundancy neutrophils and high degrees of inflammation. Recently, it was reported that there are more IL-17-producing T cells and IL-2- and IL-10-producing T cells in the lung granulomas of latent Mtb-infected macaques with a high risk of reactivation than those in low-risk animals identified by positron emission tomography CT (Lin *et al*.,2016). Therefore, high lung inflammation is associated with TB reactivation from LTBI. Indeed, exacerbated production of IL-17 appears to drive pathology by inducing S100A8/A9 proteins that recruit neutrophils into the lung. Thus, IL-17 also fits the bill as a 'goldilocks' cytokine in TB (Gopal *et al*.,2013).

1.2.12.2 Interleukin 18

IL-18 is a member of the IL-1 family of cytokines and a proinflammatory cytokine that facilitates type 1 responses. It regulates both T helper (Th) 1 and Th2 responses. It acts synergistically with IL-12 in the Th1 paradigm, whereas IL-18 without IL-12 but with IL-2, stimulates NK cells, CD4+ NKT cells .Therefore, IL-18 is a cytokine that stimulates various cell types and has pleiotropic functions. All this evidence strongly supports IL-18 as a unique cytokine able to stimulate T cells and other immune cells (Yasuda *et al.*,2019). Upon stimulation with Ag plus IL-12, naïve T cells develop into IL-18 receptor (IL-18R) expressing Th1 cells, which increase IFN-γ production in response to IL-18 stimulation (Vecchié *et al*.,2021).

IL-18 is synthesized as an inactive precursor missing a signal peptide, and the IL-18 precursor is processed intracellularly into its mature biologically form by caspase-1, the latter being activated by various canonical inflammasomes (Dinarello, 2019). The IL-18 levels are also regulated by soluble IL-18 binding protein (IL-18BP), which is a natural inhibitor of IL-18. Under physiological conditions, the concentration of plasma IL-18BP is ~20 times higher than that of IL-18, which prevents IL-18 from binding to its cellular receptor (Novick *et al*.,2013).

Interleukin -18 plays an essential role in Mtb infection. It induces NK cell cytotoxic activity and promotes the development of Th1 cell response. This mechanism is associated with the production of interferon (IFN)-γ, which is a key element in anti-mycobacterial protection. IL-18 was first described in 1989 as an "IFN-γ inducing factor" (Wawrocki *et al*.,2016).

Although IL-18 functions as an important activator of the protective immune response against intracellular pathogens, it is also implicated in pathological processes leading to tissue damage (Wawrocki and Druszczynska, 2017). The role of IL-18 in host defense against *M. tuberculosis* infection has been demonstrated in studies using IL-18 knockout and IL-18 transgenic mice. IL-18-deficient mice are susceptible to *M. tuberculosis* infection, and IFN-γ levels in the serum, spleen, lung, and liver were lower than in wild-type mice (Schneider *et al*.,2010).

1.2.12.3 Interferon gamma (IFN- γ)

IFN-γ, which forms the basis of the commercial IGRAs, is an important proinflammatory cytokine in the immune response to TB infection (Walzl *et al*.,2011). Interferon-gamma (IFN-γ), which belongs to IFN type II is exclusively produced by CD4+ T helper cell type 1 (Th1) lymphocytes, CD8+ cytotoxic lymphocytes, and natural killer cells. Following IFN-γ secretion, it binds to its cell surface receptor, which results in the dimerization of nonligand-binding receptors subunits IFNR1 and IFNR2 (Xu *et al*.,2018). Interferon-gamma (IFN-γ) and its receptor (IFN-γR) play a key role in mediating immunity against Mtb by activating macrophages , granuloma formation , phagosome-lysosome fusion and promotion of antigen recognition by enhancing major histocompatibility complex class II (MHC-II) expression on antigen-presenting cells (Ghanavi *et al*.,2021). Production of IFNγ is regulated by cytokines such as IL-12 and IL-18, which are secreted by immune surveillance cells upon ligating their pattern recognition receptors (Hume *et al*.,2004).

Production of IFNγ is a very useful diagnostic tool that is more selective than the older skin test assay. In this prominent test for Mtb exposure, Mtb antigens (selected to be unique for Mtb versus other mycobacteria) are used to stimulate IFNγ release . Recently, studies have shown that patients that have more IFNγ producing T cells are actually more likely to progress to active disease, suggesting that this test may be optimized to identify those progressing toward disease (Masood *et al*.,2020; Jung *et al*.,2021).

1.2.13 Genetic polymorphism

Genetic polymorphism is a difference in DNA sequence among individuals, groups, or populations. Polymorphism can affect individual phenotypes such as the colour of skin or eyes, susceptibility to diseases, and response to drugs, vaccines, chemicals, and pathogens. Polymorphisms arise through mutations. It occurs more often than mutations. The mutation may be due to a change from one type of nucleotide to another, an insertion or deletion, or a rearrangement of nucleotides (Teama, 2018).

1.2.13.1 Single nucleotide polymorphism (SNPs) as a Genetic marker

Single nucleotide polymorphisms (SNPs) are mostly biallelic point mutations present within a population in a frequency higher than 1%. SNPs are also believed to be the main source of variability among humans, especially when they influence gene expression or function depending on their location in the DNA sequence. Moreover, since SNPs are relatively easy to be detected and due to their high abundance in the genome , they are considered as one of the best biological markers in association or case-control studies (Pacheco and Moraes,2009; Jin *et al*.,2016)

However, Single nucleotide polymorphisms (SNPs) give rise to different alleles containing alternative bases at a given position of nucleotide within a locus. Genome-wide association studies (GWAS) search for SNPs that occur more frequently in a person with a particular disease than in a person without the disease and pinpoint genes or regions that may contribute to a risk of disease (Sukhumsirichart,2018).

SNPs are mainly formed when errors occur (substitution, insertion and deletion). SNPs may occur within gene sequences or in intergenic sequences. SNPs are mostly located in non-coding regions of the genome, and depending on where SNPs occur, it might have different consequences at the phenotypic level. Once formed, a polymorphism can be inherited like any other DNA sequence, allowing its inheritance to be tracked from parent to child (Ismail and Essawi, 2012). The majority of SNPs have two alleles, which represent a substitution of one base for another. The SNP that occurs at each allele of an individual may be different. If the SNP occurs more frequently in the general population, it is called the "major" allele. In contrast, if the frequency of the SNP exists is rare in the population, it is designated the "minor" allele. Since humans have two copies of chromosome or diploid, therefore, an individual can have various genotypes such as homozygous of major or minor alleles or heterozygous of the major and minor allele (Crawford and Nickerson,2005).

1.2.13 .2 Host genetic susceptibility and human tuberculosis

Host genetic variations could impose a significant impact on the pathogenesis of human tuberculosis. Miscellaneous genetic works through different methodological approaches, such as genome-wide association studies (GWAS), family studies, case-control and candidate gene studies highlighted the role of host genetics in shaping the disease risk (susceptibility/resistance) to human TB infection in different ethnics and populations. The interplay between variations in host genetic and TB infection has been discussed extensively (Harishankar *et al.*, 2018; Cai *et al.*, 2019).

Gene polymorphisms that involved in the recognition of *M. tuberculosis* antigens by PRRs, immune cells as well as host immune-related genes constitute the major genetic determinants to TB susceptibility and protection. Polymorphisms in genes encoding killer immunoglobulin-like receptor (KIR), Toll-like receptors (TLRs), vitamin D receptor (VDR), human leukocyte antigens (HLAs), chemokines/cytokines and associated receptors, solute carrier family 11 member 1 (SCL11A1), mannan-binding lectin (MBL), speckled 110 (SP110), nitric oxide synthase 2A (NOS2A), P2 7 receptor (P2 x7) and many other functional immune components have been reported to be genetically associated to TB infection (Van Tong *et al*., 2017).

1.2.13 .3 Genetic variation of IL-17A and IL-18 cytokines in TB

Polymorphisms in cytokine genes are known to influence cytokine levels, which may influence susceptibility to tuberculosis (TB) infection and disease. Differences in cytokine expression probably determine whether TB progresses, resolves, or becomes latent (Wu *et al.*,2019). Studies of concordance of disease in twins have shown that host genetic factors play significant roles in TB disease. Determining the specific host genes associated with TB disease may enhance the understanding of the pathogenesis of TB and further the development of treatment strategies (Naranbhai,2016).

Many studies have suggested that cytokine gene polymorphisms are associated with TB among different populations. For example, IL-18 and IL-17A polymorphisms were reported to be associated with TB risk (Mansouri *et al*.,2018; Zhen *et al*.,2019). Indeed, genetic variants in cytokine genes could affect the recognition sites of the transcription factors, leading to altered transcriptional activity, which may result in a change in cytokine production levels. The intricate interplay among lymphocytes, antigen-presenting cells and released cytokines is involved in the immune response against TB (YIM and Selvaraj, 2010).

Several association studies have been described in Th17- related cytokine genes, such as IL-17A, which are involved in the susceptibility, severity and clinical outcome of TB in humans. The human IL-17A gene is located on chromosome 6p12 and is composed of 3 exons and two introns. It is a disulphidelinked homodimeric glycoprotein consisting of 155 amino acids as a homodimer with a molecular weight of around 35 kDa (Ponce-Gallegos *et al*.,2020; Shao *et al*.,2021). Many SNPs in IL17A cytokine loci have been described and studied in tuberculosis, in particular, the rs2275913 SNP, produced by a substitution of the G by an A nucleotide base in the IL-17A gene promoter, is significantly associated with a vast number of diseases (Ocejo-Vinyals *et al*.,2013).

Numerous studies have shown that IL18 gene polymorphisms are related to many inflammatory and autoimmune diseases, such as chronic hepatitis B, asthma, coronary heart disease, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease and TB (Li *et al*.,2012; Farid *et al*.,2018). The IL-18 gene is located on the long arm of chromosome 11 (11q22.2-q22.3) and has six exons, and incorporates many genetic polymorphisms, particularly in the promotor region. The changes in IL-18 gene promotor have an influence on the production and activity of IL-18. Two of the most common single nucleotide polymorphisms in IL-18 gene are located in the promoter region: -607C/A (rs1946518) and -137G/. C (rs187238) (Zhen *et al.*,2019).

These gene variants could also serve as robust genetic predictive markers in different populations and ethnicities where variations in certain genes might be predominantly expressed in specific population and ethnic. Nevertheless, a larger and homogenous cohort of study is required to validate the efficacy and the predictive power of the genetic marker identified as well as to avoid genetic heterogeneity in a particular population. More biological studies on genetic variation in humans with different populations are also needed to assess the functional implications or consequences of genetic variations in human in tuberculosis context (Kanabalan *et al*.,2021).This can serve as a basis for translational study, which converts the genetic findings into clinical applications in future for disease control in tuberculosis. On the other hand, several epidemiological models and case studies demonstrated that about 5%–20% of subjects who have close and prolonged contact with individuals with active tuberculosis or subjects who are living in small spaces with open tuberculosis did not contract the infection or became infected transiently with early clearance of infection. This evidence strongly suggests the existence of host genetics in determining the resilience to *M. tuberculosis*. A better understanding of the genetic role of TB susceptibility enables us to elucidate immunopathological mechanisms of TB, which could serve as the basis of prevention and treatment of TB (Ringshausen *et al*., 2010).

1.2.13.4 SP110 gene polymorphisms

In 2005, Pan and colleagues found a new genetic locus in mice, designated super-susceptibility to TB 1 (sst1), which mediates innate immunity in mouse TB models. Mouse gene, intracellular pathogen resistance-1 (Ipr1), which is located in the sst1 region, might promote macrophage activities and enhance the ability of macrophages to fight against *M. tuberculosis* infection (Li *et al*.,2015).

In humans, the *Speckled 110 (SP110)* gene, the closest homology to the mouse *Ipr1* gene, the Speckled 110 (SP110) gene in humans holds 41% sequence homology to the mouse *Ipr1 gene* (Apt,2011). This gene is located on the chromosome at 2q37.1 and encodes the SP110 nuclear body protein, which is a component of cellular structures called nuclear bodies (Wu *et al*.,2015). By participating in signal transmission processes between nuclear hormone receptors, SP110 protein could regulate the biological activity of macrophages and influence the growth and proliferation of *M. tuberculosis*, which might be associated with the susceptibility to TB (Cai *et al*.,2013). The expression of both Ipr1 and SP110 genes is intensively regulated by IFNs, suggesting that the function of both genes is related to the IFN-mediated immune response. SP110 is expressed at high levels in human peripheral blood leukocytes and the spleen and lower levels in many other tissues such as the lung (Bloch *et al*.,2000).

The *SP110 gene* encodes the SP110 nuclear body protein, which has at least three isoforms, including the dominantly expressed SP110a, b, and c isoforms that are believed to be the result of alternative mRNA splicing . SP110b, which is most similar to mouse Ipr1 and is expressed more abundantly than SP110a and SP110c (Chang *et al* ., 2018). A study by Leu *et al*., (2017) provides evidence demonstrating that SP110b, suppresses IFN-γ–induced monocyte and/or macrophage cell death by modulating nuclear factor-kB (NF-kB) activity. The suppression was dependent at least in part on downregulating TNF-α production and upregulating antiapoptotic gene expression induced by NF-kB. Therefore, SP110b plays a crucial role in regulating host immunity by functioning as a "fine-tuner" of NF-kB activity to prevent over amplifying signalling of both cytokines, thereby facilitating the host's control of unwanted immunopathology, as shown in

Figure (1.5). This indicates that the protein is crucial in the control of the activation of macrophages, the reservoir for Mtb persistence .

In humans, SP110 variants have been implicated in several diseases including hepatic veno-occlusive disease with immunodeficiency (VODI) (Cliffe *et al*.,2012), viral hepatitis infection (Saito *et al*.,2004) and TB (Png *et al*.,2012), indicating that the gene plays important role in immunity. Although several genetic variants of the SP110 gene have been reported to be associated with susceptibility to human TB, the results of studies regarding the relationship between SP110 polymorphisms and TB susceptibility are inconsistent (Lei *et al*.,2012).

Transcriptome analysis revealed that Sp110 regulates genes involved in immune responses, apoptosis, defence responses, and inflammatory responses. Detailed investigation revealed that Sp110 regulates cytokines, chemokines and genes that regulate intracellular survival of *Mtb*. Moreover, Sp110 regulates miRNA expression in macrophages, with immune and apoptosis-related miRNAs under Sp110 regulation (Wu *et al*., 2016).

Although the association of the SP110 gene with TB is also racially specific and needs further confirmation, given its impact on host TB immunity, many studies have suggested that SP110b can serve as a potential target for host-directed therapy (Leu *et al*.,2017).

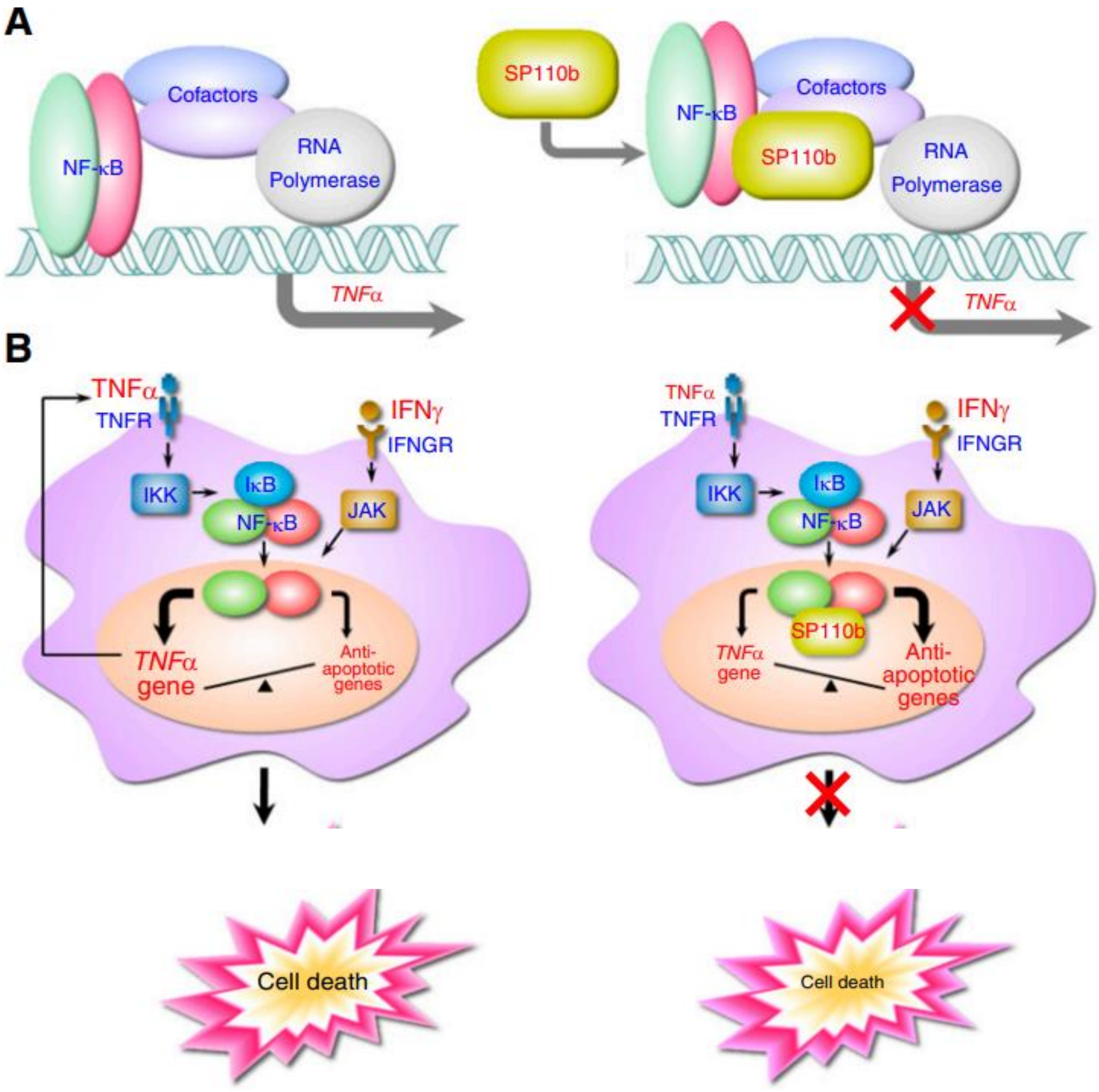


Figure (1.5): A model of SP110b function (Leu *et al*., 2017) . (A) A model of the SP110b-mediated downregulation of tumour necrosis factor-a (TNF-α) production. (B) A model of the SP110b-mediated mechanisms that are associated with cell death suppression. IFNGR = IFN-γ receptor; IkB = inhibitor of kB; IKK = inhibitor of kB kinase; JAK = Janus kinase; TNFR = tumor necrosis factor receptor.

Chapter Two

Materials and Methods

2.1 Materials

2.1.1 Laboratory Apparatus and Equipments

The Laboratory equipment that used in the present study are mentioned in table (2.1).

Table (2.1) Laboratory Apparatus and Equipments

Apparatus and Equipments	Company	Country
Autoclave	ALP	Japan
Centrifuge	Beckman	Germany
GeneXpert MTB/RIF System	Cepheid	USA
Refrigerator	Concord	Korea
Biological safety cabinet	Esco class II	Indonesia
UV-Transilluminator	Fisher Scientific	USA
Water bath	Gallenkamp	England
Water distillatory	GLF	Germany
Hot Plate	Heidoph MR Hei-standard	England
Automatic ELISA reader + Micro plate washer	Human	Germany
Vortex Genius 3	IKA	Germany
Micro Centrifuge	Labcco	Germany
PCR-Thermo cycler	Labnet	USA
Incubator + Oven	Memeret	Germany
Gel Electrophoresis	Mupid-one	Japan
Bunsen burner	Nahita	Japan
Light Microscope	Olympus	Japan
Sensitive electrical balance	Sartorius	Germany
Nanodrop spectrophotometer	Thermo Fisher Scientific	USA
Cold centrifuge	Universal Hitachi	Germany

2.1.2 Laboratory Instruments

The investigation necessitated using various disposable glassware, plastic and other materials as indicated in table (2.2).

Table (2.2): Laboratory Instruments

Instruments	Company	Country
Serum gel tube (6 ml)	AFCO	Jordan
EDTA tube (2 ml)		
Sterile Plain tube (10 ml)		
Graduated glass cylinder	Behring	Germany
Plastic tips	CAPP	Denmark
Wooden sticks	Deansgate	China
Micropipettes	Dragon	China
Medical Cotton	EMPO	Turkey
Universal Bottle, 28 ml	Favorit	Malaysia
Falcon tubes 50 ml	Greiner Bio-One	Germany
Micro-centrifuge tube	Hettich	Germany
Disposable syringe (10 ml)	JJMD	China
Disposable syringe (5 ml)	Million care	China
Sputum cups	Sivizera	Europe
Microscopic slides	Y. H. E	China
Tourniquet for blood collection	Z. H. M. D	China

2.1.3 Chemical and Biological Materials

The chemicals and biological materials used in the present study are listed in tables (2.3) and (2.4).

Table (2.3): The chemicals and biological materials ready to use in *Mtb* Diagnosis

Material	Company	Country
5% Phenol	Alfa Aesar	Germany
70% isopropyl alcohol	BDH	England
Immersion oil	G C C Diagnostics	UK
Ziehl-Neelsen (ZN) staining material		
3% HCL	AppliChem	Germany
Methylene Blue	Chem-Lab NV	Belgium
95% ethanol	Park	UK
0.3% Basic Fuchsin	Reagent world	USA
25% aqueous sulfuric solution	Thomas baker	India

Culture material		
4% NaOH	Alpha chemika	India
Glycerol	Cooper	USA
Lowenstein Jensen base consists of the following ingredients: Asparagine ; Monopotassium Phosphate ; Magnesium Sulfate , Magnesium Citrate ; Potato Flour ; Malachite Green	Becton Dickenson	USA
pH buffer	Reagent world	USA

Table (2.4): The chemicals materials used in PCR and Electrophoresis

Material	Company	Country
DNA Ladder Marker 100bp	BDH	England
Ethidium Bromide (5µg/ml)		
Agarose	Bio Basic	USA
Primers	Macrogen	Korea
GoTaq® G2 Green Master Mix*	Promega	USA
TBE buffer (10x)		
Loading dye **		
Nuclease-Free Water		

***GoTaq® G2 Green Master Mix** :It is a premixed ready-to-use solution. chemical components of Master Mix as shown in table (2.5) .

****loading dye:** It is provided in a premixed, ready-to-use form. chemical component of loading dye as shown in table (2.5).

Table (2.5):Chemical components of Master Mix and loading dye

NO.	Material
Components of GoTaq® G2 Green Master Mix:	
1.	DNA Polymerase
2.	Reaction Buffer (pH 8.5)
3.	400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP
4.	3mM MgCl2
Component of loading dye:	
1.	0.4% orange G
2.	0.03% bromophenol blue
3.	0.03% xylene cyanol FF
4.	15% Ficoll
5.	10mM Tris-HCl (pH 7.5)
6.	50mM EDTA (pH 8.0).

2.1.4 Commercial kits

The kits used in the present study are listed in table (2.6).

Table (2.6): Commercial kits

No.	Kits	Company	Country
1.	GeneXpert MTB/RIF Assay kit	Cepheid	USA
2.	Human IL-18(Interleukin 18) ELISA Kit	Elabscience	China
3.	Human IL-17A(Interleukin 17A) ELISA Kit		
4.	ReliaPrep™ Blood gDNA Miniprep system	Promega	USA
5.	QuantiFERON®-TB Gold (QFT) ELISA Kit	Qiagen	Germany

2.1.4.1 Gene Xpert MTB/RIF Assay kit

The following items included in the kit are illustrated in table (2.7).

Table (2.7): Components of Xpert MTB/RIF Assay kit

No.	items
1.	Xpert MTB/RIF Assay Cartridges with Integrated Reaction Tubes (n= 10) The cartridge contained the following materials:
A.	DNA Polymerase
B.	dNTPs (deoxynucleoside triphosphates)

C.	Primers
D.	Probes
E.	Tris Buffer
F.	EDTA (ethylenediaminetetraacetic acid)
G.	BSA (Bovine serum albumin)
H.	Sample Processing Control
I.	Surfactants
2.	Sample Reagent (8 mL per bottle): A. Sodium Hydroxide B. Isopropanol
3.	Disposable Transfer Pipettes 12

2.1.4.2 ReliaPrep™ Blood gDNA Miniprep system

Extraction of DNA from human blood required the use of ReliaPrep™ Blood gDNA Miniprep Kit, and this kit included components are listed in table (2.8)

Table (2.8): Components of Genomic DNA extraction kit

No.	Materials
1.	Two packs ReliaPrep™ Binding Columns (50/pack)
2.	Ten packs Collection Tubes (40/pack)
3.	22ml Cell Lysis Buffer (CLD)
4.	1.1ml Proteinase K (PK) Solution
5.	27.5 ml Binding Buffer (BBA)
6.	165 ml Column Wash Solution (CWD)
7.	50 ml Nuclease-Free Water

2.1.4.3 QuantiFERON -TB Gold (QFT- Plus) ELISA kit

The QFT-Plus ELISA kit includes the components listed in table (2.9).

Table (2.9): Components of QuantiFERON -TB Gold (QFT- Plus) ELISA kit

<table>
<tr><td colspan="3">1 . Blood Collection Tubes: There are four types of tubes</td></tr>
<tr><td>A.QuantiFERON Nil Tube (grey cap)</td><td>Nil</td><td>50 tube</td></tr>
<tr><td>B.QuantiFERON TB 1 Tube (green cap)</td><td>TB 1</td><td>50 tube</td></tr>
<tr><td>C.QuantiFERON TB 2 Tube (yellow cap)</td><td>TB2</td><td>50 tube</td></tr>
<tr><td>D.QuantiFERON Mitogen Tube (purple cap)</td><td>Mitogen</td><td>50 tube</td></tr>
<tr><td colspan="3">2. ELISA Components:</td></tr>
<tr><th colspan="2">Components</th><th>Quantity</th></tr>
<tr><td colspan="2">Microplate Strips (12 x 8 wells) coated with murine anti-human IFN-γ monoclonal antibody</td><td>2 sets of 12 x 8-well Microplate Strips</td></tr>
<tr><td colspan="2">Human IFN-γ Standard, lyophilized (contains recombinant human IFN-γ, bovine casein, Thimerosal)</td><td>1 x vial (8 IU/ml when reconstituted)</td></tr>
<tr><td colspan="2">Green Diluent (contains bovine casein, normal mouse serum, Thimerosal)</td><td>1 x 30 ml</td></tr>
<tr><td colspan="2">Conjugate 100X Concentrate, lyophilized (murine anti-human IFN-γ HRP, contains Thimerosal</td><td>1 x 0.3 ml (when reconstituted)</td></tr>
<tr><td colspan="2">Wash Buffer 20X Concentrate</td><td>1 x 100 ml</td></tr>
<tr><td colspan="2">Enzyme Substrate Solution (contains H_2O_2, Tetramethylbenzidine</td><td>1 x 30 ml</td></tr>
<tr><td colspan="2">Enzyme Stopping Solution (contains H_2SO_4)</td><td>1 x 15 ml</td></tr>
</table>

2.1.4.4 Human IL-17A and IL-18 ELISA Kit

IL-17A and IL-18 ELISA kits included the components listed in table (2.10).

Table (2.10): Components of Human IL-17A and IL-18 ELISA Kit

No.	Items	Quantity
1.	Micro ELISA Plate (Dismountable)	8 wells ×12 strips
2.	Reference Standard	2 vials
3.	Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 µL
4.	Concentrated HRP Conjugate (100×)	1 vial, 120 µL
5.	Reference Standard & Sample Diluent	1 vial, 20 mL
6.	Biotinylated Detection Ab Diluent	1 vial, 14 mL
7.	HRP Conjugate Diluent	1 vial, 14 Ml
8.	Concentrated Wash Buffer (25×)	1 vial, 30 mL
9.	Substrate Reagent	1 vial, 10 mL
10.	Stop Solution	1 vial, 10 mL

2.1.5 Primers of PCR used in DNA amplification

In the present study, four primers were designed to detect the SNPs of three genes under interest by sanger sequencing : the first primer pair for an amplified targeted region of gene IL17A included SNPs: rs2275913; the second primer pair targeted region of gene IL18 included: rs1946518. The third and fourth primer pair targeted two regions of the Sp110 gene, including SNPs: rs7580900, rs7580912 and rs9061, rs11556887, respectively, the primers detail information as shown in table (2.11). The primers were supplied by Macrogen Company , Korea.

All the primers that were used in this study designed with the aid of NCBI-primer BLAST online software, and then the primers pair was selected according to the criteria such as product length, the similarity of melting temperature, primers length, G/C content, etc. (Chuang *et al.*, 2013).

Other primers for detection of rs1946518 in the IL-18 by Allele specific-Polymerase chain reaction (AS-PCR) analysis were obtained from (Giedraitis *et al.*,(2001) and detection of rs2275913 in the IL-17A by AS-PCR was obtained from. The AS-PCR is based on allele-specific amplification of desired fragment using primers corresponding to each allelic variant. The primers detail information as shown in table (2.12).

Table (2.11): Sequences of primers used for Sanger sequencing method.

Gene	SNPs	Primer sequence 5′ to 3′	Amplicon Size (bp)	References
Sp110	rs9061; rs11556887	F-ACGCATGTTCTCCCTTCACA R-CCCCTTCCCCAAGTAATTCTGA	804	Designed in this study by NCBI-primer BLAST online
SP110	rs7580900; rs7580912	F- TGGGATGATGCCTGTAGTGAG R- CGCCTATGCCATACACAAGC	715	
IL-18	rs1946518	F- TTCCTGGTCACACTTCAGCA R- CACTCTGCTCTTCAAACGTTAC	567	
IL17A	rs2275913	F- ATTCACCTCAGTGGGGGTAGG R- GTGGTCACTTACGTGGCGTG	886	

F= forward, R= reverse, bp = base pair

Table (2.12) : Sequences of primers which are used for AS-PCR analysis.

gene	SNPs	Primer sequence 5′ to 3′	Amplicon Size (bp)
IL18	rs1946518 C>A	F1-GTT GCA GAA AGTGTA AAA ATT ATT AC F2-GTT GCA GAA AGTGTA AAA ATT ATT AA **Common reverse** R-TAA CCT CAT TCA GGA CTT CC **internal positive control** F-CTT TGC TAT CAT TCC AGG AA	196 301
IL-17A	rs2275913 G > A	F1-CTTCCCATTTTCCTTCAGAAGG F2- CTTCCCATTTTCCTTCAGAAGA **Common reverse** R- ATGCCCACGGTCCAGAAATAC	317

F= forward, R= reverse, bp = base pair

2.2 Methods

2.2.1 Type of study: The current study was designed as case-control study.

2.2.2 Study population

The study was performed from February to September (2021) at the National Tuberculosis Institute (NTI) / National Reference Laboratory (NRL) for Tuberculosis in Baghdad. All participants were divided into three groups :

A. Active pulmonary TB (PTB) group

One hundred and forty patients with suspected pulmonary TB were referred to the National Tuberculosis Institute (NTI) in Baghdad for diagnosis. Sputum samples of patients were received for the request of ZN microscopy, culture and GeneXpert MTB/RIF assay at the National Reference Laboratory (NRL) to confirm that they were diagnosed with tuberculosis. .

B. Latent TB infection group (LTBI).

A total of 164 subjects with suspected latent TB infection were recruited in the current study, sixty subjects who were QFT-Plus + at recruitment were defined as latently infected (LTBI). The subjects who had contact with active TB patients or TB-exposure and were judged to be at higher risk of acquiring TB infection were included in the present study. The risk of TB exposure in this group was investigated via a questionnaire survey of family history of TB, personal contact with TB patients, and healthcare workers with a high risk of TB exposure. Peripheral venous blood was collected from participants and tested by the QFT-Plus test to confirm that they were diagnosed with latent tuberculosis according to the manufacturer's instructions (Qiagen /Germany).

C. Apparently Healthy control group(AHC)

A forty healthy controls were defined as apparently healthy persons with no evidence of TB exposure . Blood specimens were collected from healthy controls (HC) and tested by a QFT-Plus test. Patients and AHC were interviewed using structured questionnaire information related to the inclusion and exclusion criteria.

2.2.3 Inclusion and Exclusion criteria

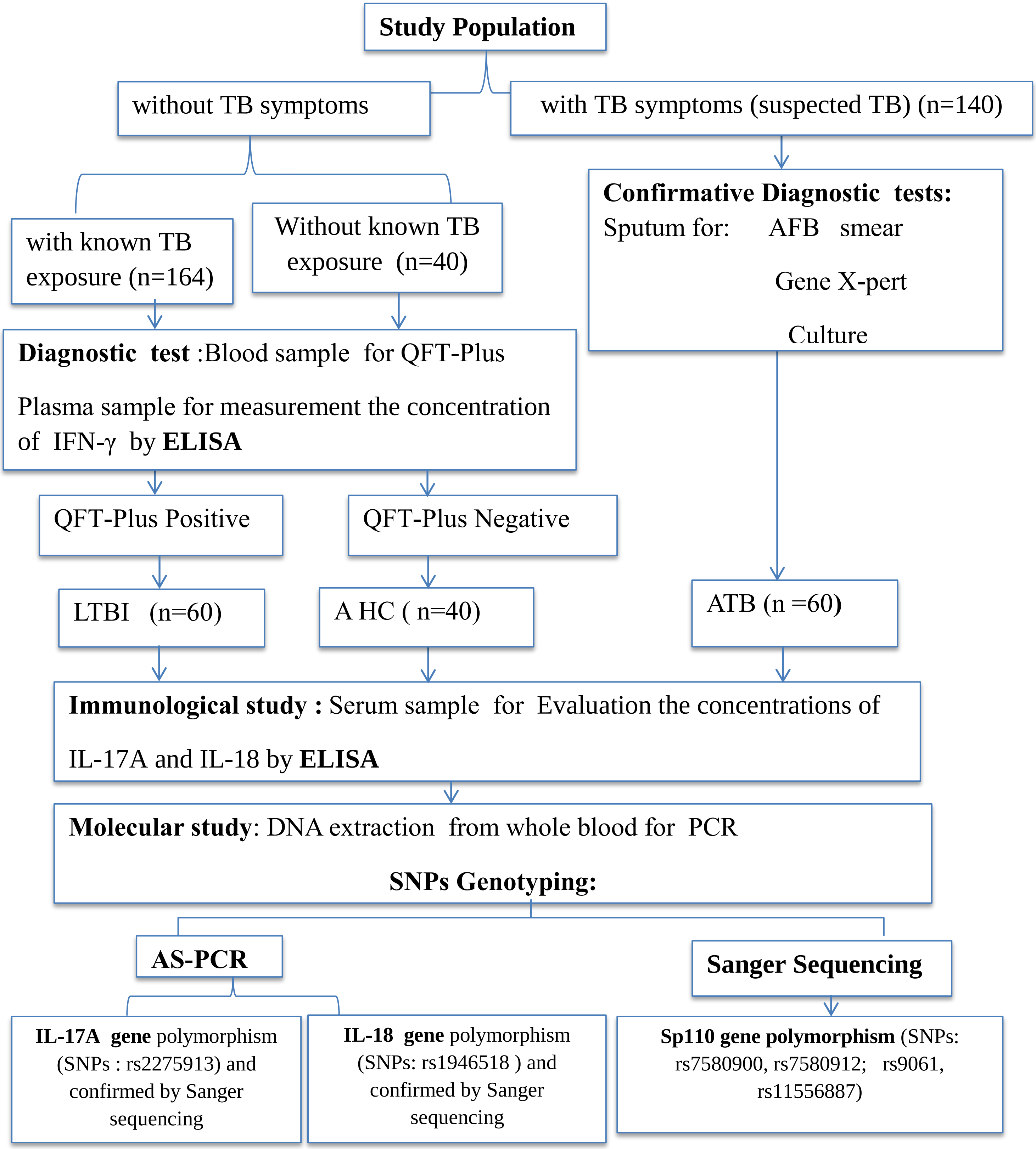
The inclusion criteria in the present study included all active pulmonary tuberculosis cases with no record of prior anti-TB treatment (ATT) and newly diagnosed patients. On the other hand, the participants who had close contact with active TB patients or TB exposure were included in the present study.

The exclusion criteria included patients with autoimmune diseases, diabetes mellitus, nephropathy, follow-up patients, extrapulmonary TB, and subjects who had close contact with TB but were QFT-Plus negative. Also, the subjects who refused to participate in this study.

2.2.4 Ethical approval

All subjects involved in this work were informed, and the agreement was obtained verbally from each one before collecting samples. This study was approved by the committee of publication ethics at the college of medicine, university of Babylon, the plagiarism committee at the College of Medicine, as well as, the ethical committee at the Public Health Directorate in Baghdad

2.2.5 Scheme of study design :



2.2.6 Collection of Specimens

2.2.6.1 Sputum Specimens Collection

The expectorated morning sputum specimens were collected from the enrolled patients. Where the patients were asked to collect the sputum specimens following the standard sputum collection procedure using the recommended sterile plastic containers. The volume of sputum was approximately 3-5 ml in each collection cup. The sputum specimens were tested by Ziehl-Neelsen staining microscopic examination, Xpert MTB/RIF assay and culture on L.J medium. A good sputum specimen should be of discharged material from the bronchial tree. Clear saliva or nasal discharge is not suitable as a TB specimen (WHO,2014).

2.2.6.2 Blood Specimens Collection

A Venipuncture was used to collect the blood. This collection method was carried out according to (WHO, 2010). Five millilitres of blood were collected for each subject with suspected latent TB infection and AHC and 1 ml of blood was placed directly into each of the QFT blood collection tubes (Nil, TB1, TB2, Mitogen) to diagnose latent tuberculosis and AHC. Additionally, Five ml of freshly venous blood were collected from each participant after diagnosis of active TB, Latent TB and AHC for the molecular and immunological study. Two ml of blood were kept in an EDTA tube treated with an anticoagulant. The tube mixed by inverting it and kept at -20°C and later were subjected to extraction of DNA (Yamagata *et al*.,2021). The remaining blood (3 ml) was put in a gel tube and centrifuged at 3000 rpm for 10 min to obtain serum. The serum samples were collected and stored at - 20 C° until analyzed by ELISA assay .

2.2.7 Bacteriological diagnosis

2.2.7.1 AFB Smear microscopy

AFB Smear microscopy was performed by the following steps (Dzodanu *et al*.,2019):

1. After smear preparation, the smears were arranged in serial order on the staining bridge, with smear side up and flooded with filtered Carbol Fuchsin. The smears were steamed and allowed to stain for 5 minutes, rinsed with water, and drained.
2. The smear were decolourized with 25% sulphuric acid for 2 minutes or acid alcohol solution, rinsed with water and drained. Then counterstained with methylene blue solution for 1 minute and rinsed with water.
3. The smear was allowed to air dry and examined microscopically using the oil immersion (100x) objective.

2.2.7. 2 Cultivation of *Mycobacterium tuberculosis*

A. Digestion and decontamination of sputum samples

Normal host microbiota contaminates sputum samples. Contaminated samples must be subjected to rigorous decontamination procedures that liquefy the organic debris and eliminate the unwanted normal flora. Digestion and decontamination using the 4% NaOH (modified Petroff) method were used. It's known Sodium hydroxide is toxic, both for contaminants and for tubercle bacilli; strict adherence to the indicated timings about is therefore essential . The Cultivation of *Mycobacterium tuberculosis* was performed according to David *et al* ., (2018) by the following procedure:

- Two ml of sputum was marked on the falcon tube and added an equal volume of 4% NaOH and tightened the screw-cap.
- The sample was mixed using a vortex to digest and allowed to stand for 15 minutes at room temperature.
- The tube was filled to within 2 cm of the top with phosphate buffer. (e.g. to the 50 ml mark on the tube) .Then the sample was centrifuged at 3000 g for 15 minutes.
- The supernatant was poured off carefully using a funnel into a discarded container containing 5% phenol or another mycobacterial disinfectant.
- The deposit was inoculated on two slopes of the L.J medium. A Micropipette was used to inoculate each slant with approximately (3–4 drops).
- All cultures were incubated at 35–37°C for 3 to 4 weeks until growth was evident or discarded as negative. Inoculated solid cultures should be incubated with caps loosened in a slanted position for at least one week to ensure an even distribution of the inoculum. Then, caps were tightened to prevent desiccation of the media.

2.2.8 Molecular diagnosis of *Mycobacterium tuberculosis*

2.2.8.1 GeneXpert MTB/RIF Assay

A. Principle

It is a fully automated cartridge-based molecular system that integrates sample processing, nucleic acid amplification and recognition of the target sequences. The assay uses nucleic acid probes that identify and report the presence or absence of the normal, rifampicin susceptible, in a sequence of the rpoB gene of MTB. Five different coloured beacons are used, each covering a separate nucleic acid sequence within the amplified rpoB gene. The assay gives different results, MTB detected and RIF resistance not detected OR MTB detected and RIF Resistance detected or MTB not detected (Fouda *et al*.,2019).

B. Procedure for the Assay

Xpert MTB/RIF Assay was performed according to the method recommended by the manufacturing company (Cepheid, USA).

1. Sputum processing

- Approximately two times the volume of the sample reagent were poured into the sputum (2:1 dilution, Sample Reagent: sputum).
- The sample was mixed using a vortex for at least 10 seconds after recapping it and then incubated for 15 minutes at 20-30°C, and this step was repeated between 5 to 10 minutes through the incubation period.

2. Loading the Xpert MTB/RIF Cartridge

The sample and reagents were added to the cartridge according to the following steps:

- The sample ID was written on each Xpert MTB/RIF Assay cartridge.
- The cartridge lid was opened and then opened the sample container.
- The homogenized sample was drawn close to the line on the pipette using the provided transfer pipette. To reduce the possibility of air bubbles generation, the sample was dispensed slowly.
- The sample reagent-treated was loaded into the sample chamber of the Xpert MTB/RIF cartridge.
- The cartridge lid was closed firmly.

3. Running the test

- After turning on the GeneXpert instrument, the GeneXpert software system started by double-clicking the GeneXpert software shortcut icon on the Windows desktop.
- The GeneXpert instrument system software was signed in using user name and password.
- When the program window was opened, clicked on Create Test
- The scan cartridge barcode dialogue box would appear.
- The barcode on the Xpert MTB/RIF Assay cartridge was scanned using a Barcode reader.
- The sample ID was written in the Sample ID box, and the start test clicked.
- The instrument module door with the blinking green light was opened and loaded the cartridge, and the door was closed.
- The test was started, and the blinking green light changed to a solid green light. When the test was finished, the light turned off.
- After the two-hour work period ends, a beep appears in the time field on the computer desktop, and the instrument automatically ejects the cartridge.
- The view results icon was clicked.
- The report button of the view results screen was clicked to view and/or generate a PDF report file.

2.2.9 Immunological tests

2.2.9.1 QuantiFERON-TB Gold (QFT- Plus) Assay.

Diagnosis of latent TB infection by QuantiFERON-TB Gold (QFT- Plus) Assay. The QFT–Plus Assay was performed according to the method recommended by the manufacturing company (Qiagen / Germany).

A. Principles of the Assay

The QFT-Plus test is performed in two stages. First, whole blood is collected into each of the QFT-Plus blood collection tubes, which include a Nil tube, TB1 tube, TB2 tube and a Mitogen tube. The QFT-Plus Blood Collection Tubes are shaken to mix antigen with the blood and should be incubated at 37°C as soon as possible and within 16 hours of collection. Following 16 to 24-hour incubation period, the tubes are centrifuged, the plasma is removed . Second, the amount of IFN-γ (IU/ml) is measured by ELISA. The Mitogen tube can be used with the QFT test as a positive control. The Mitogen tube may also serve as a control for correct blood handling and incubation. The Nil tube adjusts for background (e.g., elevated levels of circulating IFN-γ or presence of heterophile antibodies).

B. Preparation of Reagents of IFN-γ

1. Conjugate preparation

- The lyophilized conjugate 100X concentrate was reconstituted with 300 microliters of distilled water indicated on the label of the conjugate vial and mixed gradually to minimize frothing and ensure complete solubilization of the conjugate.
- Working strength conjugate was prepared by diluting the required amount of reconstituted conjugate 100X concentrate in green diluent as shown in table (2.13) and mixed thoroughly but gently to avoid frothing and Working strength conjugate should be used within 6 hours of preparation.
- Any unused conjugate 100X concentrate was stored at 4°C immediately after use.

Table (2.13): Conjugate preparation of IFN-γ

Number of strips	Volume of Conjugate 100X Concentrate	Volume of Green Diluent
2	10 µl	1.0 ml
3	15µl	1.5 ml
4	20 µl	2.0 ml
5	25 µl	2.5 ml
6	30 µl	3.0 ml
7	35 µl	3.5 ml
8	40 µl	4.0 ml
9	45 µl	4.5 ml
10	50 µl	5.0 ml
11	55 µl	5.5 ml
12	60 µl	6.0 ml

2. Standard preparation of IFN-γ

The IFN-γ standard was reconstituted with 1.52 ml of distilled water indicated on the label of the standard vial and mixed gently to minimize frothing and ensure full solubilization. Reconstitution of the IFN-γ standard to the correct volume would produce a solution with a concentration of 8.0 (IU/ml). The reconstituted standard was used to prepare a dilution series of four IFN-γ concentrations.

Steps for duplicate standard

- Four tubes were labelled “S1”, “S2”, “S3” and “S4” respectively.
- One hundred and fifty milliliter of Green Diluent was added to S1, S2, S3, S4.
- One hundred and fifty milliliter of the kit standard were added to S1 and mix thoroughly.
- Fifty milliliter from S1 to S2 were transferred and mixed thoroughly.
- Fifty milliliter from S2 to S3 were transferred and mixed thoroughly.
- Fifty milliliter from S3 were drawn and discarded

- Green dilution alone serves as the zero standard (S4). A standard curve were generated using IFN-γ concentrations as shown in table (2.14).

Table (2.14): Standard concentrations of IFN-γ

No. of standard	Concentration
Standard 1 (S1)	4 IU per ml
Standard 2 (S2)	1 IU per ml
Standard 3 (S3)	0.25 IU per ml
Standard 4 (S4)	0 IU per ml (GD alone)

C. The procedure of QFT-Plus Assay:

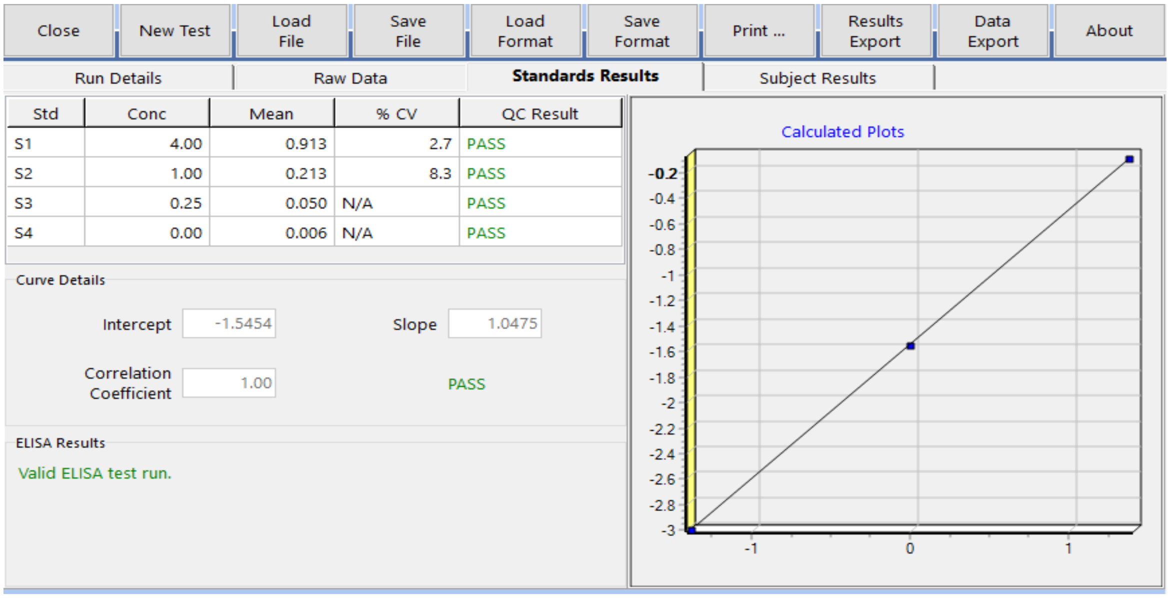
- Promptly after filing the four tubes with blood (Nil, TB1, TB2, Mitogen), the four tubes were shaken ten times just firmly enough to ensure that the entire inner surface of the tube had been coated with blood. This will dissolve antigens on tube walls.
- Prior to incubation, the tubes were maintained at room temperature (25°C) . The tubes must be transferred to a 37°C incubator as soon as possible and within 16 hours of collection.
- The QFT-Plus tubes were incubated upright at 37°C for 24 hours.
- After incubation, the tubes were centrifuged for 15 minutes at 3000 RCF (g) to allow plasma harvesting. The gel plug will separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged at a higher speed.
- Plasma samples were loaded directly from centrifuged blood collection tubes into the QFT-Plus ELISA plate.
- Fifty milliliter of test plasma samples were added to the appropriate plate wells by using the pipette.

- Finally, 50 µl each of the Standards 1 to 4 were added to the appropriate plate wells.
- Fifty milliliter of the working strength conjugate in Green dilution was added to all wells using a pipette after preparing it as explained in table (2.15) Conjugate preparation.
- ELISA plate was covered with a lid and mixed the conjugate and plasma samples/standards thoroughly by using a microplate shaker for 1 minute at 500 to 1000 rpm. Splashing should be avoided.
- ELISA plate was covered with a lid and incubated for 120 minutes at room temperature (25°C) . ELISA plates should not be exposed to direct sunlight during incubation. Deviation from a specified temperature range can lead to erroneous results.
- A working strength wash buffer was prepared during the ELISA plate incubation. One part Wash Buffer 20x Concentrate was diluted with 19 parts distilled water and mixed thoroughly. Sufficient wash Buffer 20x Concentrate had been provided to prepare 2 liters of working strength wash buffer. The wells were washed with 400 µl of working strength wash buffer for at least 6 cycles.
- ELISA plate face was tapped down on an absorbent towel to remove the remaining wash buffer.
- One hundred milliliter of Enzyme Substrate Solution was added to all wells, and the plate was covered with a lid and mixed.
- ELISA plate was covered with a lid and incubated at room temperature 25°C °C for 30 minutes
- After the 30-minute incubation, 50 µl of Enzyme Stopping Solution were added to all wells in the same order as the substrate was added and mixed.

- The Optical Density (O.D.) of all wells was measured within five minutes of stopping the reaction using a microplate reader at 450 nm .

D. Preparation of the standard curve

The optical density was obtained for standard and samples by an ELISA reader at 450 nm. QFT-Plus analysis software (version 2.71.2) was used to generate the standard curve. The software performs a Quality Control assessment of the assay, generates a standard curve and provides a test result for each subject, as shown in figure (2.1), Appendixes 1 and 2 .



Std	Conc	Mean	% CV	QC Result
S1	4.00	0.913	2.7	PASS
S2	1.00	0.213	8.3	PASS
S3	0.25	0.050	N/A	PASS
S4	0.00	0.006	N/A	PASS

Curve Details

Intercept -1.5454

Slope 1.0475

Correlation Coefficient 1.00

PASS

ELISA Results

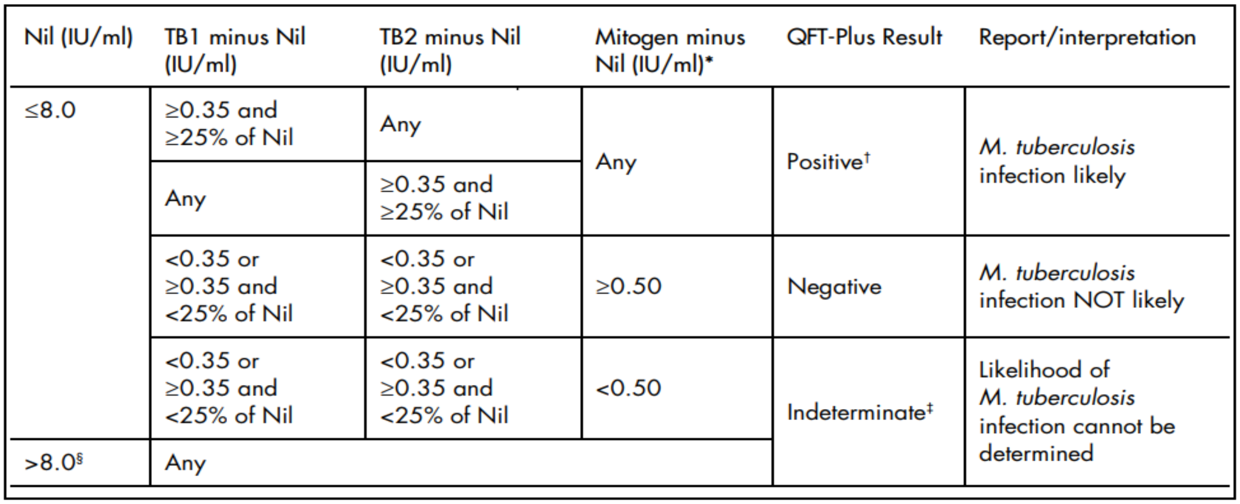
Valid ELISA test run.

Figure (2.1): Standard curve of IFN-γ concentration (IU/ml) calculated by QFT-Plus analysis software

E. Interpretation of QFT-Plus test results

QFT-Plus results are interpreted using the following criteria as shown in table (2.15).

Table (2.15): Interpretation of QFT-Plus test results



Nil (IU/ml)	TB1 minus Nil (IU/ml)	TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/interpretation
≤8.0	≥0.35 and ≥25% of Nil	Any	Any	Positive†	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥25% of Nil			
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	≥0.50	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	<0.50	Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0§	Any				

2.2.9.2 Estimation the serum levels of IL-17A and IL 18 by ELISA

The estimation of IL-17A and IL-18 was performed according to the method recommended by the manufacturing company (Elabscience / China).

A. Principle of testing

The Sandwich-ELISA principle is used in this ELISA kit. The antibody specific of IL-17A or IL-18 has been pre-coated on the micro ELISA plate included in this kits. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. After that, a biotinylated detection antibody specific of ,IL-17A or IL-18 and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well and incubated. Free components are rinsed away. The substrate solution is added to each well. Only the wells with IL-

17A or IL-18, biotinylated detection antibody and Avidin-HRP conjugate would appear blue in color. The enzyme-substrate reaction is finished by the addition of stop solution and the color turns yellow. At a wavelength of 450 nm , the optical density (OD) is measured spectrophotometrically. By comparing the OD of the samples to the standard curve, the concentration of IL-17A and IL-18 in the samples were calculated .

B. Preparation of Reagents

All reagents were brought to room temperature (25℃), and before optical density (OD) measurement, the microplate reader was preheated for 15 minutes.

1. Wash Buffer

To prepare 750 ml of wash buffer, thirty millilitres of concentrated wash buffer was diluted with 720 mL of distilled water.

2. Standard working solution

- The standard was centrifuged at 10,000×g for 1 minute.
- One millilitre of Reference Standard and Sample Diluent was added, left to stand for 10 minutes and inverted several times gently.
- After it has dissolved fully, mixed thoroughly with a pipette. This reconstitution produced a working solution of 2000 pg/mL of IL-17 A while 1000 pg/mL of IL-18. The serial dilutions were made as needed. The recommended dilution gradient of IL-17 A is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0.0 (pg/mL) while the recommended dilution gradient of IL-18 is as follows: 1000, 500, 250, 125, 62.5, 31.25,15.62, ,0.0 (pg/mL).

These concentrations were prepared by the following dilution method:

- Seven Eppendorf tubes were taken, and 500 µL of Reference Standard and sample diluent was added to each tube.

- Five hundred milliliter of the 2000 pg/mL working solution was pipetted to the first tube and mixed up to produce a 1000 pg/mL working solution of IL-17 A, while 500µL of the 1000 pg/mL working solution was pipetted to the first tube and mixed up to produce a 500 pg/mL working solution of IL-18.
- Five hundred milliliter of the solution was pipetted from the former tube into the latter one according to these steps. The last tube is regarded as a blank.

3. Biotinylated Detection Ab working solution

The required amount was calculated prior to the experiment (100 µL / well). Before use, the stock tube was centrifuged, and the 100× concentrated biotinylated detection antibody was diluted to a 1×working solution with biotinylated detection Ab diluent.

4. Concentrated HRP Conjugate working solution:

The required amount was calculated prior to the experiment (100 µL / well). The 100× concentrated HRP conjugate was diluted to 1× working solution with concentrated HRP conjugate diluent.

C. Procedure for the assay

- The Standard working solution was added (100 uL for each well) to the first two columns: Each concentration of the solution was added in duplicate to one well each, side by side. The samples were added to the other wells (100 uL for each well). Then the plate was covered with the sealer provided in the kit and incubated at 37°C for 90 minutes.
- The liquid was removed from each well without washing it. One hundred microliters of Biotinylated Detection Ab working solution were immediately added to each well. The plate was covered with the sealer. Then the plate was

gently mixed up and incubated at 37°C for 1 hour. The solution was decanted from each well

- Three hundred fifty microliters of wash buffer were added to each well. The plate was soaked for 1- 2 minutes, and the solution was decanted from each well and pat dry against clean absorbent paper. This wash procedure should be repeated three times.
- One hundred microliters of Horseradish Peroxidase (HRP) Conjugate working solution were added to each well. Then the plate was covered with the Plate sealer and incubated at 37°C for 30 minutes.
- The solution was decanted from each well and repeated the wash process five times.
- Ninety microliters of Substrate Reagent were added to all wells, and the plate was covered with a new plate sealer and incubated at 37°C for about 15 minutes. The plate was Kept away from light. The reaction time can be shortened or extended according to the actual colour shift, but it should not exceed 30 minutes.
- Fifty microliters of Stop Solution were added to all well. In the same order as the substrate solution, the stop solution should be added.
- The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

D. Calculation of the results

The optical density was obtained for standard and samples by an ELISA reader at 450 nm; a standard curve was constructed by plotting standard concentration on the X-axis and optical density (OD) values on the y-axis. The equation of the standard curve was calculated by using the Microsoft Excel office 2010 program. The concentrations of IL-18A and IL-18 (pg/ml) in the serum of the samples can be calculated by using standard curve equations as shown in figures (2.2) and figure (2.3), respectively.

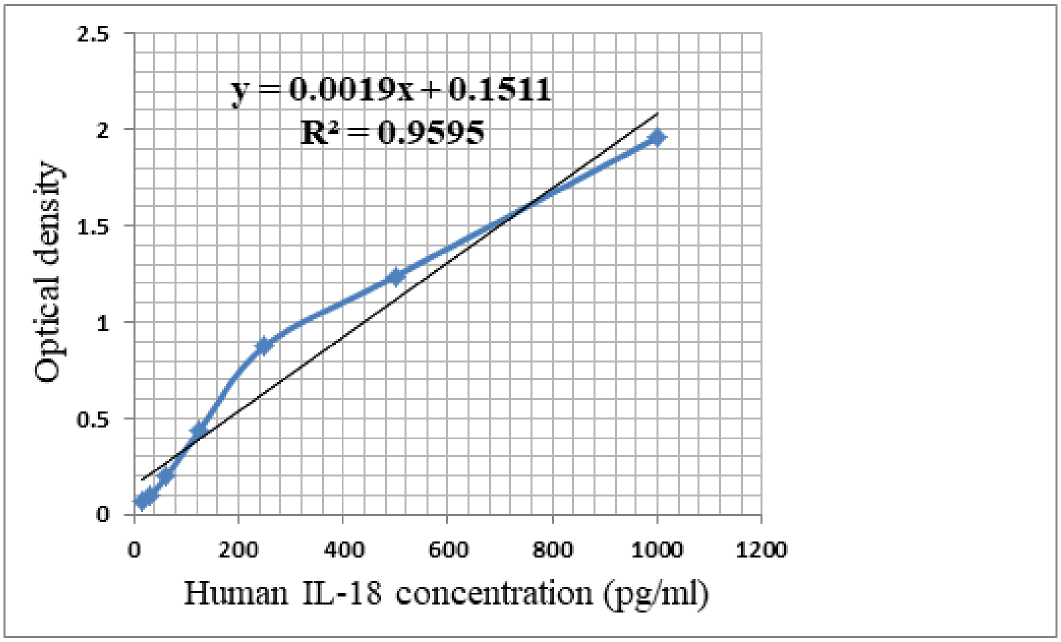


Figure (2.2): The standard curve of IL-18 concentration (pg/ml).

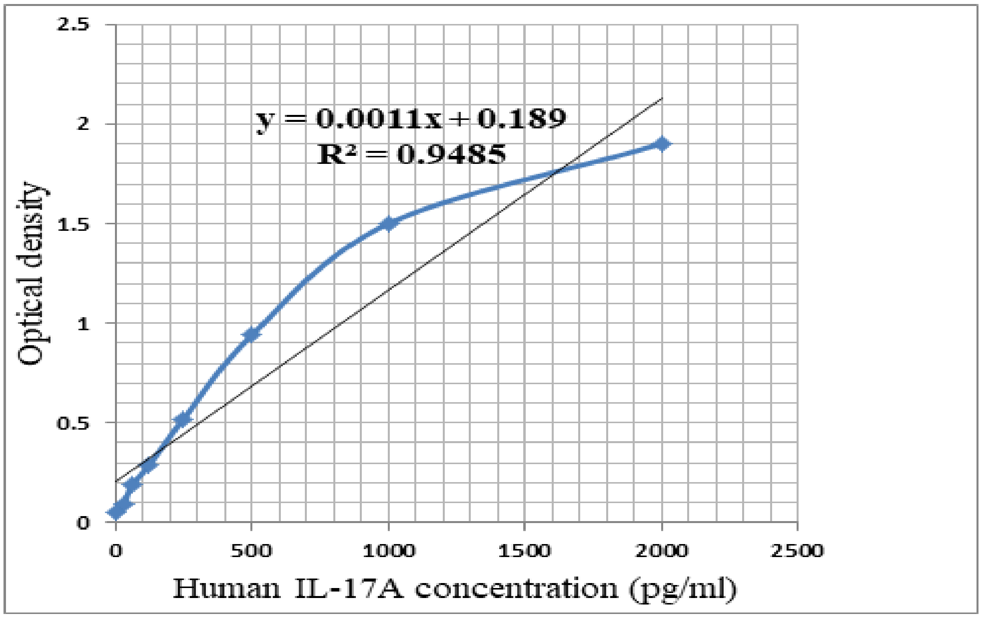


Figure (2.3): The standard curve of IL-17A concentration (pg/ml).

2.2.10 Molecular methods

2.2.10.1 Extraction of Genomic DNA

The ReliaPrep™ blood gDNA Miniprep system offers a rapid, simple technique for preparing purified and intact DNA from mammalian blood. Human genomic DNA (gDNA) was extracted from whole blood samples of active pulmonary TB, Latent TB infection, and control groups by using the Protocol for DNA extraction.

The protocol steps:

The procedure was achieved according to the method recommended by the manufacturing company (Promega/USA). ReliPrepTM Blood gDNA Miniprep system was performed as follows:

- Two hundred milliliter of blood was transferred into a 1.5ml microcentrifuge tube containing 20µl of the Proteinase K (PK) solution.
- Two hundred milliliter of RBC Lysis Buffer (RLB) was added to each sample and mixed by vortex for a minimum of 15 seconds and then incubated in the water bath at 56°C for 12 minutes.
- The ReliaPrep™Binding Column tube was placed into an empty collection tube.
- Then the samples were removed from the water bath, and 250µl of Binding Buffer (BBA) was added, and the tubes were mixed by vortex for 15seconds
- The content would show a dark green colour in the tubes.
- After that, the content of the microcentrifuge tube was added to an empty the ReliaPrep™ Binding Column and put in a microcentrifuge
- The spin column was centrifuged for 3 minutes at 10000 rpm speed; after that, checked the lysates had completely passed through the membrane.
- The collection tube was removed because it contained hazardous waste.

- The binding column was placed into a fresh collection tube, and 300µl of column wash solution (CWD) was added to the column, centrifuge for 5 minutes at 12000 rpm speed and then discarded the waste in the bottom of the collection tubes and repeated this step 3 times.
- The column was placed in a clean 1.5 microcentrifuge tube, and then 80µl of Nuclease Free Water (elution buffer) was added to the column with a centrifuge for 2 minutes at 12000rpm to elute DNA.
- The ReliaPrep™ Binding Column was discarded and saved elute.
- All DNA samples have been stored at -20 °C until use.

2.2.10.2 Measurement of DNA Concentration and Purity

The extracted blood genomic DNA was checked by using a nanodrop spectrophotometer, which measured DNA concentration (ng/µL) and checked the DNA purity by reading the absorbance at 260 /280 nm .

2.2.10. 3 DNA Electrophoresis

The process of DNA electrophoresis was carried out according to Armstrong and Schulz, (2015).

A. Principle

Electrophoresis separates charged molecules migrating in an electric field. A buffer with pH 8 to 8.3 is included in the electrophoresis chamber to counter pH changes and allow for the passage of current. As a result of this passage of current and consequent electrolysis of water at the platinum electrodes, hydrogen gas is formed at the cathode (negative side), and oxygen gas is formed at the anode (positive side). DNA molecules have regularly repeating phosphodiester linkages that carry negative charges in solutions of near neutral pH. These macromolecules migrate to the anode of the electrophoresis chamber under an applied constant voltage. In the presence of an agarose matrix, the nucleic acid polymers will electrophorese according to their sizes, with the smaller molecules sieving faster through the pores of the matrix (Armstrong and Schulz,2015).

B. Protocol of Gel Electrophoresis

- One hundred ml of the TBE buffer (1 X) was placed in a conical flask (250ml).

A concentration of 1X Tris-Borate EDTA (TBE) buffer was prepared by diluting concentrated 10X TBE Buffer. This solution was used to dissolve agarose and in the electrophoresis process. Each 100ml of 10X TBE was added to 900ml of water to give a final concentration,1X TBE (Sambrook and Russel, 2001).

- One gram of agarose was added to the buffer and heated on a hot plate to boiling point so that all of its components were solvent.
- The agarose mixture was cooled by leaving it between 50-60˚C.
- Pre-staining with ethidium bromide dye (EtBr) by adding 5 µl to the agarose-TBE buffer before hardening.
- The comb was fixed at one of the ends of the agarose gel casting tray for making wells that were used for loading DNA sample.
- Agarose was poured into the casting tray gently to prevent the formation of the bubble and left to cool at room temperature for 30 minutes.
- The electrophoresis tray was filled with TBE (1X) buffer solution covering the surface of the gel.
- Five µl of DNA product were mixed with one µl loading stain and loaded into the agarose-gel wells.
- The electrophoresis was performed at 70 V for 45 min.
- The agarose gel was exposed to a UV light transilluminator at a wavelength of 312 nm and DNA bands were visualized and documented.

2.2.10.4 Conventional Polymerase Chain Reaction (PCR)

A. Principle

PCR makes it possible to obtain, by in vitro replication, multiple copies of a DNA fragment from an extract. This amplification is based on the replication of a double-stranded DNA template. It is broken down into three phases: a denaturation phase, a hybridization phase with primers, and an elongation phase. The products of each synthesis step serve as a template for the following steps, thus exponential amplification is achieved. The polymerase chain reaction is carried out in a reaction mixture which comprises the DNA extract (template DNA), Taq polymerase, the primers, and the four deoxyribonucleoside triphosphates (dNTPs) in excess in a buffer solution. The tubes containing the mixture reaction are subjected to repetitive temperature cycles several tens of times in the heating block of a thermal cycler (Pelt-Verkuil *et al*.,2008).

B. Primers Preparation:

All primer pairs used in this study were spin down before opening the cap of primers tube. According to the instruction provided by primer manufacturer (Macrogen, Korea), a desired amount of nuclease free water was added to each primer to produce a 100 Pico-mole/microliter concentration of primer stock solution. The primer stock solution was re-suspend by transferring 10 µl to a 1.5 ml eppendorf tube that contain 90 µl of free nuclease water to yield 10 Pico-mole/microliter that used in PCR amplification and the primer stock was stored at -20 C°.

C. Reaction mixture of PCR

The Components of PCR reaction are listed in table (2.16).

Table (2.16): Components of PCR reaction mixture

Component	Volume
PCR Mater mix	12.5 µl
Forward Primer	1.2 µl
Reverse Primer	1.2 µl
Template DNA	1.6 µl
Nuclease-free water	8.5 µl
Total reaction volume	25µl

D. Polymerase Chain Reaction (PCR) Condition

The thermal cycler was programed with the desired PCR profile as shown in table (2.17).

Table (2.17): The conditions of PCR for three genes; SP110 gene, IL18 gene , IL17A gene utilized in current study

Stages	Temperature	Time	Cycles
Initial	95°C	5 min	1
Denaturation	95°C	30 sec	30
Annealing Tem.	57°C * ,55 °C **, 60°C ***	30 sec	
Extension Tem.	72°C	1 min	
Final extension Tem.	72°C	5 min	1
Cooling	4°C	∞	

*SP110 gene ,** IL18 Gene , ***IL17A Gene

E. Protocol of the PCR technique

The procedure of the PCR technique was carried out according to (Kuslich *et al* ., 2018)

- Required reagents were added into PCR tubes, including 12.5 µl of PCR master mix, 8.5 µl of nuclease-free water, 1.2 µl of primers forward and reverse and 1.6 µl of template DNA in a total reaction volume of 25 µl as shown in table (2.16).

- All the components were mixed by spinning the tubes to avoid the components attached to the wall of PCR tube.
- PCR tubes were put in a thermal cycler (PCR machine) .
- A thermal cycler was programmed for each gene under amplification, as shown in table (2.17). The PCR condition was similar except annealing temperatures (Ta).The annealing temperatures were (57 C°, 57 C° , 55 C°,60 C°) of SP110 gene (rs7580900 and rs7580912), Sp110 gene (rs9061and rs11556887), IL18 gene (rs1946518) and IL17A gene (rs2275913) respectively.
- The PCR tubes were removed from the thermal cycler. Then, four microliters of PCR products and five microliters of the ladder were loaded on 1 % agarose gel (1gram per 100mL of TBE buffer (1X) and 100 V for 45 min) recoloured with pre-stained with 0. 5 µl Ethidium bromide to ensure the presence of amplicons bands.
- The agarose gel was exposed to a UV light transilluminator at 312 nm, and amplicon size was determined by comparison with the ladder. DNA bands were visualized and documented.
- PCR products (18-20 µl) were sent to the macrogen company in South Korea to read the DNA sequencing of amplicons.

2.2.10.5 SNPs Genotyping by AS-PCR

Allele specific-polymerase chain reaction (AS-PCR) method was used to detect IL-18 rs1946518 (-607C/A) and IL-17A rs2275913 (-197G>A) SNPs .

A. Principle of the technique.

The amplification-refractory mutation system (ARMS), also known as allele-specific polymerase chain reaction (AS-PCR) or PCR amplification of specific alleles, is a simple, rapid, and reliable method for detecting any mutation involving single base changes or small deletions . A PCR primer is designed so that it can discriminate between templates that differ at a specific single nucleotide residue. It is based on the principle that Taq polymerase has no 3' to 5' exonuclease activity; therefore a mismatch at the 3' end of the primer and the template will result in its inability to function as a primer under appropriate conditions. A standard AS-PCR consists of two complementary reactions (two tubes) and utilizes 3 primers. One primer is constant and complementary to the template in both reactions , the other primers differ at their 3' terminal residues and are specific to either the wild type DNA sequence or the mutated sequence at a given base. Only one of these primers is used per tube. If the sample is of a homozygous mutant or homozygous wild type, amplification will only occur in only one of the tubes, if the sample is heterozygous, amplification will be seen in both the tubes. (Kumar and Garg .,2005).

B. Procedure

- Two sets of PCR tubes were sorted based on the number of samples under interest. The first set of tubes for the wild allele and the second set for the mutant allele.
- The final total volume of reaction as shown in table (2.18) and (2.20) .The wild forward primer was added to the first set of tubes and the mutant forward primer was added to the second set of tubes.
- A thermal cycler was programmed for each gene under amplification, as shown in table (2.19) and (2.21).
- Five microliters of AS-PCR products and five microliters of ladder marker were loaded on a 2% agarose gel (2 gram per 100mL of TBE buffer and 70V for 50 min) recoloured with pre-stained with 0. 5 µl Ethidium bromide

to ensure the presence of amplicons bands. Agarose gel was exposed to a UV light transilluminator at 312 nm, and amplicon size was determined by comparison with the ladder. DNA bands were visualized and documented.

Table (2.18) AS-PCR mixture reaction for genotyping of IL-18 rs1946518

Component	Volume
PCR Mater mix	12.5µl
Control forward primer	1 µl
Common reverse primer	1µl
Normal forward primer OR Mutant forward primer	1 µl
DNA template	2 µl
Nuclease free water	7.5 µl
Final volume	25µl

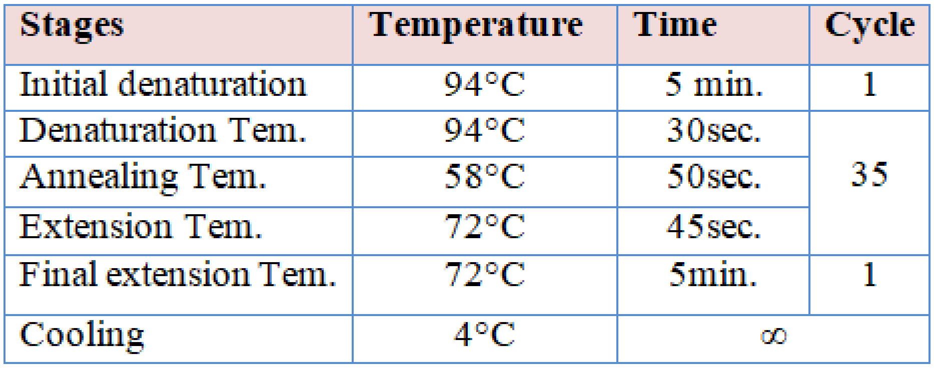
Table (2.19): AS-PCR Condition for genotyping of IL-18 rs1946518

Stages	Temperature	Time	Cycle
Initial denaturation	93°C	3min.	1
Denaturation Tem.	93°C	30sec.	33
Annealing Tem.	57°C	35sec.	
Extension Tem.	72°C	35sec.	
Final extension Tem.	72°C	3min.	1
Cooling	4°C	∞	

Table (2.20): AS-PCR mixture reaction for genotyping of IL-17A rs2275913

Components	volume
Master mix	10 µl
Common reverse Primer	1 µl
Normal forward Primer or Mutant forward Primer	1µl
DNA template	2 µl
Nuclease free water	6 µl
Total volume	20 µl

Table (2.21): AS-PCR Condition for genotyping of IL-17A rs2275913



Stages	Temperature	Time	Cycle
Initial denaturation	94°C	5 min.	1
Denaturation Tem.	94°C	30sec.	35
Annealing Tem.	58°C	50sec.	
Extension Tem.	72°C	45sec.	
Final extension Tem.	72°C	5min.	1
Cooling	4°C	∞	

2.2.10.6 SNP selection and genotyping

Candidate SNPs for the present study were selected according to the literature review of previous studies and from the National Center for Biotechnology Information (NCBI) website. SNPs were selected if they were reported to be associated with disease and/or predicted to have effects on function (Wu *et al*.,2018). SNPs were genotyped using the Sanger method of DNA sequencing of the SP110 gene, while SNPs were genotyped by using AS-PCR of the IL18 gene and IL-17A gene, then the results for this technique were confirmed by Sanger sequencing analysis.

2.2.10.7 Analysis of Sanger sequencing data:

About 18-20 µl of PCR products of all genes under interest in this study, thirty six samples were sent to the Macrogen company in South Korea to perform the sequence of DNA for detection of SNPs under interest: rs2275913; rs1946518 ,and ninety samples were sent to the Macrogen company in South Korea to perform the sequence of DNA for detection of SNPs rs7580900, rs7580912, rs9061, rs11556887. Through 15 days the data of sequencing was received by email in three formulas; pdf file, text document sheet and AB1 file

The sequencing results of the PCR products of different samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using a reading program by Geneious prime purchased version. In addition, the NCBI data tools were used to alignment the gene sequence by the BLAST tool of NCBI. The observed variations in each sequenced sample were numbered in PCR amplicons as well as in their corresponding position within the referring genome.

2.2.11 Statistical analysis :

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation after performance of the Kolmogorov- Smirnov normality test and making decision about normally and non-normally distributed variables.

One way Anova test used to study difference in mean between more than two groups provided that the variable is normally distributed. The chi-square test was used to assess whether there were significant differences between the observed and expected frequencies. The odds ratio and 95% confidence interval (95% CI) were estimated to measure risk. The difference was considered significant when the probability value (P-value) was < 0.05 (Daniel and Cross, 2018). Receiver Operator Characteristics (ROC) curves was designed to test the power of the immunological markers to predict active TB and latent TB (Hosmer and Lemeshow ,2013).

Agreement between TB1 and TB2 were assessed using Cohen’s kappa coefficients using the categorical variables (positive/negative) based on the cutoff values for IFN-γ (McHugh, 2013) . Normal distribution was ruled out for (TB1-Nil) and (TB2-Nil) antigens and calculated the median (interquartile range: IQR).

Genotypes were presented as percentage frequencies. Allele frequency of SNPs was estimated by direct gene counting method . The online Hardy-Weinberg equilibrium (HWE) calculator for two alleles was used to determine if two alleles deviated significantly from the Hardy-Weinberg equilibrium (Ad'hiah *et al*.,2019).

Chapter Three

Results and Discussion

3.1 Laboratory diagnosis of *Mycobacterium tuberculosis*

Out of 140 suspected patients with TB, 60 patients were confirmed with active Pulmonary tuberculosis (ATB) based on positive results from at least one form of microbiological evidence; staining of acid-fast bacilli, bacterial culture or from a molecular test (Xpert MTB/RIF) and clinical symptoms and signs, chest radiography (CXR) suggestive of tubercle bacilli (WHO,2010TB; Kaewseekhao *et al*., 2020).

In this study, out of 140 sputum specimens tested, laboratory results of sputum specimens showed that 38 (27.14%), 60 (42.86%), and 41 (29.28%) specimens were positive by ZN /AFB smear microscopy, GeneXpert MTB/RIF assay, and MTB culture on LJ medium, respectively as shown in table (3.1) .

Table (3.1) : Laboratory diagnostic Techniques Results

Results	**Type of methods**		
	AFB smear microscopy	Gene Xpert MTB/RIF	Culture
Positive, n (%)	38 (27.14)	60 (42.86%)	41 (29.28%)
Negative, n (%)	102 (72.86)	80 (57.14%)	99 (70.72%)
Total, n (%)	140 (100%)	140 (100%)	140 (100%)

n: number of cases

3.1.1 Efficiency of the laboratory diagnostic techniques

To assess the efficacy of the diagnostic tests, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for AFB smear microscopy and the GeneXpert, using culture of *Mycobacterium tuberculosis* from sputum specimens as the gold standard (reference method) (Luetkemeyer *et al*., 2016, WHO,2021) as illustrated in table (3.2). Gene Xpert MTB/RIF assay gave high sensitivity (100%) and specificity

(80.8%), respectively. AFB smear microscopy had high specificity (86.9%) but significantly lower sensitivity (61%) in comparison with Xpert MTB/RIF assay.

There was a significant difference (P < 0.001) in both sensitivity and specificity between AFB smear microscopy, Gene Xpert MTB/RIF assay and culture technique, as illustrated in table (3.2).

Table (3.2): Sensitivity, Specificity, PPV and NPV of AFB smear microscopy and Gene Xpert MTB/RIF assay compared to culture.

Type of test		***Culture***		**Sen.**	**Spec.**	**PPV**	**NPV**	**Accuracy**	**P value**
		P	**N**						
AFB smear microscopy	**P**	**25**	**13**	**61.0%**	**86.9 %**	**65.8%**	**84.3%**	**79.29%**	**< 0.001¥**
	N	**16**	**86**						
Gene Xpert MTB/RIF	**P**	**41**	**19**	**100%**	**80.8 %**	**68.3%**	**100%**	**86.43%**	**<0.001¥**
	N	**0**	**80**						

P: Positive; N: Negative; P-value: probable value, PPV: positive predictive values, NPV: negative predictive values, ¥: Chi-square test.

The overall sensitivity of Xpert MTB/RIF was 100%. The sensitivity observed in this study was comparable to Kumar *et al* .,(2021), who reported 100 % in India. The results of current study do not differ from other studies conducted in different places, which have demonstrated a sensitivity ranging from 95.6 to 100 (Asfar *et al.*,2018). Faria *et al*.,(2021) showed that the use of GeneXpert substantially increased the detection of TB cases, with sensitivity ranging from 68% to 100%, superior to sputum smear microscopy.

The specificity of GeneXpert in comparison with sputum smear microscopy was (80.8 % vs. 86.9 %). Some studies concluded that both exams showed similar performance concerning the mentioned aspect (Mollel *et al.*,2017; Griesel *et al.*,2018). The results of several studies were near to the results of the current study. Mustafa *et al*. (2021) found that the specificity of Gene Xpert in diagnosing pulmonary tuberculosis was 81.3%. In a related matter, Muia *et al.*,(2017) documented a specificity of 84.3 % for smear microscopy as compared to the reference culture method.

According to the current results, the sensitivity of AFB smear microscopy was lower than those of GeneXpert MTB/RIF assay (61% vs. 100%). Ahmed *et al* ., (2021) showed that the sensitivity of ZN smear microscopy was 63.63%. While, Umair *et al.*,(2020) reported that Sputum ZN smear microscopy is a highly specific but moderately sensitive test (60%) for the diagnosis of pulmonary tuberculosis. However, there is a wide variation in the reported sensitivity of ZN smear microscopy, ranging from 20% to 80% (Dzodanu *et al*.,2019).

The current study illustrated the diagnostic accuracy was calculated as (86.43 *vs.* 79.29 %) for Gene Xpert MTB/RIF assay and smear-microscopy, respectively. It was near to study by Elbrolosy *et al.*, (2021) in Egypt showed the results of accuracy for AFB smear microscopy and Gene Xpert MTB/RIF assay were 78.8%, 86.92%, respectively. The result of the NPV value of GeneXpert (100%) is similar to the results of previous studies from other countries (93.8%-100%) (Saifullah *et al.*,2016) but the results of the NPV value of AFB smear microscopy (84.3%) is high in this study in comparison to the study of Deng *et al.*,(2021) in which it was 61.4% . PPV value for AFB smear microscopy and GeneXpert was 65.8% and 68.3%, respectively. These results were near to the results provided by Agrawal *et al.*,(2016).

The variations reported in studies regarding sensitivity, specificity, PPV, NPV and accuracy may be explained by differences in sample collection, standard and quality of laboratories, and samples testing times.

Rapid early detection of MTB requires the use of highly sensitive and specific techniques. An explanation for the lower sensitivity of AFB smear microscopy is the higher detection threshold in comparison with the GeneXpert MTB/RIF assay; it requires 5000–10,000 bacilli per mL of sputum in specimens to show a positive result. (Habte *et al*.,2016). Moreover, this test requires a 3-day early morning sputum specimen collection protocol to enhance its sensitivity. In addition to the lower sensitivity of sputum smear microscopy, it cannot differentiate MTB from the MTB complex (Riaz *et al*.,2016).

ZN staining is the most widely used method for the initial diagnosis/screening of patients for TB infection, it is an inexpensive but less sensitive test for the diagnosis of TB (Caulfield and Wengenack,2016). AFB smear microscopy missed 22 (15.71%) cases when compared with the sputum GeneXpert MTB/RIF test in this study. Therefore, a missed diagnosis of smear-negative pulmonary TB can have significant financial implications for individuals, families, and the country as a whole. The GeneXpert MTB/RIF assay is a sensitive and specific test for the diagnosis of smear-negative pulmonary tuberculosis (Rasheed *et al*.,2019).

A culture technique using Lowenstein Jensen (LJ) medium for mycobacterial growth is considered as the gold standard method for TB detection, The biggest drawback of tuberculosis culture is the long culture cycle, which can’t meet the needs of clinical diagnosis it takes a longer amount of time, which is usually 3–4 weeks with high sensitivity (Sambarey *et al*., 2017) . The efficiency of MTB culture using LJ medium has been demonstrated to detect MTB when 10 viable bacilli per mL of sputum were present (Munir *et al*.,2015). AFB culture can be detected at lower bacillary load and can also be influenced by other factors, such as specimen collection and processing, and this explains the reasons for the negative results in culture. MTB culture assay is prone to cross-contamination, which may appear to be another factor affecting the lower recovery rate by culture, while GeneXpert MTB/RIF assay is not prone to cross-contamination (Shi *et al* ., 2018).

GeneXpert assay has shown a much higher sensitivity for the detection of MTB than ZN smear microscopy in pulmonary samples is reasonable due to the detection threshold, where a minimum number of 131 mycobacterial colony-forming units is required to be present in the specimen to produce a positive Xpert MTB/RIF reading (Atehortúa *et al*.,2015).

WHO recommends sputum GeneXpert MTB/RIF testing for all suspected cases of pulmonary tuberculosis or newly diagnosis patients (Gilpin *et al*.,2018). Compared with culture, the Gene-Xpert MTB/RIF assay can detect *M. tuberculosis* and the presence of rifampicin resistance simultaneously and directly from clinical specimens within 2 hours . Nucleic acid amplification tests (NAATs) are widely used to diagnose tuberculosis (TB) but cannot discriminate live bacilli from dead bacilli. Live bacilli can be isolated by cultural methods, but this is time-consuming (Wang *et al*.,2020).

In summary, the GeneXpert assay is considered a valuable diagnostic tool for the rapid detection of MTB with simultaneous detection of RR-TB. This early detection facilitates controlling the disease transmission and timely start of TB treatment therapy, and hence the introduction of the GeneXpert assay results in a significant reduction of MDR-TB cases.

3.1. 2 Detection of bacillary load and rifampicin resistance TB (RR-TB) by GeneXpert MTB/RIF assay

Xpert MTB/RIF gives a semi-quantitative result (as very low, low, medium, and High) when the test detects MTB. Interpretation of the results was performed with GeneXpert software version 4.8. from measured fluorescence signals and embedded calculation algorithms and are displayed in the view results window as indicated in table (1) , appendixes-1 and 2.

In this study, among 60 specimens that were positive by GeneXpert MTB/RIF assay, the results showed 11 (18.3%) specimens had a high semi-quantitative result (average cycle threshold (CT) value of 14.3), 25 (41.7 %) specimens had a medium result (average cycle threshold (CT) value of 20.8), 15 (25.0 %) specimens had a low result (average cycle threshold (CT) value of 25.4), and 9 (15.0%) specimens demonstrated a very low semi-quantitative result (average cycle threshold (CT) value of 30.1) as shown in the table (3.3) and table (3.4).

Table (3.3): Frequency of MTB and Rifampicin Resistance detected by GeneXpert MTB/RIF assay in patients with Active Pulmonary TB.

MTB DETECTED by GeneXpert MTB/RIF assay			
	MTB detected	No.	%
1.	high, *n* (%)	11	18.3
2.	low, *n* (%)	15	25.0
3.	Medium, *n* (%)	25	41.7
4.	very low, *n* (%)	9	15.0
5.	total, *n* (%)	60	100
Rif. Resistance detected by GeneXpert Dx. System			
1.	Rif.R, , *n* (%)	7	11.67
2.	Rif.S, *n* (%)	53	88.33
3.	Total	60	100.0

Table (3.4):Average cycle threshold (CT) value in GeneXpert MTB/RIF assay

N=60	Mean	Std. Deviation	Minimum	Maximum
Low	25.40	1.96	22.10	29.90
Medium	20.82	1.73	17.30	23.20
Very low	30.11	2.71	22.50	34.30
High	14.36	1.79	9.20	17.20

Ct values for each of the five probes were recorded. The five probes (A, B, C, D and E) were used to quantify bacilli by Xpert and the latter was reported as the Mean Ct value (Najjingo *et al*.,2019) .These results were consistent with other published studies. A Study by Fradejas *et al*.,(2018) demonstrated that semiquantitative mycobacterial load results were as follows: very low (cycle threshold (Ct) > 28), low (Ct 22–28), medium (Ct 16–22) or high (Ct < 16) and Geleta *et al*.,(2015) reported a high semi-quantitative mycobacterial load result (average cycle threshold (CT) value of 14.5), a medium semi-quantitative result (average CT value of 19.1), a low semi-quantitative result (average CT value of 27.0), a very low semi-quantitative result (average CT value of 33.1). Singh *et al*.,(2016) showed that Semi- quantitation of bacillary load by Xpert assay is determined by threshold-cycle (Ct) ; High Ct value is <16; Medium Ct value is 16-22; Low Ct value is 22–28; Very Low Ct value is >28.

An inverse correlation between mycobacterial load and GX Ct (r = −0.94). Bodmer and Ströhle (2012) reported that lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template. Measurements of bacterial load have long played an important role in tuberculosis diagnostics. Semiquantitative measures of the number of *Mycobacterium tuberculosis* bacilli present within a clinical sample have been clinically useful for determining disease severity, and assessing transmission risk (Blakemore *et al*.,2011).

GeneXpert MTB/RIF is an automated Real-Time polymerase chain reaction assay targeting the rpoB gene for the detection of *Mycobacterium tuberculosis* (MTB) that estimates mycobacterial load by measuring the threshold-cycle (Ct) of multiple probes targeting the *rpoB gene* (Boakye-Appiah *et al*.,2016). The primers in the Xpert MTB/RIF assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. Five differently coloured fluorogenic nucleic acid hybridization probes, called molecular beacons, interrogate the entire 81-bp core (El-Hajj *et al*.,2001). Each molecular beacon was designed to be so specific that it does not bind to its target if the target sequence differs from the wild-type rpoB sequence by as little as a single nucleotide substitution. Since molecular beacons fluorescence only when they are bound to their targets, i.e. wild-type *rpoB* sequence, the absence of any one of the five probes in the assay differentiates between the conserved wild-type sequence and mutations in the core region that is associated with RIF resistance (Alame-Emane *et al*.,2017).

Among 60 specimens with MTB detected by GeneXpert, rifampicin resistance was detected in 11.67% (7/ 60) of specimens. RIF resistance not detected (Rif.S) results were recorded in 88.33 % (53/60) specimens, as shown in table (3-3). The frequency of rifampicin resistance in the current study was consistent with a study conducted in the Baghdad governorate by Aljanaby *et al*.,(2022), in which they demonstrated that the prevalence of RR-TB was 11.1% in patients with active tuberculosis . However, this rate differs to a considerable extent from the prevalence rate reported by Al-Obaidy *et al* .,(2013) in Baghdad (14.3%). Al-Mussawi *et al*.,(2017) documented the Rifampicin-resistance prevalence rate in the Basra province, south of Iraq, was (7.56%). This difference needs more research in Iraq to determine whether this difference is a real one or due to differences in methodology.

Rifampicin (RIF) plays a pivotal role in the treatment of tuberculosis due to its bactericidal effects. Because the action of RIF is on the rpoB gene encoding RNA polymerase subunit, 95% of RIF resistant mutations are present in the rpoB gene. The majority of the mutations in the rpoB gene are found within an 81 bp RIF-resistance determining region (RRDR) (Zaw *et al*.,2018).

The detection of rifampicin resistance serves as a surrogate marker for detecting Multi-drug Resistant Tuberculosis (MDR-TB), which means resistance to at least isoniazid and rifampicin (Dagnra *et al*.,2015). The rifampicin resistance mechanism involves missense mutations in the rifampin resistance-determining region (RRDR) of the rpoB gene and a delay in recognition of drug resistance results in a delay in the initiation of treatment or effective therapy, which is the major factor that contributes to MDR-TB outbreaks (Masenga *et al*.,2017).

Although RIF resistant mutation is difficult to occur when compared with any other anti TB drugs, the rate of RIF resistance is increasing due to its wide usage . The acquired rifampicin resistance can occur when there is a history of incomplete treatment regimens lasting at least 1 month (Mulisa *et al*.,2015). Prior inappropriate anti-TB regimen only suppresses the growth of susceptible bacilli but does not affect other resistant strains, leading to suitable conditions for the dominant multiplication of pre-existing drug-resistant mutants (Mekonnen *et al*.,2015). RR-TB cases which will lead to the occurrence of MDR-TB. Additionally, the association between contact with a known TB patient and MDR-TB was a significant factor, as observed in several other studies that supported the hypothesis that contact with a known TB patient is linked with rifampicin resistance due to exposure to resistant TB strains (Desissa *et al*.,2018). Early detection of rifampicin resistance may reduce the incidence of multidrug resistance (MDR) strains among tuberculosis patients.

3.2 The diagnosis of latent TB infection (LTBI)

3.2.1QuantiFERON -TB Gold (QFT- Plus) Assay

An OFT-Plus ELISA kit was used to quantify the IFN-γ in the plasma samples. Data are presented as IU per millilitre of IFN-γ; the cutoff value for a positive test was ≥ 0.35 (IU/ml). IFN-γ concentrations (IU/mL) were calculated by the QFT-TB-analysis Software as shown in Appendixes -3 and 4.

In total, 164 subjects with suspected latent TB were tested with the QFT-Plus test. Overall, 60 subjects (36.6%) displayed positive results in the QFT-Plus were defined them as latently TB infected (LTBI) consisting of (37 males and 23 females) with ages ranging from 18 – 66 years and 104 samples (63.4%) were negative as shown in appendix (3). Forty healthy persons without known TB exposure (individuals with no TB infection or disease) who were negative by the OFT-Plus test included 22 males and 18 females with ages ranging from 15 - 70 years, as shown in appendix (4).

The positivity rates of QFT-Plus TB1 antigen tube, and QFT-Plus TB2 antigen tube were 27.4%%, and 30.5%, respectively. There were no indeterminate cases. There was no significant difference in positivity rate between TB1 and TB2 antigen tubes (P=0.6), as shown in table (3.5). When comparing TB1 and TB2 results, 139 (84.8%) were concordant, the κ coefficient (kappa coefficient) was 0.630, and 25 (15.2%) were disconcordant as indicated in table (3.6). 15 (25.0 %) of the subjects with latent TB had a negative result in TB1 and a positive in TB2, while 10 (16.7%) of the subjects with latent who had a positive result in TB1 and negative in TB2, 35 (58.3 %) of the subjects with latent who had positive results of both TB1 and TB2 in QFT-Plus as indicated in table (3.7).

Table (3.5): The positivity rates of the QFT-Plus test

Test	Positive		Negative		(95% CI)
	n	%	*N*	%	
QFT-Plus	60	36.6%	104	63.4%	(0.955-2.435)
TB1 tube	45	27.4%	119	72.6%	(0.410-1.046)
TB2 tube	50	30.5%	114	69.5%	(0.719-1.87)
P-value	0.60 ¥				

***n*: number of cases; ¥: Chi-square test; NS: not significant at $P \geq 0.05$**

Table (3.6): The concordance rate of TB1 and TB2 tubes in QFT-Plus

Type of test		TB2 tube		Total
		Positive	**Negative**	
TB1tube	**Positive**	35	10	45
	Negative	15	104	119
Total		50	114	164

The κ coefficient was 0.630, and the concordant rate of TB1 and TB2 was high (n=139, 84.8%).

Table (3.7) Positive results of TB1 tube and TB2 tube in QFT-Plus

TB1 alone	10 (16.7%)
TB2 alone	15(25.0 %)
TB1+ and TB2+	35 (58.3 %)
Total	60 (100%)

The QFT-Plus test is the fourth generation in QuantiFERON-TB testing technology assessing cell-mediated response through quantitative measurement of IFN-γ released in a whole blood sample responses to mycobacterial proteins. These proteins, ESAT-6 and CFP-10. Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of IFN-γ. The detection and subsequent quantification of IFN-γ form the basis of this test (Andersen *et al*.,2000) .

In the Netherlands, Pieterman *et al*.,(2018) reported outcomes were similar in TB1 and TB2, resulting in an agreement of 97%. Igari *et al*.,(2019) showed that there was no significant difference in the percentages of positive between TB1 and TB2, and the concordance rate was 99.3% between TB1 and TB2. Buonsenso *et al*.,(2020) found that TB1 and TB2 responses had 100% concordance. The results of the current study were in agreement with these results, but the concordance rate in these studies was higher than in the present study.

The higher positivity rate in TB2 than TB1 because TB2 tube contains an additional set of peptides targeted to the induction of CMI responses from CD8+ cytotoxic T lymphocytes and this leads to a difference in the amount of IFN-γ produced in TB1 and TB2 tube (Tsuyuzaki *et al*.,2019).

This study observed 10 discordant results, which were TB1 positive and TB2 negative, and 15 discordant results, which were TB2 positive and TB1 negative. Based on the assumption that TB1 only elicits a CD4+ T-cell reaction and TB2 a CD4+ and a CD8+ T-cell reaction, these observations seem to contradict this assumption and might therefore be due to variability of the test (Metcalfe *et al*.,2013).

Interestingly, among the sixty positive patients in that study, only 35 had concordant positive results between tube 1 and tube 2 QFT-Plus antigen tubes. Siegel *et al*.,(2018) believed that enrollees with concordant tube 1/tube 2 results are more likely to have LTBI without TB-exposure . The lack of a gold standard for LTBI prevents the proper assessment of QFT-Plus performance for the LTBI group. Nevertheless, it is remarkable that a total of 60/164 subjects (36.6 %)

scoring positive for QFT-Plus, and therefore diagnosed with LTBI had at least one risk factor for TB infection (known exposure to TB), supporting *M. tuberculosis* infection (Nguyen *et al*., 2019).

An initial recent study certifies the new generation QFT-Plus as delivering results with good sensitivity and specificity in the diagnosis of latent TB infection (LTBI). QFT-Plus is an LTBI diagnostic tool with more sensitivity in comparison with QFT-GIT(Takasaki *et al*.,2018; Theel *et al*.,2018). Whereby requiring positivity in both tubes could maximize specificity and potentially minimize false-positive results. However, if the patient had a risk of exposure and a high risk of progression, then accepting either one as positive in defining a positive assay result would provide maximal sensitivity (Moon *et al*.,2017).

It has been reported that QFT-Plus showed improved sensitivity to identify MTB infection, especially recent infections (Hoffmann *et al.*,2016). The suggested advantage of QFT-Plus is the added CD8 antigens as a biomarker for intracellular TB burden in LTBI, more likely to improve performance in patients recently exposed to TB .

In this study, the overall prevalence of LTBI among 164 subjects in contact with active pulmonary TB was 36.6% by QFT-Plus. In correspondence to this study, other studies from Iraq using IGRA showed a higher LTBI prevalence rate ranging from 36.94% to 41.3% (Hussein *et al*., 2019). In Mosul City, Al-Ghreer *et al*., (2015) showed that the prevalence of LTBI is 35.7% among the 140 contacts tested by QFT-Plus assay. In a study conducted in South Korea recruiting 308 subjects with a household-contact history with patients with active TB, 38.6% of examined samples were positive (Lee *et al*.,2014). Another study from Egypt showed that the prevalence of LTBI was 33.3% (Abu Taleb *et al.*, 2011) . In a meta-analysis study analyzing 203 published studies, it was found that the prevalence of LTBI in contacts in low-income countries was 51.5%, whereas the prevalence was 28.1% in high-income countries (Fox *et al*.,2013).

Although the sample sizes in the studies mentioned above were small, the explanation of the discrepancy in the results is not clear at this stage; however, further studies with larger sample sizes are warranted to better understand the trends of LTBI . Finally, the results of the current study showed that the QFT-plus test tended to be more positive among subjects in contact with active pulmonary TB. It is, therefore, reasonable to pay particular attention to TB contacts individuals during the contact investigation of active TB and this might help in the early detection of disease or infection for the better control of the TB program.

3.2.2 The predictive value of QuantiFERON-TB Gold Plus

The immune responses to TB-specific antigens would change in the phases of active and latent TB infection . QFT-Plus provides an additional opportunity for analyzing the immune response caused by CD8 T cells. The analysis of IFN-γ production in both the first antigen tube (TB1) and the second antigen tube (TB2) would lead to further analysis of the TB immune response, especially that caused by CD8 T-cells (Petruccioli *et al*.,2016).

The median IFN-γ release of TB2 was insignificantly higher (p = 0. 142) compared to TB1 in the QFT-Plus positive results, with a median of 1.32 IU/mL (IQR: 2.05) versus 0.64 (IU/mL) (IQR: 2.69). The difference in median concentration was 0. 68(IU/ml) as shown in table (3.8).

Table (3.8): The median IFN-γ release in TB1 and TB2 tubes

Characteristic	**TB1 tube** ***n* =60**	**TB2 tube** ***n* = 60**	***P*-value**
Median (IQR)	0.64 (2.69)	1.32 (2.05)	0.142† NS
Range	-4.95_8.80	-1.28_7.84	

n: number of cases; †:Mann-Whitney Test; NS: not significant at P ≥ 0.05.

To assess the specific contribution of CD8+ T-cells ,the present study subtracted the quantitative value of the first antigen tube expressed in IU·mL−1 (TB1), which stimulates the CD4+ population only, from the value provided by the second antigen tube (TB2), in which a combined CD4+ and CD8+ T-cell stimulation occurred (TB2-TB1) and used a difference of 0.6 IU·mL−1 to define positive results to reduce the bias of the intrinsic variability of the test (Metcalfe *et al*.,2013) or A cut-off value was set at 0.6 IU·mL−1 to exclude small variations due to inter test variability. After analyzing the differences between IFN-γ production in TB1and in TB2 (TB2-TB1). Normal distribution was ruled out and the median was calculated (interquartile range: IQR) of 0.21 (1.82). The results showed that 22 (36.7%) out of 60subjects with latent TB had a difference between TB2 and TB1 >0.6 IU·mL−1 and 38 (63.3 %) of subjects with latent TB was < 0.6 as shown in the table (3.9).

Table (3.9): Differences of interferon-γ productions responding to TB-specific antigens

Characteristic	TB2-TB1
Range	-2.67_6.52
Median (IQR)	0.21 (1.82)
< 0.6, *n* (%)	38 (63.3 %)
> 0.6, *n* (%)	22 (36.7 %)

n: number of cases. IQR: interquartile range

The median IFN-γ release of TB2 was insignificantly higher than TB1 in the QFT-Plus, and the difference in median concentration was 0. 68 (IU/ml). This result was near to a study conducted by Telisinghe *et al.,(*`2017), who documented the median IFN-γ concentration was higher in TB2 than in TB1. They found that the median TB1-nil and TB2-nil concentrations were 1.61 and 2.47(IU/ml), respectively, and the difference in median concentration was 0.86 (IU/ml). In contrast, Barcellini *et al*.,(2016) reported that the median TB1 QFT-Plus antigen IFN-γ level was higher than the IFN-γ level in TB2 (0.74 IU·mL−1 vs. 0.67 IU·mL−1).

The present study observed that a greater TB2 antigen response (TB2−TB1 difference >0.6 IU·mL−1) in 22 (36.7 %) individuals with latent TB from all QFT-Plus positive. The results of the current study were also in agreement with Tsuyuzaki *et al*.,(2020) .The difference in response between the TB2 and TB1 antigens tubes in the QFT-Plus (TB2-TB1>0.6 IU·mL−1) meant a higher antigenic burden in latent TB infection, leading to the prediction of developing active TB. Also, the difference in response between the TB2 and TB1 tubes could be used as a surrogate marker of recent exposure and not to previous cumulative *M. tuberculosis* exposure. If validated, this may prove to be a surrogate marker of recent infection, which, having the highest risk of progression to active TB, may enable QFT-Plus to distinguish recent infection from long-lasting reactivity and hence allow better-targeted delivery of preventive therapy (Barcellini *et al*. 2016).

In MTB infection, CD4+ T cells play a critical role in immunological control through their secretion of the IFN-γ and the subjects with latent TB infection had immune responses mainly caused by CD4 T-cells ; the role of CD8 T-cells is restricted in latent TB without active TB cases (Tsuyuzaki *et al*.,2020). Several studies have described that CD8+ T-cells play a unique function in the recognition and containment of intracellular infection with MTB, an increase of the CD8 T-cell responses has been observed to be associated with MTB load. Additionally, a strong CD8+ T-cells response has been identified as well among recently exposed contacts of patients with active TB(Rozot *et al*.,2013 ; Lee *et al*., 2019).`

M. tuberculosis-specific CD8+ T-cells have been more frequently detected in individuals with active TB when compared with LTBI and correlated with the increasing antigenic burden, suggesting that the presence of CD8+ T-cells in a small proportion of latently infected individuals may be predictive of *M. tuberculosis* active replication and more likely disease progression (Chiacchio *et al*.,2014).

However, to date, there are no tools for direct evaluation of *M. tuberculosis* antigenic burden as current LTBI tests rely on the (indirect) measurement of a specific immune response. Observational studies found the difference between the two antigen tubes (TB2-TB1), used as an indirect estimate of specific CD8+ T-cell activation, is associated with factors indicating increased MTB exposure , suggesting a possible role in identifying individuals with recent infection who are at greater risk of progression to active TB (Zhang *et al*.,2019).

The difference in IFN-γ productions in TB1 and TB2 would be a marker for estimating both CD4 T-cell and CD8 T-cell immune responses. Finally, The predictive value of QFT-Plus to predict latent TB infection who will progress to develop active TB needs to be further investigated.

3.3 Demographic characteristics of studied groups

Table (3.10): Demographic characteristics of ATB, LTBI patients and Apparently healthy controls in this study.

	Number Sex			**Age (years)**	
Group	Total	Male (%)	Female (%)	Mean ± SD	Range
AHC	40	22(55.0%)	18 (45.0 %)	38.46 ±14.59	15- 70
ATB	60	39 (65.0%)	21 (35.0%)	36.16 ±14.16	16 - 68
LTBI	60	37 (61.7 %)	23(38.3 %)	42.45±13.62	18 – 66

Abbreviations: HC, Apparently healthy controls; LTBI, latent TB infection; ATB, Active pulmonary TB .

3.3.1 Age distribution among active pulmonary tuberculosis (ATB) group

In the present study, the age of Active pulmonary tuberculosis patients (ATB) were divided into five groups including 15-24 years, 25-34 years, 35-44 years, 45-54 years and ≥ 55 years, respectively, as shown in the table (3.11).The age groups (25-34years) showed higher frequencies of TB cases (33.3 %) than other age groups and followed by the age group (15 - 24) years (25.0 %) and 35-44 years (16.7 %), but the lowest recorded age groups were observed in (45-54) and ≥ 55 years with percentage 11.7 %, 13.3 % respectively, with highly significant differences between all age groups ($p = 0.001$).

Table (3.11): Age and Sex distribution among active pulmonary tuberculosis (ATB) group

Characteristic	Patients n = 60	P-value	X2
Age groups			
15-24, *n* (%)	15 (25.0 %)	0.001 ¥ S	20.8
25-34, *n* (%)	20 (33.3 %)		
35-44, *n* (%)	10 (16.7 %)		
45-54, *n* (%)	7 (11.7 %)		
≥ 55, *n* (%)	8(13.3 %)		
Sex			
Male, *n* (%)	39 (65.0 %)	0.020 ¥ S	5.40
Female, *n* (%)	21 (35.0 %)		
Male: female ratio	1.85:1		

n: number of cases; SD: standard deviation; ¥: Chi-square test; S: significant at P < 0.05

The results of the present study were in agreement with Merza, (2020) who found the most common affected groups in order were aged (15–24) years and (25–34) years in Duhok province. Sbayi *et al*.,(2020) who demonstrated that the mean age was (31.74 ± 18.83) years, and tuberculosis affects particularly the young population whose age group(15-34) years in Morocco. In Nigeria, Nwokoye,(2021) demonstrated that the mean age of participants was (39.8 ± 15.5) years, and the most common age group was 25 – 34 years. In contrast , In Pakistan, Qadeer *et al*.,(2016) reported a higher TB prevalence in the elderly. Ahmad *et al*.,(2021) found that the mean age of study participants was (38 ±7.1) years and the most frequent age group were above 50 years.

The most commonly affected groups were the younger age groups which was consistent with the findings of most developing countries (Dogar *et al*.,2012). This was an expected finding as TB is a disease of the labour force population (Mokdad *et al*.,2014). Conversely, in developed countries, elder people account for a significant proportion of the cases, and the reason behind this is claimed to be multi-factorial, particularly the increase of latent tuberculosis infection (LTBI) in the geriatric age group (Jappar and Low,2015).

Tuberculosis infection involves all age groups, more than 90% of global TB cases and deaths occur in the developing world, where 75% of patients are in the most economically productive age group. The high infection rate in these age groups may be returned to many factors like smoking and conditions that weaken immunity, such as malnutrition, and alcoholism (Bialvaei *et al*.,2017). Al-Kadhimi and Dawood, (2011) found that TB is significantly more in the younger age group in the province of Baghdad; this may be due to the high number of young population of Iraq, also maybe the patients of these ages are more active and met many people make possible predispose to infections.

3.3.2 Sex distribution among active pulmonary tuberculosis (ATB) group

out of 60 patients with active pulmonary tuberculosis (ATB), 21 (35.0 %) were females, and 39 (65.0 %) were males, as shown in table (3-11). The male-to-female ratio was 1.85:1 (39/21), and there was a statistically significant difference in the incidence of active pulmonary tuberculosis (ATB) among males and females (P = 0.02).

The results of the current study were in agreement with many local studies regarding gender distribution among pulmonary tuberculosis cases. In Baghdad province, Ahmed *et al* .,(2018) indicated a higher prevalence of tuberculosis among males (62.5%) than females (37.5%) with an overall male to female ratio was 1.7; this difference was highly significant. This sex disparity among the reported PTB case findings corresponds to the findings by Fernandes *et al*.,(2018) . Horton *et al*.,(2016) have identified that this disease affects the men population more than the women population, with a ratio close to 2 as reported by worldwide case notification rates By employing epidemiological surveys. In contrast to this study, few reports from the EMR such as Pakistan and Iran, showed a higher incidence of TB cases among women in certain geographic locations; however, the exact cause for that finding was not conclusive (Dogar et al.,2012).

The prevalence of tuberculosis disease (TB) is higher in males (Fernandes *et al*.,2018). This phenomenon may be since men are more active in the community and more exposed to the carriers, the nature of work that the males in various fields' non-sanitary and crowded areas, especially in the cases of poor ones. In addition, this result could be attributed to behavioural factors such as smoking which is a predisposing factor to TB, with more males being smokers than females, alcohol consumption, malnutrition and delay in seeking medical treatment, especially by males (Mohajeri *et al*.,2014). Instead, Narasimhan *et al*.,(2013) reported that females are subjected to greater stigmas, biological characteristics, socioeconomic and barriers that limit access to health care. At present, shisha smoking, also known as hookah or narghile, is increasingly common among youth, and the use of shared mouthpieces during smoking could lead to TB transmission, and therefore according to Krishnan *et al*.,(2014) , more than 20% of TB incidence may be attributable to smoking.

The immune response to tuberculosis may also be closely related to differences between females and males in the type of sexual hormones. Sexual hormones may play a part in the protection/susceptibility to TB. The study by WHO,(2014) found that the sex steroid hormones may render men even more susceptible to pulmonary TB than women and also found the influence of estradiol sexual hormone on immune functions acts as an enhancer of cell-mediated immunity response through increasing the number of T lymphocytes and causes lower susceptibility for tuberculosis against females. Testosterone represses T-helper cells and can impact macrophage stimulation and humoral immune responses, which play an important role in tuberculosis immune responses.

3.3.3 Risk factors associated with QFT-Plus positive results

The mean age of the Latent TB infection (LTBI) group was 42.45±13.62 years (range: 18 – 66 years). The males and females ratio was 1.60:1. The most frequent age group was 45-54 years (30.0%), followed by the age groups ≥ 55 (23.3%) and were associated significantly with positive QFT-Plus (OR: 3.8, 95% CI :1.21-11.95, P=0.022 and OR : 4.03 , 95% CI :1.20 - 13.52; P=0.024) respectively. Males (61.7 %) were more frequent than females (38.3 %) of the Latent TB infection (LTBI) group and associated significantly with positive QFT-Plus (OR : 2.19, 95% CI :1.15 - 4.20, *P*=0.018*)* as shown in table (3.12).

Table (3.12): Risk factors associated with QFT-Plus positive results.

QFT-Plus	**Positive N=60**	**Negative N=104**	**OR (95% CI)**	**P-value**
Sex				
Male, *n* (%)	37 (61.7 %)	44 (42.3%)	2.19 (1.15 -4.20)	***0.018***
Females, *n* (%)	23 (38.3%)	60 (57.7%)		
Age groups				
15-24, *n* (%)	6 (10%)	19 (18.3%)	**Reference**	**Reference**
25-34, *n* (%)	10 (16.7 %)	35 (33.7%)	0.90 (0.28 - 2.87)	0.865 ¥ *NS*
35-44, *n* (%)	12 (20.0%)	24 (23%)	1.58 (0.50 - 5.00)	0.434 ¥ NS
45-54, *n* (%)	18(30.0%)	15 (14.4%)	3.80(1.21 - 11.95)	***0.022¥ S***
≥ 55, *n* (%)	14 (23.3%)	11 (10.6)	4.03(1.20 - 13.53)	***0.024 ¥ S***

n: number of cases; ¥: Chi-square test; NS: not significant at P > 0.05 ; S: significant at P < 0.05;OR:Odd ratio.

Ageing is a defined risk factor for acquiring LTBI, which is in line with several studies (Pareek *et al*., 2013; Wu *et al*.,2021). A local study in Dohuk Governorate by Abdulkareem *et al*.,(2020) found that the prevalence of infection was higher in males compared to females and subjects aged ≥ 45 years were strongly associated with LTBI. In Mosul city, Al-Ghreer *et al*.,(2015) found the rate of LTBI increased with an increase in age and is more common in males (60.71%) than in females (39.29%). Chen *et al*., (2015) demonstrated that the LTBI rates were increasing with age (ORs were in significance from 6.60 to 20.92), and the male significantly increased the risk of LTBI by 1.52 fold in Eastern China. A Study in Vietnam by Nguyen *et al.*,(2019) found the positivity of QFT-Plus appeared to increase with older age, with a significant difference in univariate analysis. The high positive rates of IGRA in the elderly population in South Korea (Yoo *et al*.,2016).

These results partially correspond with Çekiç *et al*.,(2015), who reported that the mean age was 42.0 years, while 69.7% were male and 30.3% were female of individuals with latent tuberculosis in Turkey. On the other hand, Taxonera *et al*.,(2017) found that the mean age was 42.6 years and 55.3% were male in Spain. In contrast, Almufty *et al*.,(2019) found the mean age of the LTBI was 33.4, and the younger ages were the risk of LTBI in Iraq.

Ting *et al*.,(2014) found that the proportion of LTBI was significantly higher in men than in women (32.6% vs. 25.2%). The proportion of LTBI significantly increased with increasing age in both men and women, and the sex disparity was most prominent in the elderly populations (age ≥55 years), which was partially consistent with the results of the current study. In contrast, several studies recorded significant positive Tuberculin skin test among male close contact but not IGRA positivity (Campbell *et al*., 2015).

The effect of ageing is directly related to its cumulative effect on acquiring TB infection. A longer time of exposure or repeated exposure to Mtb may play a role in increasing the odds of having a positive QFT-Plus result. The most published literature stated that the risk of LTBI is directly linked with increasing age (Christopher *et al*.,2010). As a consequence, further research is warranted to better understand the association of LTBI with age. Eom *et al*.,(2018) highlighted a dilemma in making treatment decisions for LTBI in elderly contacts because elderly contacts have vulnerable immunity and therefore develop TB more easily, and they may also have frequent adverse drug reactions during LTBI treatment.

Males have a higher probability of having TB infection with active disease progression. Moreover, men may have more social contact than women and thus leading to an increased risk of exposure to the contagious case. Several potential confounding factors like smoking, drug use and air pollution exposure can be associated with Sex disparity (Slama *et al*.,2007). Although men have a higher proportion of LTBI than women, with an odds ratio of 2.19, male sex may not be an independent factor associated with LTBI after adjusting for other risk factors. Sex differences in LTBI were also not studied much. More data will be needed to clarify the influence of Sex on the incidence of LTBI .

3.4 Immunological analysis results

3.4.1 Distinguishing cytokine distribution among LTBI, ATB and AHC groups.

3.4.1.1 Serum IL-18 level in the studied groups

The mean serum concentration of IL-18 was highly significantly higher in patients with active pulmonary TB 412.74 ± 74.54 (pg /ml) compared to the latent TB group and apparently healthy control respectively,(346.39 ± 70.55 and 269.02±

55.14 pg /ml) (p<0.001), also the mean serum concentration of IL-18 were significantly higher in latent TB group which reached 346.39 ± 70.55 (pg/ml) compared to apparently healthy control (269.02 ± 55.14 pg /ml) , (p<0.001) as shown in figure (3.1) and table (3.13).

Table (3.13): Serum levels of Interleukin-18 among studied groups.

	Groups			P value		
IL-18 pg/ml	ATB n = 60	LTBI n = 60	AHC n=40	ATB vs. AHC	LTBI vs. AHC	ATB vs. LTBI
Mean	412.74±74.54	346.39±70.55	269.02±55.14	< 0.001 † HS	< 0.001 † HS	< 0.001 † HS
SD	74.54	70.55	55.14			
Range	302.55- 710.75	206.04 – 482.9	191.0–358.39			

n: number of cases; SD: standard deviation; †: one way anova; ATB: Active TB; LTBI: Latent TB infection; AHC: Apparently Healthy controls; HS: highly significant at P≤ 0.001

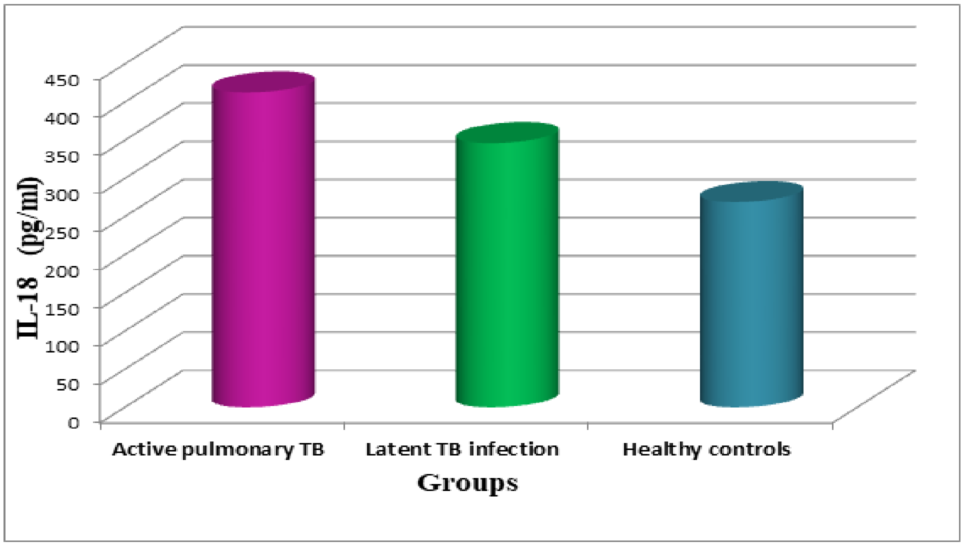


Figure (3.1): Distribution of active pulmonary TB patients , Latent TB infection and apparently healthy controls groups according to the mean level of serum IL-18.

The present study performed ROC analysis to estimate the discriminatory power of serum IL-18 in distinguishing active TB or latent TB from apparently healthy control as well as ATB vs. LTBI. As shown in figure (3.2), IL-18 exhibited significant discriminatory power with high AUC values, sensitivity and specificity in distinguishing between active TB vs. apparently healthy control (IL-18 Cutoff > 318.58 , sensitivity 92% , specificity 90% , AUC= 0.952(excellent), P-value <0.001), active TB vs. latent TB comparison , IL-18 Cutoff value was >365.21 with sensitivity 85 % , specificity 80 %, AUC=0.877(good) and P-value <0.001. Latent TB vs. apparently healthy control comparison , IL-18 Cutoff value was > 319.12 with sensitivity 88 % , specificity 83 % , AUC=0.903 (good) and P-value <0.001.

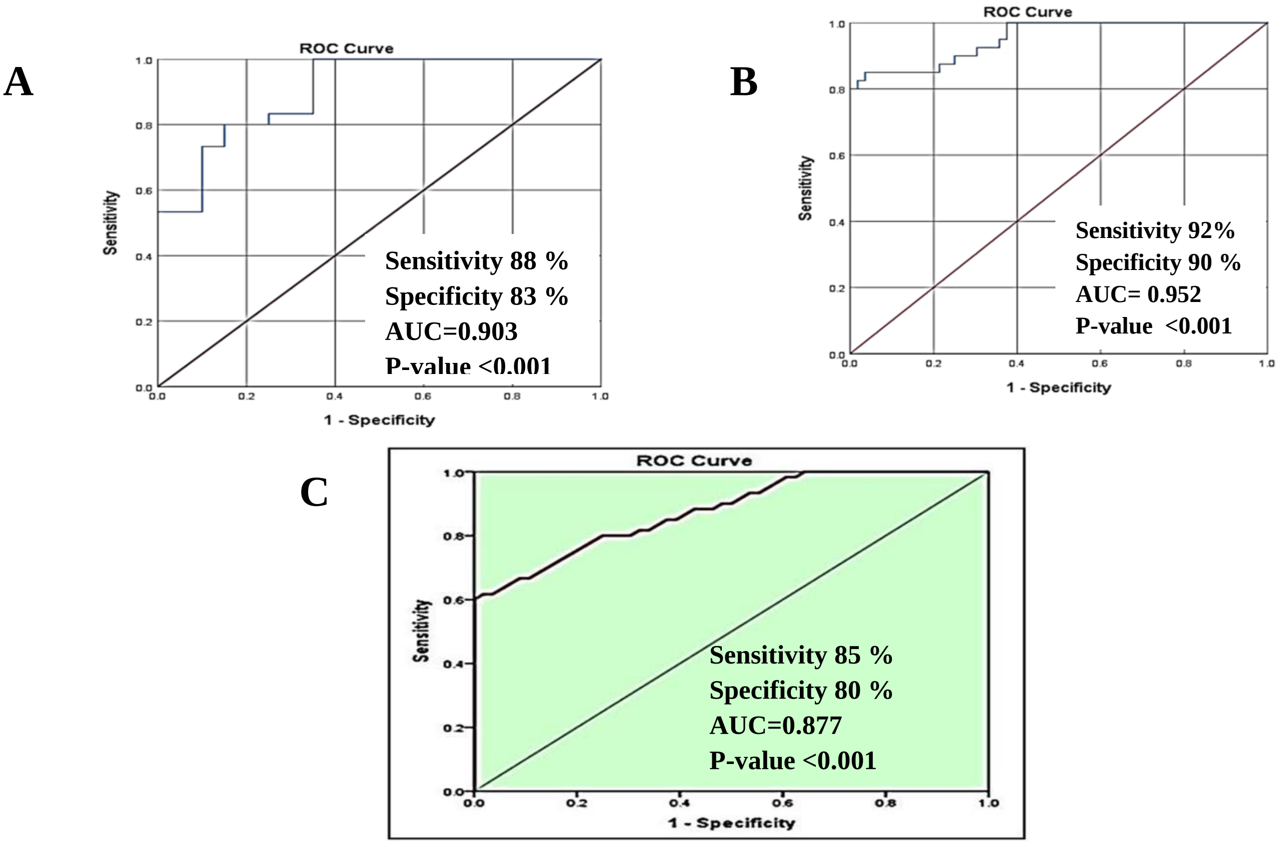


Figure (3.2): ROC analysis to estimate the sensitivity, specificity and AUC was performed using IL-18 to estimate the capacity of this parameter to distinguish individuals with, (A)latent TB vs. apparently healthy control , (B) active TB vs. apparently healthy control , (C) active TB vs. Latent TB.

When comparing the group of patients with AHC group, the results of this study corroborated with a local study in Baghdad province by Utba ,(2019), Wang *et al*.,(2015) in China, and Abhimanyu *et al*.,(2016) in India . They all found that serum IL-18 concentrations were greater in TB cases than in AHC group and this difference was statistically significant . The circulating levels of IL-18 used as markers of disease activity in pulmonary tuberculosis and IL-18 can also be used to monitor the efficiency of treatment (Gamal and Ahmed, 2008). A recent study in China by He *et al*.,(2020) has been reported that IL-18 significantly upregulated in TB patients compared to AHC group. In addition, they initially examined the levels of IL-18 in LTBI, and it was found that IL-18 was significantly higher in the LTBI group than in the AHC group, while this was lower than in the TB group. Moreover, a study by Wawrocki *et al*.,(2019) showed a significant increase in the serum levels of IL-18 in ATB patients compared to LTBI or AHC individuals in Poland.

The results of the current study were partially consistent with Wawrocki *et al*.,(2020) reported that a significantly higher relative level of IL-18 mRNA expression was observed in LTB individuals as compared to AHC group without Mtb infection. The difference in values for ATB and AHC group was not significant. On the contrary, the study by Kathamuthu *et al*.,(2017) observed the serum concentrations of IL-18 were significantly lower in TB compared to LTB individuals.

This study documented increased levels of circulating IL-18 in patients with tuberculosis. The levels of circulating IL-18 paralleled disease activity (Yamada *et al*.,2000).The role of IL-18 has been actively studied in the pathogenesis of many diseases, including cancers and digestive diseases (Caligiuri *et al*.,2005). IL-18 signalling complex might be exploited by *M. tuberculosis* to expand the clinical manifestation of pulmonary TB (Wawrocki *et al*., 2019). In addition, IL-18 has also been known as interferon-gamma inducing factor following infection. Therefore, it was supposed that IL-18 parallels to the extent of lung lesions (He *et al.*, 2020).

Furthermore, IL-18 is an essential mediator of the activation of macrophages that are involved in controlling Mtb. IL-18 is essential for the optimal production of IFNγ from T cells during TB and in some instances, its absence can result in increased susceptibility to Mtb (Domingo-Gonzalez *et al.*,2016). In fact , a study in Saudi Arabia by Mukhtar, (2017) showed a positive significant correlation between serum levels of IL-18 and IFN-γ. IL-18 has a similar tendency similar to IFN-γ, which has long been considered one of the markers to assess the state of tuberculosis infection.

The levels of interleukin-18 in cases with latent tuberculosis were lower than in active TB but higher than in AHC. Interleukin-18, a regulatory cytokine, with increased production plays a potentially central role in promoting the reactivation of TB (Akgun *et al.*,2005). Wawrocki *et al.*, (2020) found that the increase in IL-18 gene expression and the reduced expression of IL-18R gene in cases with latent TB infection can, at least partially, prevent the development of a pathological inflammatory reaction and promote the maintenance of homeostatic conditions between host immunity and Mtb infection.

On the other hand, ROC analysis results showed that IL-18 owned the highest area under curve (AUC=0.952) for predicting active TB disease, when comparing the active tuberculosis group with the findings were consistent with Wawrocki *et al.*,(2019) who found the high discriminative capacity of IL-18 for the diagnosis of active TB. In addition, an area under curve (AUC) was good for predicting latent TB. These results were consistent with He *et al.*, (2020) who indicated that it is among a group of cytokines, IL-18 could be used to discriminate latent TB from apparently healthy control. Sudbury *et al.*,(2020) found that several cytokines have shown promise as stage-specific markers of TB infection, there is little consistency between studies and much heterogeneity in the changes reported for different cytokines. Further verification would contribute to obtaining more effective immunological markers for active and latent TB.

3.4.1.2 Serum IL-17A level in the studied groups

The present study found that the mean serum concentration of IL-17A showed an insignificant variation in patients with active pulmonary TB (107.95±41.18 pg/ml) compared to AHC group (122.14 ±39.80 pg/ml), (p=0.069). In contrast, the mean serum concentration of IL-17A was significantly higher in the latent TB infection group (147.14±43.99 pg/ml) compared to the AHC group (122.14 ±39.80 pg/ml) (p=0.002). Also, the mean serum level of IL-17A was significantly lower in active pulmonary TB (107.95±41.18 pg/ml) compared to latent TB infection(147.14±43.99 pg/ml), (p<0.001) as shown in the table (3.14) and figure (3.3).

Table (3.14): Serum levels of Interleukin-17A among studied groups

	Groups			P-value		
IL-17A pg/ml	ATB N=60	LTBI N=60	AHC N=40	ATB vs. AHC	LTBI vs. AHC	ATB vs. LTBI
Mean	107.95	147.14	122.14	0.069† NS	0.002† S	<0.001 †HS
SD	41.18	43.99	39.80			
Range	51.76- 202.46	90.59-300.38	60.30-215.64			

SD: standard deviation; †: one-way ANOVA; ATB: Active TB; LTBI: Latent TB infection; AHC: Apparently Healthy controls; HS: highly significant at P ≤ 0.001 , NS: not significant at P ≥ 0.05.

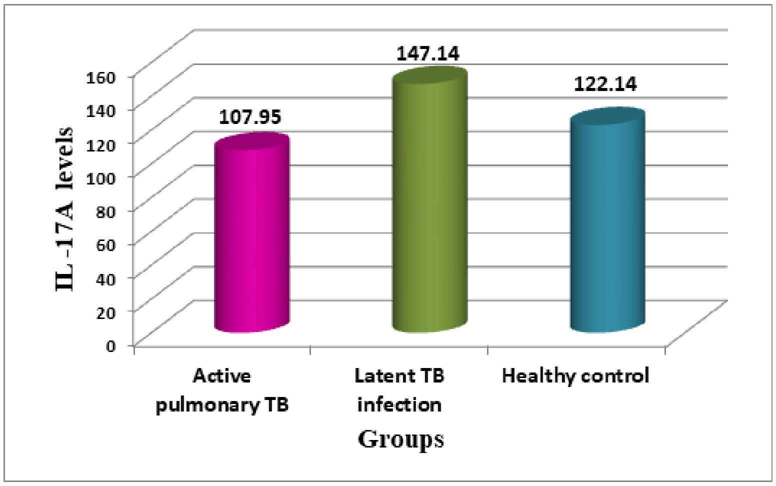


Figure (3.3): Distribution of patients with TB , Latent TB infection and apparently healthy controls groups according to the mean level of serum IL-17A.

ROC analysis was performed to determine the discriminatory power of serum IL-17A. As shown in figures (3.4), IL-17A showed significant discriminatory power with high AUC values, sensitivity and specificity in discriminating latent TB from apparently healthy control (IL-17 A cutoff >81.50, sensitivity 98 % , specificity 95 % , AUC=0.993(excellent), P-value= 0.002) . ATB vs. LTBI comparison , the IL-17 A cutoff value was <117.50 with sensitivity, specificity and Area under curve of 60%, 71%, and 0.756(fair), respectively .In addition, IL-17A displayed no significant discriminatory power between active TB vs. apparently healthy control (IL-17 A cutoff ≤ 116,sensitivity 65 % , specificity 50 % , AUC=0.608,P=0.06).

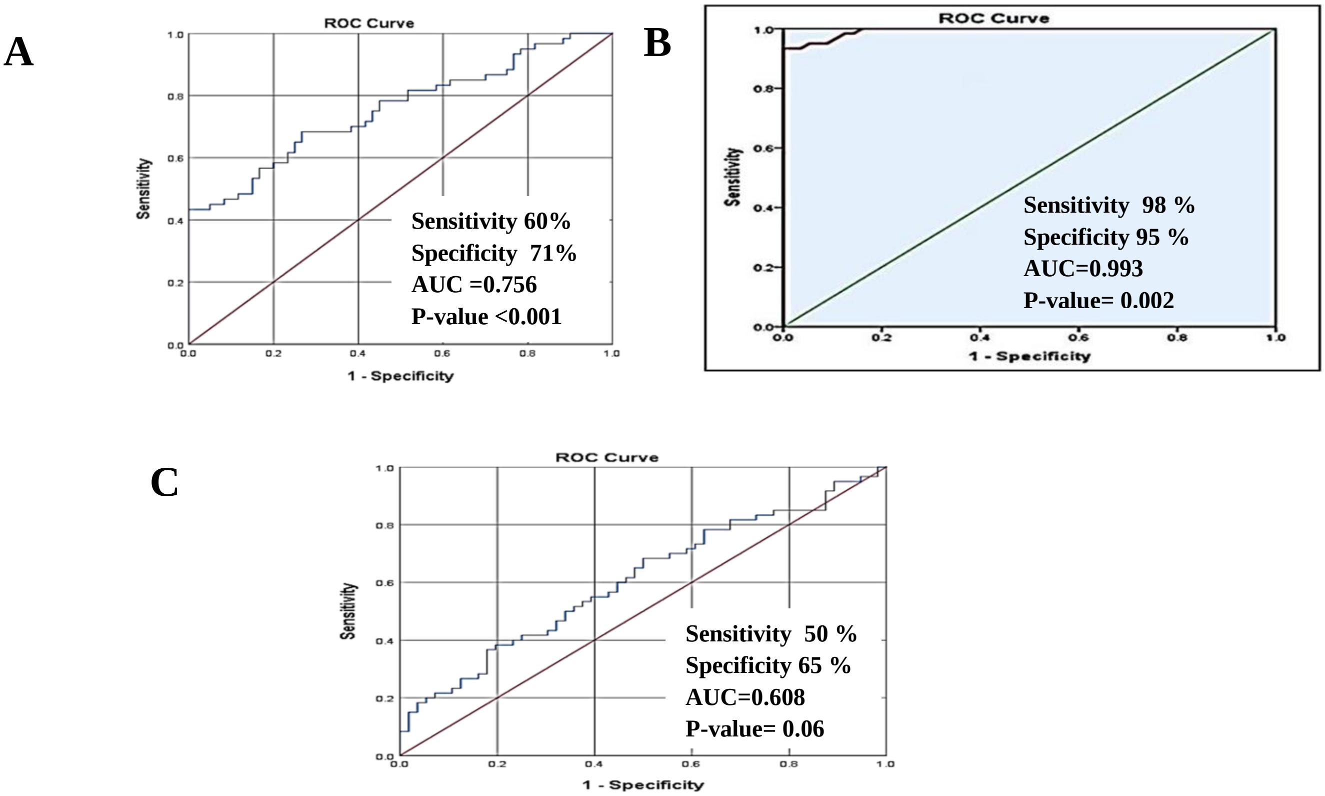


Figure (3.4) : ROC analysis to estimate the sensitivity, specificity and AUC was performed using IL-17A to estimate the capacity of this parameter to distinguish individuals with (A) ATB vs. LTBI (B) LTBI vs. AHC (C) ATB vs. AHC.

Interleukin 17 A cytokine is a proinflammatory cytokine that primarily acts as a mediator of resistance to extracellular bacteria and fungi. However, this cytokine has also been linked to host immunity to tuberculosis and the production of autoimmune and inflammatory disorders. IL-17A plays a role in the formation and maintenance of granulomas by stimulating the production of chemokines, which in turn assists in the recruitment of inflammatory cells that are migrating to Mtb-infected sites (Shen and Chen,2018; Matsuzaki and Umemr, 2018).

In line with the present results when comparing active TB with the apparently healthy control, local studies by Al-Dolaimi, (2019) and Hafid and Ismael, (2021) showed that an insignificant variation between pulmonary tuberculosis patients and apparently healthy control in the serum level of IL17A. In addition to this, no difference in IL17 production has been reported between apparently healthy control and patients with active tuberculosis (Marín *et al*.,2013).

The present findings showed that IL-17A levels were increased in LTBI group compared to those of apparently healthy control. This was compatible with Tekla *et al*.,(2018). A study in India by Kumar *et al*.,(2019), where they are found that the serum levels of IL-17A in LTBI groups increased compared to the apparently healthy controls . In line with present findings when comparing Active TB vs. LTBI groups, a study from Malawi showed higher soluble IL-17 in LTBI compared to active TB (Hur *et al*.,2013). In Iran, a study by Heidarnezhad *et al* .,(2016) displayed that the level of IL-17 is significantly lower in active TB patients in stimulated and non-stimulated PBMCs compared to latent TB in the apparently healthy control. Coulter *et al*.,(2017) reported significantly lower levels of both cell types (Th1/Th17) in active TB pretreatment compared to LTBI, supporting previous findings that IL-17 is increased during early TB infection (i.e., before disease progression).

In contrast with the present results, a local studies by Alfatlawi *et al*.,(2021), Kumar *et al*.(2021) and reported that IL-17A was significantly increased in active TB in comparison with apparently healthy control. Wang *et al* .,(2014) who showed that the following factors may be important: The varied immunity of the selected experimental subjects may play a part in the inconsistency; different sampling times may also be an important factor. Sampling from subjects may happen before treatment, after treatment or during treatment, which could also lead to the discrepancy in results; The choice of different experimental methods also had a certain influence on the results.

It is unclear why latent and active TB-infected subjects respond differently to Mtb. One of the possible explanations for this issue is that CD4 T producing IL-17 cells from individuals with LTB and active TB recognize different antigens at different phases of the disease (Voskuil *et al*.,2003). It has also been shown that in LTB individuals, depletion of regulatory T cells increased the production of IL-17 and IL-23, suggesting regulatory T cells might be involved in the differences in the levels of IL-17 between active and LTB subjects. Thus, the differences in the level of IL-17 in different studies in TB might be attributed to the stage of disease and the number of regulatory T cells and the production of CTLA-4 and FOXp3 (Bandaru *et al*.,2014). The differences in the environmental exposure to Mtb should also be taken into account.

Several reports have demonstrated the protective role of IL-17A during the early stages of TB infection, contributing to the recruitment of neutrophils and IFN-γ secreting cells to the site of infection to establish an effective memory response (Etna *et al*.,2014). The higher serum level of IL-17A in latent TB infection groups compared to active pulmonary TB and apparently healthy control indicate its importance in immunity against TB, and about 90% of MTB-exposed individuals can change MTB infection to latent TB infection (LTBI) (Heidarnezhad *et al*., 2016).

In addition, serum levels of IL-17 were lower in patients with active TB than in those with LTBI again suggesting that the lack of IL-17 may either predispose to active TB disease or be a consequence of it . IL-17 can activated localized tissue cells to secrete the chemokine CXCL8 which attracts neutrophils ,phagocyte and T-cells to the site of infections . Thus, the role of Th17 cells during primary Mtb infection remains unclear it (Li *et al*., 2012). The decrease of IL-17A production correlated with the exhaustion of T cells due to the overexposure to Mtb antigens and hyper expression of the exhausted marker programmed death1 (PD-1) (Khan *et al.*,2017). Marin *et al.*,(2010) reported the frequency of regulatory T cells increased in patients who suffered from activate TB infection in comparison with latent tuberculosis infection. It seems that Treg cells inhibited a protective response and resulted in bacteria spreading.

Furthermore, the results showed that IL-17A had a high discriminative power in the ROC analysis to discriminate between Latent TB and apparently healthy control. These findings were in agreement with Teklu *et al.*,(2018) who documented that IL-17A may be a complementary or alternative marker for LTBI diagnosis. To generalize the importance of IL-17A, and IL-18 as markers differentiating TB disease states and/or LTBI from apparently healthy individuals, the cohorts will need to be expanded and diversified. If such studies confirm some of these preliminary results, biomarker validation studies with thousands of clinical samples can be conducted. The ultimate goals are specific, less expensive, point-of-care immunological tests for the diagnosis of TB in symptomatic and asymptomatic stages.

3.5 Molecular study

3.5.1 Purity and concentration of DNA extracted from studied groups

The extracted human DNA genome from whole blood of all samples showed a DNA concentration from 80- 170 (ng/ µL) with a purity (1.8 -2.0). Then, human genomic DNA was electrophoresed with agarose gel to detect DNA integrity; electrophoresis had been at 70 volts for 45 minutes on 1 % agarose gel, as indicated in the figure (3.5).

Figure (3.5): Gel-electrophoresis of gDNA extracted from blood of active TB group (Lane:1-10) , latent TB groups (Lane:11 -17) and apparently healthy control (Lane:18-23) , electrophoresis was done on on 1% agarose, TBE buffer (1X) ,70 V for 45 min.

3.5.2 SNPs genotyping of IL-18, IL-17A and SP110 genes

3.5.2.1 Screening of Single nucleotide polymorphism at IL-18 -607C/A (rs1946518) by AS-PCR

The AS-PCR technique detected the distribution of IL18-607C/A (rs1946518) polymorphism. The results of AS-PCR showed polymorphism in IL18-607C/A; three genotypes were observed in active patients, the Latent group and the apparently healthy control group: CC, CA and AA genotypes. To examine the results, the first band was to observe the internal control. The internal control band must be present in all reactions, and the PCR product size of internal control was 301 bp. Where the positive amplification of both PCR reactions exhibited a CA genotype, while positive amplification in only the first PCR reaction revealed a CC genotype, the positive amplification in the second reaction only showed a AA genotype of IL-18 rs1946518 and the PCR product size was 196 bp as shown in figure (3.6), (3.7) and (3.8).

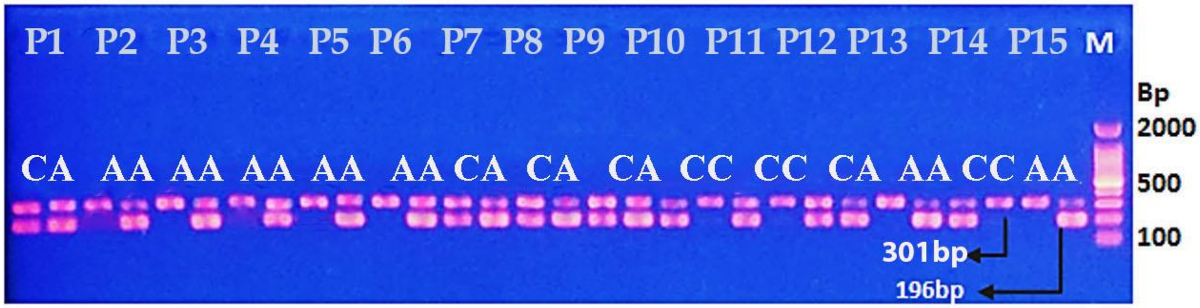


Figure (3.6): Agarose gel electrophoresis image that showed the AS-PCR product analysis of IL-18 -607C/A (rs1946518) gene polymorphism of active TB patient group. The 196 bp represents the amplification of IL-18-607C/A, while the 301 bp representing the internal positive control. Every two adjacent lanes represent a single sample. Gel electrophoresis carried on agarose gel (2%), 70 V for 50 min., M: 100 bp DNA marker for the first step.Lanes:P1 ,P7,P8,P9,P12 ; CA genotype ; lanes: P10,P11,P14; CC genotype ; lanes: P2,P3,P4,P5, P6,P13,P15; AA genotype .

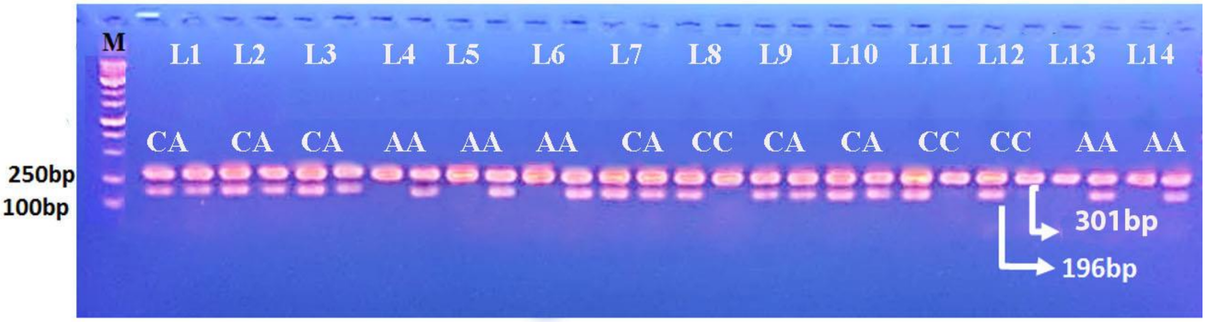


Figure (3.7): Agarose gel electrophoresis image that showed the AS-PCR product analysis of IL-18 -607C/A (rs1946518) gene polymorphism of latent TB group. The 196 bp represents the amplification of IL-18-607C/A, while the 301 bp representing the internal positive control. Every two adjacent lanes represent a single sample. Gel electrophoresis carried on agarose gel (2%), 70 V for 50 min., M: 100 bp DNA marker for the first step. Lanes:L1,L2,L3, ,L7,L9,L10; CA genotype ; lanes:,L8,L11,L12; CC genotype ; lanes: L4,L5,L6,L13,L14; AA genotype.

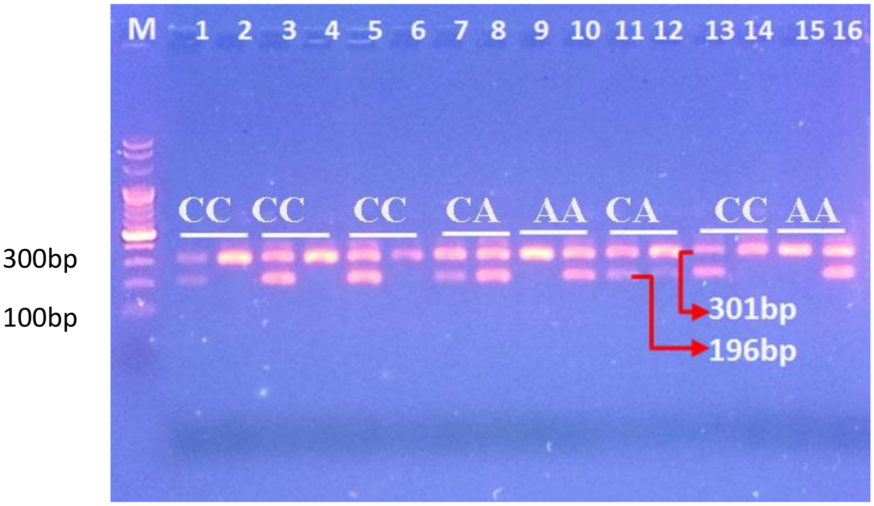


Figure (3.8): Agarose gel electrophoresis image that showed the AS-PCR product analysis of IL-18 -607C/A (rs1946518) gene polymorphism of apparently healthy group. The 196 bp represents the amplification of IL-18-607C/A, while the 301 bp representing the internal positive control. Every two adjacent lanes represent a single sample. Gel electrophoresis carried on agarose gel (2%), 70V for 50 min., M: 100 bp DNA marker for the first step. Lanes:7-8,11-12; CA genotype ; lanes:1-2,3-4,5-6,13-14; CC genotype ; lanes 9-10,15-16; AA genotype.

To confirm the results, thirty six samples were sent to the macrogen company (South Korea) to read the DNA sequencing of amplicons for SNPs detection by the sanger method after the amplified targeted region of IL-18 by conventional PCR. The results of conventional PCR showed success in the primer pair efficiency to amplify partial sequences of IL-18 gene, including SNPs: rs1946518 C>A, the amplification region with flanking primers and PCR products size was 567bp, as shown in the figure (3.9).AS-PCR genotyping assay results were confirmed by sanger sequencing analysis, as shown in figures (3.10).

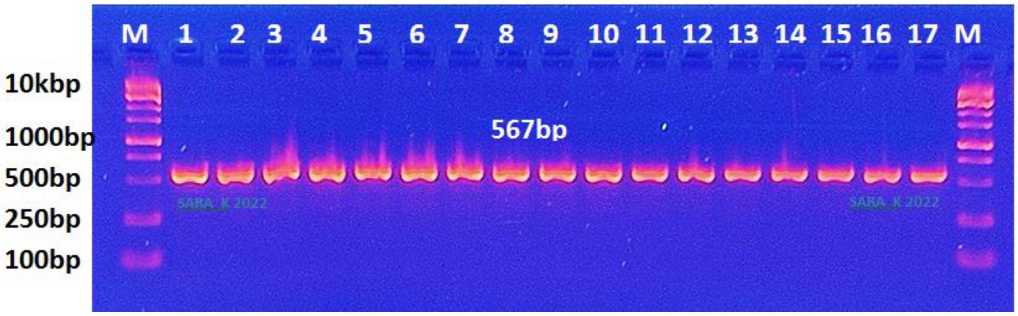
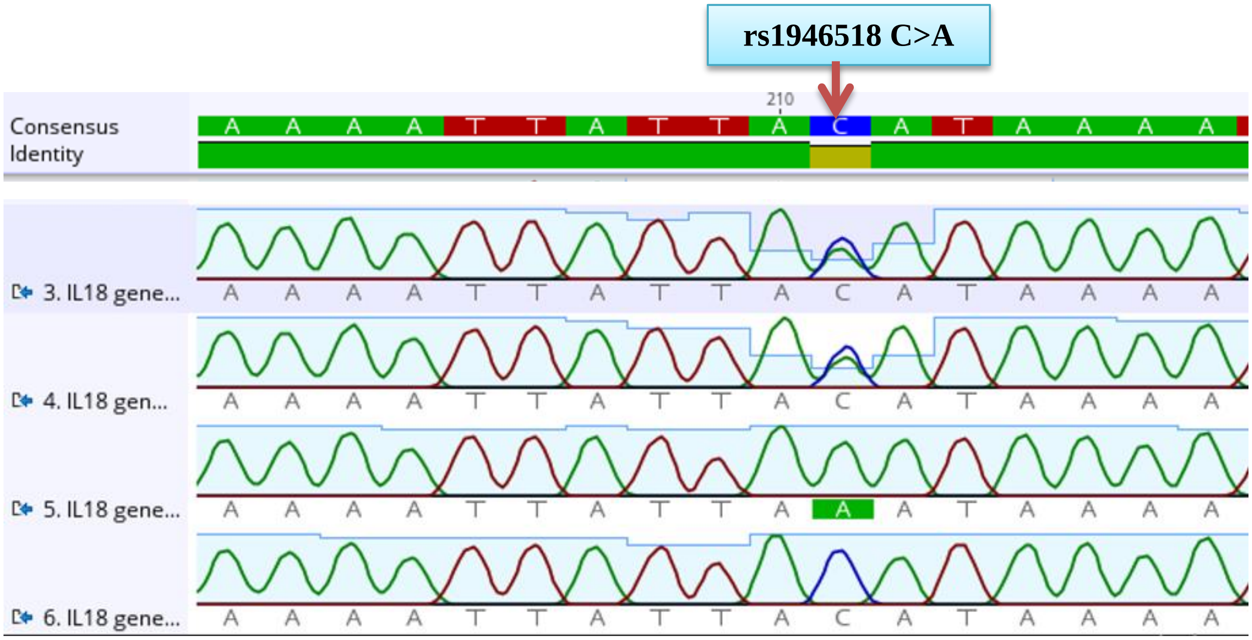


Figure (3.9): Image of an agarose gel electrophoresis displaying the PCR products obtained from amplifying of partial sequence of IL18 included SNPs: rs1946518 C>A, PCR product size is 567 bp, M: molecular marker 100bp for the first step, Lane:1-10 for Active TB group, Lane:11-17for Latent TB group. Electrophoresis was done on 1% agarose, TBE buffer (1X), and 100 V for 45 min.



Figure(3.10): Sanger sequencing results. Multiple alignment Chromatograms of partial sequences of IL-18 gene included SNPs: rs1946518 C>A. Alignment performed by Geneious prime software.

3.5.2.2 Genotype and allele distribution of polymorphisms in the *IL-18* promoter region among the studied cohort

To determine the association between IL-18 -607C/A (rs1946518) and susceptibility to active and latent TB infection, Genotypic & allelic frequencies of the studied cohorts were calculated. In the apparently healthy control, the genotype distribution had no deviation from Hardy-Weinberg equilibrium (P >0.05), as shown in the table (3.15) and table (3.16). The frequencies of CC, CA and AA genotypes at position -607 C/A were 42.5%,37.5% and 20% ; respectively in apparently healthy control, compared to 31.7% ,45% and 23.3% ; respectively in latent TB infection (LTBI) group .The frequencies of CC, CA and AA genotypes were 16.7% ,46.7% and 36.6%; respectively, in patients with active pulmonary tuberculosis (ATB).

In active TB patients versus apparently healthy control, the A- allele of rs1946518 frequency was more common in TB patients than in healthy control (60% vs. 38.75%) with OR value of 2.37 and this difference was statistically significant (p=0.003) as shown in table (3.15). The AA genotype (OR: 4.67,95% Cl : 1.52 - 14.40, p=0.007) and CA genotype (OR: 3.17,95% CI: 1.17 - 8.64, P=0.024) were significantly associated with active TB risk . In the LTBI group versus the apparently healthy control, the results showed that the A- allele was more frequent in the latent TB group than apparently healthy control (45.83% vs. 38.75%) with OR value of 1.34, but this difference did not reach statistical significance (p=0.322), and C allele had lower frequency (54.17% vs. 61.25 %) in latent TB group than in apparently healthy control. Also, the frequency of the genotypes at a position -607C/A (rs1946518) did not show significant differences between LTBI groups and apparently healthy control, as shown in table (3.16).

In active TB patients vs. LTBI group, the results found that TB patients had a higher frequency of the A- allele than latent TB group (60% vs. 45.83%) with OR value of 1.77 and this difference was statistically significant (P = 0.028). The mutant homozygous genotype AA of rs1946518 was significantly associated with active TB risk (OR: 2.99, 95% CI: 1.08 - 8.26, p = 0.035), as shown in table (3.17). The frequency of the C -allele was 61.25%, 54.17% and 40% in healthy individuals, latent TB , and active TB, respectively and C -allele had been used as a reference for comparison.

Table (3.15): Association between IL-18- 607C>A (rs1946518) genotypes and TB risk in Active TB patients vs. apparently healthy control and allele frequency distribution.

SNPs	Genotype	Active TB (n=60)	AHC (n=40)	OR (95%CI)	P-value
IL-18-607C/A rs1946518	CC	10 (16.7%)	17 (42.5%)	**Reference**	
	CA	28 (46.7%)	15 (37.5%)	3.17(1.17 - 8.64)	***0.024***
	AA	22 (36.6%)	8 (20%)	4.67 (1.52 - 14.40)	***0.007***
	HWE-*p*	0.82	0.18		
Allele frequency	C	48 (40%)	49 (61.25%)	**Reference**	
	A	72 (60%)	31 (38.75%)	2.37(1.33 - 4.23)	***0.003***

OR: odds ratio; CI: confidence interval; S: significant at P < 0.05. Values in boldface are statistically significant. HWE-p: P value for Hardy Weinberg Equilibrium. AHC: apparently healthy control.

Table (3.16): Association between IL-18- 607C>A (rs1946518) genotypes and LTBI risk in LTBI cases vs. apparently healthy control and allele frequency distribution.

SNPs	Genotype	Latent TB (n=60)	AHC (n=40)	OR(95%CI)	P-value
IL-18-607C/A rs1946518	CC	19 (31.7%)	17 (42.5%)	**Reference**	
	CA	27 (45%)	15 (37.5%)	1.61(0.65 - 4.00)	0.304
	AA	14 (23.3%)	8 (20%)	1.57(0.53 - 4.65)	0.419
	HWE-*p*	0.4679	0.18		
Allele frequency	C	65 (54.17%)	49 (61.25%)	**Reference**	
	A	55 (45.83%)	31 (38.75%)	1.34 (0.75 -2.38)	0.322

OR: odds ratio; CI: confidence interval; S: significant at P < 0.05. HWE-*p* :P Value for Hardy Weinberg Equilibrium. AHC: apparently healthy control.

Table (3.17): Association between IL-18- 607C>A (rs1946518) genotypes and TB risk in Active TB patient vs. LTBI cases and allele frequency distribution.

SNPs	Genotype	Active TB (n=60)	Latent TB (n=60)	OR(95%CI)	P-value
IL-18-607C/A rs1946518	CC	10 (16.7%)	19 (31.7%)	**Reference**	
	CA	28 (46.7%)	27 (45%)	1.97(0.78 - 5.00)	0.153
	AA	22 (36.6%)	14 (23.3%)	2.99 (1.08 -8.26)	***0.035***
Allele frequency	C	48 (40%)	65 (54.17%)	**Reference**	
	A	72 (60%)	55 (45.83%)	1.77 (1.06 - 2.96)	***0.028***

OR: odds ratio; CI: confidence interval; S: significant at P < 0.05. Values in boldface are statistically significant.

Polymorphisms in genes encoding cytokines determine susceptibility to *Mycobacterium tuberculosis* (*M.tb*) infection. In particular, interleukin-18 (IL-18), an inducer of interferon-gamma (IFN-γ), playing an essential role in anti-mycobacterial immune responses, may influence the risk of developing active tuberculosis (TB). The previous study has shown that genetic Polymorphism of IL-18-607C/A (rs1946518) in the promoter of the IL-18 gene may affect the immune response and is associated with the risk of several kinds of diseases, such as tuberculosis, nasopharyngeal carcinoma, Takayasu arteritis and stroke (Tsai *et al*.,2013; Huang *et al*., 2018;Wen *et al*.,2020;).

Numerous studies have evaluated the association between interleukin-18 promoter gene polymorphism (rs1946518) and tuberculosis (TB) risk. However, the results remain conflicting (Li *et al*.,2013).

The results of the present study were consistent with Han *et al*.,(2011). They found that allele A of rs1946518 confers a 1.47-fold increased risk of developing TB, and the AA+CA genotypes of rs1946518 are significantly associated with TB risk.

On the other hand , Hasan and Naif (2017) reported that IL-18-607AA mutant genotype and A alleles were significantly associated with a high risk of HBV infection in the Iraqi population. Zakariya *et al*.,(2022) showed that Iraqi women with the A allele had a 5.03-fold increased susceptibility to breast cancer. Li *et al*.,(2012) found that individual carriers of the A allele at position -607 have a 28% increased risk of chronic hepatitis B, and the risk of chronic hepatitis B is increased by about 53% in individuals with the AA genotype at position 607.

In contrast, these observations are inconsistent with the results obtained by Harishankar *et al*. (2007) in South India, Taheri *et al*., (2012) in Southeast Iran and Zhou *et al*.,(2015) in Chinese Han populations. Furthermore, Zhen *et al*.,(2019) conducted a meta-analysis of five case-control studies involving 1293 TB cases and1724 healthy subjects for the IL-18 (rs1946518) Polymorphism. They found no association between this variant and TB susceptibility.

The discrepancies between the findings of this study and those of previous studies could be due to many factors. First, the limited sample size in the genetic analysis is always a major issue, resulting in little power to detect minor effects of the various genetic factors studied. A second reason for the discrepancies may be ethnicity-related differences in gene polymorphisms. The third possibility is differences in gene-environment interactions among different ethnic groups.

The SNP at position -607 was predicted to lie within a potential cAMP-responsive element binding protein (CREB) binding site, The cAMP-response element binding protein (CREB) is localized in the nucleus and acts as a transcription factor, which binds to the cAMP response element (CRE) of the promoters of its target genes. Once CREB is activated and CREB-binding protein (CBP) is recruited, transcription is initiated, with the A allele possibly reducing the binding specificity of this motif. Indeed variation at –607 C/A of IL-18 gene disrupts cAMP-responsive element-binding protein binding site, influence the quantity of transcribed IL-18 mRNA (Giedraitis *et al*.,2001). As was shown, IL-18 deficiency increases the susceptibility to mycobacterial infection. Kinjo *et al*., (2002) demonstrated that IL-18 knockout mice, characterized by attenuated production of IFN-γ, were more prone to Mtb infection than wild-type individuals. Therefore, genetic variations within the IL-18 gene might potentially predispose to the development of TB in humans through their influence on the expression of the cytokine and, subsequently, IFN-γ-mediated Th1 response (Wawrocki *et al*.,2019).

IL-18 gene polymorphisms are associated with TB susceptibility and this suggests that the common allelic variant of IL-18 is likely to contribute to TB susceptibility . Further work on deep re-sequencing of the IL-18 gene is needed to identify the causal variant(s) and reveal the molecular mechanism underlying the association. Such information, together with the known common susceptibility variants, may increase the discriminative value of genetic risk factors and push it towards a threshold that is clinically useful. It will be of utmost interest to validate these findings with higher sample size and to perform functional studies assessing the transcriptional activity of the IL-18 gene (Han *et al*., 2011).

Finally, IL-18 gene polymorphisms are associated with active TB susceptibility and showed the potential to predict the risk of active tuberculosis .Where the individuals carrying the AA +CA genotypes at position IL-18-607 C > A (rs1946518) were more likely to have a significantly increased risk of active TB when compared with the CC genotype.

3.5.2.3 Detection of IL17A (rs2275913 G>A) polymorphism

The AS-PCR technique detected the distribution of IL17A (rs2275913 G>A) polymorphism. At this locus, there were three genotypes; GG, GA and AA, in which The wild-type homozygote genotype showed only G allele amplification at 317 bp product size. The mutant homozygote genotype showed only A allele amplification at 317 bp product size. The heterozygote genotype showed A and G alleles amplification at 317 bp product size, respectively, as shown in the figures (3.11) and (3.12).

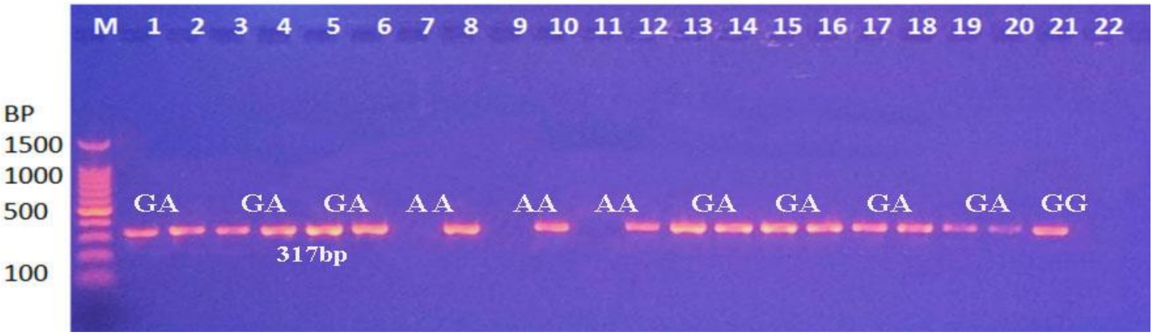


Figure (3.11): Agarose gel electrophoresis image that showed the AS-PCR product analysis of IL-17A (rs2275913 G > A) gene polymorphism of active TB patient group. The presence of the G or A allele was observed at 317 bp product size. Every two adjacent lanes represent a single sample. Gel electrophoresis was carried on agarose gel (2%), 70 V for 50 min, Where M: 100 bp DNA marker for the first step. Lanes: 1-2,3 -4,5 -6,13-14,15-16,17-18,19-20; GA genotype ; lanes:7-8,9 -10,11-12; AA genotype ; lanes:21-22; GG genotype.

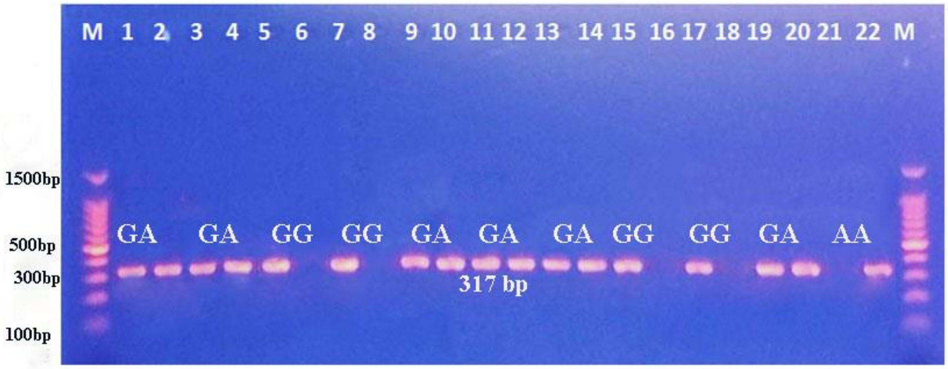


Figure (3.12): Agarose gel electrophoresis image that showed the AS-PCR product analysis of IL-17A (rs2275913 G > A) gene polymorphism of latent TB group. The presence of the G or A allele was observed at 317 bp product size. Every two adjacent lanes represent a single latent sample. Gel electrophoresis carried on agarose gel (2%), 70 V for 50 min, Where M: 100 bp DNA marker for the first step. Lanes: 1-2,3-4,9-10,11-12,13-14,19-20; GA genotype ; lanes:21-22; AA genotype ; lanes: 5-6,7-8,15-16,17-18 ; GG genotype.

To confirm the results, thirty six samples from each genotype's shape were sent to Macrogen company (South Korea) to read the DNA sequencing of amplicons for SNPs detection by the Sanger method after amplifying the targeted region of IL-17A by conventional PCR. The results of conventional PCR showed success in the primer pair efficiency to amplify the partial sequence of IL17A gene, including SNPs: rs2275913 G>A, the amplification region with flanking primers and PCR products size was 886bp, as shown in the figure (3.13). AS-PCR genotyping assay results were confirmed by Sanger sequencing analysis, as shown in the figures (3.14), (3.15), and (3-16).

Figure (3.13): Image of an agarose gel displaying the PCR products obtained from amplifying of partial sequence of IL17A included SNPs:rs2275913 G>A, PCR product size is 886bp, M: molecular marker 100bp for the first step, Lane:1-7 for active TB group, Lane:8 -14 for Latent TB group, Lane:15-20 for the apparently healthy control. Electrophoresis was done on 1% agarose, TBE buffer (1X), and 100 V for 45 min.

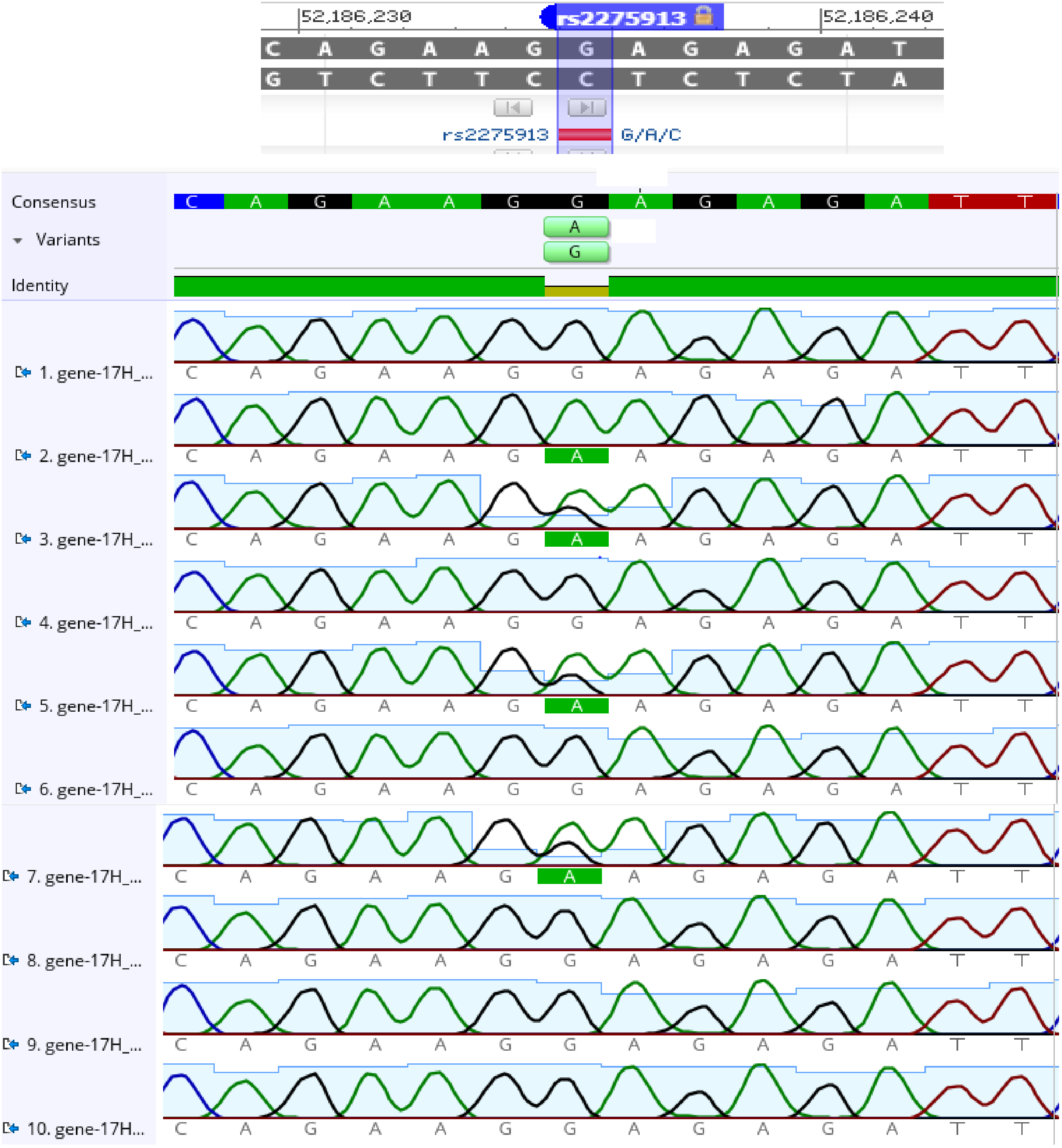


Figure (3.14): Sanger sequencing results .The multiple alignments of chromatograms of partial sequences of the IL-17A gene shown SNPs: rs2275913 G>A. 1-10 apparently healthy control group. Alignment performed by Geneious prime software.

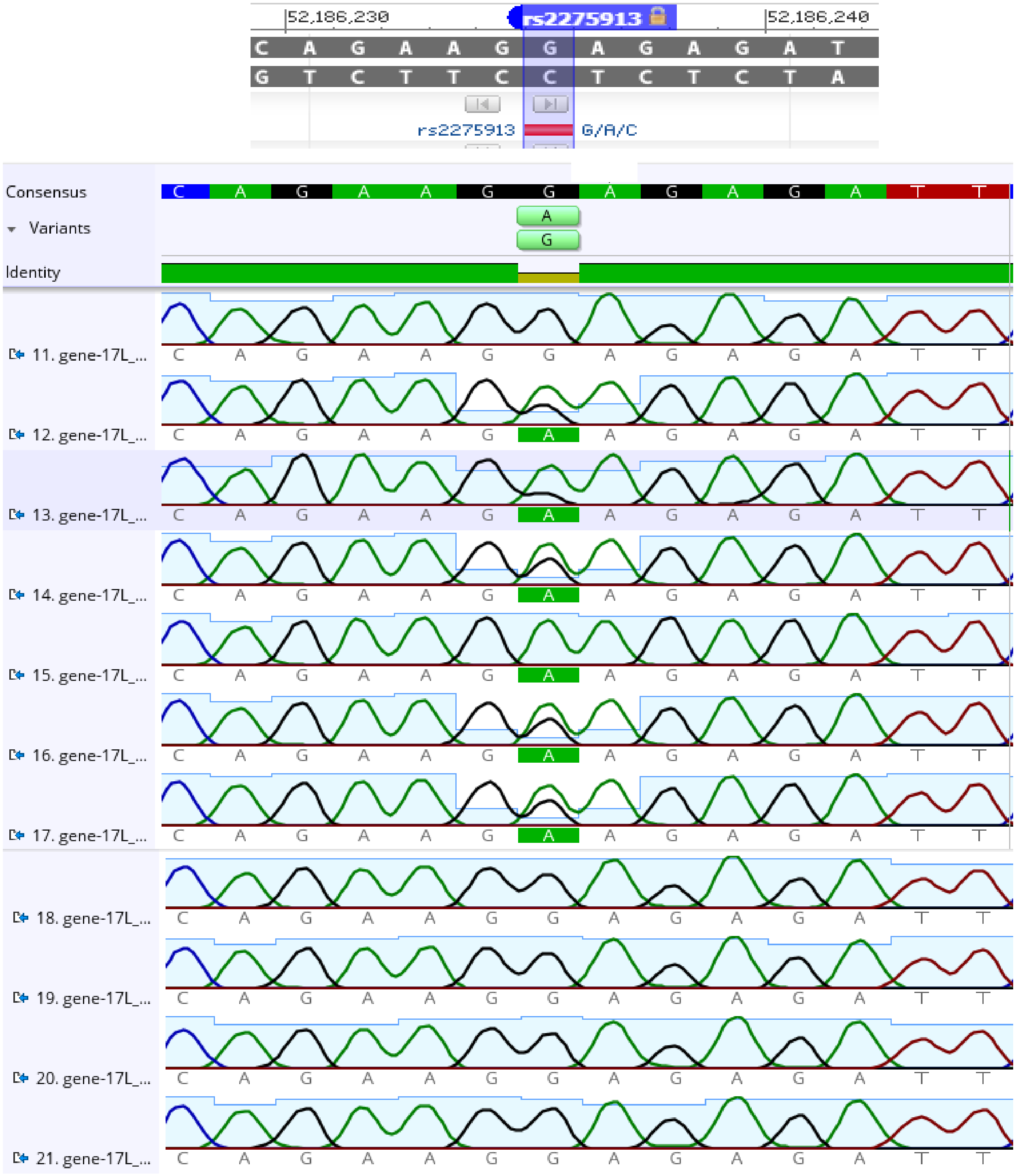


Figure (3.15): Sanger sequencing results. The multiple alignments of chromatograms of partial sequences of the IL-17A gene showed SNPs: rs2275913 G>A. 1-21 Latent TB group. Alignment performed by Geneious prime software.

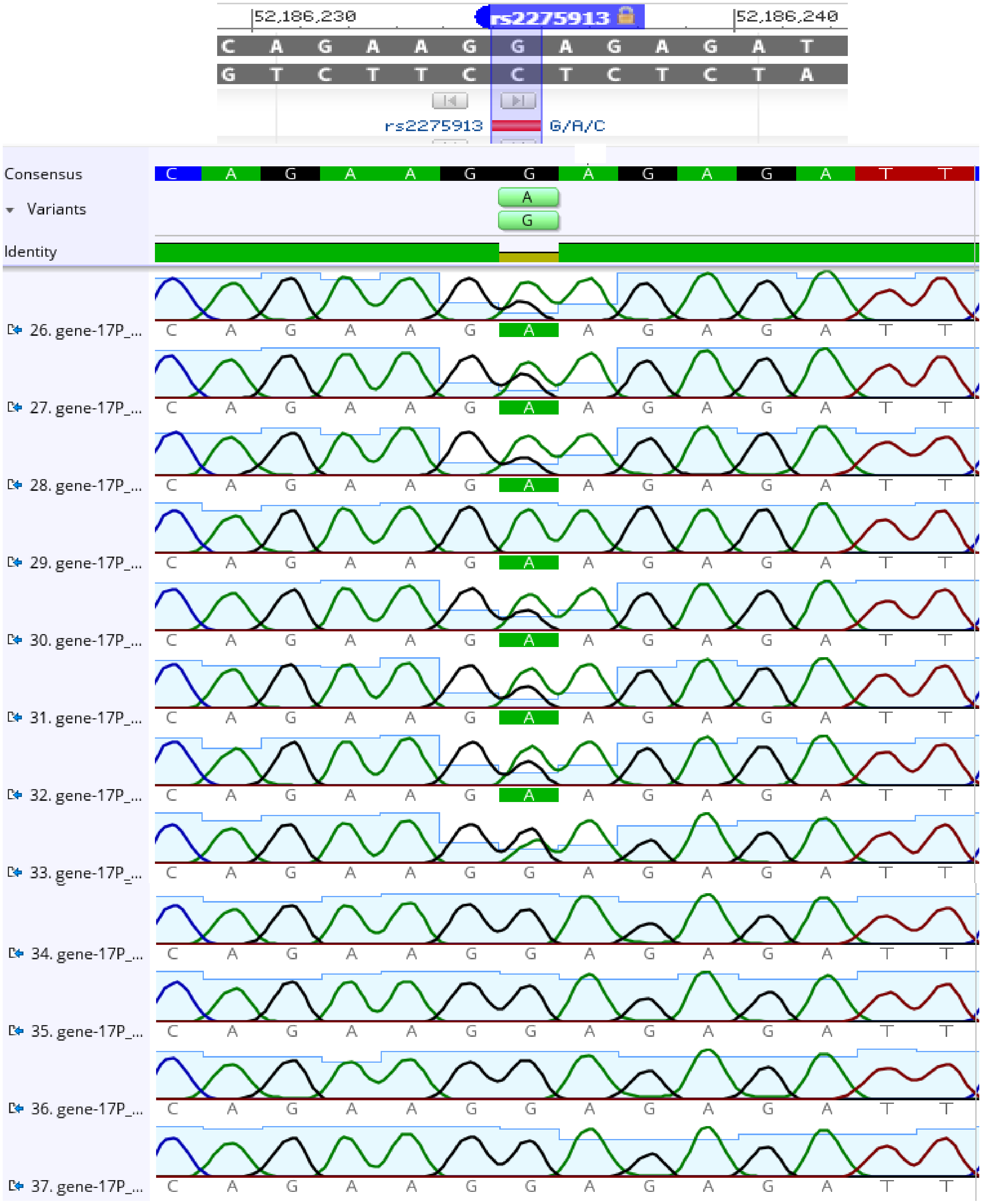


Figure (3.16): Sanger sequencing results. The multiple alignments of chromatograms of partial sequences of the IL-17A gene showed SNPs: rs2275913 G>A. 26-37Active TB group. Alignment performed by Geneious prime software.

3.5.2.4 Genotype and allele distribution of polymorphisms in the IL-17A promoter region among the studied groups

Genotypic and allelic frequencies of the studied cohorts were calculated to evaluate the association between IL-17A rs2275913 polymorphisms of the active and latent TB infection. In control, the genotype distribution had no deviation from Hardy-Weinberg equilibrium (P>0.05).

The results of the present study showed that the frequencies of GG, GA and AA genotypes were 50 %, 35% and 15%, respectively, in apparently healthy controls, compared to 36.6%,46.6% and 16.7%, respectively, in latent TB infection (LTBI) groups. At the same time, the frequencies of GG, GA and AA genotypes were 35%, 38.3% and 26.7%, respectively, in patients with active pulmonary tuberculosis (ATB). On the other hand, the frequency of the A allele of rs2275913 was 32.5 %, 40%, and 45.83%, respectively, in apparently healthy controls, LTBI group and active patients. While the frequency of G -allele in apparently healthy controls, LTBI and active patients was 67.5%, 60%, and 54.17%, respectively and was considered as a reference group as shown in Tables (3-18), (3-19) and (3-20).

In active TB patients versus apparently healthy controls, A-allele had a higher frequency (45.83% vs. 32.5 %) in TB patients than the apparently healthy controls with OR value of 1.76, but the difference did not reach statistical significance (p=0.061), and G had a lower frequency (54.17% vs. 67.5%) in TB patients than apparently healthy controls. In Latent TB versus apparently healthy controls , the frequency of the A allele of rs2275913 was more common (40% vs. 32.5 %) in the LTBI group than in the apparently healthy controls with OR value of 1.38 but the difference did not reach statistical significance (p=0.283) and G had a lower frequency (60% vs. 67.5%) in latent TB group than apparently healthy controls as shown in (3.18) and (3.19).

The present study found there was no statistically significant relationship between genotypes or alleles frequency of IL-17A (rs2275913) in the active TB patients and latent TB infection (LTBI) group compared to the healthy control, as shown in tables (3.18) and (3.19). Also, there was no statistically significant relationship between genotypes or alleles frequency of IL-17A (rs2275913) in the active TB patients compared to the latent TB infection (LTBI) group as shown in table (3.20).

Table (3.18): Association between IL17A (rs2275913 G>A) genotypes and TB risk in active TB patient vs. apparently healthy controls and allele frequency distribution.

SNPs	**Genotype**	**Active TB (n=60)**	**AHC (n=40)**	**OR (95%CI)**	**P-value**
rs2275913 G>A	GG	21(35%)	20 (50 %)	**Reference**	
	GA	23(38.3%)	14 (35%)	1.56 (0.63 - 3.86)	0.332
	AA	16 (26.7)	6 (15%)	2.54 (0.83 -7.79)	0.103
	HWE-*p*	0.07	0.2		
Allele frequency	G	65 (54.17%)	54 (67.5%)	**Reference**	
	A	55 (45.83%)	26 (32.5%)	1.76 (0.97 - 3.17)	0.061

OR: odds ratio; CI: confidence interval; S: significant at $P < 0.05$;HWE-*p* :P value for Hardy Weinberg Equilibrium, AHC: apparently healthy control.

Table (3.19): Association between IL17A (rs2275913 G>A) genotypes and LTBI risk in LTBI vs. apparently healthy controls and allele frequency distribution.

SNPs	**Genotype**	**Latent TB (n=60)**	**AHC (n=40)**	**OR (95%CI)**	**P-value**
rs2275913 G>A	GG	22 (36.7 %)	20 (50 %)	**Reference**	
	GA	28 (46.6 %)	14 (35%)	1.82 (0.75 - 4.39)	0.184
	AA	10 (16.7%)	6 (15%)	1.52 (0.47 - 4.93)	0.490
	HWE-p	0.83	0.2		
Allele frequency	G	72 (60%)	54 (67.5%)	**Reference**	
	A	48 (40%)	26 (32.5%)	1.38 (0.76 to 2.51)	0.283

OR: odds ratio; CI: confidence interval; S: significant at $P < 0.05$;. HWE-*p* :P value for Hardy Weinberg Equilibrium, AHC: apparently healthy control.

Table (3.20): Association between IL17A (rs2275913 G>A) genotypes and TB risk in active TB patients vs. LTBI and allele frequency distribution.

SNPs	Genotype	Active TB (n=60)	Latent TB (n=60)	OR (95%CI)	P-value
rs2275913 G>A	GG	21 (35%)	22 (36.6%)	**Reference**	
	GA	23 (38.3%)	28 (46.6%)	0.86 (0.38 -1.94)	0.717
	AA	16 (26.7)	10 (16.7%)	1.68 (0.62 - 4.51)	*0.307*
Allele frequency	G	65 (54.17%)	72 (60%)	**Reference**	
	A	55 (45.83%)	48 (40%)	1.27 (0.76 -2.12)	*0.362*

OR: odds ratio; CI: confidence interval; S: significant at P < 0.05.

Several studies have reported that Th17-related gene polymorphisms are associated with inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, asthma, Grave's disease, ulcerative colitis, cancer and TB (Kaabachi *et al.*,2014; Shi and Zhang., 2015; Agonia *et al*.,2020).

The present study found no significant association between genotypes or alleles of rs2275913 and TB development. These observations are consistent with many studies. A local study by Ameen *et al*.,(2018) found no significant associations between IL-17A rs2275913 polymorphism and the risk of TB in the Iraqi population, also Peng *et al.*, (2013) found no significant association between rs2275913 polymorphisms and TB risk in the Chinese population , as well as in the Indian population, Tiwari *et al*., (2014) reported that the IL17 gene polymorphisms were not associated with TB.

In contrast, the local study by Shaban *et al*.,(2021) reported that A-alleles of IL-17A (rs2275913) are associated with asthma risk in Iraqi patients. Furthermore Heshem *et al*., (2021) indicated that the A-allele of IL-17A rs2275913G>A polymorphism was associated with breast cancer in Iran women. For association with TB, In the northern Spain population, Ocejo-Vinyals *et al*., (2013) investigated an association between IL-17 rs2275913 polymorphism and susceptibility to TB and suggested that the G-allele of IL-17A rs2275913 was associated with an increased risk of pulmonary tuberculosis.

On the other hand, the A allele of rs2275913 (–197G/A) is associated with TB protection in the Brazilian population (Milano *et al*.,2016) in Argentinean and Caucasian populations (Yu *et al*.,2017; Rolandelli *et al*.,2017). The results confirmed that there are discrepancies in the outcome of the studies, which may be due to differences in genetic backgrounds, study design and sample size.

Espinoza *et al*.,(2011) demonstrated that the rs2275913 SNP is a functional polymorphism that modifies the binding of the transcriptional factor nuclear factor of activated T-cells(NFAT) to the IL-17A promoter. Thus, it affects the amount of RNA transcribed. Indeed, several previous studies reported on the role of IL-17 in TB; however, the factors that influence the disease outcome are unclear. The interaction of Mtb components with innate and adaptive cells produces higher levels of IL-17, which induces tissue damage and the release of other cytokines and chemokines. On the other hand, IL-17 recruits immune cells at the site of infection and restrict pathogenesis (Rapolu *et al*.,2021).

In summary, the present study results suggest that IL-17A rs2275913 (G>A) polymorphisms may not be the risk factors for susceptibility to TB. It will be utmost interesting to validate these findings with a higher sample size and to perform functional studies assessing the transcriptional activity of the IL-17A gene.

3.5.2.5 Association of single-nucleotide polymorphisms in SP110 gene with susceptibility to latent TB infection

A case-control study was conducted to investigate the association of SNPs (rs7580900, rs7580912, rs9061, rs11556887) in the SP110 gene with susceptibility to latent TB infection. SNP genotyping was performed by the Sanger sequencing method in 50 individuals with latent TB infection and 40 apparently healthy controls after amplifying the targeted region of the SP110 gene by conventional PCR.

The results of conventional PCR showed success in the primer pair efficiency to amplification target DNA region of SP110 included two SNPs: rs7580900 T> C and rs7580912 T>C, the amplification region with flanking primers and PCR product size was 715bp, as shown in the figure (3.17).

On the other hand, Amplifying the second targeted region of Sp110 included SNPs: rs9061 G>A and rs11556887C >T. The results of conventional PCR showed success in the primer pairs efficiency to amplification the target DNA region of Sp110, the amplification region with flanking primers and PCR product size was 804bp, as indicated in figure (3,18).

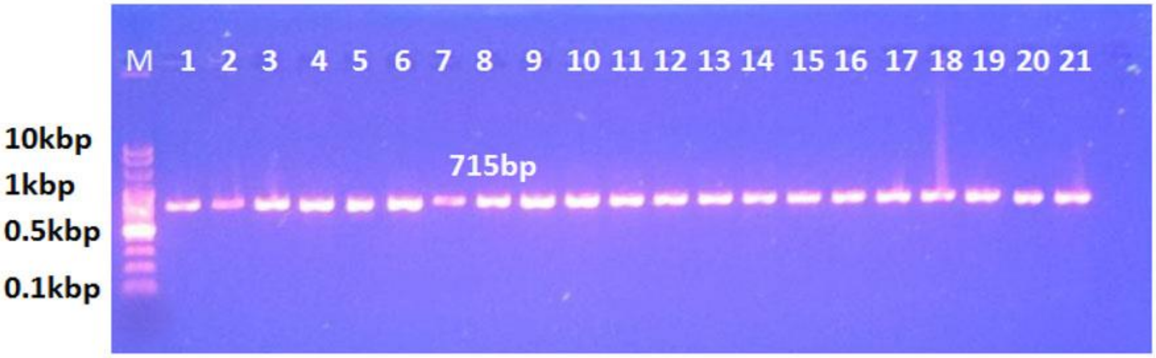


Figure (3.17): Image of an agarose gel electrophoresis displaying the PCR products obtained from amplifying of partial sequence of Sp110 gene included two SNPs: rs7580900 T> C, rs7580912 T>C, PCR product size is 715bp, M=molecular marker 100bp for the first step, Lane:1-12 for latent TB group, Lane:13-21 for apparently healthy controls, electrophoresis was done on 1% agarose, TBE buffer (1X),100 V for 45 min.

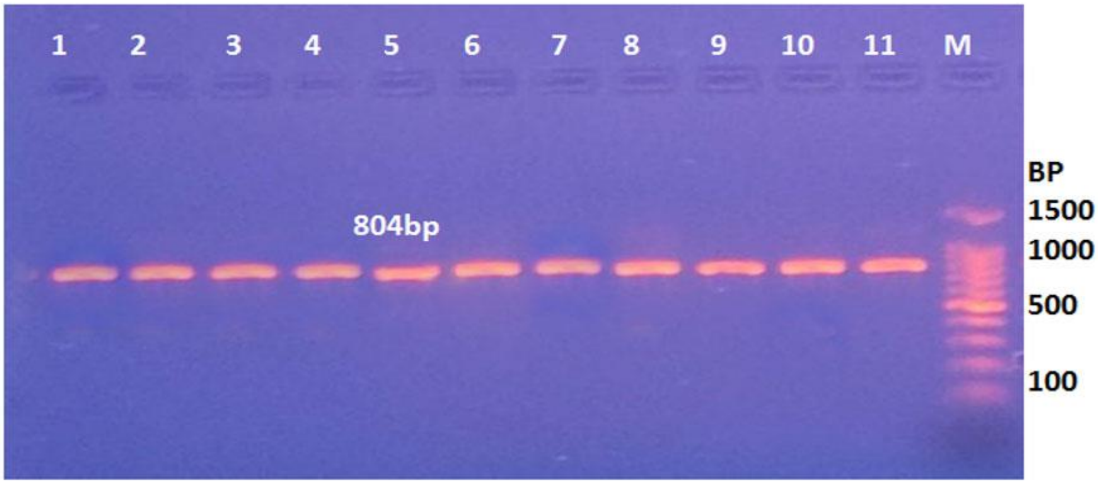


Figure (3.18): Image of an agarose gel electrophoresis displaying the PCR products obtained from amplifying of partial sequence of Sp110 gene included SNPs: rs9061 G>A and rs11556887C >T, PCR product size is 804 bp, M=molecular marker 100bp for the first step, Lane:1 -6 for Latent TB infection (LTBI) group, Lane:7 -11 for apparently healthy controls, electrophoresis was done on 1% agarose, TBE buffer (1X),100 V for 45 min.

According to NCBI data, the SP110 gene (Gene ID: 3431) is located on chromosome:2 on reference sequence NO. NC_000002.12. DNA sequencing in the first region of the SP110 gene revealed two SP110 gene variants: rs7580912 (C>T) and rs7580900 (C>T), with three genotypes, TT, TC and CC, as shown in figures (3.19), (3.20), (3.21) and (3.22). While, DNA sequencing in the second region of the SP110 gene revealed two genetic variants: rs9061 G>A and rs28930679 C>T, while the variant rs11556887 that were not polymorphic and were not further included in the analysis, and then rs9061(G>A) was analyzed. DNA sequencing showed rs9061 G>A with three genotypes GG, GA, AA as shown in figures (3.23) , (3.24), (3.25) and (3.26).

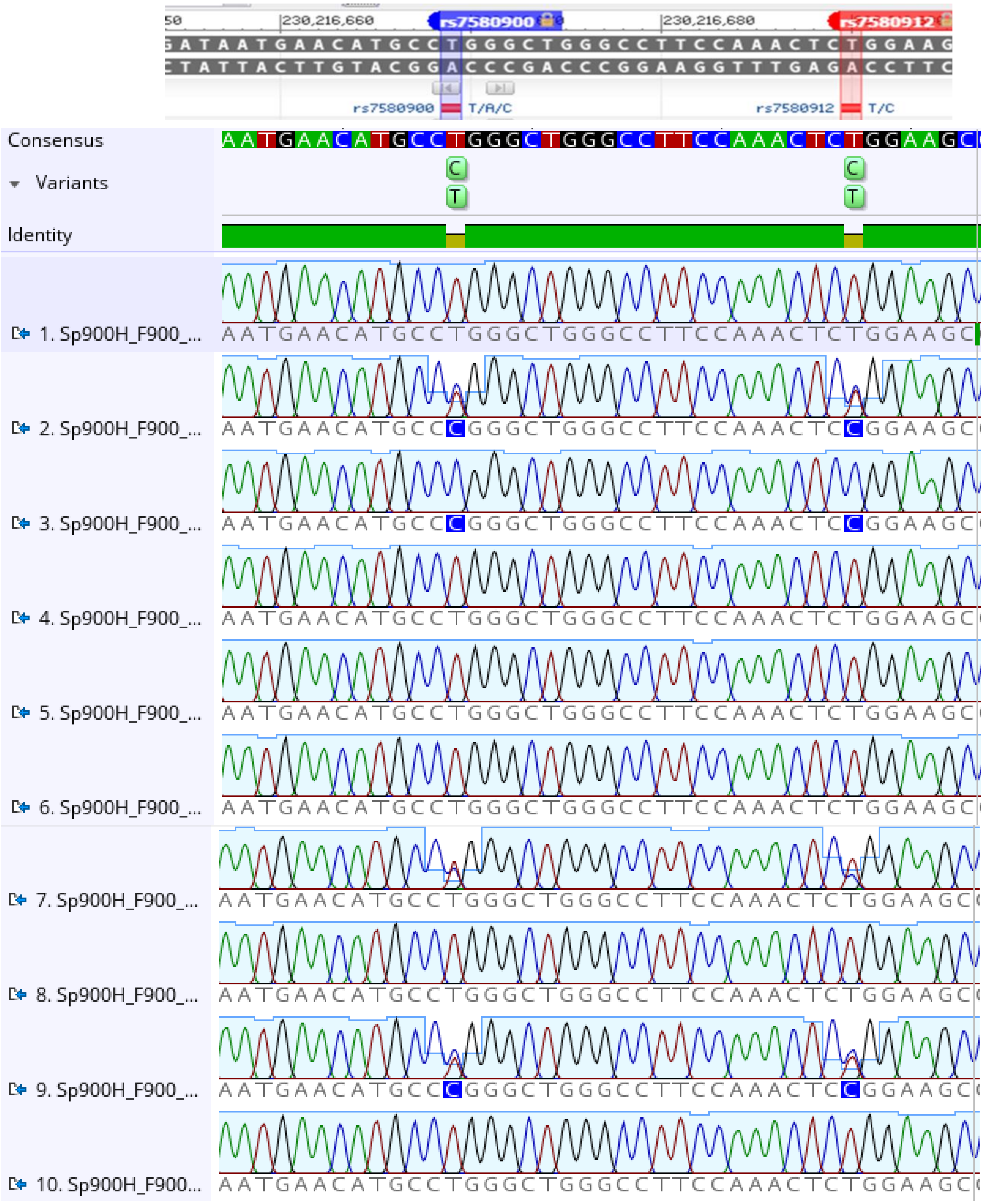


Figure (3.19): Sanger sequencing results . Multiple alignment Chromatograms of partial sequences of Sp110 for the apparently healthy controls. Two SNPs were observed: SNPrs7580912 T>C and rs7580900 T> C. Alignment was performed by Geneious prime software.

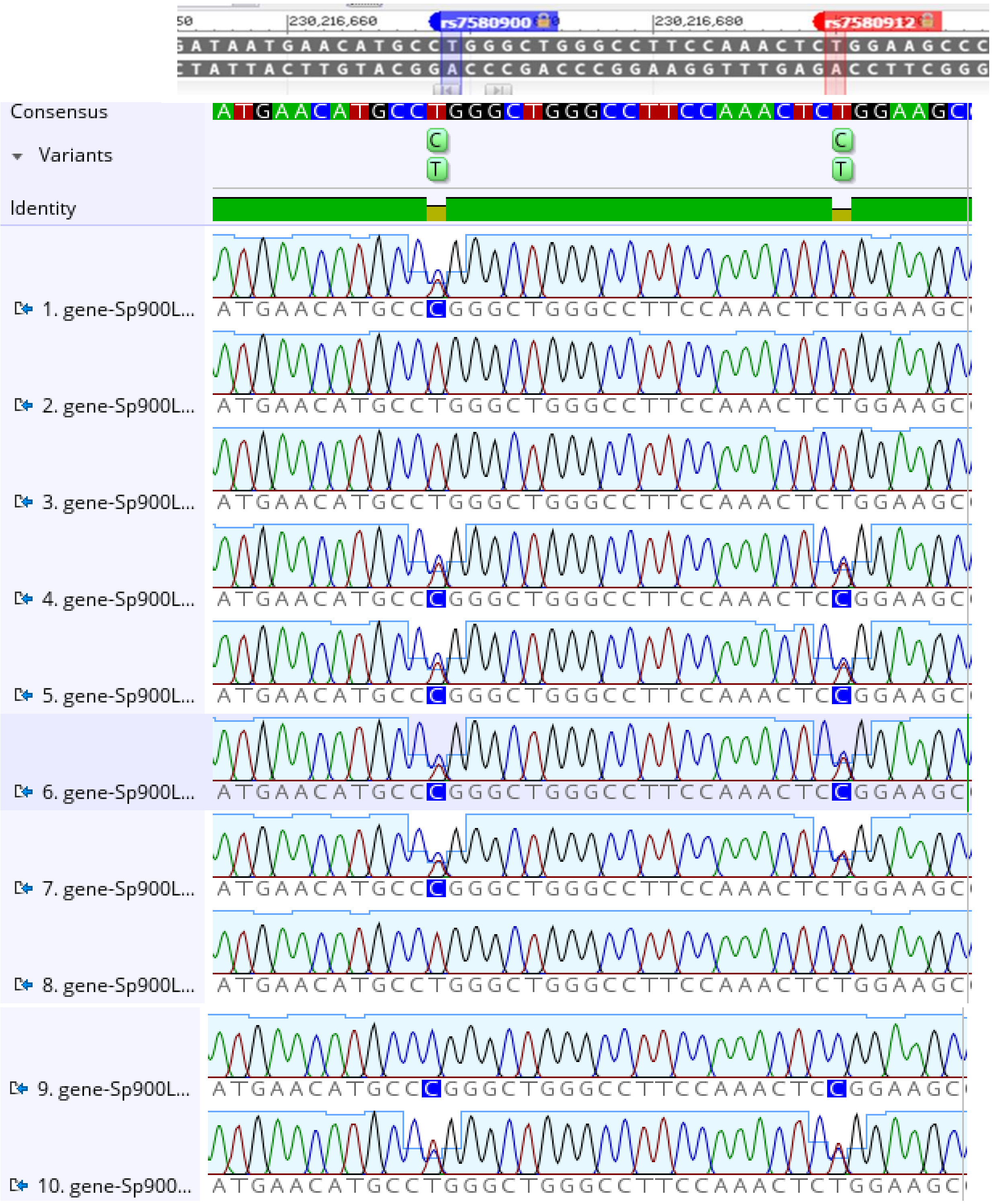


Figure (3.20): Sanger sequencing results. Multiple alignment Chromatograms of partial sequences of Sp110 for latent TB group. Two SNPs were observed: SNPrs7580912 T>C and rs7580900 T> C. Alignment was performed by Geneious prime software.

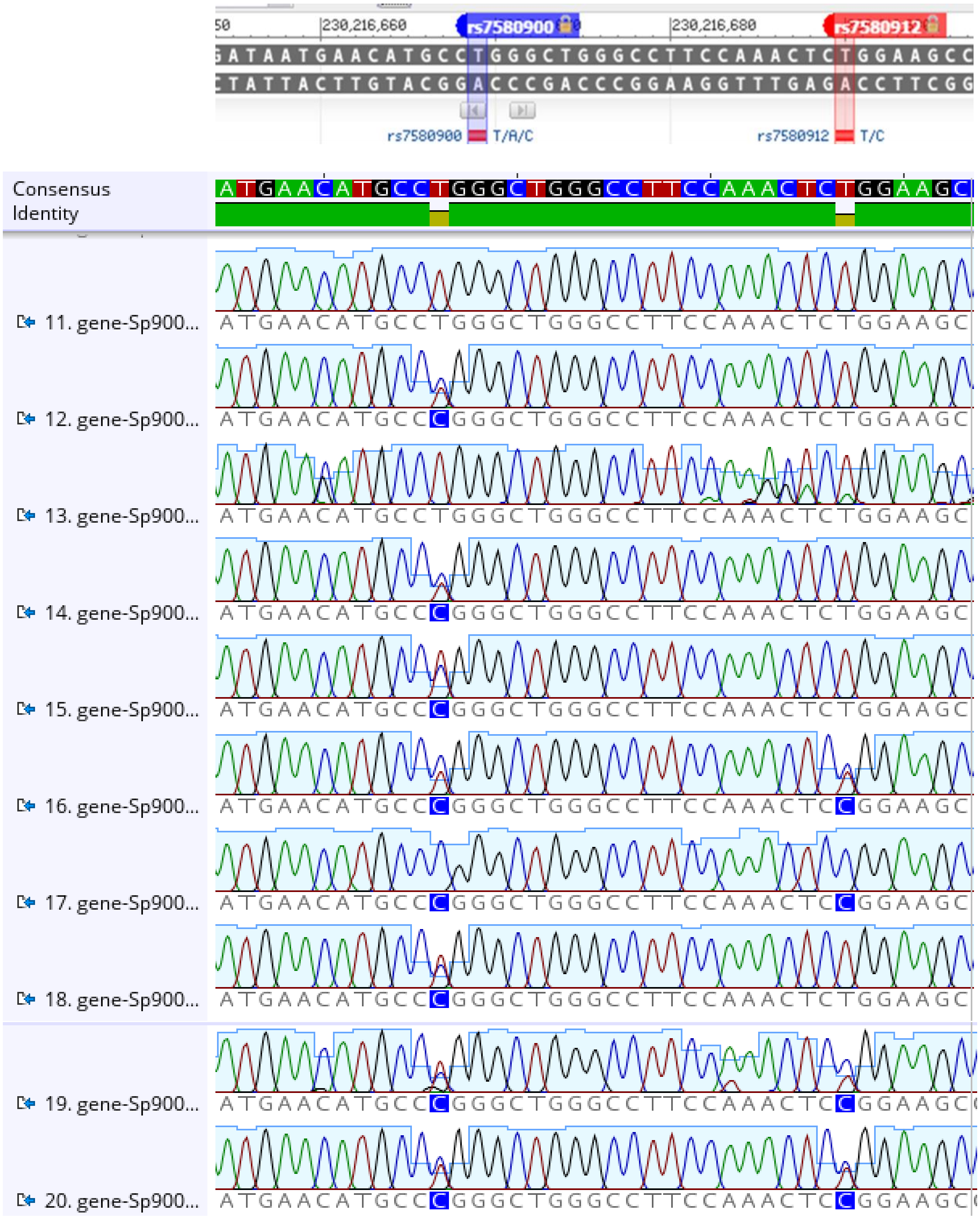


Figure (3.21 continue): Sanger sequencing results. Multiple alignment Chromatograms of partial sequences of Sp110 for latent TB group. Two SNPs were observed: SNPrs7580912 T>C and rs7580900 T> C. Alignment was performed by Geneious prime software.

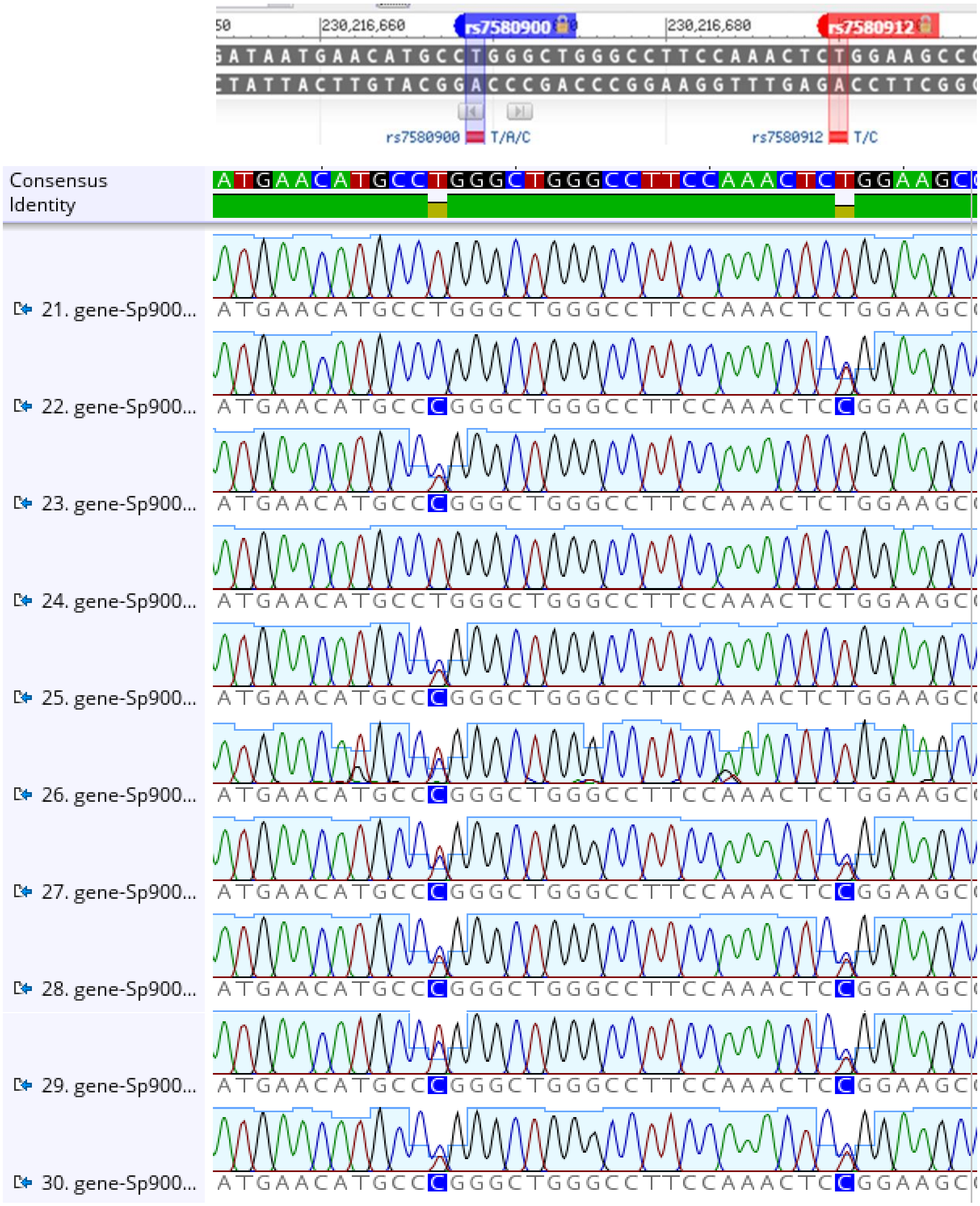


Figure (3.22 continue): Sanger sequencing result. Multiple alignment Chromatograms of partial sequences of Sp110 for latent TB group. Two SNPs were observed: SNPrs7580912 T>C and rs7580900 T> C. Alignment was performed by Geneious prime software.

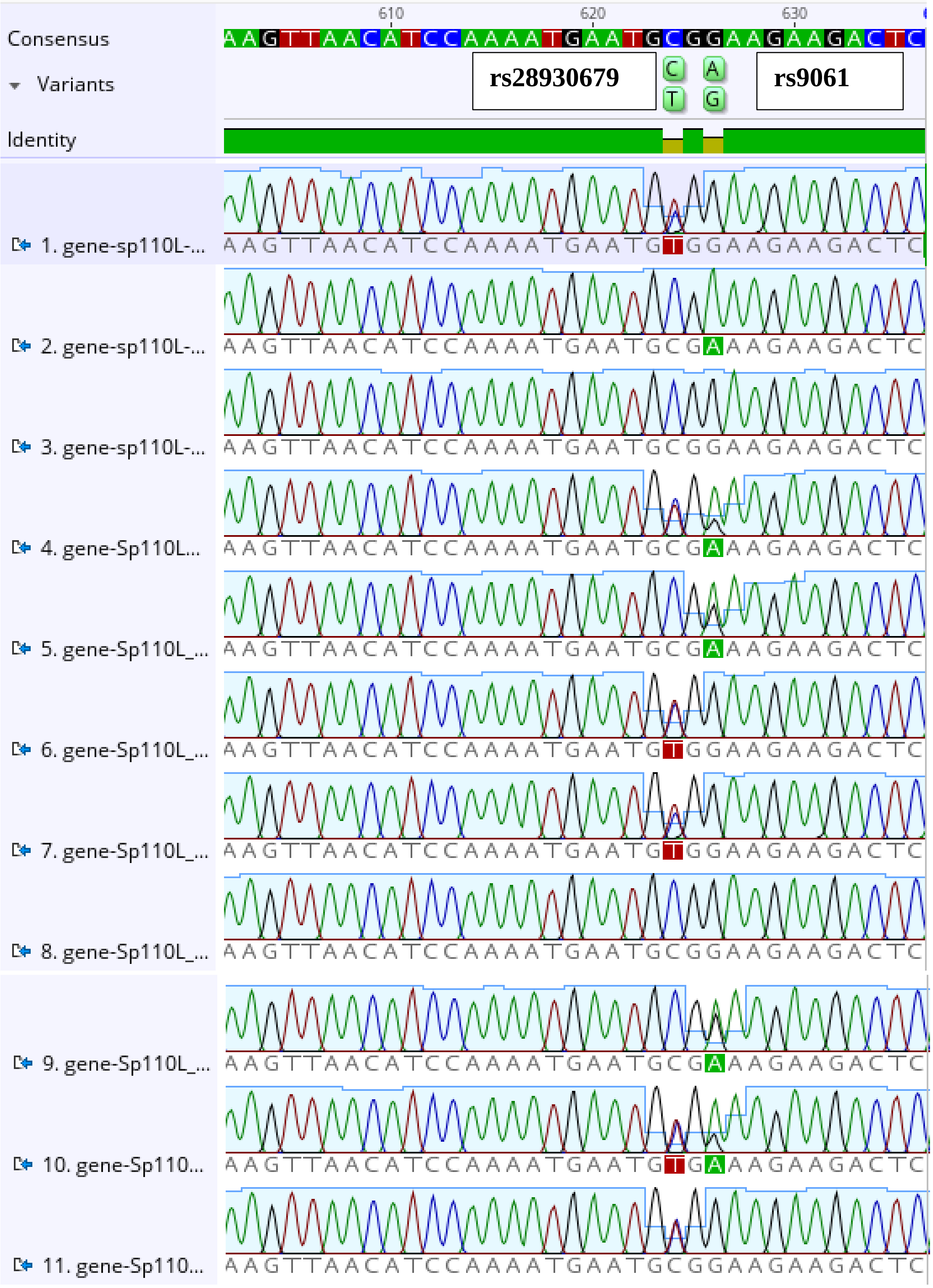


Figure (3.23): Sanger sequencing results. Multiple alignments of partial Sp110 sequences of latent TB group, based on Chromatograms peaks. Two SNP were observed: rs9061 G>A, rs28930679 C> T, and alignment performed by Geneious prime software.

Figure (3.24 Continue): Sanger sequencing results .Multiple alignments of partial Sp110 sequences of latent TB group, based on Chromatograms peaks. Two SNP were observed: rs9061 G>A, rs28930679 C> T , alignment performed by Geneious prime software.

Consensus AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC

23. gene-Sp110... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC

24. gene-Sp110... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC

25. gene-Sp110... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC

26. gene-Sp110... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC

27. gene-Sp110... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC

28. gene-Sp110... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC

29. gene-Sp110... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC

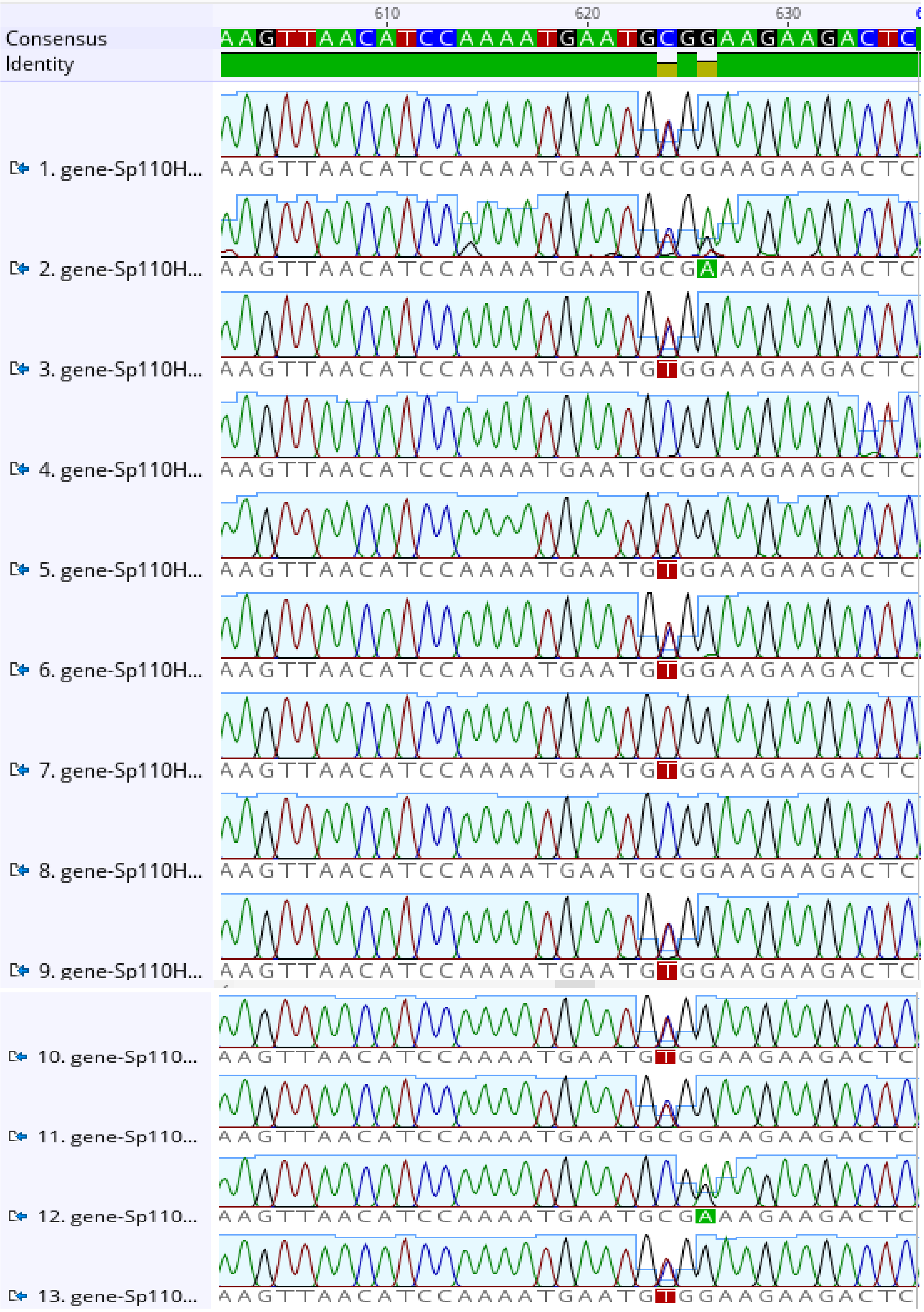
30. gene-Sp110... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC

31. gene-Sp110... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC

32. gene-Sp110... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC

33. gene-Sp110... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC

Figure (3.25 Continue): Sanger sequencing results. Multiple alignments of partial Sp110 sequences of latent TB infection group, based on Chromatograms peaks. Two SNP were observed: rs9061 G>A, rs28930679 C> T, and alignment performed by Geneious prime software.



Consensus AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC
1. gene-Sp110H... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC
2. gene-Sp110H... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC
3. gene-Sp110H... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC
4. gene-Sp110H... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC
5. gene-Sp110H... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC
6. gene-Sp110H... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC
7. gene-Sp110H... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC
8. gene-Sp110H... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC
9. gene-Sp110H... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC
10. gene-Sp110... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC
11. gene-Sp110... AAGTTAACATCCAAAATGAATGCGGAAGAAGACTC
12. gene-Sp110... AAGTTAACATCCAAAATGAATGCGAAAGAAGACTC
13. gene-Sp110... AAGTTAACATCCAAAATGAATGTGGAAGAAGACTC

Figure (3.26): Sanger sequencing results .Multiple alignments of partial Sp110 sequences of the apparently healthy controls, based on Chromatograms peaks. Two SNP were observed: rs9061 G>A, rs28930679 C> T, and alignment performed by Geneious prime software.

The associations between SP110 genotypes and susceptibility to LTBI were then analyzed .The allele and genotype frequencies of the two SP110 SNPs (rs7580900, rs7580912) in the LTBI group and healthy control are shown in table (3.21). In the apparently healthy controls, the genotype distributions of SP110 SNPs (rs7580900, rs7580912) were consistent with Hardy- Weinberg equilibrium (P>0.05). The frequency of T-allele in LTBI group and apparently healthy controls was 53% vs. 63.75% in rs7580900 and 56% vs. 72.5% in rs7580912 , respectively and was considered as a reference group.

The results showed that the mutant allele C of rs7580900 and rs7580912 frequency was more common in LTBI group than in apparently healthy controls (47% vs.36.25% ,44% vs. 27.5%), respectively . The allele C of rs7580912 confers a 2.07 –fold increased risk of latent TB infection (P = 0.023; OR: 2.07; 95% CI:1.10 - 3.89) compared to T wild type allele. The mutant heterozygous genotype "TC" in rs7580900 and rs7580912 were more frequently in the latent TB group compared to healthy control (58% vs. 32.5% and 48% vs. 30%) , respectively . The genotypes “TC” in rs7580912 (P=0.02, OR:2.88; 95% CI: 1.12 - 7.37) and “TC” in rs7580900 (P=0.011, OR: 3.53; 95% CI: 1.33 -9.36) were associated with LTBI risk are shown in table (3.21).

Table (3.21): Association between SP110 SNP (rs7580900 T>C, rs7580912 T>C) genotypes and LTBI risk in LTBI cases vs. apparently healthy control.

SNP ID	**Location**	**Genotype**	**Latent TB (n=50)**	**AHC (n=40)**	**OR (95%CI)**	**P-value**
rs7580900	Intron 2-3	TT	12 (24%)	19 (47.5%)	**Reference**	
		TC	29 (58%)	13 (32.5%)	3.53 (1.33 -9.36)	***0.011***
		CC	9 (18%)	8 (20%)	1.78 (0.54 -5.89)	0.344
		HWE-*p*	0.245	0.06		
Allele frequency		T	53 (53%)	51(63.75%)	Reference	
		C	47 (47%)	29(36.25%)	1.56 (0.85 -2.85)	*0.148*
rs7580912	Intron 2-3	TT	16 (32%)	23(57.5%)	**Reference**	
		TC	24 (48%)	12(30%)	2.88 (1.12 -7.37)	***0.02***
		CC	10 (26%)	5 (12.5%)		
		HWE-*p*	0.854	0.117		
Allele frequency		T	56 (56%)	58(72.5%)	**Reference**	
		C	44 (44%)	22(27.5%)	2.07(1.10 - 3.89)	***0.023***

OR: odds ratio; CI: confidence interval; S: significant at P < 0.05. Values in boldface are statistically significant. HWE-p: P value for Hardy Weinberg Equilibrium. AHC: apparently healthy control.

On the other hand, the allele and genotype frequencies of the SP110 rs9061 in the LTBI group and apparently healthy control are shown in table (3.22). Both populations agreed with HWE for all polymorphisms tested (P > 0.05). The frequency of G-allele in LTBI group and healthy control was 73% vs. 86.25%, respectively and was considered as a reference group. The results found that the A-allele of rs9061 was more frequent in the LTBI group compared to the apparently healthy control (27% vs. 13.75%) with OR value of 2.32 and this difference was statistically significant (p=0.033). Also, the mutant heterozygous genotype "GA" of rs9061 was more common in LTBI group compared with apparently healthy control (34% vs.15%) respectively, and this genotype was significantly associated with LTBI risk (P=0.03; OR: 3.14; 95%CI: 1.08 -9.07).

Table (3-22): Association between SP110 SNP (Rs9061 G>A) genotypes and LTBI risk in LTBI group vs. apparently healthy control.

SNP ID	**Location**	**Genotype**	**Latent (n=50)**	**AHC (n=40)**	**OR (95%CI)**	**P-value**
Rs9061	Exon5	GG	28 (56%)	31 (77.5%)	**Reference**	
		GA	17(34%)	6 (15%)	3.14 (1.08 - 9.07)	***0.03***
		AA	5 (10%)	3 (7.5%)	1.85 (0.40 - 8.44)	*0.430*
		HWE-*p*	*0.33*	0.097		
Allele frequency		G	73 (73%)	69 (86.25%)	**Reference**	
		A	27(27%)	11 (13.75%)	2.32(1.07 - 5.03)	***0.033***

OR: odds ratio; CI: confidence interval; S: significant at P < 0.05. Values in boldface are statistically significant. HWE-p: P value for Hardy Weinberg Equilibrium. AHC: apparently healthy control.

There is growing evidence that the SP110 polymorphism plays a role in tuberculosis. SP110 play an essential role in microbial immunity and adjusting the biological activity of macrophages, which could protect from TB. The genetic studies suggest that the SP110 gene is essential in modulating susceptibility to latent and active tuberculosis infection. SP110b protein acts as proinflammatory cytokines regulator, thereby reducing tissue damage caused by excessive inflammation (Leu *et al*.,2017). Although many studies have investigated the SP110 gene polymorphism association with TB susceptibility in various populations, the results have been inconclusive.

The results of the current study were compatible with a study conducted in Taiwan by Chang *et al*.,(2018), in which they demonstrated that rs7580912 and rs7580900 were significantly associated with LTBI risk. In addition, the current study indicated that genetic variant rs9061 in SP110 were linked to latent TB risk. These results were partially compatible with a study conducted in China, which found that the genetic variant rs9061 in the SP110 gene may increase the risk of TB (Zhang *et al*.,2017).

In the West African population, Tosh and colleagues reported that SNPs in the SP110 gene were linked to TB (Tosh *et al.* 2006). Other studies identified the association of SP110 polymorphisms with TB by a case-control design (Cong *et al*., 2010; Cai *et al*., 2013;). In contrast, some studies reported no significant association between rs9061 and TB risk in Asians (Lei *et al*.,2012), Indonesians (Png *et al*., 2012), Vietnamese (Fox *et al*., 2014) and Han Chinese population (Jiang *et al*. 2016). Ethnic differences may contribute to the discrepancies between the previous results and the current data.

Deng *et al*. (2017) demonstrated that Exonic Single nucleotide polymorphism has a direct effect on protein properties. The SNP rs9061(G>A) is located in the exon region. The substitution G to A at codon position 207 of the SP110 gene results in a change in amino acid from glutamic acid to lysine. The conversion of an acidic amino acid to an essential amino acid may result in a change in the protein structure or posttranslational modification of the SP110 protein. It has been proposed that the A allele may cause differences in the secondary structure of the SP110 protein's alpha helices and beta-sheets compared to the G allele (Chang *et al*.,2018). Thus, a change in amino acid affects the function of the SP110 protein, which in turn affects the development of tuberculosis (Bellamy, 2005). Moreover, SNPs within introns and untranslated regions can affect the expression and splicing of mRNA (Wang *et al*.,2013; Wang *et al*.,2014). More functional studies are needed to fully comprehend the implications of the single nucleotide polymorphism (rs9061G>A) on the SP110 gene and illness risk.

The genetic variants in the SP110 (rs7580900 T>C, rs7580912 T>C rs9061 G>A) displayed the ability to predict the risk of latent TB infection and subsequent TB disease progression. Global TB eradication efforts have concentrated on detecting and treating LTBI cases to control and eradicate TB. Therefore, several SNPs in SP110 were shown in this study to be linked with LTBI susceptibility and may serve as predictive markers for latent TB infection in the Iraq population. Also, this study may result in a better method for identifying those more susceptible to the disease. On the other hand, the present study did have certain restrictions. The study's limitation was the use of relatively small sample sizes. Additional large-scale studies must confirm the prognostic usefulness of the SP110 SNPs.

Conclusions and Recommendations

Conclusions

1. The Gene Xpert MTB/RIF assay gave a high sensitivity and specificity, making it a valuable diagnostic tool for the rapid detection of MTB and also detecting RR-TB at the same time.
2. Early detection of rifampicin resistance may contribute to reducing the incidence of (MDR) strains among tuberculosis patients.
3. The frequency of active tuberculosis disease (TB) was higher in males and more in the younger age groups. In comparison, the elderly males were risk factors associated with latent TB infection.
4. The difference in IFN-γ productions between the two antigen tubes in the QFT-Plus with greater TB2 antigen response could be used as a surrogate marker of recent exposure or higher antigenic burden in latent TB infection, who are at greater risk of progression to active TB.
5. Serum levels of IL-17A and IL18 play differential roles in two phases of tuberculosis infection and can potentially be used to develop novel diagnostics.
6. The distribution of SNPs genotypes are all in Hardy-Weinberg equilibrium.
7. IL-18 gene polymorphisms are associated with active TB susceptibility and showed the potential to predict the risk of active tuberculosis where the individuals carrying the AA +CA genotypes at position IL-18-607 C > A (rs1946518) were more likely to have a significantly increased risk of active TB when compared with the CC genotype.
8. No significant associations were found between the IL-17A rs2275913 polymorphism and susceptibility to active and latent TB.
9. Genetic variants of the SP110 are linked to susceptibility to latent TB, and the heterozygous mutant genotype TC in rs7580900 and rs7580912 as well

as GA of rs9061 displayed the potential to predict the risk of latent TB infection and may be associated with subsequent TB progression.

Recommendations

1. Study whole sequencing genome for strain of *Mycobacterium tuberculosis* especially RR-TB, MDR-TB and XDR-TB.
2. Investigation of the association between the SP110 rs9061 SNP and the TNFα.
3. The QFT-plus test tended to be more positive among subjects in contact with active pulmonary TB; therefore, reasonable to pay particular attention to those individuals during the contact investigation of active TB.
4. Assessment of the transcriptional activity of IL-18 and IL17A in TB infection.

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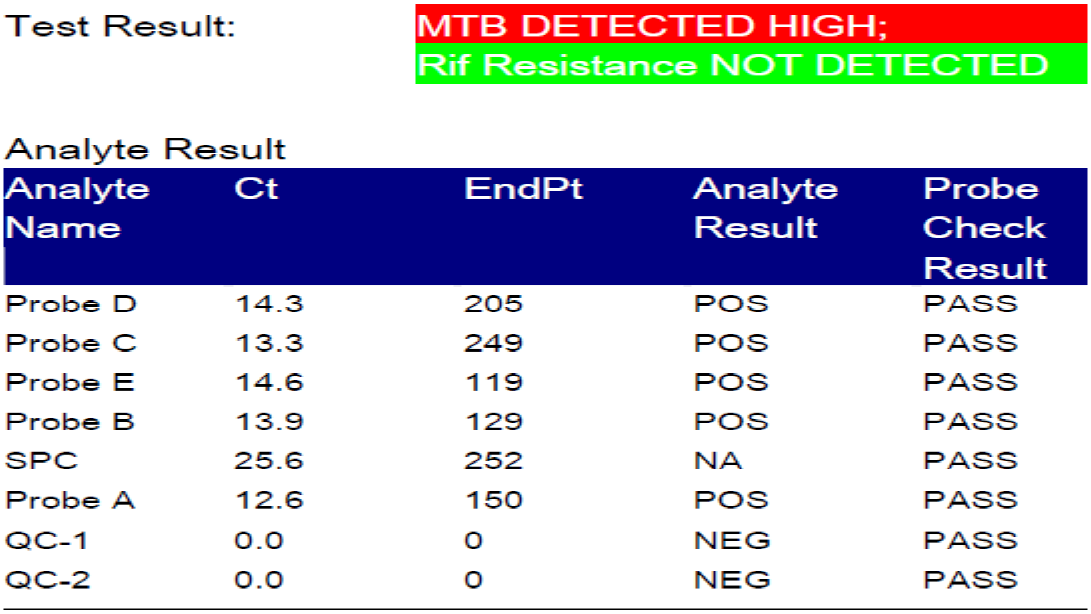
Appendices

Appendices

Appendix (1): MTB DETECTED and RIF Resistance NOT DETECTED results by GeneXpert MTB/RIF assay

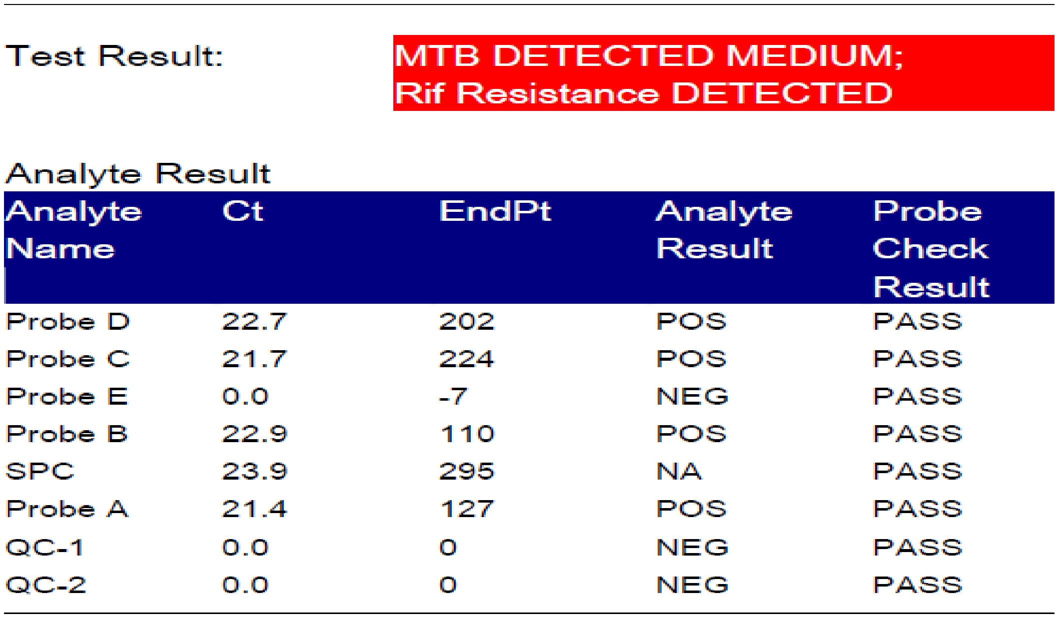


Test Result: MTB DETECTED HIGH; Rif Resistance NOT DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	14.3	205	POS	PASS
Probe C	13.3	249	POS	PASS
Probe E	14.6	119	POS	PASS
Probe B	13.9	129	POS	PASS
SPC	25.6	252	NA	PASS
Probe A	12.6	150	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

Appendix (2): MTB DETECTED and RIF Resistance DETECTED result by GeneXpert MTB/RIF assay



Test Result: MTB DETECTED MEDIUM; Rif Resistance DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	22.7	202	POS	PASS
Probe C	21.7	224	POS	PASS
Probe E	0.0	-7	NEG	PASS
Probe B	22.9	110	POS	PASS
SPC	23.9	295	NA	PASS
Probe A	21.4	127	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

Appendix (3): QFT-Plus test results for Latent TB infection Group.

Subject ID	Nil	TB1	TB2	Mitogen	TB1-Nil	TB2-Nil	Mitogen-Nil	Result
ID 1	0.06	0.38	1.10	8.39	0.32	1.04	8.33	Positive
ID 2	0.19	0.67	0.65	9.32	0.48	0.46	9.13	Positive
ID 3	0.07	0.24	0.43	5.10	0.17	0.36	5.03	Positive
ID 4	0.02	0.63	0.12	3.47	0.61	0.10	3.45	Positive
ID 5	0.05	0.99	0.09	8.85	0.94	0.04	8.80	Positive
ID 6	5.57	8.66	5.99	6.62	3.09	0.42	1.05	Positive
ID 7	0.05	2.35	2.53	9.74	2.30	2.48	9.69	Positive
ID 8	1.85	5.06	8.18	8.09	3.21	6.33	6.24	Positive
ID 9	0.66	0.78	2.44	9.29	0.12	1.78	8.63	Positive
ID 10	0.36	2.83	1.94	8.32	2.47	1.58	7.96	Positive
ID 11	0.32	1.82	0.25	8.85	1.50	-0.07	8.53	Positive
ID 12	0.31	3.65	2.29	9.37	3.34	1.98	9.06	Positive
ID 13	0.33	3.56	4.35	8.36	3.23	4.02	8.03	Positive
ID 14	0.28	9.08	6.66	6.09	8.80	6.38	5.81	Positive
ID15	0.23	0.78	1.03	4.00	0.55	0.80	3.77	Positive
ID 16	0.26	2.27	3.60	1.53	2.01	3.34	1.27	Positive
ID17	0.28	1.41	1.32	7.69	1.13	1.04	7.41	Positive
ID 18	0.32	5.14	5.41	8.72	4.82	5.09	8.40	Positive
ID 19	0.29	0.83	1.13	6.02	0.54	0.84	5.73	Positive
ID 20	0.15	7.42	6.78	8.78	7.27	6.63	8.63	Positive
ID 21	0.26	3.31	0.78	7.37	3.05	0.52	7.11	Positive
ID 22	1.73	5.74	5.55	7.87	4.01	3.82	6.14	Positive
ID23	0.08	0.69	0.65	7.23	0.61	0.57	7.15	Positive
ID24	0.12	0.93	0.40	4.04	0.81	0.28	3.92	Positive
ID25	0.10	1.37	2.43	7.16	1.27	2.33	7.06	Positive
ID26	0.81	1.48	0.16	7.02	0.67	-0.65	6.21	Positive
ID27	0.41	2.43	1.69	1.08	2.02	1.28	0.67	Positive
ID28	0.36	3.31	3.55	6.64	2.95	3.19	6.28	Positive
ID29	0.15	0.70	0.70	7.35	0.55	0.55	7.20	Positive
ID30	1.73	5.74	5.55	7.87	4.01	3.82	6.14	Positive
ID31	0.04	0.50	1.80	9.93	0.46	1.76	9.89	Positive
ID32	0.07	0.52	1.84	>10	0.45	1.77	9.95	Positive
ID33	0.21	0.31	0.86	>10	0.10	0.65	>10	Positive
ID34	0.05	0.52	1.87	>10	0.47	1.82	>10	Positive
ID35	0.22	0.32	0.87	>10	0.10	0.65	>10	Positive
ID36	0.19	0.30	0.84	>10	0.11	0.65	9.93	Positive
ID37	0.07	0.45	0.10	>10	0.38	0.03	>10	Positive
ID38	0.02	0.56	0.10	>10	0.54	0.08	>10	Positive
ID39	0.15	0.67	0.27	>10	0.52	0.12	9.96	Positive
ID40	6.06	9.33	8.55	>10	3.27	2.49	6.29	Positive
ID41	0.25	5.16	4.02	>10	4.91	3.77	>10	Positive

ID42	0.05	0.52	1.89	>10	0.47	1.84	>10	Positive
ID43	0.25	0.33	0.90	>10	0.08	0.65	>10	Positive
ID44	0.06	0.53	1.86	>10	0.47	1.80	>10	Positive
ID45	0.22	0.31	0.86	>10	0.09	0.64	>10	Positive
ID46	0.38	1.85	1.75	>10	1.47	1.37	>10	Positive
ID47	0.27	3.69	8.11	>10	3.42	7.84	>10	Positive
ID48	0.08	1.89	1.84	>10	1.81	1.76	>10	Positive
ID49	0.54	7.37	7.92	>10	6.83	7.38	>10	Positive
ID50	0.53	1.32	1.19	>10	0.79	0.66	>10	Positive
ID51	1.09	7.47	6.84	>10	6.38	5.75	>10	Positive
ID52	1.26	1.87	2.10	>10	0.61	0.84	>10	Positive
ID53	1.84	3.19	0.56	>10	1.35	-1.28	8.66	Positive
ID54	3.58	0.58	7.10	>10	-3.00	3.52	7.09	Positive
ID55	2.01	0.41	2.97	>10	-1.60	0.96	9.77	Positive
ID56	4.86	0.31	6.33	>10	-4.55	1.47	5.83	Positive
ID67	5.00	0.05	6.36	>10	-4.95	1.36	5.19	Positive
ID68	0.85	0.88	3.53	>10	0.03	2.68	>10	Positive
ID69	1.64	0.54	2.57	>10	-1.10	0.93	>10	Positive
ID60	2.44	0.33	3.37	>10	-2.11	0.93	9.46	Positive

Appendix (4): QFT-Plus test results for appeareantly healthy control Group

Subject ID	Nil	TB1	TB2	Mitogen	TB1-Nil	TB2-Nil	Mitogen-Nil	Result
ID 1	0.15	0.08	0.11	4.44	-0.07	-0.04	4.29	Negative
ID 2	0.08	0.02	0.04	6.80	-0.06	-0.04	6.72	Negative
ID 3	0.19	0.08	0.06	7.92	-0.11	-0.13	7.73	Negative
ID 4	0.14	0.10	0.11	7.74	-0.04	-0.03	7.60	Negative
ID 5	0.48	0.57	0.49	6.17	0.09	0.01	5.69	Negative
ID 6	0.17	0.10	0.11	7.44	-0.07	-0.06	7.27	Negative
ID 7	0.17	0.16	0.21	6.74	-0.01	0.04	6.57	Negative
ID 8	0.32	0.16	0.15	6.83	-0.16	-0.17	6.51	Negative
ID 9	0.12	0.09	0.07	8.55	-0.03	-0.05	8.43	Negative
ID 10	0.16	0.27	0.36	6.51	0.11	0.20	6.35	Negative
ID11	0.13	0.20	0.12	8.64	0.07	-0.01	8.51	Negative
ID 12	0.08	0.11	0.05	3.65	0.03	-0.03	3.57	Negative
ID 13	3.14	3.25	3.14	5.15	0.11	0.00	2.01	Negative
ID 14	0.08	0.11	0.12	7.56	0.03	0.04	7.48	Negative
ID 15	0.06	0.23	0.22	6.89	0.17	0.16	6.83	Negative
ID 16	0.12	0.13	0.14	5.89	0.01	0.02	5.77	Negative
ID 17	0.06	0.06	0.30	1.66	0.00	0.24	1.60	Negative
ID 18	0.19	0.12	0.19	5.04	-0.07	0.00	4.85	Negative
ID 19	0.08	0.05	0.06	3.04	-0.03	-0.02	2.96	Negative
ID 20	0.19	0.27	0.19	8.14	0.08	0.00	7.95	Negative

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ID 21	0.27	0.21	0.21	6.44	-0.06	-0.06	6.17	Negative
ID 22	0.05	0.08	0.20	8.98	0.03	0.15	8.93	Negative
ID 23	0.07	0.05	0.03	9.91	-0.02	-0.04	9.84	Negative
ID 24	0.16	0.35	0.18	9.12	0.19	0.02	8.96	Negative
ID 25	0.04	0.06	0.21	9.78	0.02	0.17	9.74	Negative
ID 26	0.08	0.05	0.05	8.56	-0.03	-0.03	8.48	Negative
ID 27	0.03	0.02	0.04	9.09	-0.01	0.01	9.06	Negative
ID 28	1.19	0.86	1.50	>10	-0.33	0.31	8.96	Negative
ID 29	0.52	0.21	0.14	>10	-0.31	-0.38	>10	Negative
ID 30	2.63	1.12	2.44	3.45	-1.51	-0.19	0.82	Negative
ID 31	1.10	0.21	0.21	9.81	-0.89	-0.89	8.71	Negative
ID32	3.56	0.19	1.37	7.77	-3.37	-2.19	4.21	Negative
ID33	0.95	0.15	0.54	9.60	-0.80	-0.41	8.65	Negative
ID34	0.65	0.35	0.37	8.50	-0.30	-0.28	7.85	Negative
ID35	2.44	0.27	0.85	>10	-2.17	-1.59	9.43	Negative
ID36	7.53	1.06	7.88	>10	-6.46	0.35	2.66	Negative
ID37	2.96	1.45	0.29	>10	-1.51	-2.67	9.14	Negative
ID38	0.04	0.15	0.06	3.66	0.11	0.02	3.62	Negative
ID39	0.07	0.15	0.14	8.88	0.08	0.07	8.81	Negative
ID40	0.02	0.01	0.05	8.16	-0.01	0.03	8.14	Negative

الخلاصة

لا يزال مرض التدرن يمثل مشكلة صحية عامة على الرغم من برامج المكافحة الدولية ، الأفراد المصابون بعدوى التدرن الكامنة يزيدون بشكل كبير من عدد حالات التدرن النشط وهم معرضين لخطر الإصابة بالمرض مدى الحياة. هدفت الدراسة الحالية الى تحديد بعض المؤشرات المناعية والوراثية في مرحلتين أثناء تطور مرض التدرن النشط وعدوى التدرن الكامنة وتقييم دورها كواسمات تنبؤية في مرضى التدرن النشط والكامن.

صُمِمت الدراسة الحالية كدراسة الحالات والشواهد وأجريت خلال الفترة من شباط إلى أيلول (٢٠٢١) في معهد التدرن الوطني / المختبر المرجعي للتدرن في بغداد. جمعت 140 عينة بلغم من مرضى يشتبة اصابتهم بلتدرن الرئوي ، وتم تأكيد إصابة 60 مريض بناءً على النتائج الإيجابية من خلال تصبيغ العصيات أو الزرع البكتيري أو من الاختبار الجزيئي Gene Xpert. تًضمنت البيانات الديموغرافية لمرضى التدرن الرئوي النشط فئات عمرية تراوحت من 16 إلى 68 عامًا بمعدل اعمار(36.16 ± 14.16) سنة , وكان عدد الاناث 21 (35.0٪) و 39 (65.0٪) ذكورآ وكان هناك فرق معنوي في حدوث التدرن الرئوي النشط بين الذكور والإناث (P = 0.02). أظهرت الفئة العمرية (25-34) سنة تكرارا أعلى في مرضى التدرن (33.3٪) من الفئات العمرية الأخرى ، مع وجود فروق ذات دلالة إحصائية بين جميع الفئات العمرية (p = 0.001). تم حساب كفاءة الاختبارات التشخيصية باستخدام طريقة الزرع كمعيار ذهبي حيث أعطى اختبار Gene Xpert حساسية ونوعية عالية بلغت 100٪ و 80.8٪ على التوالي . من بين 60 عينة موجبة تم تشخيصها بواسطة GeneXpert ، أظهرت النتائج أن سبع عزلات (11.67٪) من العصيات الدرنية كانت مقاومة للمضاد الحيوي الريفامبيسين.

شُخص المشتركين المشتبة اصابتهم بالتدرن الكامن ومجموعة الافراد الاصحاء بواسطة QuantiFERON-TB Gold (QFT- Plus assay) وتبين ان من مجموع 164 مشتركا مشتبة اصابتهم بلتدرن الكامن,أظهر 60 شخصًا (36.6 ٪) نتائج إيجابية لهذا الاختبار وتم تعريفهم على أنهم مصابون بالتدرن الكامن ،تضمنوا 37 ذكرًا و 23 إناثًا تراوحت أعمارهم بين (18 – 66) عامًا ، وكانت 104 عينة (63.4 ٪) سلبية.وتبين ان 40 شخصًا سليمًا لم يتعرضوا لمرض التدرن واعطوا نتيجة سلبية لهذا الاختبار ،تضمنوا 22 ذكرًا و 18 أنثى و تراوحت أعمارهم بين (15 – 70) عامًا. كانت معدلات الايجابية في انبوب المستضد الاول TB1 و انبوب المستضد الثاني هيTB2 (27.4٪ و 30.5٪) على التوالي. كذلك ، لم يكن هناك فرق معنوي في معدل الإيجابية بين TB1 و TB2 . كان معدل التوافق بينهما حوالي 84.8٪

، معامل كابا 0.630. كانت هنالك زيادة غير معنوية في معدل تركيز الانترفيرون كاما (IFN-γ) المتحرر في TB2 (p = 0. 142) مقارنة بـ TB1 بين العينات الإيجابية لاختبار QFT-Plus.

لتقييم المساهمة الخاصة للخلايا التائية السامة CD8 + T, تم استخدام القيم التفاضلية TB2 - TB1 كتقدير غير مباشر لتحفيز CD8 مع مستضدات TB2. لوحظ في الدراسة الحالية وجود استجابة أكبر لمستضد TB2 (TB2−TB1 >0.6 IU/mL) في 22 (36.7٪) فردًا مصابًا بالتدرن الكامن من مجموع العينات الموجبة لهذا الاختبار .أما فيما يتعلق بعوامل الخطر المرتبطة بالنتائج الإيجابية لاختبار QFT-Plus ، كانت الفئة العمرية الأكثر شيوعًا هي(45-54) سنة بنسبة 30.0٪ ، تليها الفئة العمرية (55) سنة بنسبة 23.3٪ وارتبطوا معنويا بالنتائج الإيجابية لهذا الاختبار(OR: 3.8, , P=0.022) و (OR: 4.03, P=0.024) على التوالي . كان الذكور(61.7٪) الاكثر تكرارًا من الإناث (38.3٪) في مجموعة التدرن الكامن وارتبطوا معنويا بالنتائج الإيجابية لهذا الاختبار (OR: 2.19, P=0.018).

بينت نتائج المقايسية الامتصاصية المناعية للانزيم المرتبط (ELISA) أن هنالك زيادة معنوية في معدل تركيزالحركي الخلوي IL-18 في مصل مجموعة التدرن الرئوي النشط مقارنة بمجموعة التدرن الكامن و الافراد الاصحاء (P <0.001) ، كذلك كانت هنالك زيادة معنوية في معدل تركيزالحركي الخلوي IL-18 في مصول مجموعة التدرن الكامن مقارنةً بمجموعة الافراد الاصحاء (P<0.001). في حين ، كان هنالك زيادة معنوية في معدل تركيزالحركي الخلوي IL-17A في مصول مجموعة التدرن الكامن مقارنةً بمجموعة الافراد الاصحاء(P = 0.002) ومجموعة التدرن النشط (p <0.001). تم إجراء تحليل ROC باستخدام IL-17A و IL-18 لتقدير قدرة هذه المعلمات على التنبؤ بالتدرن النشط والتدرن الكامن. حيث أظهرالحركي الخلوي IL-17A قوة تمييزية كبيرة مع قيم عالية من AUC والحساسية والخصوصية في تمييز مجموعة التدرن الكامن من مجموعة الافراد الاصحاء (الحساسية 98٪ ، الخصوصية 95٪ ، AUC= 0.993).بينما أظهر الحركي الخلوي IL-18 قوة تمييزية كبيرة مع قيم عالية من AUC والحساسية والخصوصية في التمييز بين التدرن النشط مقابل مجموعة الافراد الاصحاء (الحساسية 92٪ ، الخصوصية 90٪ ، AUC = 0.952)

تضمنت الدراسة الجزيئية،تحديد التغاير الوراثي rs1946518 C> A لجين IL-18 و التغاير الوراثي rs2275913 G> A لجين IL17A في مجموعة التدرن النشط والكامن بواسطة تفاعل البلمرة المتسلسل المتخصص بالاليل allele-specific polymerase chain reaction وأكدت نتائج هذا التحليل بواسطة طريقة سانجر Sanger sequencing method، لدراسة الارتباط بين التغايرات الوراثية اعلاة وقابلية

الإصابة بالتدرن النشط والكامن ، تم حساب تكرار الطرز الوراثية والأليلات للمجموعات المدروسة. حيث تبين في مجموعة الافراد الاصحاء ، لم يكن لتوزيع الطرز الوراثية أي انحراف عن Hardy-Weinberg equilibrium (P> 0.05). اما فيمايتعلق بالتغاير الوراثي (C/A (rs1946518 في جين IL-18 ،فقد أوضحت النتائج أن ألاليل A كان أكثر تكراراً في مرضى التدرن النشط مقارنة بمجوعة الافراد الاصحاء (60٪ مقابل 38.75٪) بقيمة أرجحية (OR: 2.37) وكان هذا الاختلاف ذا دلالة إحصائية (P = 0.003) ، وارتبطت الطرز الوراثية الطافرة (AA (OR: 4.67, p=0.007 و CA (OR: 3.17, P=0.024) بخطر الإصابة بالتدرن النشط ؛ على التوالي. كذلك ، تبين ان لدى مرضى التدرن تكراراً أعلى للأليل A مقارنة بمجموعة التدرن الكامن (60 ٪ مقابل 45.83 ٪) بقيمة أرجحية (OR: 1.77) وكان هذا الاختلاف ذا دلالة إحصائية (P = 0.028) وارتبط الطراز الوراثي متماثل اللواقح الطافرة AA لـ rs1946518 معنوياً بخطر الاصابة بالتدرن النشط (OR: 2.99,p = 0.035) مقارنة بمجموعة التدرن الكامن.لم تجد الدراسة الحالية أي علاقة ذات دلالة إحصائية بين تكرار الطرز الوراثية أو الأليلات لـلتغاير الوراثي(A G> rs2275913) لجين IL-17A في مجموعة مرضى التدرن النشط ومجموعة التدرن الكامن مقارنة مع مجموعة الافراد الاصحاء.

تم التنميط الوراثي للتغايرات الوراثية (rs7580900, rs7580912 rs9061) لجين Sp110 في مجموعة التدرن الكامن بواسطة طريقة سانجر Sanger Sequencing. بينت النتائج أن ألاليل الطافر C في rs7580900 و rs7580912 كان أكثر تكراراً في مجموعة التدرن الكامن مقارنة بمجموعة الافراد الاصحاء (47٪ مقابل 36.25٪ ، 44٪ مقابل 27.5٪) ، على التوالي. كان الطراز الوراثي الطافر متغاير اللواقح "TC" في rs7580900 و rs7580912 أكثر تكرارًا في مجموعة التدرن الكامن مقارنة بمجموعة الافراد الاصحاء وارتبط معنويا بخطر الاصابة بعدوى التدرن الكامنة (OR: 3.53 ، P = 0.011) و (P = 0.02 ، OR: 2.88) ، على التوالي. بالاضافة الى ذلك، كان النمط الوراثي الطافر متغاير اللواقح "GA" لـ rs9061 أكثر شيوعًا في مجموعة التدرن الكامن مقارنة بمجموعة الافراد الاصحاء (34٪ مقابل 15٪) على التوالي ، وكان هذا الطراز الوراثي مرتبطا معنويا بخطر الاصابة بعدوى التدرن الكامنة (P=0.03; OR: 3.14) .

أن الكشف المبكر لمقاومة المضاد الحيوي الريفامبيسين قد يسهم في تقليل حدوث سلالات التدرن المقاوم للادوية (MDR) بين مرضى التدرن. تلعب مستويات المصول من الحركي الخلوي (IL-17A) والحركي الخلوي (IL-18) أدوارًا تفريقية في مرحلتين من تطور مرض التدرن ويمكن استغلالها لإنشاء تشخيصات جديدة ، من ناحية أخرى ، ارتبط تعدد الأشكال الوراثية لجين IL-18 (IL-18-607 C>A) rs1946518 والتغايرات الوراثية في جين SP110 (rs7580900 ، rs7580912 ، rs9061) بقابلية الاصابة بالتدرن الكامن ، وأظهرت إمكانية التنبؤبخطر الاصابة بالتدرن النشط والكامن على التوالي . علاوة على ذلك، تم تشخيص الأفراد المصابين بالتدرن الكامن وتم تحديد الأشخاص الاكثر خطورة لتطور مرض التدرن النشط في المستقبل بناءً على الموشرات المناعية والجزيئية وبالتالي يمكن للواسمات الحيوية للمضيف ان تحسن من تشخيص مرض التدرن.

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دراسة مناعية وجزيئية لمرضى التدرن الرئوي

اطروحة مقدمة الى مجلس كلية الطب – جامعة بابل
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من قبل

سارة كساب شنداوي حمزة

بكلوريوس علوم حياة (احياء مجهرية)/كلية العلوم-جامعة بابل-٢٠٠٨
ماجستير علوم حياة (احياء مجهرية)/كلية العلوم-جامعة بابل-٢٠١١

بأشراف

الاستاذ الدكتور
جواد كاظم طراد

الطبيب الاستشاري
د. احمد اسمر منخي

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