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Relationship between some virulence Genes Expression of *Mycobacterium tuberculosis* and Interleukins Levels in Tuberculosis Patients

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بسم الله الرحمن الرحيم

﴿ الْحَمْدُ لِلَّهِ الَّذِي لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ وَلَهُ الْحَمْدُ فِي الْآخِرَةِ ۚ وَهُوَ الْحَكِيمُ الْخَبِيرُ ﴾

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" has been prepared under my supervision at the college of Science, University of Misan as a partial fulfillment of the requirements for the degree of Master of Biology"

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Dedication

To my beloved family, who have always supported me with their love and prayers. To my dear friends, who have encouraged me and shared my joys and sorrows. To my respected teachers, who have inspired me and guided me throughout my academic journey. To my esteemed supervisor, who has mentored me and challenged me to grow as a researcher. To all of you, I dedicate this thesis with gratitude and admiration. You have made this possible. **Thank you**.

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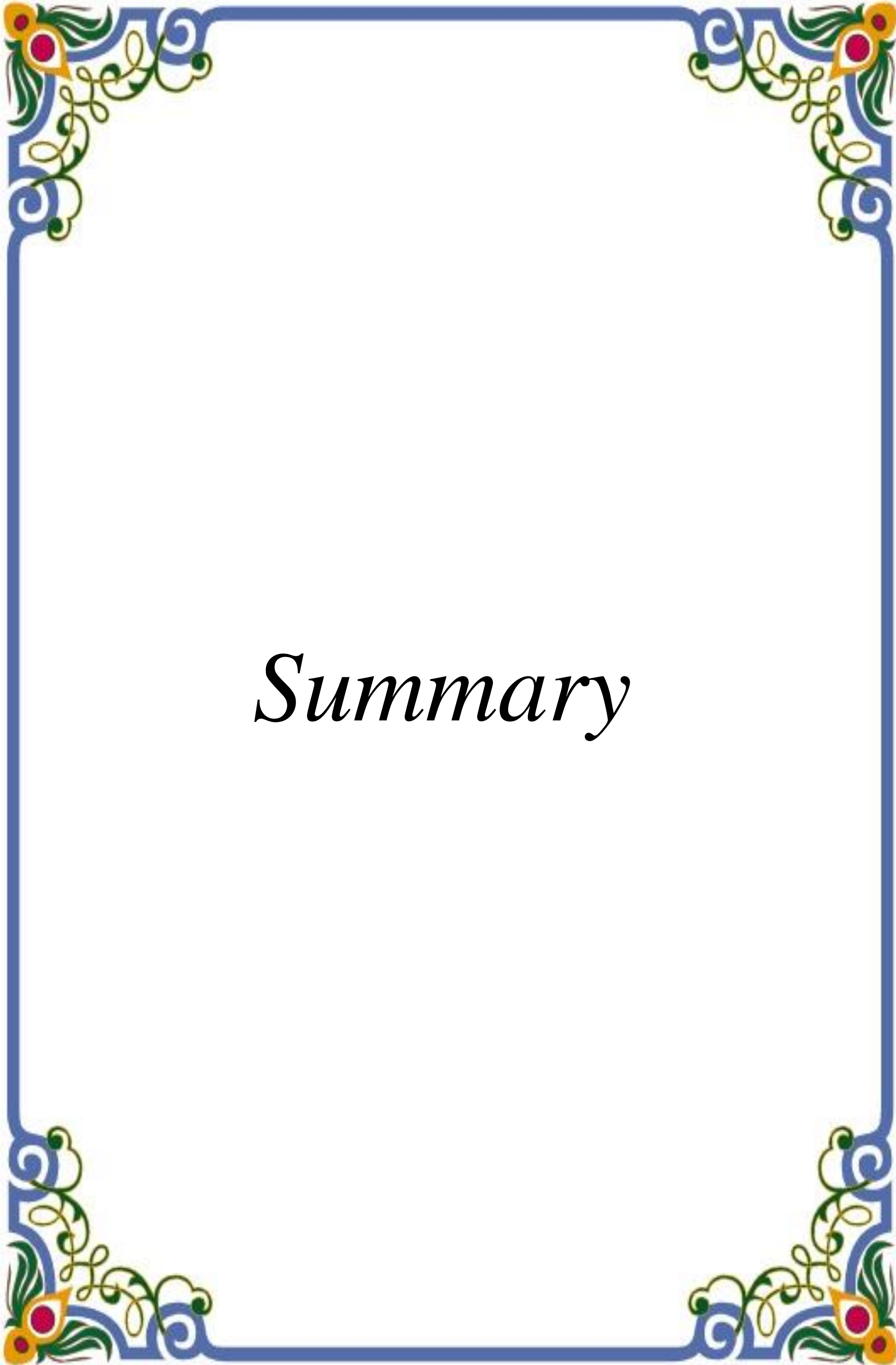

Summary

Summary

Tuberculosis (TB) is a major public health problem that has not been stopped by many efforts. A lot of work has been done by TB control programs, but the control of the disease is made harder by raising the problem of multi-drug resistant(MDR) tuberculosis, which needs new solutions to reduce its impact, new TB control programs that can deal with MDR-TB in a better way are needed all over the world. The objective of this thesis is to study the association between the expression levels of four genes and four cytokines in various TB groups. These genes are *whiB3, whiB7, pknF,* and *fbpA*, which play a role in the pathogenicity and drug resistance of *Mycobacterium tuberculosis* (M.tb), and the effect of some cytokines which are IL-4, IL-6, IL-10, and IL-12, which play a role in the immune response of the host to M.tb infection.

The study used 120 serum samples of participants who were recruited from TB center in Misan City, Iraq, during the period (2022/8/23 to 2023/1/20). They were divided into four groups according to their TB status: G1 (sensitive pulmonary tuberculosis), G2 (sensitive extra-pulmonary tuberculosis), G3 (sensitive TB patients under treatment), G4 (multidrug-resistant tuberculosis patients) in addition to healthy control. Serum levels of IL-4, IL-6, IL-10, and IL-12 in each group were quantified by enzyme-linked.immunosorbent assay (ELISA). Gene expression levels of four genes were measured by quantitative polymerase chain reaction (qPCR) for (24) bacterial samples, (12) sensitive M.tb and (12) MDR-TB.

Serum levels of IL-4, IL-6, and IL-10 were significantly different among the four groups ($P < 0.0001$). G2 had the highest IL-4 level (905.36 pg/mL), while G3 had the lowest (421.96 pg/mL). Moving into IL-6, G3 had

the lowest IL-6 level (23.96 pg/mL), while G1 had the highest (42.88 pg/mL). G4 had significantly higher IL-10 levels than all other groups (41.45 pg/mL), while G1 and G2 had comparable levels (13.32 pg/mL; 13.51 pg/mL), and IL-12 levels showed no significant difference among groups (P = 0.3362), ranging from (15.54 pg/mL in G4 to 31.02 pg/mL in G3). Fold change values of *fbpA, whiB3* and *whiB7* genes were significantly different between sensitive TB and MDR-TB strains (P=0.0003, P=0.0037, and P= 0.0082, respectively), while fold change values of *pknF* gene showed no significant difference (P=0.2934). In sensitive TB, *whiB3* and *whiB7* expressions were positively correlated with the IL-10 level (r = 0.596, P= 0.002; r = 0.637, P= 0.001 respectively), In MDR-TB, *whiB3* expression was negatively correlated with IL-12 level (r = -0.61, P= 0.001), and positively correlated with the IL-10 level (r = 0.764, P= 0.0001). *whiB7* expression was negatively correlated with the IL-12 level (r = -0.60, P= 0.001), and positively correlated with the IL-10 level (r = 0.490, P= 0.015). While *fbpA* expression was positivly correlated with IL-6 in both sensitive and MDR-TB (r = 0.460, P = 0.023; r = 0.514, P = 0.01 respectively).

The results indicated different gene expression levels and cytokine profiles in patients with sensitive TB and MDR-TB. The study identified higher expression levels of *fbpA, whiB3*, and *whiB7* genes in MDR-TB strains compared to sensitive TB strains. The study revealed significant correlations between gene expression levels and cytokine levels in both TB groups, with some genes modulating the cytokine production and influencing the disease outcome. Measurement of the serum levels of IL-4, IL-6, IL-10, and fold change values of *whiB3, whiB7*, and *fbpA* useful as biomarkers for distinguishing different TB groups and thus contribute to TB management and monitoring treatment response.

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List of Abbreviations

Abbreviations	*Meaning*
AFB	Acid-fast bacilli
AMs	Alveolar macrophages
APCs	Antigen-Presenting Cells
BCG	Bacillus Calmette-Guérin
CD4	Cluster of differentiation 4
CFP-10	Culture Filtrate Protein-10
DCs	Dendritic Cells
DST	Drug susceptibility testing
ELISA	Enzyme-linked immunosorbent assay
ESAT-6	Early Secreted Antigenic Target-6

Abbreviations	***Meaning***
fbpA	Fibronectin binding protein A
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HIV	human immunodeficiency virus
IFN-y	Interferon-gamma
IGRA	Interferon-gamma release assay
IL	Interleukin
INH	Isoniazid
LJ medium	Löwenstein–Jensen medium
M1	Classical macrophage
M2	Alternative macrophage
MDR-TB	Multiple drug resistance TB
MHC	Major Histocompatibility Complex
MTB	*Mycobacterium tuberculosis*
MTBC	Mycobacterium tuberculosis complex
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NK	Natural Killer
NO	Nitric oxide
NOX2	NADPH oxidase type 2
PAT	Polyacyltrehalose
PCR	Polymerase chain reaction
PDIM	Phthiocerol dimycocerosate
PGL-1	Phenolic Glycolipid-1
pknF	Protien kinase F
RD-1	Region of differentiation1
RIF	Rifampicin
ROS	Reactive Oxygen Species
RR-TB	Rifampicin-resistant tuberculosis
qPCR	Quantitative polymerase chain reaction
STPKs	Serine/Threonine Protein Kinases
TB	Tuberculosis
TDM	Trehalose Dimycolates
TDR	Totally drug resistance

Abbreviations	***Meaning***
TGFβ	Transforming growth factor-beta
Th1	T helper type 1
TLR	Toll-Like Receptors
TMM	Trehalose Monomycolate
TNF-α	Tumor necrosis factor alpha
Tregs	Regulatory T cells
TST	Tuberculin Skin Test
whiB3	WhiB-like regulatory protein 3
whiB7	WhiB like regulatory protein 7
WHO	World Health Organization
XDR	Extensive drug resistance

CHAPTER ONE
INTRODACTION

1-1 Introduction:

Tuberculosis (TB) is a serious infectious disease caused by *Mycobacterium tuberculosis* (M.tb). M.tb is a rod-shaped, aerobic, non-spore forming, and non-motile. It is spread by the air once an infected individual coughs or sneezes. TB can infect any part of the body, but it mostly invades the lungs (Asfaw *et al*., 2010).

In the face of the availability of effective certain medications, TB remains an important global health challenge. In 2020, there were an estimated 9.9 million new cases of TB and 1.5 million deaths. Multi-drug resistant (MDR) strains of TB, which are not response to the two most powerful anti-TB drugs, isoniazid and rifampicin reflect a major concern. MDR-TB is more challenging to treat and has a greater death rate than drug susceptible TB (DS-TB) (WHO, 2021; Espinosa-Pereiro *et al.,* 2022).

The virulence of M.tb and MDR-TB is a multifaceted phenomenon that implicates numerous factors, including the expression of virulence genes and the host immune reaction to the disease (Cerezo-Cortés *et al.,* 2022). Some of the fundamental virulence genes in M.tb and MDR-TB are *whiB3, whiB7, pknF,* and *fbpA*. These genes have been presented to play vital roles in the pathogenesis of the disease. The regulation of these genes is multifaceted and is assumed to be influenced by the host immune response such as the production of cytokines (Cushman *et al*., 2021; Rastogi *et al*., 2021; Artama *et al.,* 2021).

Interleukins (ILs) are signaling molecules secreted by immune cells in response to infection. They show a critical role in the immune response to M.tb and MDR-TB by regulating the activity of other immune cells and organizing the immune response to the infection (Waghmare *et al*., 2019).

IL-4, IL-6, IL-10, and IL-12 are significant cytokines that have been presented to be involved in the host immune response to M.tb and MDR-TB (Heitmann *et al*., 2014; Wong *et al*., 2020; Sivakumaran *et al.,* 2022).

1-2 The aims of study:

- To isolate and identify of sensitive TB and resistant TB from sputum samples
- To study the gene expression of *whiB3, whiB7, pknF*, and *fbpA* genes in drug-sensitive TB and MDR-TB and their association with disease severity.
- To evaluate the levels and roles of IL-4, IL-6, IL-10, and IL-12 cytokines in the host immune response to M.tb, MDR-TB infection and patients under the treatment.
- To find the correlation between the genes expression levels and interleukins levels.

CHAPTER TWO

LITERTURE REVIEW

2 Literature Review

2-1 History of Tuberculosis

The ancient human remains presented signs of TB infection were found in an ancient settlement in the eastern Mediterranean called (Atlit Yam), back to the Neolithic era, nearby (9,000) years ago. DNA examinations confirmed that these individuals had *Mycobacterium tuberculosis* complex (MTBC) in their bones (Jäger *et al* ., 2022). The genus Mycobacterium is believed to have evolved in the environment roughly (150) million years ago, and a primary form of M.tb appeared in East Africa about (3) million years ago. The spread of TB from animals to humans have happened by several ways such as domestication, hunting, or interact with animal products (Jordan *et al*., 2023).

TB has had diverse terms and meanings in different cultures and religions through history. In ancient Greece, TB was recognized as (phthisis), which means consumption or wasting away and was realized as a genetic illness. In ancient Rome, it was named as (tabes), which means decay or rottenness, and was accused on the poor living environments of the city residents. In ancient Hebrew, it was interpreted into (schachepheth), which refer to corruption, and was noticed as a divine punishment for human sinners. In the Mid Ages, TB which infected the neck and lymph nodes was termed "scrofula or king's evil", and was believed to be cured by the touch of a king or a saint. In the (1700s), TB was named the white plague because of the paleness of the patients, and also called consumption due to its damaging effects on the body (Jain, 2020; Rai, 2020).

The leading scientific progress in the understanding of TB happened in (1720), after the English doctor Benjamin Marten advocated that TB disease caused via "wonderfully minute living creatures" that possibly be delivered from person to person by air or contact (Mousa *et al*., 2019). However, his concept was not broadly conventional until over a century later on, once the German scientist Robert Koch discovered the M.tb as the cause of TB on "March 24, 1882". This day is now celebrated as "World TB Day" to increase consciousness about the global problem of TB and the urgent necessity for their elimination. Koch's finding encouraged more research and innovation by other scientists and institutions (Hasnain, 2019; Heffernan, 2021).

2-2 The Causative agent of Tuberculosis

For several ages, the causative agent for TB continued as unknown and confusing among medical specialists. However, this puzzle was eventually unraveled by the groundbreaking effort of well-known scientist Robert Koch in (1882), when he identified the tubercle bacillus, marking an important announcement in medical history (Cain *et al.,* 2015). Then, the pathogen was primarily mentioned as tubercle bacillus then later re-named into *M. tuberculosis* (Gurjav *et al*., 2015).

There is an inclusive classification system that reveals the complicated nature of this deceptive pathogen (Dara *et al*., 2012; Hirpa *et al*., 2013). This group mentions as *Mycobacterium tuberculosis* complex (MTBC), which is a multifaceted group that comprises diverse lineages and strains of mycobacteria, including: *M.tuberculosis, M.bovis, M. africanum, M. microti, M. canetti, M..caprae,* and *M. pinnipedi,* each facing unique challenges to human and animals health (Tesfay *et al*., 2016). Among them, M.tb which is the most main species linked with TB disease in

humans is categorized by its rod- shape, aerobic, non-spore forming, and non-motile bacteria, giving an important obstacle for medical researchers in their mission to improve active Defensive strategies (Asfaw *et al*., 2010).

2-3 Clinical manifestations of Tuberculosis

TB is a bacterial infection that can affect any part of the body, but it frequently affects the lungs, and can be diffused over the air when a sickness individuals with active TB coughs or sneezes. TB disease can be categorized into two types: active and latent. Active TB happens when the bacteria multiply and cause disease symptoms, and it can be transmitted to others. Latent TB occurs when the bacteria are existing in the body but do not cause any symptoms and it is not spreadable. However, latent TB can develop into active form at any given time if the immune system is declining for certain reasons such as human immunodeficiency virus (HIV) infection, diabetes, malnutrition, alcohol abuse, chronic kidney illness, cancer, and organ transplantation (Peto *et al.,* 2009).

The symptoms of active TB vary reliant on which portion of the body is infected. The most common form of TB is pulmonary TB, which affects the lungs. The symptoms of pulmonary TB include a cough that continues for more than three weeks, chest pain, fever, night sweats, weight loss, fatigue and loss of appetite (Alshoabi *et al*., 2022). An additional form of TB is extra-pulmonary TB, which affects additional parts of the body such as the lymph nodes, bones, joints, abdomen, brain, kidneys or skin. The symptoms of extra-pulmonary TB determined by the spot of infection such as swollen lymph nodes, bones or joint pain, abdominal pain, meningitis (inflammation of the membranes that surround the brain and spinal cord), kidney disease or skin lesions (Gopalaswamy *et al*., 2020; Alshoabi *et al.,* 2022).

2-4 *Mycobacterium tuberculosis* transmission

The transmission of M.tb happens through airborne droplets released when those with active TB performance particular activities such as cough or sneeze (Patterson *et al*., 2021). These droplets can stay for about 20 minutes to 6 hours in the atmosphere, reliant on other factors as temperature, humidity, size, concentration, and air circulation. Which carrying the highly infectious M.tb bacilli. Researchers estimate that an individual with active TB has the capacity to transfer the pathogen to (3-10) individuals per year (Nardell, 2016).

Healthy individuals who come into touching TB patients have a 10% risk of developing active TB, which can result in severe consequences, including death (Pai *et al*., 2016). In fact, approximately half of those infected with active TB possibly will not live with the disease. However, treatment with antibiotics can mainly lead to a period of latency long-lasting into 6 months, for the period of that the bacteria are successfully suppressed (Chee *et al*., 2018).

2-5 Epidemiology of Tuberculosis

TB is a serious infectious disease that poses a major challenge for Iraq's public health system. Even with the efforts of the World Health Organization (WHO) and other participants, Iraq still has a high TB burden and a low case recorded rate. According to the WHO, Iraq reported 41,000 TB cases in 2019, with an expected incidence rate of 42 per 100,000 population. However, this number could be an underestimation, as the case detection rate for all TB cases was only 54%, meaning that several TB cases go unreported or undiagnosed. The treatment success rate for new detected and relapse TB cases was 83%, which is below the global target of

90%. These statistics designate that Iraq faces multiple challenges in monitoring and stopping TB spread and resistance (WHO,2020).

Related to other countries in the Eastern Mediterranean region, Iraq has a moderate TB burden ranking seventh among 22 countries in terms of the incidence rate. However, Iraq's performance in terms of case detection and treatment success is lower than the regional average. Iraq also accounts for 3% of all TB patients in the Eastern Mediterranean region (WHO,2022).

WHO reported alarming numbers about TB in 2020. An estimated 9.9 million people suffered from this transmittable disease, resulting in 1.5 million deaths (WHO, 2021), and these statistics are expected to grow as a result of the surge in MDR-TB cases. MDR-TB is responsible for 3.6% of new TB cases and 20.2% of relapse TB cases globally. Asia and Africa have the highest incidence of TB, with an estimated 58% and 27% of cases respectively. India and China accounted for a staggering 38% of all reported cases (Sulis *et al.,* 2014). The ostracized communities are more vulnerable to TB due to their inadequate access to healthcare, which includes prisoners, homeless people, migrants/refugees, and people with HIV. Despite centuries of research, the knowledge of host-M.tb interaction remains incomplete (Langer *et al*., 2019).

2-6 Diagnosis of tuberculosis and multi-drug resistance tuberculosis (MDR-TB)

TB is a multifaceted disease with diverse indicators, encompassing active TB disease and latent TB infection (LTBI). LTBI is characterized by persistent bacterial viability, immune control, and the absence of clinical symptoms, rendering its diagnosis a challenging endeavor. Presently, a

variety of diagnostic methods are employed for both LTBI and active TB detection, each with its own strengths and limitations (Zumla *et al.,* 2013; Getahun *et al*., 2015):

- **Tuberculin Skin Test (TST):** This method is used to diagnose latent tuberculosis infection (LTBI). However, it's not specific particularly in populations immunized with Bacille Calmette Guérin (BCG) (Pia *et al*., 2014).
- **Interferon-Gamma Release Assay (IGRA):** Has more specificity for LTBI diagnosis but IGRA needs laboratory equipment and higher costs compared to TST (De Keyser *et al.,* 2014).
- **Sputum Smear Microscopy**: A rapid and cost-efficient method for diagnosing active TB disease, but it exhibits low sensitivity, particularly among children and individuals with compromised immunity (WHO, 2010).
- **Sputum Culture:** Considered as the confirmatory gold standard for TB diagnosis, sputum culture is obstructed by the slow growth rate of the bacteria leading to delays in obtaining results (Sia and Wieland, 2011).
- **Molecular Diagnostics** (e.g., Xpert® MTB/RIF): This type offers faster and more precise results compared with sputum smear microscopy. Additionally, it can identify rifampicin resistance (RR) and enhancing the diagnostic process (WHO, 2016).
- **Phenotypic or Genotypic Methods**: These particular techniques provide a detection of drug-resistant tuberculosis strains such as MDR-TB and extensively drug-resistant TB (XDR-TB) (Dheda *et al.,* 2017).
- **Genotype MTBDRplus Assays**: Permitted by the WHO for immediate identification of rifampicin, isoniazid, fluoroquinolones, and second-line

injectable drug resistance MDR-TB and XDR-TB (Dinnes *et al.,* 2007; WHO, 2015).

2-7 Tuberculosis treatment

The management of TB usually includes a grouping of four standard or first-line anti-TB drugs, namely isoniazid, rifampin, pyrazinamide, and ethambutol. This treatment is effective in curing the majority of TB cases, and the treatment length generally spans 6 to 9 months. However, it is important to accept that certain TB bacteria able to develop resistance to these drugs which is in need necessitating more intricate treatment methods (Gafar *et al*., 2023).

MDR-TB is a form of TB that does not respond to as a minimum two of the most effective TB drugs isoniazid and rifampin. MDR-TB necessitates treatment with second-line drugs, including fluoroquinolones and injectable agents such as (amikacin, kanamycin, or capreomycin). The treatment length for MDR-TB typically prolongs from 18 to 24 months (Pontali *et al.,* 2019).

Extensively Drug-Resistant TB (XDR-TB) is a rare variant of MDR-TB, categorized by resistance to fluoroquinolones and at least one of three injectable second-line drugs. XDR-TB poses substantial treatment tasks and brings a heightened risk of mortality. The treatment for XDR-TB is an extended course, spanning 24 to 36 months (Singh *et al*., 2020).

Total Drug-Resistant TB (TDR-TB) is a term used in specific regions to define TB strains that exhibit resistance to almost all existing anti-TB drugs. It's essential to note that the WHO does not formally categorize TDR-TB as a separate type of drug-resistant TB. The treatment for TDR-TB absences a standard protocol and needs individualized therapy based on drug

susceptibility testing (DST) results (Khawbung *et al*., 2021; Imran *et al*., 2021).

2-8 *Mycobacterium tuberculosis* genome and virulence factors:

The M.tb genome comprising around (4.41) million base pairs and about (4000) genes (Behir *et al.,* 1999), it has a critical pathogenicity region identified as region of difference-1 (RD-1) presented in clinical isolates of virulent reference strain H37Rv and virulent strains of *M. bovis* but lacking in BCG vaccine (Samten *et al.,* 2011).

The development and spread of MDR-TB is critical as this disease poses a severe threat to worldwide health safety. MDR-TB is generally caused by insufficient or incorrect usage of drug-susceptible TB (Hang *et al.,* 2013; Mulu *et al.,* 2015). However, various results are inconsistent and contradictory, and the geographic locality restrictions in these studies limit their findings. Therefore, an international systematic review and meta-analysis were achieved to detect independent causes that predict MDR-TB versus drug-susceptible TB (Shin *et al*., 2020).

Actinobacteria possess *whiB* genes (Alhadlaq *et al.,* 2021), making them remarkable targets for medications. In mycobacteria, WhiB proteins show countless roles in cell division, redox homeostasis, virulence, and antibiotic resistance (Mehta and Singh, 2019).

whiB3 is a unique factor that engages in M.tb pathogenesis, as it stimulates the synthesis of virulence lipids that act as a redox sink throughout infection (You *et al.,* 2019). It also has redo-sensitive DNA-binding activity and sense intracellular redox levels to modify metabolic activities (Mahatha *et al*., 2020). A potential *whiB3* binding site screening proposes that it activates genes linked with fatty acid metabolism and stress

responses. Additionally, it is greatly upregulated through the late stationary phase and acidic stress (Larsson *et al*., 2012).

The role of *whiB3*-M.tb and its influence on cytokine levels has been inspected in numerous studies. Upon inhalation into the lungs, M.tb elicits the release of different cytokines such as IL-1, IL-6, IL-8, TNF-α, GM-CSF, and TGF-β via phagocytic cells (Ma *et al*., 2021). These cytokines have immune-regulatory effects facilitated clinical manifestations of infection, including tissue necrosis, fibrosis, and fever. However, immune-suppressive cytokines for instance, IL-10 and TGF-β are also secreted to decrease acute inflammation and tissue damage (Nair *et al.*, 2018).

Previous studies have observed the correlation between microorganism load and host survival, proposing that specific mycobacterial factors modulate the equilibrium between pro-inflammatory and inhibitory cytokines to weaken the host survival (Etna *et al.*, 2014).

whiB7 is predominantly remarkable as it provides resistance to several antibiotic classes (Cushman *et al*., 2021). Together with antibiotic treatment, *whiB7* is upregulated in response to certain physiological stresses such as iron starvation, hypoxia, nitric oxide, heat shock, and entry into stationary phase. It acting in virulence since it is one of the genes upregulated in the M.tb complex within inactive or activated murine macrophages together with *whiB3* (Burian *et al*., 2012). Temporal investigation of the M.tb transcriptome throughout macrophage infection showed that *whiB7* is one of the first genes expressed once entry into the hostile environment (Lee *et al.,* 2022).

The *whiB7* transcriptional activator shows a role in inducible drug resistance and persistence of M.tb in infected cells (Lilic *et al*., 2021). The

redox environment of the bacterium effects the action of *whiB7* and alters in this environment arise during exposure to antibiotics while existing in macrophages and granulomas, which are low-oxygen, low-nutrient environments (Mahatha *et al*., 2022). WhiB7 protein is considerably up-regulated as soon as M.tb infected phagocytic cells, probably because of nutrient starvation, and is responsible for triggering genes that respond to the effects of stressful situations. For example, iron starvation and entry into the stationary phase (Nguyen and Thompson, 2006).

Besides its role in inducing drug resistance, *whiB7* plays a role in pushing harmful metabolic leftover out of the M.tb cytoplasm. This is an actual importance since lipid eating contributes to reduce hassle and generates toxic derivatives for example, propionyl-CoA (Pollock, 2014). There are presently 12 recognized genes that are regulated by *whiB7*, including *Rv2416c (eis:*enhanced intracellular survival) which is associated with kanamycin resistance and modulation of cytokine secretion, *Rv1258c* this gene encoded a protein named as conserved integral membrane transport, this protein responsible for pump out drugs mostly macrolides across the membrane, and *Rv1988* which are a member of the mycobacterial membrane protein large (Mmpl), which is thought to be associated with inducible drug resistance (Reeves *et al.*, 2013; Lilic *et al*., 2021).

The *fbpA* gene is a key element in the biosynthesis of mycobacterial cell walls (Mandato and Chai, 2018). The mycolic acids in the cell walls of mycobacteria are primarily esterified to trehalose in arrangements that can be extracted by organic solvents or covalently linked to the arabino-galactan in the cell wall (Nguyen *et al*., 2005). The *fbpA* gene is part of a group of at least three enzymes which were initially considered as major antigens of

M.tb identified as the antigen-85 complex. Later, they were found to be fibronectin-binding proteins (*Fbp*) assisted the phagocytosis in macrophages (Jiang *et al.,* 2015; Artama *et al*., 2021).

These proteins were found to catalyze a mycolyltransferase reaction that is crucial for cell wall biogenesis. Genetic studies displays that all three M.tb *fbp* genes could be disrupted separately and they play roles in cell wall biosynthesis. The fact that any defect in a synthetic analog of an Fbp substrate was capable of inhibiting growth and biosynthesis of the cell wall demonstrates that these proteins have similar activities and are essential and attractive targets for new anti-mycobacterial drugs (Piubelli *et al.,*2013: Zarif *et al*., 2013). Targeting the *fbpA* gene possibly will be one of an effective approaches to interrupt the cell wall biosynthesis and prevent the growth of mycobacteria (Nguyen *et al.*, 2005).

Protein phosphorylation is a vital mechanism for transforming environmental signals into intracellular in both prokaryotes and eukaryotes (Barthe *et al.*, 2009). In M.tb, Serine/Threonine protein kinases (STPKs) are necessary for environmental sensing. STPKs not just alter protein function but also boost protein interactions through Phosphorylation-Dependent mechanisms (Panni, 2019). In M.tb, there are eleven STPKs contributing in various substrates involved in all biological processes of the bacterium (Cabarca *et al.*, 2021).

pknF STPK (*Rv1746*) plays a vital role in numerous physiological processes as a result of its different range of substrates (Wagner *et al*., 2019). *pknF* is involved in cell division, arabinoside production, mycolic acid production, peptidoglycan production, tricarboxylic acid cycle, methionine cycle, chaperone, and transportation (Richard-Greenblatt and

Av-Gay, 2017). The conservation of STPK genes throughout evolution and the upregulation of the members of this family for the duration of M.tb infection showed their importance in pathogenesis.

Extensive exploration has been conducted on the functions of *pknF* in M.tb. Previous studies have recommended that *pknF* regulates growth, cellular morphology, glucose transference, sliding movement, and biofilm formation. (Spivey *et al.*, 2011; Rastogi *et al*., 2021).

2-9 Innate immune response to M.tb :

According to a growing body of evidence, innate immunity plays a vital role in the immune response against M.tb. Innate immune cells have been shown to facilitate communication with adaptive immunity and thus play a unique role in determining the balance between protective and pathogenic immune responses in human TB (Ferluga *et al*., 2020). Macrophages, dendritic cells, natural killer cells, and neutrophils are the principal innate immune cells involved in responding to M.tb (Liu *et al*., 2017). However, recent studies have also highlighted the contribution of other cells including airway epithelial cells in the immune response function against M.tb in human (de Waal *et al.,* 2022). Therefore, it is clear that innate immunity is a critical component of the immune response against TB, and its various cell types play vital roles in shaping the immune response to M.tb (Shamaei and Mirsaeidi, 2021).

2-9-1 Neutrophils:

Neutrophils play a role in the innate immune response and are essential in protecting against TB infection. These immune cells are responsible for various functions such as chemotaxis, phagocytosis, and activation of other immune cells, all of which are important in eliminating invading microbes

(Dallenga *et al.,* 2018). Despite their significance in M.tb infection, neutrophils are often overlooked as crucial responders compared to other components of the human immune system, possibly due to the challenges in working with them. However, recent research has provided insights into the role of neutrophils during TB in both animals and humans (Dallenga and Schaible, 2016).

Studies have shown that neutrophils make a significant contribution in the early defense against mycobacterial infection in animal models, and they are the most abundant inflammatory cell type during early murine pulmonary TB. It has also been shown that recruiting neutrophils to the site of infection improves outcome, but this is believed to only occur when neutrophils are recruited early during infection (Kruger *et al*., 2015).

Neutrophils employ mechanisms of action such as phagocytosis and oxidative burst to control pathogens such as M.tb. Compared to macrophages, neutrophils have higher levels and intensity of phagocytosis and oxidative respiratory response (Cerdeira *et al*., 2022). During phagocytosis, microbes are engulfed into phagosomes, which rapidly fuse with intracellular granules to form phago-lysosomes. Neutrophils then generate reactive oxygen species (ROS) and release them from granules which contributing to the control of pathogens such as M.tb (Nwongbouwoh Muefong *et al.,* 2022). Oxidative burst/killing of ROS includes superoxide and hydrogen peroxide, involving the assembly of the (NOX2-containing) (NADPH-Oxidase complex) at the phago-lysosomal membranes. However, there are contradictory reports about the phagocytic potential and oxidative burst capacity of neutrophils in TB infection. Depending on mycobacterial virulence and immune status of the host, even if phagocytosis occurs M.tb can still escape the immune response. M.tb exploits neutrophilic

inflammation to preferentially replicate at sites of tissue damage that promote contagion, and nitric oxide primarily affects this neutrophilic influx by repressing an IL-1 (Bylund *et al*., 2010; Mishra *et al*., 2017; Zemskov *et al.,* 2019).

Neutrophils employ an arsenal of proteolytic enzymes stored in their granules, such as neutrophil elastase, proteinase-3, and cathepsin-G, to control pathogens such as M.tb. These enzymes are stored in large quantities in neutrophil cytoplasmic azurophilic granules (Jena *et al.,* 2012; Thorpe *et al*., 2018). The controlled activation and release of these enzymes are crucial, as they have the potential to be highly destructive to normal tissues. Once activated, they act in combination with (ROS) to help degrade engulfed microorganisms inside phago-lysosomes. M.tb strains have been shown to influence the neutrophil enzyme secretion. Neutrophils play a major role in the development of lung lesions during TB disease, and hypoxia and hypoxia-induced factors inside granulomas may impact neutrophil function and TB pathophysiology (Steinwede *et al.,* 2012; Remot *et al*., 2019). The activation of oxidative burst in neutrophils by M.tb regulates the inflammatory response by induction of apoptosis, and apoptotic neutrophils can augment the pro-inflammatory response activated in human macrophages during M.tb infection (Andersson *et al.*, 2014; Allen and Criss, 2019).

2-9-2 Macrophage

Macrophages are an integral part of the innate immunity responsible for identifying and eliminating foreign materials in the body. These white blood cells are found throughout the body and can differentiate into specialized cells based on their location. Macrophages perform several crucial functions, including phagocytosis of pathogens, antigen presentation,

and cytokine production (Fu *et al*., 2021; Aegerter *et al.,* 2022). Macrophages act as a bridge between innate and adaptive immunity, making them major regulators of the inflammatory response during infection (Brady *et al.,* 2016).

When M.tb enters the body, macrophages are the first line of defense. These immune cells recognize and engulf the bacteria, attempting to eliminate them through phagocytosis. However, M.tb is a challenging pathogen that can evade the immune system and survive inside macrophages, leading to the establishment of chronic infection that can persist for years (Schifano and Woychik, 2017; Singh *et al*., 2022).

In response to M.tb infection, macrophages undergo a series of changes collectively known as macrophage activation. There are two broad types of macrophage activation: M1 (classical macrophage) and M2 (alternative macrophage). M1 macrophages are activated by cytokines produced by other immune cells in response to M.tb infection. Once activated, M1 macrophages produce nitric oxide and other toxic molecules that can kill the bacteria. M1 macrophages also produce pro-inflammatory cytokines that help recruit other immune cells to the site of infection (Wang *et al.*, 2020; Das, 2021).

M2 macrophages, on the other hand, are activated by cytokines produced by the infected macrophages themselves, as well as by other factors such as tissue damage, or anti-inflammatory signals. These cells are involved in tissue repair and play a role in regulating the immune response to prevent excessive inflammation. However, M2 macrophages are also associated with promoting the growth and persistence of M.tb, which can lead to chronic infection (Mily *et al*., 2020; Cho *et al*., 2020).

The balance between M1 and M2 macrophages is crucial for controlling M.tb infection. Too much M2 activation can lead to chronic infection, while too much M1 activation can cause tissue damage and exacerbate inflammation. Thus, understanding the mechanisms that regulate macrophage activation is crucial for developing new therapies to treat tuberculosis (Yu and Tang, 2019).

One potential approach is to target the host immune response rather than the bacteria itself. Several drugs that target specific immune pathways have been shown to improve outcomes in TB patients. These drugs modulate the immune response by promoting M1 activation and suppressing M2 activation, enhancing bacterial clearance and reducing the risk of chronic infection (Tardito *et al*., 2019).

2-9-3 Natural Killer (NK):

NK cells are a type of granular innate cell that originates from the lymphoid lineage and possesses the cytolytic capacity (Kucuksezer *et al*., 2021). Unlike macrophages and dendritic cells, NK cells are not exclusively MHC-restricted and rely on receptor-ligand interactions for activation (Ravindranath *et al.,* 2019). NK cells have been shown to have the ability to liaise M.tb-infected macrophages (Paidipally *et al*., 2018). Additionally, studies have shown that NK cells can inhibit the intracellular growth of M.tb by promoting phagolysosomal fusion through the production of IFN-gamma and IL-22 (Dhiman *et al.,* 2009). Furthermore, Vankayalapati *et al*. (2004) demonstrated that NK cells can stimulate the production of IFN-gamma from CD8 T cells by promoting the production of IL-15 and IL-18 from M.tb-infected monocytes.

2-9-4 Dendritic Cells:

Dendritic cells serve as an important component in the connection between innate and adaptive immunity. One of the main roles of dendritic cells is to act as antigen-presenting cells enable adaptive responses through antigen presentation, co-stimulatory capacity, and the release of T-helper cytokines (Xu *et al*., 2022).

2-10 Adaptive Immune Response to M.tb:

The immune system's ability to counteract M.tb counts on effectual interactions between antigen presenting cells (APCs) and several T cell subsets located in the lung draining lymph node (Ankley *et al.,* 2020). Circulating naïve T cells distinguish M.tb antigens by the MHC on the APCs surface (Morgan *et al*., 2021). Stimulated T cells then move to the infection spot and assist to regulate the immune response by secreting pro-inflammatory cytokines such as IFN-γ, IL-2, and TNF-α, which increase macrophages' anti-mycobacterial action. This containment process comprises both CD4+T cells and CD8+ T cells (Cooper, 2009).

2-10-1 CD4-T Cells

CD4 T cells detect M.tb peptide antigens exhibited on MHC-class II molecules. As soon as activated naïve CD4+T cells differentiate into main T cell subsets into T helper Th1, Th2, Th17, and regulatory T cells (Tregs), giving to exact transcription factor expression and cytokine secretion (Luckheeram 2012; Sakai *et al*., 2014). Precursor Th0 cells differentiate under the effect of cytokine such as IL-12, IFN-γ, IL-4, and TGF-β (Kara *et al.,* 2014). The kind of CD4+ T cell subgroup generated decides the outcome of M.tb infection, leading to pro-inflammatory, anti-inflammatory, or regulatory responses (Richardson *et al*., 2015).

CD4+ Th1 cells are the crucial players in M.tb immune control, which secrete cytokines such as IFN-γ, IL-2, and TNF-α, causing macrophage and CD8+ T cell stimulation, along with M.tb infection controls. IFN-γ which is a significant pro-inflammatory cytokine secreted by activated CD4+ Th1 cells and other lymphocytes (Prezzemolo *et al*., 2014).

Conversely, the CD4+ Th2 cell has been connected with the TB progression due to their cytokine secretion such as IL-4, IL-5, and IL-10 (McLaughlin *et al*., 2020). Th2 cytokines inhibit Th1 cell immune responses, leading to loss of M.tb immune control and TB disease progression. This indicates that a balance between Th1 and Th2 cell is required for active M.tb infection control (Ashenafi *et al.,* 2014).

2-10-2 CD8-T Cells

CD8+ T cells also play a part in immune protection against M.tb (Lin and Flynn, 2015). Generally, CD8+ T cells identify M.tb antigens processed in the cytosol and presented onto MHC-class I molecules (Mott *et al*., 2022). Activated CD8+ T cells produce cytotoxic and anti-microbial molecules as granzymes, granulysin, and perforin to eradicate infected cells or directly destroy M.tb (Aerts *et al.,* 2019). Additionally, CD8+ T cells secrete cytokines as IFN-γ, TNF-α, and IL-2, which boost immune cell stimulation and trigger of apoptosis in infected cells (Prezzemolo *et al.,* 2014).

In humans, higher proportions of M.tb-specific CD8+ T cell responses have been detected in TB patients, linking with increased M.tb antigen burden and granulomas compared to those with latent TB infection (LTBI) and healthy individuals (Sharan *et al.,* 2021). Furthermore, differences in phenotypic and functional features of M.tb-specific CD8+ T cells have been noted in active TB and LTBI patients, representing that M.tb-specific CD8+

T-cell responses vary with the activity and clinical demonstration of M.tb infection (Rozot *et al*., 2013).

2-10-3 B-Cell:

The role of B-cells and antibodies in TB patients is a topic of interest that can be explored in three main ways. Firstly, B cells can contribute to the host's response to TB by secreting various cytokines and chemokines that can influence the differentiation of CD4+ naive T cells by releasing Th1-Th2 type cytokines (Lu *et al*., 2016; Mattos *et al.*, 2016). Secondly, B cells have been shown to improve the functions of the effector immune cells. For instance, B cells adjust the neutrophil recruitment in the lung throughout TB infection (Zeng *et al*., 2020). Moreover, B cells show TB antigens to T cells for effectively stimulating these cells (Rijnink *et al*., 2021). Thirdly, precise antibodies produced by B-cells against M.tb stimulate anti-microbial activity after being noticed by FC receptors of effector immune cells (Irvine *et al*., 2022).

2-11 Cytokines

The host immune system organizes a collection of proteins identified as cytokines to fight infectious diseases. These signaling molecules are low-molecular weight secreted by leukocytes and other cells in response to several stimuli, and they simplify interaction between cells. Cytokines contain numerous molecules, including interleukins, interferons, tumor necrosis factor, and chemokines, which set the recruitment of immune cells to diverse locations. Throughout TB infection, the recognition of M.tb-antigens by phagocytic cells lead to the activation and secretion of cytokines

that enhance innate immune responses and initiate adaptive immunity (Dorhoi and Kaufmann, 2016; Waghmare *et al.,* 2019).

Cytokines secreted through M.tb infection such as pro-inflammatory, which is useful for adjusting the pathogen but can also cause tissue damage if the response is extreme, or anti-inflammatory which will weaken the immune response for M.tb. Pro-inflammatory cytokines such as IFN-γ, IL-1β, IL-6, IL-12, IL-17, and TNF-α, stimulate inflammation, induce the immune response, stimulate macrophages and T cells at the spot of infection, and improve host immune resistance against the pathogen (Ronacher *et al*., 2018; Sivakumaran *et al.,* 2022).

Intracellular infections as TB affected as well by cytokines such as **Interleukin-4**, which inhibits IFN-γ production and macrophage activation. In TB infected mice, increased production of IL-4 is linked to disease progressive and TB reactivation, although it remains uncertain whether IL-4 correlated with human TB severity (Heitmann *et al*., 2014; Chatterjee *et al*., 2017).

Interleukin-6 is a pleiotropic cytokine produced by numerous cell lineages and has been involved with various human chronic inflammatory diseases (McElvaney *et al*., 2021). In the case of TB, IL-6 subsidizes to the host resistance by stimulating a pro-inflammatory response and regulating additional cytokine secretion. It has been revealed to play a protective role for M.tb infection and the initiation and maintenance of acquired immunity (Ritter *et al*., 2020; Boni *et al*., 2022). Genetic variations in the IL-6 promoter have been connected with susceptibility to TB, and studies using IL-6 deficient mice propose that it plays a serious role in protective immune response against mycobacterial infection (Chen *et al.,* 2012).

Cytokines with anti-inflammatory or regulatory functions such as **Interleukin-10** suppresses the pro-inflammatory response to M.tb, which can assist TB development. IL-10 suppresses macrophage activation, down-regulates IL-12, and inhibits Th1-cell development and IFN-γ secretion. IL-10 also inhibits T-cell proliferation and pro-inflammatory cytokine production, and inhibit cytotoxic T-cell function in granuloma (Shi *et al*., 2016; Wong *et al*., 2020).

Interleukin-12 is another cytokine that plays a vital role in anti-TB cell-mediated immunity and is the main cytokine responsible for guiding Th1 differentiation in vitro and in vivo (O'Garra *et al.*, 2013). Therefore, IL-12 shows a crucial role in host protection against intracellular pathogens such as M.tb. It is mainly produced by dendritic cells and macrophages, and it directs the immune response against the M.tb infection (Domingo-Gonzalez *et al*., 2016).

2-12 Granuloma Formation

A TB granuloma characterizes a distinctive structure, mainly located in the lung and composed of a group of M.tb-infected and uninfected immune cells that limit M.tb growth (Martinot, 2018). The granuloma principally comprises of macrophages, T-cell subsets, along with other cells such as dendritic cells, neutrophils, B-cells, giant cells, plasma cells, and central necrotic macrophages (Russell *et al*., 2009). This immune microenvironment shows a fundamental role in coordinating the interactions of these cells to limit bacterial spreading and replication in uninfected tissues (Ashenafi and Brighenti, 2022).

Granuloma formation creates when inhaled M.tb bacteria are engulfed by macrophages and unable to eliminate the bacteria, allowing the bacteria to persist within the infected macrophages (Santucci *et al*., 2016). Leading to triggers apoptosis and subsequently attracts more innate immune cells, including neutrophils, dendritic cells, and monocyte-derived macrophages to the infected area (Figure 1), thereby promoting inflammation and the initial establishment of M.tb infection within the granuloma. Adaptive immunity, mainly driven by Th1 cells that secrete pro-inflammatory cytokines such as IFN-γ and TNF-α, and chemokines that recruit T-cells to the infection site, which are significantly contributing to granuloma formation and the sustained containment of M.tb infection (Zuniga *et al.,* 2012).

While granulomas can control bacterial extension at the infection site, M.tb inside the granuloma constantly activates host immune cells. If this process is weakened under immunosuppressive situations, it could lead to the spread of the infection, mostly in individuals with HIV infection and other risk factors (Pagán and Ramakrishnan, 2015). In such cases, the granuloma assists as a site for bacterial re-activation from dormancy causing the progress of primary TB disease. However, in around (90)% of infected and immune-competent individuals remains inactive for entire life without developing active TB forms (Saunders, 2022).

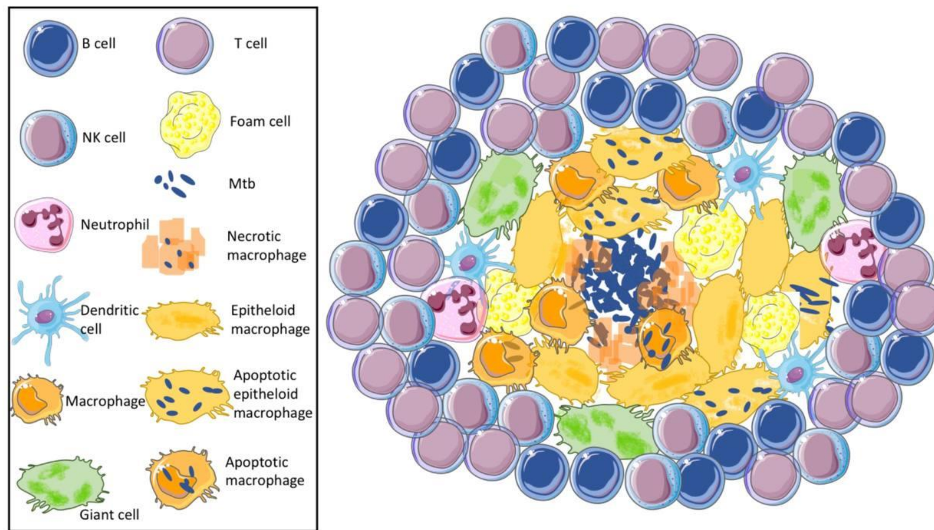


Figure: (2-1) A visual representation displaying the diverse cells and arrangement of a granuloma in tuberculosis (McClean and Tobin, 2016)

CHAPTER THREE

MATERIALS AND METHODS

3- Materials and Methods

3-1 Subjects

The present study included (168) sample were collected from different clinical cases and healthy control. They were distributed between (120) blood samples included 24 sample with sensitive pulmonary TB (G1), 24 sample with sensitive extra-pulmonary TB (G2), 24 sample TB patients under treatment (G3), and 24 sample multi-drug resistance TB (G4). In addition to 24 healthy control. Also the study included 24 Sputum sample with 12 sensitive M.tb and 12 sample MDR-TB, during the period from (2022/8/23 to 2023/1/20) from Tuberculosis Center Misan/Al-Amarah city.

3-2 Selection Criteria

1. **Patient Selection**: Select patients who had been diagnosed with either sensitive TB or MDR-TB. Ensure that the patients are at a similar stage of the disease and have not received any prior treatment for TB. However, patients under treatment duration have been taken as well to monitor the interleukins levels.
2. **Age and Sex**: Age and sex can affect the immune response to TB infection. Therefore, this study considered the age and sex when selecting patients to ensure that they are matched between the sensitive TB and MDR-TB groups.
3. **Geographic location**: All patients were selected from Misan province in South Iraq and the study selected two groups of patients which lived in urban and lived in rural.
4. **Virulence factors**: This study chose virulence factors that were known to be associated with the virulence of TB and had been well-studied in previous research.

5. **Collection Time**: Samples were collected at specific time points which is the diagnosed stage of the disease to capture changes in gene expression and cytokine levels, also a group of patients under treatment took as well in this study.

6. **Clinical parameters**: Collected relevant clinical parameters such as chest X-rays as seen in Appendix(4), sputum Smear as is seen in Appendix(1), and drug resistance profiles.

7. **Informed Consent**: Ensured that all patients gave informed consent for sample collection and analysis, and that ethical guidelines for human subjects research are followed.

3-3 Ethical Approval

This study was conducted in compliance with the ethical considerations outlined in the ethical guidelines approved by the Committee on Ethical Standards in the College of Science at Misan University.

3-4 Questionnaire Sheet

Complete clinical histories and patient information were directly obtained from each case. This information was then organized into a detailed and informative data collection form as in appendix (3).

3-5 Equipment's and their suppliers

Table (3-1): List of equipments

Instrument	Company	Country
Autoclave	Hirayama	Japan
Biosafety cabinet	Kimo,24700Montpon	France
Centrifuge	Heraeus	England
Cooling Centrifuge	Hettich	Japan
Distillator	GFL	Germany
ELISA multi-well reader plate	Bio-Rad	India
Flask (250-500) ml	Schott	Germany
Hot Plate	GallenKamp	England
Incubator	Memmert	Germany
Light microscope	Olympus	Japan
Micopipettes (0.5-10), (5-50), (100-1000)	Gilson instrument	France
Oven	Memmert	Germany
Quantus Fluorometer	Promega	USA
Refrigerator	Diora	Turkey
SaCycler-96 Real Time PCR SYSTEM	Sacace	Italy
Sensitive Balance	Sartorius	Germany
Thermostatic Incubator	Zxinstrument	Chain
Vortex	Fisher Scientific	USA

3-6 Consumer Materials

Table (3-2): List of consumer materials and their suppliers

Consumer	Company	Country
Cotton	SHAKEEB	Malaysia
Cover slips	MEHE	China
Disposible gloves	SHAKEEB	Malaysia
Eppendorf tubes	BDH	UK
Forceps	Hebson	India
Gel tubes	AL-Rayaan	China
Pasteur pipette	BioMerieux	France
PCR tubes	BDH	UK
Pipette tips	BOENMED	China
Plain tube	RITTAL	China
Plastic cups	Shangai Blopak	China
Screw capped tube	Schott	Germany
Slides	MEHE	China
Spatula	Hebson India	India
Syringe	CHANGZHOU	China

3-7 Laboratory kits

Table (3-3): Type of kits applied in the current study and their company

Name of kits	Manufacture	Country
AFB stain	Himedia	India
Favor Prep Blood/ Cultured Cell Total RNA Mini Kit	FAVORGEN	Korea
Go Taq® q PCR Master Mix	Promega	USA
Interleukin-10	Elabscience	Swedish
Interleukin-12	Elabscience	Swedish
Interleukin-4	Elabscience	Swedish
Interleukin-6	Elabscience	Swedish
Luna Script Reverse Transcriptase	Bio lab	England

3-8 Design of experimental study

The general steps for study are shown in the Figure below (3-1)

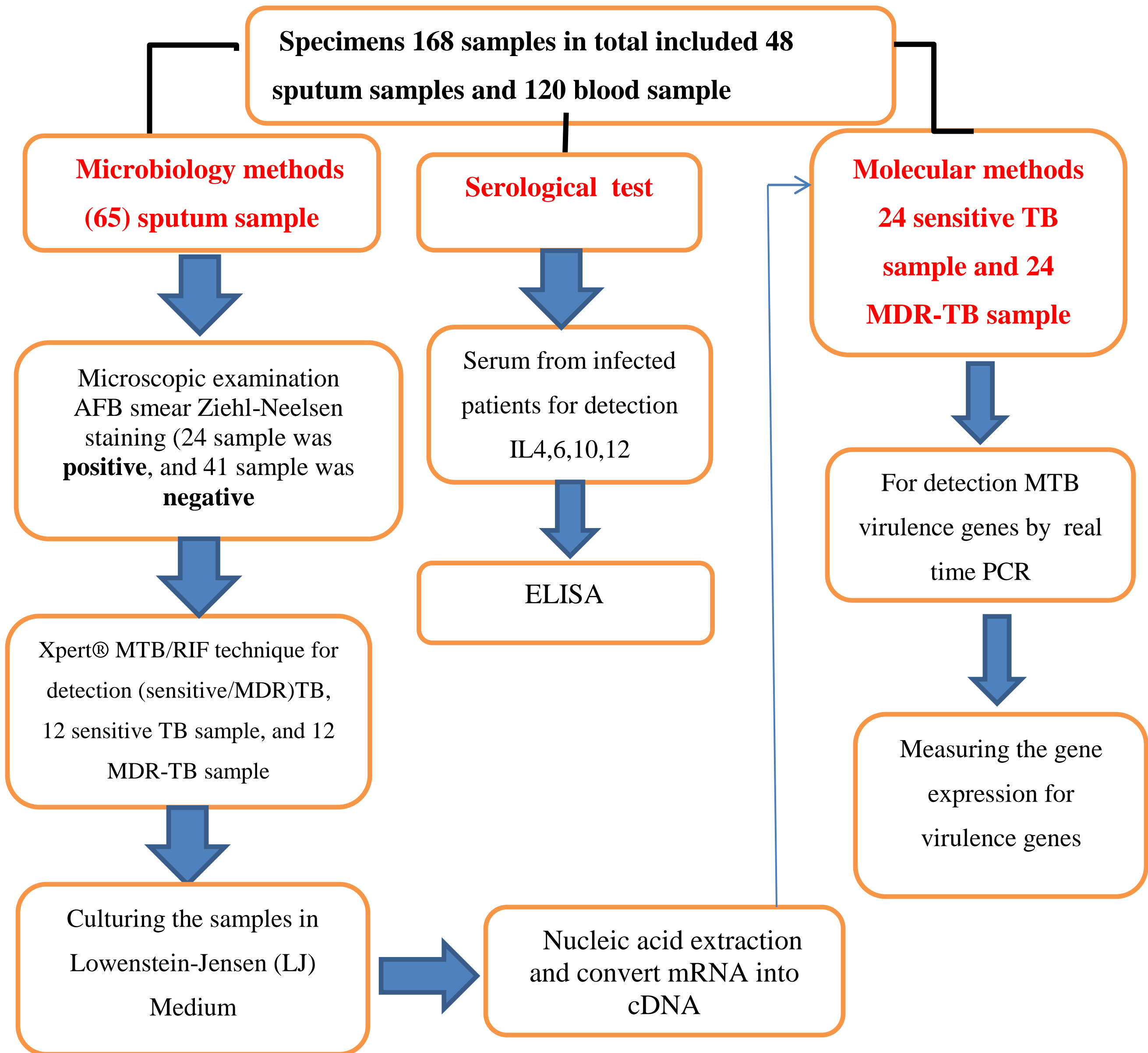


Figure (3-1): Experimental diagram

3-9 Sterilization Methods

3-9-1 Sterilization by Autoclaving

The culture media were sterilized by autoclave at 121° C for (15) minutes under pressure 15 Psi.

3-9-2 Sterilization by Dry heat

The glassware tools (Volumetric flask, Conical flasks, and others tools) were sterilized by oven at 180 ºC for 1.5-2 hours.

3-10 Acid Fast Stain Procedure

1. A thin layer of Sputum was fixed on clean slide by using heat
2. Flooded the slide with Carbolfuchsin stain then Steamed the slide with a Bunsen burner for (5) minutes then Rinse with water.
3. Flooded the slide with Acid Alcohol for (30) seconds then Rinse with water.
4. Counterstained by drowning the slide with Methylene Blue stain for (30) seconds then Rinse with water.
5. Let the slide dry and then Viewed organisms using the oil immersion objective of the microscope.

3-11 Culture Media Preparation

The preparation of the following media was carried out and employed in accordance with the method outlined in the preparation kit manufactured by HIMEDIA/India as follows:

1. (37.3) gm of the Löwenstein-Jensen medium was dissolved in (600) ml of distilled water containing (12) ml of glycerol.
2. The resulting mixture was heated to achieve complete dissolution of the medium.

3. Subsequently, autoclaving was performed at (121°C) for (15) minutes.
4. (1000) ml of a uniform suspension of fresh eggs was prepared under aseptic conditions. It is important to note the avoidance of introducing air into the suspension during the collection and mixing process.
5. The (1000) ml of egg suspension was aseptically combined with (600) ml of the sterile Lowenstein-Jensen Medium (LJ Medium) cooled to 50 – 60°C, with care taken to prevent the formation of air bubbles.
6. The finished medium was dispensed into sterile screw-cap test tubes.
7. Finally, the tubes were positioned in a slanted orientation and subjected to heating at 85°C for (45) minutes.

3-12 Cultivation of *Mycobacterium tuberculosis*

Petroff's method for culturing the sputum of M.tb, a decontamination and concentration technique, involved the following steps:

1. A minimum of (5) ml of sputum was obtained in a clean container.
2. The sputum was mixed with the same volume of (4%) NaOH solution.
3. The mixture was left at room temperature for (15 to 20) minutes and was occasionally shaken.
4. (40%) HCl solution was added to the mixture until the pH was between (6.8) and (7.2).

5. The mixture was spun at (3000) rpm for (15) minutes, and the liquid on top was discarded.
6. The solid at the bottom was dissolved in (1 to 2) ml of sterile saltwater or phosphate buffer.
7. A small amount of the solution was transferred to solid or liquid culture media and was maintained at (37°C).

3-13 Assay Procedures of Measuring Interleukins (4,6,10, and 12)

1. (100) µL of standard or sample was added to the wells. The incubation was carried out for (90) minutes at 37°C.
2. The liquid was discarded, and (100) µL of Biotinylated Detection Ab working solution was immediately added to each well. The incubation continued for 60 minutes at 37°C.
3. The plate was washed three times.
4. (100) µL of HRP-conjugate working solution was added, and incubation was performed for (30) minutes at (37°C).
5. The plate was washed five times.
6. (90) µL of Substrate Reagent was added, followed by incubation for (15) minutes at (37°C).
7. (50) µL of Stop Solution was added.
8. The plate was read at 450nm immediately.

Molecular Methods

3-14 Laboratory Procedure For Xpert MTB/RIF Assay

1. Initially, (2) ml of buffer was added to (1) ml of the fresh sample, and it was shaken well.
2. The mixture was allowed to stand for (10) minutes, then shaken again and left to stand for another (5) minutes.
3. (2.5) ml of the mixture was transferred to the Xpert cartridge and scanned.
4. The test was then run, and the result was read after (2) hours (Global Laboratory Initiative, 2014).

3-15 Primers used in the study : The following below primers used in this study showed in figure(3-4)

Table (3-4): The sequence of the primers used in this study

Primer	Sequence	Primer sequence 5'- - 3'	Reference
whiB7	**F**	**TATCTCACCACCCCAAACGC**	**(Zenteno, 2018)**
	R	**CGATCTGTGGTTCGCCGATA**	
whiB3	**F**	**ACGCAGACATCTGGAACTGG**	**(Zenteno, 2018)**
	R	**ATTTCCTTGGCGCGTTGTTC**	
pknF	**F**	**GTGGTGATCAGCCAGCATCT**	**In this study**
	R	**AATCTCCTCGCGACATTCCC**	
fbpA	**F**	**GCTTCATAGCGTTGAGCTGC**	**In this study**
	R	**AGCTTGTTGACAGGGTTCGT**	
TB16SRNA	**F**	**GGCGTGCTTAACACATGCAA**	**(Patel *et al.,* 1997)**
	R	**AGTCTGGGCCGTATCTCAGT**	

3-16 Nucleic Acid Extraction of M.tb:

1. Up to (1×10^9) cells of a well-grown bacterial culture were transferred to a (2) ml screw centrifuge tube.

2. The bacterial cells were descended at 4°C by centrifuging at 18,000 x g for 2 min, and all the supernatant was removed.
3. A lysozyme reaction solution (100 µl) was added. The cell pellet was resuspended by pipetting up and down, and the sample was incubated at 37°C for 10 min.
4. FARB Buffer (350 µl) and ß-Mercaptoethanol (3.5 µl) were added.
5. Acid-washed glass beads (250 mg, 500~700 µm) were added, and the cells were disrupted by vortexing vigorously for 5 min.
6. The mixture was centrifuged at 18,000 x g for 2 min to spin down insoluble material. The supernatant was transferred to a microcentrifuge tube, and the volume of the supernatant was measured.
7. RNase-free ethanol (1 volume) was added, and the mixture was vortexed well. The ethanol-added sample mixture, including any precipitate, was transferred to the FARB Mini Column in a Collection Tube.
    - (Centrifuge) Centrifuged at 18,000 x g for 30 sec. Discarded the flow-through and returned the FARB Mini Column back to the Collection Tube.
    - (Vacuum) Applied vacuum at -6 inches Hg until the column emptied. Switched off the vacuum and released vacuum from the manifold.
8. Wash Buffer 1 (500 ml) was added to the FARB Mini Column.
    - (Centrifuge) Centrifuged at 18,000 x g for 30 sec. Discarded the flow-through and returned the FARB Mini Column back to the Collection Tube.
    - (Vacuum) Applied vacuum at -6 inches Hg until the column emptied. Switched off the vacuum and released vacuum from the manifold.

9. Wash Buffer 2 (750 µl) was added to the FARB Mini Column.
    - (Centrifuge) Centrifuged at 18,000 × g for 30 sec. Discarded the flow-through and returned the FARB Mini Column back to the Collection Tube.
    - (Vacuum) Applied vacuum at -6 inches Hg until the column emptied. Switched off the vacuum and released vacuum from the manifold.
10. Repeated step 9 for one more washing.
11. Dried Column:
    - Centrifuged at 18,000 × g for 3 min. Discarded the flow-through and returned the FARB Mini Column back to the Collection Tube.
    - Important step! This step prevented subsequent enzymatic reactions from inhibition by the residual wash buffer.
12. Placed the FARB Mini Column in an Elution Tube.
13. RNase-free ddH20 (30-50 µl) was added to the membrane center of the FARB Mini Column. The FARB Mini Column was stood at room temperature for 1 min.
    - Important Step! Ensured that RNase-free ddH2O was dispensed on the membrane center and absorbed completely.
14. The FARB Mini Column was centrifuged at 18,000 × g for 30 sec to elute RNA. The RNA was stored at -70°C.

3-17 RNA concentration and purity:

The RNA of 24 sampled was measured using Quantus Fluorometer, Promega USA, and the concentration and purity recorded as seen in appendix (5).

3-18 Conversion of RNA to cDNA:

LunaScript Reverse Transcriptase RT reagent Kit is designed to perform the qRT-PCR. It uses RTase, which features excellent extendibility and makes fast, efficient cDNA template synthesis for Real Time PCR.

A. Principle

The efficient synthesis of cDNA templates was made fast by Real Time PCR possible. This kit is best suited for two step real-time RT-PCR. Gene Specific Primers were used for extension the total RNA and to convert it to cDNA.

B. Protocol:

1. The following reaction mixture was placed on ice. After dispensing aliquots of this mixture into the micro tubes, the RNA sample was added as in Table (3-5).
2. The reaction mixture was incubated under 25℃, 2 minutes (Primer Annealing) 55℃, 15 min (reverse transcriptase, cDNA synthesis) followed by 90℃ for 1 min (Heat inactivation).

Table (3-5): Reverse transcription reaction mix for cDNA synthesis

Reagents	Volumes (µl)
LunaScript RT SuperMix (5X)	2 µl
Random primers	1ul
total RNA	5 µl
RNase Free dH2O	up to 20
Total Volume	Total 20 µl

< For 1 sample reaction >

3-19 Performing qPCR

A. Product Description

The NEB Luna Universal qPCR Master Mix is an optimized 2X reaction mix for real-time qPCR detection and quantitation of target DNA sequences using the SYBR/FAM channel of most real-time qPCR instruments. It contains Hot Start Taq DNA Polymerase and has been formulated with a unique passive reference dye that is compatible across a variety of instrument platforms (including those that require a high or low ROX reference signal). It also features dUTP for carryover prevention and a non-fluorescent, visible dye to monitor reaction setup. This dye does not spectrally overlap with fluorescent dyes used for qPCR and will not interfere with real-time detection. The master mix formulation is supplied at 2X concentration and contains all PCR components required for amplification and quantitation of DNA except the primers and DNA template. Genomic DNA or cDNA of interest can be quantitated with Luna qPCR and existing as well as commercial qPCR assay primer sequences can be used as in figure (3-6).

B. Protocol

Any existing qPCR assay performed efficiently using standard cycling conditions may be converted to a fast qPCR assay with Luna Universal qPCR Master Mix. Typically, minimal re-optimization of reaction parameters is required.

C. Reaction Component

1. All reaction components were properly thawed and mixed.
2. Master Mix was prepared according to the appropriated volume of all reaction components common to all or a subset of reactions to be performed.
3. The required volume was calculated of each component based on the following tables as in Table (3-6).

Table (3-6): The qRT-PCR reaction components for amplification

Components	20µL (Total volume)	Total concentration
Template cDNA Sample Volume	5 µL	1pg-100ng
Luna Universal qPCR Master Mix(2x)	10µL	2x
Forward primer	0.5µL	0.2µM
Reverse primer	0.5µL	0.2µM
Nuclease-free water	Up to 20µL	

Table (3-7): qPCR Cycling Program for cDNA amplification and detection

Cycle Step	Temperature	Time	Cycle
Initial Denaturation	95°C	60 seconds	1
Denaturation	95°C	15 seconds	40-45
Extension	60°C	30 seconds	
Melting Curve	60-95°C	Various	1

The fold change was calculated by the following equations (Livak and Schmittgen, 2001):

1. ΔCT = CT of target gene – CT of reference gene.
2. $\Delta\Delta CT$ = ΔCT of each sample - average control (Sensitive TB) ΔCT.
3. Fold change = $\mathbf{2^{-\Delta\Delta Ct}}$

3-20 Statistical Analysis

GraphPad 5.0 & 7.0 Prism software was used for statistical analyses to perform an unpaired, 2-tailed Student’s t test of the difference between two means and to perform One-way ANOVA for multiple group comparison, followed by post-Tukey analyses of the differences between means of each column. Differences were considered significant at $P<0.05$.

CHAPTER FOUR

RESULTS

4- Results

4-1 Clinical Data obtained from the Study Population

A detailed overview of the survey data is presented in table (4-1). It covers various categories such as age, gender, housing, tuberculosis type, BCG vaccination, COVID-19 infection, and chronic diseases among participants. Numerical values and ratios are used to represent data.

Table(4-1): A Survey of Questions and Categories Related to Medical Conditions in Four Study groups

No.	Questions	Categories	Control	G1	G2	G3	G4
1.	Age		49.1 (26-62)	30.4(18-51)	38.1(24-60)	56.3(40-64)	62.2(50-71)
2.	Sex	Male	12 (50%)	13 (54%)	10 (42%)	6 (25%)	4 (17%)
		Female	12 (50%)	11 (46%)	14(58%)	18 (75%)	20 (83%)
3.	Housing	Urban	10 (43%)	12 (50%)	10 (40%)	11 (46%)	15 (63%)
		Rural	14 (57%)	12 (50%)	14 (60%)	13 (54%)	9 (37%)
4.	Tuberculosis type	Pulmonary	0 (0%)	24(100%)	0 (0%)	18 (75%)	24(100%)
		Extra-Pulmonary	0	0 (0%)	24(100%)	6 (25%)	0 (0%)
5.	BCG	Yes	24 (100%)	23 (96%)	8 (33%)	12 (50%)	24(100%)
		No	0 (0%)	1 (4%)	16 (67%)	12 (50%)	0 (0%)
6.	Covid-19 infected	Yes	9 (37%)	11 (45%)	12 (50%)	9 (37%)	24(100%)
		No	15 (63%)	13 (55%)	12 (50%)	15 (63%)	0 (0%)
7.	Chronic diseases	Diabetes	0 (0%)	1 (4%)	7 (29%)	5 (20%)	0 (0%)
		Hypertension	0 (0%)	1 (4%)	0 (0%)	4 (17%)	8 (33%)
		Diabetes& Hypertension	0 (0%)	1 (4%)	3 (13%)	4 (17%)	16 (67%)
		Non	24 (100%)	21 (88%)	14 (58%)	11 (46%)	0 (0%)

G1: Active pulmonary TB
G2: Active extra pulmonary TB
G3: TB patients under the treatment
G4: MDR-TB patients

Table (4-1) showed the **mean age** conditions of four groups of TB patients and healthy individuals. G4 was the oldest group with a mean age of 62.2, while G1 was the youngest group with a mean age of 30.4. In **Sex** distribution G1 was the highest participants 13 (54%) males while G4 was 20 (83%) females. Most of the G4 participants resided in urban areas 15 (63%), while most of the G2 participants resided in rural areas 14 (60%).

The **tuberculosis type** for each group was also determined. G1 had the highest proportion of cases with pulmonary TB 24 (100%), while G2 had the highest proportion of cases with extra-pulmonary TB 24 (100%).

The **BCG vaccine and COVID-19 infections** were also assessed for each group. G4 had the highest proportion of participants who had received the BCG vaccine and COVID-19 infection 24 (100%).

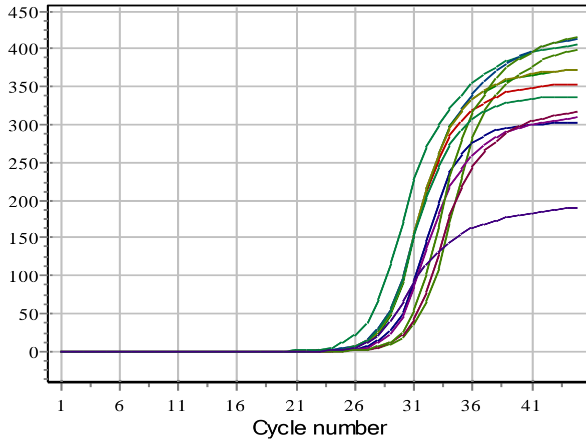
The **chronic diseases**, such as diabetes and hypertension, was also evaluated for each group. G2 had the highest proportion of patients with diabetes 7 (29%), while G4 had the highest proportion of patients with hypertension 8 (33%). G4 also had the highest proportion of patients with both diabetes and hypertension 16 (67%), while G1 had the highest proportion of patients without any chronic diseases 21 (88%).

4-2 Gene Expression Levels between Sensitive TB and MDR-TB Strains

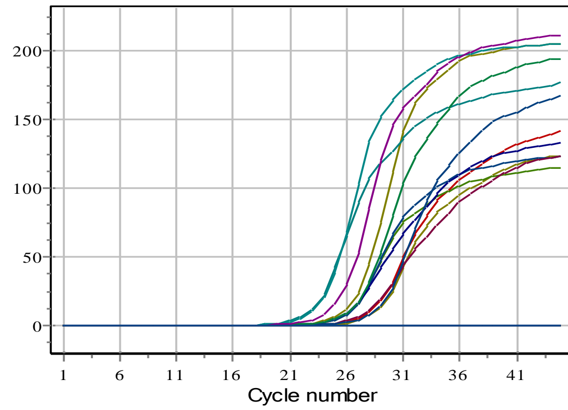
The gene expression levels of four genes (*whiB3, whiB7, pknF, fbpA*) and 16sRNA between sensitive and MDR-TB strains were measured by using RT-PCR, the data were collected from 24 samples (12 samples sensitive TB and 12 samples MDR-TB). The results were analyzed according to Livak method, the ΔCT and ΔΔCT methods to calculate the fold change (2^-ΔΔCT).

4-2-1 Gene expression level of 16s RNA

Figures (4-1),(4-2) showed the CT value of 16S RNA was measured by qPCR in sensitive TB and MDR-TB. In sensitive TB, the mean CT value of 16SRNA was 25.6 (SD = 0.474) as in table (4-2), while in MDR-TB, the mean CT value of 16S RNA was 24.6 (SD = 0.786) as in the table (4-3).



Figure(4-1): Gene expression for 16sRNA in sensitive TB



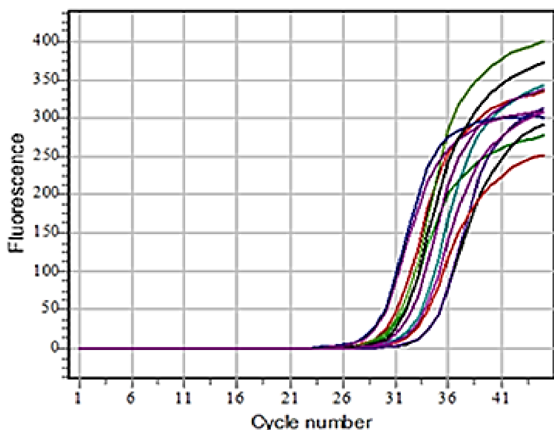
Figure(4-2): Gene expression for 16sRNA in MDR-TB

Table (4-2): CT values of 16s RNA in sensitive TB and MDR-TB

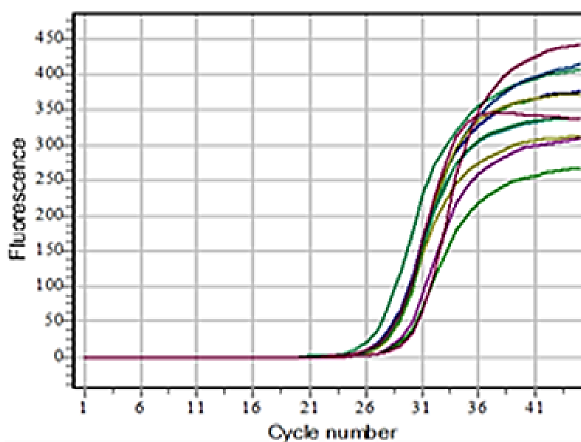
CT values for 16s RNA in Sensitive TB	CT values for 16s RNA in MDR-TB
25.8	22.6
26.4	25.6
25.6	24.8
26.2	25.4
25.4	24.6
26	25.2
25.2	24.4
25.8	25
25	24.2
25.6	24.8
24.8	24
25.4	24.6
Mean CT value= 25.6 (SD = 0.474)	Mean CT value= 24.6 (SD = 0.786)

4-2-2 Gene expression levels of *whiB3*

The expression levels of *whiB3* gene in sensitive TB and MDR-TB strains were measured by using qRT-PCR, as in the figure (4-3),(4-4), also ΔCT, ΔΔCT, and fold change for each sample was presented in the table (4-3) for sensitive TB and table (4-4) for MDR-TB.



Figure(4-3): Gene expression of *whiB3* in sensitive M.tb group



Figure(4-4): Gene expression of *whiB3* in MDR-TB group

Table (4-3): the values of CT, ΔCT, ΔΔCT, and fold change for *whiB3* gene in sensitive TB group

CT of *whiB3* in sensitive TB	ΔCT	ΔΔCT	Fold change (2^-ΔΔCT)
31.2	5.4	1.75	0.2973
30.6	4.2	0.55	0.68302
30	4.4	0.75	0.5946
29.4	3.2	-0.45	1.36604
28.8	3.4	-0.25	1.18921
28.2	2.2	-1.45	2.73208
27.6	2.4	-1.25	2.37841
27	1.2	-2.45	5.46416
26.4	1.4	-2.25	4.75683
31.2	5.6	1.95	0.25882
30.6	5.8	2.15	0.22531
30	4.6	0.95	0.51763

Table (4-4): the values of CT, ΔCT, ΔΔCT, and fold change for *whiB3* gene in MDR-TB group

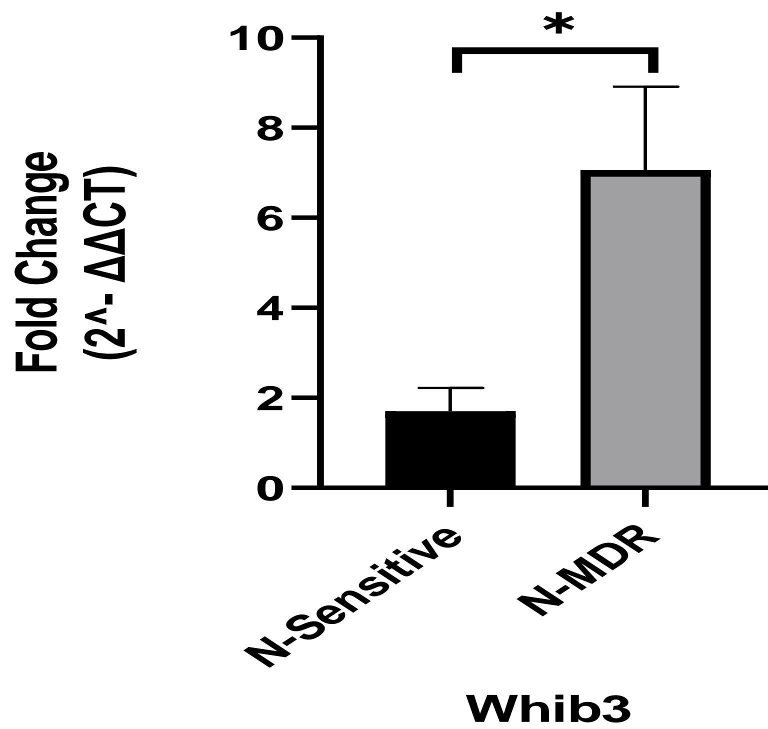
CT of *whiB3* in MDR-TB	ΔCT	ΔΔCT	Fold change (2^-ΔΔCT)
27.5	4.9	1.25	0.42045
27.9	2.3	-1.35	2.54912
28.4	3.6	-0.05	1.03526
26.7	1.3	-2.35	5.09824
26.1	1.5	-2.15	4.43828
25.5	0.3	-3.35	10.1965
24.9	0.5	-3.15	8.87656
24.3	-0.7	-4.35	20.393
23.7	-0.5	-4.15	17.7531
28.5	3.7	0.05	0.96594
24.9	0.9	-2.75	6.72717
25.6	1	-2.65	6.27667

Table (4-5): The mean ΔCT, ΔΔCT, and fold change of *whiB3* gene expression in sensitive TB and MDR-TB

Genes	Groups	ΔCT (Mean ± SE)	P-value	ΔΔCT (Mean ±SE)	P value	Fold Change ($2^{-\Delta\Delta CT}$) Mean ±SE	P-value
WhiB3	Sensitive TB	3.65±0.46	0.006	0.001±0.46	0.006	1.72±0.52	0.001*
	MDR-TB	1.57±0.50		-2.08±0.50		7.06±1.86	

(P < 0.05*)

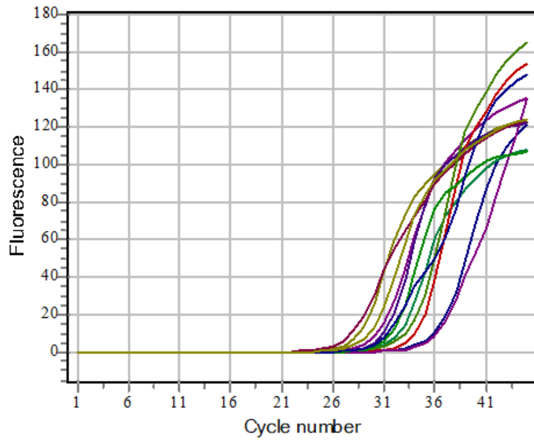
As seen in the table (4-5) The mean ΔCT value for *WhiB3* were lower in MDR-TB 1.56 than in sensitive TB 3.65. Also the mean ΔΔCT value was lower in MDR-TB -2.08 than in sensitive TB 0.001. Moving to the mean fold change in MDR-TB 7.06 which was 7-folds higher than sensitive TB 1.72 indicated higher expression levels of this gene in MDR-TB group compared to sensitive TB group figure (4-5).



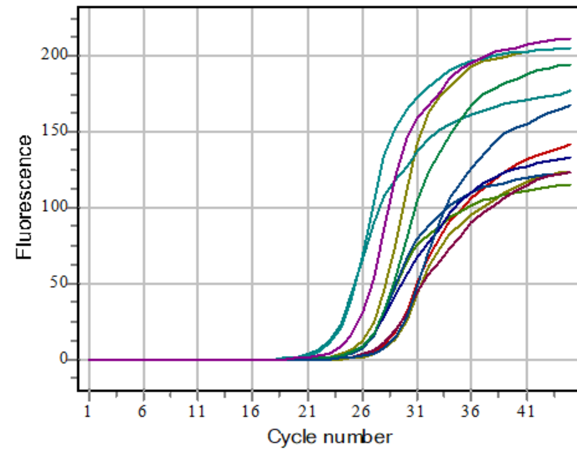
Figure(4-5): *whiB3* gene expression changes in sensitive TB and MDR-TB strains.

4-2-3 Gene expression levels of *whiB7*

The expression levels of *whiB7* gene in sensitive TB and MDR-TB strains were measured by using qRT-PCR, as in the figure (4-5),(4-6), also ΔCT, ΔΔCT, and fold change for each sample was presented in the table (4-6) for sensitive TB and table (4-7) for MDR-TB.



Figure(4-6): Gene expression of *WhiB7* in Sensitive M.tb group



Figure(4-7): Gene expression of *WhiB7* in MDR-TB group

Table (4-6): the values of CT, ΔCT, ΔΔCT, and fold change for *whiB7* gene expression in sensitive M.tb group

CT of *whiB7* in sensitive TB	ΔCT	ΔΔCT	Fold change (2^-ΔΔCT)
30.6	4.8	0.8	0.57435
31	4.6	0.6	0.65975
30.4	4.8	0.8	0.57435
29.8	3.6	-0.4	1.31951
29.2	3.8	-0.2	1.1487
28.6	2.6	-1.4	2.63902
28	2.8	-1.2	2.2974
27.4	1.6	-2.4	5.27803
26.8	1.8	-2.2	4.59479

31.6	6	2	0.25
28	3.2	-0.8	1.7411
30.4	5	1	0.5

Table (4-7): the values of CT, ΔCT, ΔΔCT, and fold change for *whiB7* gene expression in MDR-TB

CT of *whiB7* in MDR-TB	**ΔCT**	**ΔΔCT**	**Fold change (2^-ΔΔCT)**
28.3	5.7	2	0.25
27.7	2.1	-1.6	3.03143
27.1	2.3	-1.4	2.63902
26.5	1.1	-2.6	6.06287
25.9	1.3	-2.4	5.27803
25.3	0.1	-3.6	12.1257
24.7	0.3	-3.4	10.5561
24.1	-0.9	-4.6	24.2515
23.5	-0.7	-4.4	21.1121
28.3	3.5	-0.2	1.1487
27.7	3.7	-4.4	21.1121
27.1	2.5	-1.2	2.2974

Table (4-8): The mean ΔCT, ΔΔCT, and fold change of *whiB7* gene expression in sensitive TB and MDR-TB

Gene	Groups	ΔCT (Mean ± SE)	P-value	ΔΔCT (Mean ±SE)	P value	Fold Change ($2^{-\Delta\Delta CT}$) Mean ±SE	P-value
whiB7	Sensitive TB	3.72±0.39	0.009*	-0.28±0.39	0.008*	1.80±0.48	0.008*
	MDR-TB	1.75±0.56		-2.32±0.57		9.16±2.49	

(P < 0.05*)

As seen in the table (4-8) The mean ΔCT value for *WhiB7* was significantly lower in MDR-TB 1.75 than in sensitive TB 3.72 (P-value= 0.009). Also the mean ΔΔCT value was significantly lower in MDR-TB -2.32 than in sensitive TB -0.28 (P-value= 0.008). Moving to the mean fold change in MDR-TB 9.16 which was 9-folds significantly higher than sensitive TB 1.80 (P-value= 0.008), as in the figure (4-7) indicated higher expression levels of this gene in MDR-TB group compared to sensitive TB group.

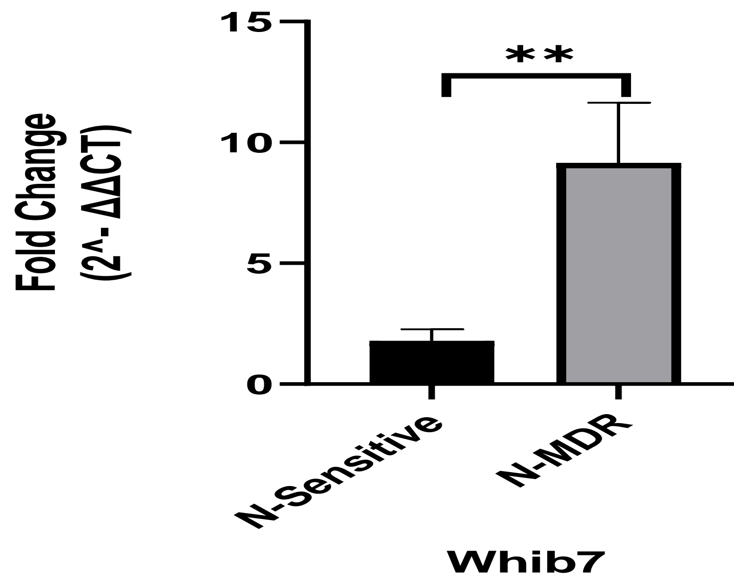


Figure (4-8): *whiB7* gene expression changes in sensitive TB and MDR-TB strains.

4-2-4 Gene expression levels of *fbpA*

The gene expression levels of *fbpA* in sensitive TB and MDR-TB strains were measured by using qRT-PCR, as in the figure (4-7),(4-8), also ΔCT, ΔΔCT, and fold change for each sample was presented in the table (4-9) for sensitive TB and table (4-10) for MDR-TB.

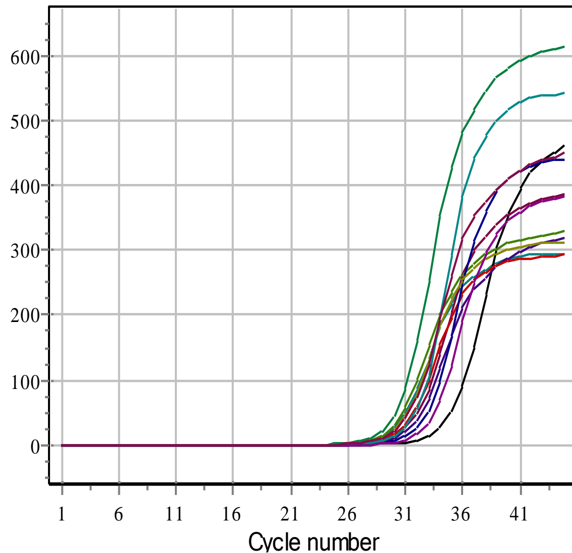


Figure (4-9): Gene expression of *fbpA* in sensitive M.tb group

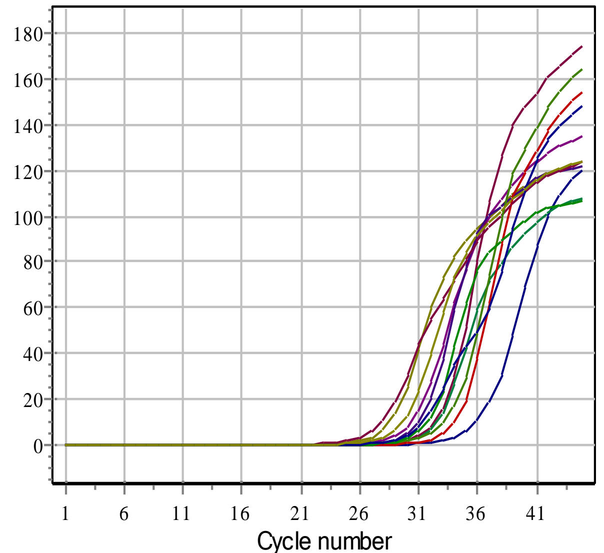


Figure (4-10): Gene expression of *fbpA* in MDR-TB group

Table (4-9): the values of CT, ΔCT, ΔΔCT, and fold change for *fbpA* gene expression in sensitive TB

CT of *fbpA* in sensitive TB	ΔCT	ΔΔCT	Fold change (2^-ΔΔCT)
31	5.2	-0.2	1.1487
31.5	5.1	-0.3	1.23114
30.9	5.3	-0.1	1.07177
31.4	5.2	-0.2	1.1487
30.8	5.4	0	1
31.3	5.3	-0.1	1.07177
30.7	5.5	0.1	0.93303

31.2	5.4	0	1
30.6	5.6	0.2	0.87055
31.1	5.5	0.1	0.93303
30.5	5.7	0.3	0.81225
31	5.6	0.2	0.87055

Table (4-10): the values of CT, ΔCT, ΔΔCT, and fold change for *fbpA* gene expression in MDR-TB

CT of *fbpA* in MDR-TB	**ΔCT**	**ΔΔCT**	**Fold change (2^-ΔΔCT)**
28.6	6	0.6	0.65975
29.1	3.5	-1.9	3.73213
28.5	3.7	-1.7	3.24901
29	3.6	-1.8	3.4822
28.4	3.8	-1.6	3.03143
30.4	5.2	-0.2	1.1487
28.3	3.9	-1.5	2.82843
31.6	6.6	1.2	0.43528
28.2	4	-1.4	2.63902
28.7	3.9	-1.5	2.82843
28.1	4.1	-1.3	2.46229
27.8	3.2	-2.2	4.59479

Table (4-11): The mean ΔCT, ΔΔCT, and fold change of *pknF* gene expression in sensitive TB and MDR-TB

Gene	Groups	ΔCT (Mean ± SE)	P-value	ΔΔCT (Mean ±SE)	P value	Fold Change ($2^{-\Delta\Delta CT}$) Mean ±SE	P-value
fbpA	Sensitive TB	5.40±0.05	0.001*	0.001± 0.05	0.001*	1.01±0.037	0.003*
	MDR-TB	4.30±0.31		-1.11±0.31		2.59±0.36	

(P < 0.05*)

As shown in the table (4-11) The mean ΔCT value for *fbpA* was significantly lower in MDR-TB 4.30 than in sensitive TB 5.40 (P-value= 0.001). Also the mean ΔΔCT value was significantly lower in MDR-TB -1.11 than in sensitive TB 0.001 (P-value= 0.001). Moving to the mean fold change in MDR-TB 2.59 which was significantly higher than sensitive TB 1.01 (P-value= 0.003), as in the figure (4-7) indicated higher expression levels of this gene in MDR-TB group compared to sensitive TB group.

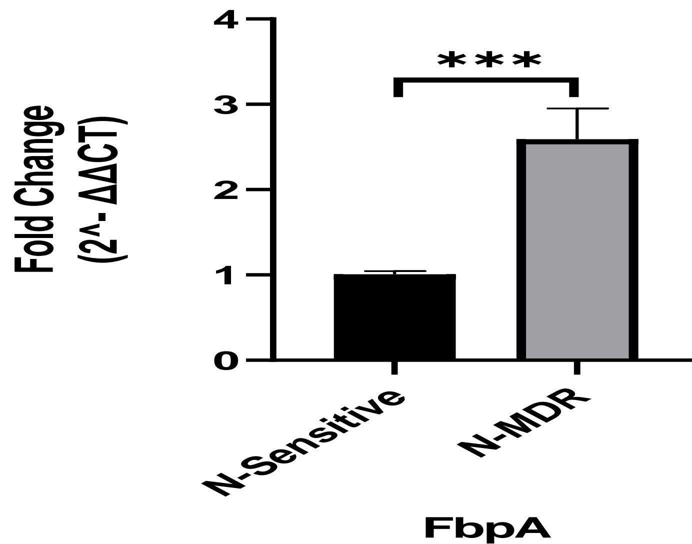
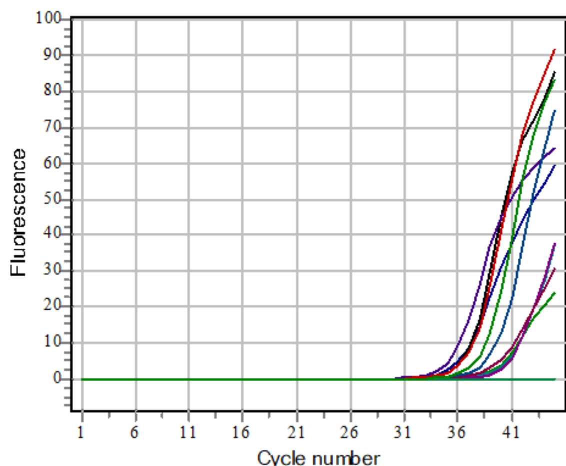


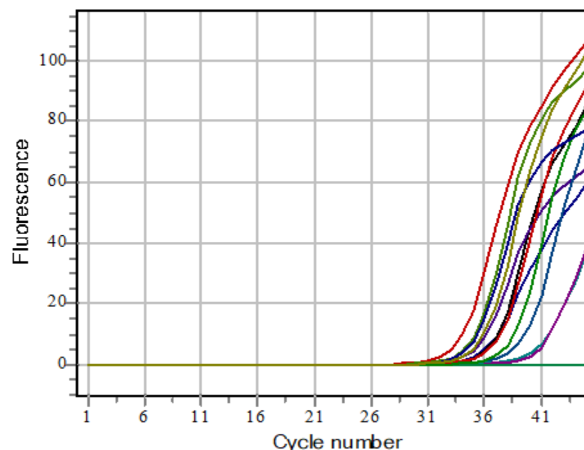
Figure (4-11) : *FbpA* gene expression changes in sensitive TB and MDR-TB strains.

4-2-5 Gene expression levels of *pknF*

The gene expression levels of *pknF* in sensitive TB and MDR-TB strains were measured by using qRT-PCR, as in the figure (4-12),(4-13), also ΔCT, ΔΔCT, and fold change for each sample was presented in the table (4-12) for sensitive TB and table (4-13) for MDR-TB.



Figure(4-12): Gene expression of *pknF* in sensitive M.tb group



Figure(4-13): Gene expression of *pknF* in MDR-TB group

Table (4-12): the values of CT, ΔCT, ΔΔCT, and fold change for *pknF* gene expression in sensitive TB

CT of *pknF* in sensitive TB	ΔCT	ΔΔCT	Fold change (2^-ΔΔCT)
39.8	14	0.4	0.75786
38.4	12	-1.6	3.03143
39.6	14	0.4	0.75786
38.2	12	-1.6	3.03143
41.3	15.9	2.3	0.20306
38	12	-1.6	3.03143
41.1	15.9	2.3	0.20306
37.8	12	-1.6	3.03143
40.6	15.6	2	0.25

37.6	12	-1.6	3.03143
40.4	15.6	2	0.25
37.4	12	-1.6	3.03143

Table (4-13): the values of CT, ΔCT, ΔΔCT, and fold change for *pknF* gene expression in MDR-TB

CT of *pknF* in sensitive TB	**ΔCT**	**ΔΔCT**	**Fold change (2^-ΔΔCT)**
38.4	15.8	2.2	0.21764
37	11.4	-2.2	4.59479
38.2	13.4	-0.2	1.1487
36.8	11.4	-2.2	4.59479
39.9	15.3	1.7	0.30779
36.6	11.4	-2.2	4.59479
39.7	15.3	1.7	0.30779
36.4	11.4	-2.2	4.59479
39.2	15	1.4	0.37893
36.2	11.4	-2.2	4.59479
39	15	1.4	0.37893
36	11.4	-2.2	4.59479

Table (4-14): The mean ΔCT, ΔΔCT, and fold change of *pknF* gene expression in sensitive TB and MDR-TB

Gene	Groups	ΔCT (Mean ± SE)	P-value	ΔΔCT (Mean ±SE)	P value	Fold Change ($2^{-\Delta\Delta CT}$) Mean ±SE	P-value
pknF	Sensitive TB	13.5± 0.51	0.602	-0.02±0. 51	0.6029	1.72±0.40	0.2891
	MDR-TB	13.1±0.56		-0.42± 0.56		2.53±0.62	

(P < 0.05*)

As shown in the table (4-14) The mean ΔCT value for *pknF* was non-significantly lower in MDR-TB 13.1 than in sensitive TB 13.5 (P-value= 0.602). Also the mean ΔΔCT value was non-significantly lower in MDR-TB -0.42 than in sensitive TB -0.02 (P-value= 0.602). Moving to the mean fold change in MDR-TB 2.53 which was non-significantly higher than sensitive TB 1.72 (P-value= 0.289), as in the figure (4-7).

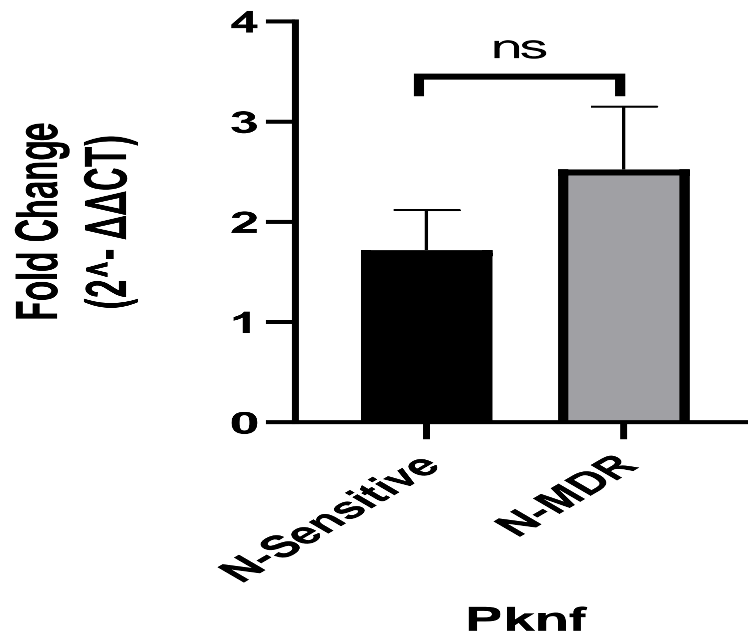


Figure (4-14): *PknF* gene expression changes in sensitive TB and MDR-TB strains.

4-3 Interleukins Levels in different Groups of TB Patients

4-3-1 IL-4 levels

In this study, IL-4, 6, 10, and 12 was evaluated in four patient groups: sensitive pulmonary TB (G1), sensitive extra-pulmonaryTB (G2), TB patients under treatment (G3), TB patients with MDR-TB strains (G4), and a group of healthy control. The findings of IL4 were presented in Table (4-15).

Table (4-15): Descriptive and inferential statistics for IL-4 levels

Parameters	Groups	Numbers	Mean	Standard deviation
IL-4 (pg/mL)	Control group	24	106.67*	45.9
	G1: Sensitive pulmonary TB	24	673.45*	275.6
	G2: Sensitive extra-pulmonary TB	24	905.36*	417.2
	G3: TB patients under treatment	24	421.96*	118.4
	G4: MDR-TB patients	24	602.54*	252.2
	F-statistic	**10.641**	-	-
	P-value	**<0.0001**	-	-

Note: Means with (*) are significantly different at P < 0.05 according to Tukey's post hoc test.

In the table (4-15), there were significant differences in mean IL-4 levels among groups (F= 10.641)(P < 0.0001). Group G2 was the highest mean IL-4 level (905.36 pg/mL), while group G3 had the lowest (421.96 pg/mL).

4-3-2 IL-6 levels

IL-6 also measured in the four patients groups and healthy control group and the results were shown in table (4-16).

Table (4-16): Descriptive and inferential statistics for IL-6 levels

Parameters	Groups	Numbers	Mean	Standard deviation
IL-6 (pg/mL)	Control group	24	9.22*	1.27
	G1: Sensitive pulmonary TB	24	42.88*	14.06
	G2: Sensitive extra-pulmonary TB	24	29.23*	13.62
	G3: TB patients under treatment	24	23.96*	12.6
	G4: MDR-TB patients	24	30.91*	10.3
	F- statistic	**12.799**	-	-
	P- value	**<0.0001**	-	-

Note: Means with (*) are significantly different at $p < 0.05$ according to Tukey's post hoc test.

The table (4-16) presented a significant difference in the mean IL-6 levels among groups (F= 12.799)(P < 0.0001). G3 was the lowest mean IL-6 level (23.96 pg/mL), while group G1 was the highest (42.88 pg/mL).

4-3-3 IL-10 levels

The level of IL-10 was measured in four different patient groups and healthy control. The results showed in the table (4-17).

Table (4-17): Descriptive and inferential statistics for IL-10 levels

Parameters	Groups	Numbers	Mean	Standard deviation
IL-10 (pg/mL)	Control group	24	15.22*	2.49
	G1: Sensitive pulmonary TB	24	13.32*	1.90
	G2: Sensitive extra-pulmonary TB	24	13.51*	1.14
	G3: TB patients under treatment	24	21.37*	8.20
	G4: MDR-TB patients	24	41.45*	1.61
	F- statistic	**106.456**	-	-
	P- value	**<0.0001**	-	-

Note: Means with (*) are significantly different at $p < 0.05$ according to Tukey's post hoc test.

Table (4-17) revealed a significant difference in mean IL-10 levels among groups (F= 106.456) (P< 0.0001). G4 was significantly higher mean IL-10 levels (41.45 pg/mL)(p < 0.0001). However, Groups G1 was the lowest (13.32 pg/mL).

4-3-4 IL-12 levels:

IL-12 was evaluated in four patients groups and control group. The results showed in the table (4-18).

Table (4-18): Descriptive and inferential statistics for IL-12 levels

Parameters	Groups	Numbers	Mean	Standard deviation
IL-12 (pg/mL)	Control group	24	16.00	5.2
	G1: Sensitive pulmonary TB	24	18.66	8.4
	G2: Sensitive extra-pulmonary TB	24	23.00	7.61
	G3: TB patients under treatment	24	31.02	15.8
	G4: MDR-TB patients	24	15.54	2.49
	F- statistic	**1.151**	-	-
	P- value	**0.3362**	-	-

Note: Means without (*) are not significantly different at p < 0.05 according to Tukey's post hoc test.

The table (4-18) revealed no significant differences in mean IL-12 levels among groups (F= 1.151)(P= 0.3362). The mean IL-12 levels ranged from (15.54 pg/mL) in group G4 to (31.02 pg/mL) in group G3.

4-4 Correlation between gene expression levels and interleukin levels

The Pearson correlation coefficients and the corresponding P-values were calculated to assess the linear relationship between gene expression levels of *whiB3* and *whiB7* with IL-10 and IL-12, as in the tables (4-19)(4-20). Also *fbpA* and *pknF* with IL-4, IL-6, as in the tables (4-21)(4-22).

Table (4-19): Correlation between *WhiB3* genes and interleukins IL-10 and IL-12

No	*WhiB3* S-TB (fold change)	IL-10 S-TB	IL-12 S-TB	*WhiB3* MDR-TB (fold change)	IL-10 MDR-TB	IL-12 MDR-TB	Correlation (r) S-TB	P- value
1	0.22531	7.78	21.25	0.42045	40.67777	22.7	IL-10 0.596	0.002*
2	0.25882	9.38	13.33	2.54912	39.58	13.33	IL-12 0.353	0.093
3	0.2973	14.71	12.08	1.03526	43.06	22.7	**Correlation (r) MDR TB**	
4	0.51763	12.5	17.13	5.09824	36.33	20.45		
5	0.5946	13.44	13.656	4.43828	37.228	18.6		
6	0.68302	14.58	15.94	10.1965	47.06	14.8		
7	1.18921	13.33	30.42	8.87656	41.94	18.9	IL-10 0.7645	0.0001*
8	1.36604	15.28	10.654	20.393	50.33	13.43	IL-12 -0.610	0.001*
9	2.37841	10.97	13.75	17.7531	50.33	13.33		
10	2.73208	15.21	11.573	0.96594	38.94	18.75		
11	4.75683	15.42	22.08	6.72717	35.71	13.33		
12	5.46416	17.22	24.7654	6.27667	43.52	13.33		

(P < 0.05*)

Table(4-20): Correlation between *WhiB7* genes and interleukins IL-10 and Il-12

no	*WhiB7* STB (fold change)	IL-10 STB	IL-12 STB	*WhiB7* MDR-TB (fold change)	IL-10 MDR	IL-12 MDR	Correlation (r) S-TB	P- value
1	0.25	7.78	10.654	0.25	35.71	14.012	IL-10 0.637	0.001*
2	0.5	9.38	11.573	3.03143	36.33	13.33	IL-12 0.337	0.107
3	0.57435	10.97	12.08	2.63902	37.228	22.7		
4	0.57435	12.5	13.33	6.06287	38.94	15	Correlation (r) MDR TB	
5	0.65975	13.33	13.656	5.27803	39.58	13.33	IL-10 0.490	0.015*
6	1.1487	13.44	15.94	12.1257	40.67777	20.45	IL-12 -0.602	0.001*
7	1.31951	14.58	13.75	10.5561	41.94	14.3223		
8	1.7411	14.71	17.13	24.2515	43.06	13.43		
9	2.2974	15.21	22.08	21.1121	43.52	18.9		
10	2.63902	15.28	24.7654	1.1487	47.06	18.75		
11	4.59479	15.42	21.25	21.1121	50.33	14.8		
12	5.27803	17.22	30.42	2.2974	50.33	18.6		

(P < 0.05*)

Table(4-21): Correlation between *FbpA* gene and IL- 4 and IL- 6

No	*FbpA* DS-TB (fold change)	IL-4 DS-TB	IL-6 DS-TB	*FbpA* MDR-TB (fold change)	IL-4 MDR-TB	IL-6 MDR-TB	Correlation (r) DS-TB	P-value
1	1.1487	1442.14	44.29	0.65975	1503.57	36.5	IL-4 -0.16	0.455
2	1.23114	950.71	50.83	3.73213	893.57	53.57	IL-6 0.46	0.023*
3	1.07177	977.86	43.83	3.24901	861.43	49.92	**Correlation (r) MDR TB**	
4	1.1487	957.86	40.92	3.4822	861.43	48.96		
5	1	977.86	38.08	3.03143	1539.29	46.70		
6	1.07177	978.57	48.88	1.1487	1785.467	44.63		
7	0.93303	932.86	49.46	2.82843	789.14	11.08	IL-4 -0.37	0.075
8	1	978.57	41.54	0.43528	827.14	17.43	IL-6 0.514	0.01*
9	0.87055	878.57	39.25	2.63902	798.57	49.92		
10	0.93303	954.29	30.67	2.82843	1539.29	53.57		
11	0.81225	1695	34.17	2.46229	811.14	44.54		
12	0.87055	932.86	47.58	4.59479	893.57	50.79		

($P < 0.05$*)

Table(4-22): Correlation between *PknF* gene and IL- 4 and IL- 6

No	*PknF* S-TB (fold change)	IL-4 S-TB	IL-6 S-TB	*PknF* MDR-TB (fold change)	IL-4 MDR-TB	IL-6 MDR-TB	Correlation (r) S-TB	P-value
1	0.75786	1442.14	44.29	0.21764	1503.57	36.5	IL-4 -0.38	0.067
2	3.03143	950.71	50.83	4.59479	893.57	53.57	IL-6 0.18	0.185
3	0.75786	977.86	43.83	1.1487	861.43	49.92	**Correlation (r) MDR TB**	
4	3.03143	957.86	40.92	4.59479	861.43	48.96		
5	0.20306	977.86	38.08	0.30779	1539.29	46.70		
6	3.03143	978.57	48.88	4.59479	1785.467	44.63		
7	0.20306	932.86	49.46	0.30779	789.14	11.08	IL-4 0.088	0.682
8	3.03143	978.57	41.54	4.59479	827.14	17.43	IL-6 0.21	0.60
9	0.25	878.57	39.25	0.37893	798.57	49.92		
10	3.03143	954.29	30.67	4.59479	1539.29	53.57		
11	0.25	1695	34.17	0.37893	811.14	44.54		
12	3.03143	932.86	47.58	4.59479	893.57	50.79		

($P < 0.05$*)

- Table (4-19) showed a significant positive correlation between *WhiB3* gene expression in sensitive TB and IL-10 level (r = 0.596) (P = 0.002). Additionally, *WhiB3* gene expression in MDR-TB was significantly positive correlated with IL-10 (r = 0.764) (P = 0.0001). While IL-12 showed a significant negative correlation in MDR-TB with *WhiB3* (r = -0.610) (P = 0.001).
- Table (4-20) revealed a significant positive correlation between *WhiB7* gene expression in sensitive TB and IL-10 level (r = 0.637) (P = 0.001). Also, *WhiB7* gene expression in MDR-TB was significantly positive correlated with IL-10 (r = 0.490) (P = 0.015). While IL-12 showed a significant negative correlation in MDR-TB with *WhiB7* (r = -0.602) (P = 0.001).
- In Table (4-21) there was a significant positive correlation between *FbpA* gene expression in sensitive TB and IL-6 level (r = 0.46) (P = 0.023), and between *FbpA* gene expression in MDR-TB and IL-6 level (r = 0.514) (P = 0.01). This means that higher expression of these genes was associated with higher level of IL-6.

CHAPTER FIVE

DISCUSSION

5 .DISCUSSION

TB remains the highest causative agent of mortality between all infectious diseases universally with above (1.5) million deaths stated in (2021) only (WHO, 2022). In spite of these accessible drugs, it is still a problem to totally eradicate M.tb in specific infected individuals since these drugs are less effective against slow dividing M.tb (Kanehiro *et al*., 2018). Moreover, M.tb has established numerous resistance mechanisms that can assistance its survival inside phagocytic cells, for instance the capability to prevent the phagosome-lysosome fusion. The quick increase of multi-drug resistant strains of M.tb is also additional question. Therefore, there is a pressing necessity for the development of medications that target the host so as to enhance its anti-mycobacterial activity while reducing a destructive inflammatory response (Liu *et al.,* 2017) .

5-1 Risk Factors

In TB patients, age is the a risk factor for infection, as older adults have a higher prevalence of latent TB infection (LTBI) and other comorbidities that compromise their immune response (Gardner Toren *et al.,* 2020). Moreover, aging is connected with a deterioration in the creation and function of immune cells, particularly T-lymphocytes which are critical for controlling TB infection. Therefore, older adults who have been exposed to the TB bacillus are more prone to developing active disease than younger adults and need close monitoring for initial diagnosis and treatment (Pangrazzi and Weinberger, 2020).

The mean age of pulmonary TB patients in this study was (30.43) years, with 13 (54)% male and 11 (46)% female. This agreed with existing research signified a positive correlation between the age and sex distribution.

For instance, in India, Gajalakshmi and Peto, (2009) observed the age as a risk factor for TB patients in (949) new pulmonary TB, which the ratio was (Male 2.11; Female 1) aged between (35-64) years and (1963) healthy individuals. Another study agreed with the present results by Kolappan *et al.* (2007) investigated the age and sex distribution of the study population in India, which consisted of (93945) participants, of whom 45.5% were female and 54.5% were male. The majority of the study 70% belonged to younger age group (15-45).

For extra-pulmonary TB the results presented the mean age were 38.13, 10 (41.6)% male and 14 (58.3)% females which are agreed with Al-Ghafil *et al.* (2019) a study explored the age of extra-pulmonary TB patients in Saudi Arabia, among (902) patients 312 (34.6)% were between 35-60 years, followed by 279 (30.9)% aged 25-35. Another study agreed the present results by Lin *et al.* (2013) explored the sex and concurrent extra pulmonary TB among patients with pulmonary TB in Taiwan, based on a population-based database. The study stated that females had a higher proportion of PTB developed into EPTB than males (9.3% vs. 7.3%, P=0.013), and this association was stronger among older females (45 years and older) than older males (9.0% vs. 6.8%, P=0.016).

In MDR-TB patients the mean age was 62.25 and the majority of patients was females 20 (83)% and 4 (17)% males which is agreed with A study by Seifert *et al*. (2021) used Xpert MTB/RIF assay results from Myanmar to characterize M.tb test positivity and rifampicin resistance by sex and age, and to evaluate risk factors associated with rifampicin resistance. The study stated that a greater proportion of females (11.4)% with rifampicin resistant as compared to males (9.3)%. These studies also attributed the higher rates of TB among older people to the decreased

function of macrophages, the central cellular defense against TB, and the increased probability of reactivation of LTBI because of reduced immunity and decreasing protection from BCG vaccine (Byng-Maddick and Noursadeghi, 2016).

Location is another objective of this study to examine, the spatial distribution of TB cases between the **city** and **rural** in different groups. Location is an important factor that impacts on the epidemiology and control of TB, as different places have diverse levels of exposure, risk factors, health services, and social conditions that affect TB transmission and outcomes (Napier *et al*., 2020; Zhou *et al*., 2022).

In this study, the distribution varied among different groups (Table 4-1), the highest percentage of patients living in the city was observed in Multi-drugs resistance TB group (G4) 15 (63)%, and this results agreed to a study by Wang W *et al*. (2023) they found that most of the TB patients were from city areas 68.7% compared to rural areas 60.4%. Also Mutembo *et al.* (2019) reported that the recurrence of MDR-TB was higher in city areas 15.3% than in rural areas 11.3% in Southern Province, Zambia. Another study agreed the present results done by Li *et al.* (2022) reported that city populations have higher recurrent infections with MDR-TB rates than rural populations in China, as they have more repeated exposure to TB re-infection. The proportion of city area patients was higher than among in rural areas, with 15.8% (3566/25533) of the cases being from city areas and 11.1% (2889/25533) of the cases being from rural areas. However, the highest percentage of TB infections in rural areas in this study was reported in extra-pulmonary TB group (G2) as in the table (4-1), which was 14 (60)% of the patients whom been living in rural, that agreed with previous study by Sikalengo *et al*. (2018) they observed that TB cases were more common in

rural than city regions 7.8% vs. 5.4%, and those rural patients had a higher proportion of positive cases, lower HIV prevalence, and lower mortality than city patients.

BCG vaccine was explored as another objective of this study. BCG is a vaccine for TB disease that is used in many countries with a high prevalence of TB to prevent childhood tuberculosis and miliary TB disease. However, the use of BCG in the United States is not generally recommended because of the low risk of infection with M.tb, and the variable effectiveness of the vaccine against adult pulmonary TB, and the possible interfering the vaccine with tuberculin skin test reactivity (Koster *et al.,* 2021; Patil *et al.*, 2023).

In this study, the highest percentage of BCG vaccinated people who infected with tuberculosis was seen in active pulmonary TB (G1) 23 (96)% and (G4) 24 (100)%, and this results disagreed with several studies for example, a study by Nguipdop-Djomo, (2017) followed 83421 BCG-unvaccinated and 297905 BCG-vaccinated people for 41–44 years and notice a lower TB rate in vaccinated than in unvaccinated people were found, and the overall effectiveness of BCG vaccination against TB was 49%, but the efficiency of the vaccine reduced over time and became insignificant after 20 years. Also, a meta-analysis of cohort studies that evaluated the effect of BCG vaccine on TB disease in humans was conducted by Martinez *et al.* (2022) they observed About 18% reduction in the odds of TB infection.

The COVID-19 pandemic has had enormous effects on human health, society and economy in 2020 and 2021. One of the indirect consequences of the pandemic is the disturbance of TB services, which has caused in a upsurge in TB cases and deaths worldwide. TB is a bacterial infection that

mostly affects the lungs and can be deadly if not treated appropriately (Dheda *et al*., 2022).

The results of this study showed that (G4) group had the highest percentage of COVID-19 infection 24 (100)% which is agree with a study by WHO (2022) that the COVID-19 pandemic has caused disturbances to the access to vital TB services, leading to an increase of about 100 000 in the global number of TB deaths between 2019 and 2020. The report also displayed a considerable drop of 18% in the global number of TB case announcements between 2019 and 2020, suggesting that numerous people with TB were undiagnosed and untreated. The report estimates that the total TB occurrence raised up to 4.5% between 2020 and 2021, from 10.1 million to 10.6 million. Another study agreed the results of the present study by Filardo *et al*. (2022) showed that in the USA, TB incidence during 2021 increased by 9.4%. The study advocates that greater fluctuations in reported TB have occurred during the COVID-19 pandemic, affecting both the sources and demand sides of TB diagnosis and treatment.

TB cases and chronic diseases such as diabetes and hypertension were another factor included in this study the results expressed that the highest percentage, 16 (67)% of patients who have MDR-TB having both diabetes and hypertension. This result agreed with previous study was done by WHO (2022) revealed that diabetes is a major risk factor for TB disease, death, relapse and drug resistance. People with diabetes have a two to three times higher chance of developing TB, a twofold higher chance of dying during TB treatment, a four times higher chance of having TB again after being cured and a twice higher chance of having MDR-TB. In 2020, there are about 370 000 new TB cases worldwide having diabetes. In 2019, the proportion of patients with TB who also had diabetes was more than 15%

totally, compared with 9.3% among the general adult population aged 20-79 years. This means that about 1.5 million people with TB and diabetes needed coordinated care and follow-up to manage both conditions effectively.

5-2 Virulence factors of M.tb

Tuberculosis (TB) is a contagious infection caused by members of the *Mycobacterium tuberculosis* complex (MTBC), which comprises closely related species that infect mutually humans and animals (Forrellad *et al*., 2013). The pathogenesis and clinical indicators of TB rely on the interaction between the host immune system and the virulence elements of the mycobacteria (Chandra *et al.,* 2022). Virulence factors are defined as the bacterial genes or proteins that are essential for the infection, survival, multiplication and transmission of the pathogen (Leitão, 2020).

To better understand the molecular mechanisms of M.tb pathogenesis, this study selected the following virulence genes *whiB3, whiB7, pknF,* and *fbpA* These genes are involved in host cell invasion, phagosomal escape, and immunogenicity (Cushman *et al.,* 2021; Chandra *et al.,* 2022). The study aimed to investigate their expression levels, and interactions with immune system, in clinical isolates from sensitive TB and MDR-TB patients.

5-2-1 *whiB3*

whiB3 is a cytoplasmic protein in M.tb that senses and familiarizes to fluctuations in the redox state of mycothiol, the major thiol in M.tb. *whiB3* modifies the expression of genes that take part in lipid biosynthesis, secretion, and redox homeostasis in M.tb. This gives two benefits to M.tb: firstly, it increases M.tb’s resistance to acidic and oxidative tension; secondly, it prevents phagosomal development and decreases the expression

of innate immune cell genes, so will helping M.tb's persistence and survival inside the host cells (Naeem *et al*., 2021; Joshi *et al.,* 2021).

This study examined the difference in the gene expression levels of *whiB3*, the results revealed that the gene expression level of *whiB3* was significantly higher in MDR-TB (7.06) than in sensitive TB (1.70) (P-value = 0.0037). This findings is agreed with a previous study by Liu *et al.* (2016) they investigated the gene expression levels of 24 genes, including *WhiB3*, in sensitive TB and MDR-TB under both aerobic and anaerobic environments. Then they informed that the gene expression level of *whiB3* was 6-fold higher in MDR-TB than in sensitive TB under hypoxic circumstances. They also observed that M.tb displayed increased drug resistance in hypoxia, which was not discovered by routine drug susceptibility analysis. Therefore, they advised that drug susceptibility tests must also be implemented in hypoxic environments to eliminate hypoxia resistance in M.tb, particularly for cases shown initial therapeutic failure. Another study done by Mehta *et al*., (2016) to quantify the redox state of M.tb in acidic tension. They found that *whiB3* helps M.tb manage with acidic tension by adaptable its redox metabolism. *whiB3* expression was induced by acidic pH and was necessary for the reductive change in redox state of M.tb within macrophages. They found that 35-fold increased expression of *whiB3* as matched with 16S rRNA at (pH 4.5). Steyn *et al.,* 2002 Assumed that Reducing *whiB3* levels will compromise the existence and adaptation of M.tb to the aggressive environment by weakening its capability to sense and respond to host-generated nitric oxide (NO) and low levels of O2. Therefore, *whiB3* levels have associations with the diagnosis, prognosis, and treatment of patients (Singh *et al.,* 2009).

5-2-2 *whiB7*

whiB7 belongs to the WhiB-Like (Wbl) family, a group of proteins that are exclusively found in the Actinobacteria phylum and contain iron-sulfur (4Fe-4S) groups (Wan *et al.,* 2021). This protein shows a vital role in the resistance of M.tb to several antibiotics by modifying its own expression and many genes associated with drug resistance and redox balance when exposed to antibiotics (Lilic *et al*., 2021; Cushman *et al.*, 2021). *whiB7* in M.tb works by binding to domain 4 of the main sigma factor (σA4) in the RNA polymerase and stimulating genes associated with multiple drug resistance and redox stability (Elchennawi and Ollagnier de Choudens, 2022).

The present study estimated the gene expression levels of *whiB7* in the sensitive TB group and MDR-TB group. The results showed that the gene expression in MDR-TB was 9-folds higher than in sensitive TB (P-value= 0.0083). This result agreed with a previous research by Kim *et al.* (2017) they showed that the expression of *whiB7* was significantly higher in MDR-TB strains than in the standard strain H37Rv. The increase fluctuated from (1.01 to 1. 49) times, with a P-value of less than (0.001). In contrast, Pasca *et al.* (2004) disagreed with the present study they found that the genes *whiB7* involved in the efflux of fluoroquinolones and contributed to the resistance to these antibiotics in mycobacteria. However, the expression of *whiB7* did not change significantly.

5-2-3 *fbpA*

Is a protein that belongs to the antigen 85 complex (Ag85), a group of fibronectin-binding proteins (Fbps) secreted by M.tb (Kuo *et al*., 2019). *fbpA* catalyzes the transfer of mycolic acids to the cell wall and is assumed to display a part in the virulence of M.tb, as it possibly be mediated host attachment, immune escape and cell wall synthesis (Avellan *et al.,* 2022; Pal *et al*., 2022).

The results showed that *fbpA* was significantly higher in MDR-TB (2.59) than in sensitive TB (1.01) (P-value= 0.0003). These results agreed with lacobino *et al*., (2020) they found that *fbpA* expression levels are higher in MDR-TB than in sensitive TB, and this can be effect on resistance to isoniazid (INH) and rifampicin (RIF). Increasing expression of *fbpA* in M.tb is connected with virulence and immunogenicity and affect the transmission and resistance of MDR-TB strains. *fbpA* bind to fibronectin to host cells and help bacterial attachment and invasion (Rivas-Santiago *et al*., 2023).

5-2-4 *pknF*

A gene that codes for a serine/threonine protein kinase (STPK) that has various roles in the cellular functions of M.tb (Nilkanth and Mande, 2022). For example, the synthesis of cell wall components, such as arabinose and mycolic acids, additionally binds to numerous substrates that are involved in metabolic pathways, signaling, transport, and cell division (Richard-Greenblatt and Av-Gay, 2017; Cabarca *et al.,* 2021).

The expression level of *pknF* genes in M.tb has numerous significances for the bacterium and the host. Higher *pknF* expression alters the cell wall structure and morphology, shifting its susceptibility to antibiotics and host defenses (Deol *et al*., 2005). Elevated *pknF* expression

also modifies the glucose transport and metabolism of M.tb, changing its growth rate and persistence (Shamma *et al.,* 2022).

The study results showed that the expression level in MDR-TB (2.52) was higher compared to sensitive TB (1.72) but not significantly different (P= 0.2934). These findings agree with a study by Gupta *et al.* (2014) examined the mRNA levels of the kinases by qPCR. They found that the expression levels of the kinases were either similar (*pknE* and *pknF*) or significantly lower (*pknB*) in comparison to the standard strain H37Rv. The mRNA levels of *pknE* and *pknF* did not change much throughout the bacterial growth cycle, with a fold change approximately (1) and P-value of less than (0.05).

5-3 Dynamics of cytokine profiles in different TB population groups

The results from this study have revealed several differential patterns in IL-4, IL-6, IL-10, and IL-12 profiles that merit attention. In general, the results indicate that serum cytokine levels for each cytokine varied among sensitive TB, MDR-TB, TB patients under the treatment and the control groups. The results of this study showed significantly different levels of IL-4, IL-6, and IL-10 between sensitive TB, MDR-TB, TB patients under the treatment and the control groups. In addition, no significant difference was observed for IL-12 in all four groups.

5-3-1 Interleukin-4 levels (IL4)

IL-4 is one of the cytokines that play a key role in the immune response to M.tb infection. IL-4 is mostly produced by Th2 cells, which is linked with a weak cytotoxic activity and decreased macrophage-mediated

killing of M.tb (Geffner, 2011). IL-4 can also control the balance between Treg cells and Th1 cells, which are important for adjusting M.tb infection (Pooran *et al.,* 2019).

In this study, the results showed a highest level of IL-4 was in G2 (extra-pulmonary TB group) (IL-4= 905.36 pg/ml) (P-value <0.0001). This result agreed with a previous study by Liu *et al.* (2020) they compared the levels of IL-4 in 17 patients with extra pulmonary TB and 17 healthy individuals. They revealed that the extra pulmonary TB group had significantly higher IL-4 concentrations (59.06 ± 39.82 pg/mL vs. 5.07 ± 2.66 pg/mL, P < 0.05). This advocates that IL-4 plays a role in the pathogenesis of extra pulmonary TB by Prompting arginase-1 expression, which competes with nitric oxide synthase for the substrate L-arginine and reduces nitric oxide production, a significant anti-microbial against MTB (Heitmann *et al.,* 2014). And inhibiting Th1 polarization which leads to declined production of IFN-γ, a cytokine that stimulates macrophages and increases their bactericidal activity (Pooran *et al*., 2019). Also Promoting IL-4 secretion by CD4+ T cells which generates a positive response loop that increases Th2 cell responses and suppresses Th1 cell responses (Ma *et al*., 2020). Another study by Sharma *et al*. (2018) to quantify the levels of IL-4 in the vitreous fluid of extra pulmonary TB patients and healthy individuals. They found that the extra pulmonary TB group had significantly higher IL-4 concentrations (8.41 ± 4.25 pg/mL vs. 7.46 ± 5.41 pg/mL, P < 0.05), which agreed with the current results of the study.

The present results showed that IL-4 was significantly higher in G4 (MDR-TB) patients 602.54 pg/ml (P-value< 0.0001) and this results agreed with a study by Tan *et al.* (2012) when they measured the levels of IL-4 in the serum of individuals with multiple drug resistance-TB (MDR-TB) and

healthy individuals. They found that the MDR-TB group had significantly higher IL-4 concentrations (82.87 ± 32.69 pg/mL vs. 3.72 ± 1.61 pg/mL, P < 0.05). This indicates that IL-4 involved in the pathogenesis of MDR-TB.

5-3-2 Interleukin-6 levels (IL-6)

IL-6 is a pleiotropic cytokine that adjusts many aspects of the immune and inflammatory responses to M.tb infection. IL-6 can exert both protective and harmful effects on the host, depending on the timing, degree, and the condition of its production and signaling (Howard and Khader, 2020).

In this study, the serum levels of IL-6 were measured in different groups of TB patients and healthy controls. The results found that IL-6 levels were significantly higher in patients with sensitive TB, multidrug-resistant TB (MDR-TB), and patients under treatment than in healthy controls (P<0.001). These results agreed to a study by Correia *et al.* (2009) they measured the levels of IL-6 in the serum of individuals with sensitive TB (S-TB), multidrug-resistant TB (MDR-TB), and healthy individuals. They found that the TB group had significantly higher IL-6 concentrations (median = 4.3 pg/ml, range 0.5–24) than the healthy group (median = 0.5 pg/ml, range 0–2.8), (P < 0.001). Moreover, both sensitive TB (median = 4.3 pg/ml, range 0.5–24) and MDR-TB (median = 5.1 pg/ml, range 0.5–12) groups had increased IL-6 concentrations compared with the healthy group (P < 0.001). This indicated that IL-6 involved in the infection and immune response to TB, regardless of the drug resistance status. Additional study done by Nagu *et al.* (2018) when they measured the levels of IL-6 in the serum of patients with TB and healthy individuals. They found that the TB group had significantly higher IL-6 concentrations (3863 pg/ml) than the healthy group (11 pg/ml). Moreover, higher IL-6 levels were linked to

survival (RR = 0.95; 95% CI 0.91 - 0.98; P < 0.01) and severe lung injury (RR 1.03; 95% 1.01 - 1.05; P = 0.02) in TB patients. Among TB patients with severe lung injury, every (100) pg/ml rise in baseline IL-6 levels was linked with (6)% increased threat for residual lung damage after TB treatment (RR = 1.06; 95%CI 1.01 - 1.13). Anti-TB treatments decreased serum cytokine levels except for IL-6, which remained high with treatment (P = 0.003), and that agreed with the patient under treatment group of the current study (23.96 pg/ml) compared with the healthy group (9.22 pg/ml) (P-value <0.0001).

5-3-3 Interleukin 10 levels (IL-10)

IL-10 is an anti-inflammatory cytokine that inhibit pro-inflammatory responses of both innate and adaptive immune cells. IL-10 has an immune-suppressive influence and weakens the host resistance against M.tb disease (Cohen *et al.,* 2018; Jiang *et al.*, 2021).

In this study, the serum levels of IL-10 were measured in different groups of TB patients and healthy controls. Results found that IL-10 levels were significantly higher in patients with MDR-TB (41.45 pg/ml) than in sensitive TB patients (13.32 pg/ml) (P <0.0001), while they were significantly higher in patients under treatment than in healthy controls (Table 4-13) (P<0.001).The results of this study agreed to a study published by Basingnaa *et al.,* 2018 they investigated the plasma levels of IL-10, IFN-γ and TNF-α in (83) participants (49 MDR-TB and 34 DS-TB patients). They observed that the MDR-TB group had higher levels of all three cytokines than the DS-TB group. The mean level of IL-10 (7.8 ± 3.61 ρg/mL) in the MDR-TB group was significantly higher (P = 0.0022) than the mean level of IL-10 (4.8 ± 4.94 ρg/mL) in the DS-TB group. They suggested that IL-10 secretion is increased throughout the infection enabling TB reactivation. Too

much production of this cytokine also harms the control of the infection, which clarify why IL-10 was elevated in the MDR-TB participants. A similar finding of high IL-10 production in patients with disease including MDR-TB was reported in Turkey by Deveci *et al.* (2005) they evaluated the serum levels of IL-10 in the serum of individuals with MDR-TB and healthy controls (HC). They found that the MDR-TB group had significantly higher IL-10 concentrations (mean = 5.9 ± 1.9 pg/mL) than the HC group (mean = 3.5 ± 2.1 pg/mL), ($P < 0.05$). Furthermore, MDR-TB groups had reduced IL-10 concentrations after anti-tuberculous treatment (ATT) for (4 and 6) months (mean = 3.5 ± 2.1 pg/mL and 3.1 ± 1.7 pg/mL), respectively compared with the baseline level (mean = 5.9 ± 1.9 pg/mL), ($P < 0.05$), which is agreed to the results of IL-10 of patients under treatment of the present study (G3) (IL-10= 21.37± 8.20 pg/mL). This suggests that IL-10 involved in the inflammation and immune response of TB, and ATT decrease its production. This high IL-10 production in MDR-TB also show suppression of the immune response, leading to an imbalance of pro- and anti-inflammatory cytokines (Jiang *et al.*, 2021).

5-3-4 Interleukin 12 levels (IL-12)

IL-12 is a cytokine which induces the expression of IFN-γ and activate antigen-specific lymphocytes that are essential for eradicating intracellular bacteria such as MTB. IL-12 also contributes to the adaptive immunity to TB by stimulating the differentiation of Th1 cells that secrete IFN-γ (Cooper *et al.,* 2007; Yin *et al*., 2021).

In this study, the serum levels of IL-12 were measured in different groups of TB patients and healthy controls. The results found that the highest level of IL-12 was observed in TB patients under the treatment (G3) (31.02 pg/ml) and the lowest level was in G4 (15.54 pg/ml) but not

significantly different between any of the groups (P= 0.3362). These results agreed with Mankhi *et al.* (2009) when they calculated the serum levels of IL-12. They found that there is no statistically difference between TB group concentrations (mean = 82.1±3.6 pg/ml) compared with the control group (mean = 48.9±1.36 pg/ml). They clarified that IL-12 involved in the anti-microbial host defense against MTB and is vital for inducing IFN-γ production. They also advised that IL-12 has the capability to induce the differentiation of naive CD4+ T cells into Th1 cells, which are central for TB immunity. In contrast, Abohashrh *et al*. (2022) disagreed with the current results by tested the mRNA expression level of IL-12 in the blood of individuals with active TB and healthy controls. They found that the TB group had significantly higher IL-12 mRNA expression (13.01-fold) than the control group ($P < 0.05$). They observed that the TB cases with fever, night sweat, weakness, sputum with blood had a higher IL-12 mRNA expression than the other TB cases. This suggests that IL-12 involved in the inflammation and immune response to TB infections.

5-4 Correlation between the virulence factors of M.tb and cytokines level:

M.tb can cause and keep the infection in the host through avoiding and manipulating the immune system. It has numerous elements that help the bacteria to live and grow secretly inside phagocytic cells, alters the inflammatory responses, and interrupt the exhibition of antigens. (Khan *et al.*, 2022). Some of these factors are components of the cell wall such as lipids and glycolipids. Also secreting systems secrete proteins and enzymes that metabolize nutrients. The immune system reacts to M.tb infection by making cytokines which are central for balancing and protection in TB. The correlation between M.tb virulence factors and cytokine levels in patients with sensitive and MDR-TB is not entirely clear (Imperiale *et al.*, 2021; Jøntvedt Jørgensen *et al.*, 2021).

This study investigated the comparison between virulence factors of sensitive and MDR-TB with cytokine levels in the serum samples of these patients. The study focused on four virulence factors: *whiB3, whiB7, pknF*, and *fbpA*, and four interleukins : IL-4, IL-6, IL-10, and IL-12.

From the tables (4-19) (4-20), *whiB3* and *whiB7* gene expression are positively correlated with IL-10 levels in sensitive TB patients (r= 0.596, P-value= 0.002) (r= 0.637, P- value= 0.0001), respectively. Patients who infected with MDR-TB strains are overexpress *whiB3* and *whiB7* exhibited higher levels of IL-10 production (r= 0.7645, P-value= 0.001) and moderate level of IL-10 for *whiB7* (r= 0.490, P-value= 0.015). These results come to an agreement with several studies in this area for instance, Mahatha *et al.,* (2020) investigated the role of *RegX3*, a regulator for *WhiB3* in granuloma formation in vitro. The study used human PBMCs (peripheral blood

mononuclear cells) to induce granuloma formation in vitro. They infected (1) million PBMCs with M.tb, They detected that the wild type bacterium induced the formation of granulomas (>100 µm) in PBMCs. However, when PBMCs were infected with a mutant strain that lacked *regX3* (Δ*regX3*), there was no granuloma formation. These results indicated that *RegX3* and *whiB3* is obligatory for granuloma formation in vitro and this process is dependent on *whiB3*. Another study agreed the current study done by Singh *et al.* (2009) they investigated the effect of *whiB3* on the cytokine release from macrophages infected with M.tb. They infected macrophages with wild type M.tb and a mutant strain that lacked *whiB3* (MtbΔ*whiB3*). They measured the levels of IL-2, IL-4, IL-5, IL-10, IL-12(p70), GM-CSF, IFN-γ and TNF-α in the culture supernatant of macrophages (24) h post infection. They found that the MtbΔ*whiB3* group had a significantly higher imbalance the concentrations of both pro- and anti-inflammatory cytokines than the wild type M.tb group ($P < 0.05$). This indicates that *whiB3* modulates the cytokine release from macrophages and that its absence impairs the immune suppressing capacity of M.tb. Finally, Mehta *et al.* (2019) study the role of *whiB3* in granuloma formation in human models, they used an in vitro model and infected (1,000,000) human PBMCs with either wild type M.tb and a mutant strain that lacked *whiB3* (MtbΔ*whiB3*) and incubated them for (6 to 9) days. They detected that human PBMCs shaped cellular masses in the presence of wild type M.tb at (6) and (9) days post-infection, and that the size of the masses enlarged with greater numbers of PBMCs. However, when human PBMCs were infected with M.tbΔ*whiB3*, there was no granuloma formation.

While the results suggest that there is a positive correlation between the expression amount of *whiB3* and *whiB7* in MDR-TB with interleukin-10, a

decrease in the level of IL-12 had been observed in MDR-TB patients for both *whiB3* and *whiB7* (IL-12= -0.610) (IL-12= -0.602) (P- value= 0.001) compared to sensitive TB (IL-12= 0.353) (IL-12= 0.337). And these results agreed by Gupta *et al*. (2010) they investigated the effects of *whiB3* protein coded from M.tb on the production of IL-12 in dendritic cells (DCs) and macrophages. They transfected DCs with M.tb from day-1 to day-5 of infection then They measured the IL-12 levels in the supernatants and found that they were reduced from (380 pg/ml) to (200 pg/ml), a day-5 was the most effective in suppressing DC activation and was also detected in vivo in infected mice. Therefore, they selected *whiB3* for further experiments with mouse peritoneal macrophages. They observed that *whiB3* also significantly inhibited TLR2-induced IL-12 in mouse macrophages from (340 pg/ml) to (180 pg/ml). Steyn *et al.,* (2002) was reported that as IL-12 is induced by microbial products and regulates the development of adaptive immune cells, *whiB3* is one of the M.tb products which plays a significant role in the host immune response by regulating specific mycobacterial factors that modulate the balance between pro-inflammatory and inhibitory cytokines to diminish host survival.

fbpA stimulates Th1 cytokines and cytotoxic T lymphocytes (CTLs) activity to clear intracellular bacteria such as MTB. *fbpA* is part of the Ag85 complex, which makes the mycobacterial cell wall. *fbpA* can bind to fibronectin, a host protein, and help the bacteria enter the host cells. (Mukhopadhyay *et al.,* 2012). *fbpA* activates APCs such as dendritic cells and macrophages, which present *fbpA* peptides to T cells. *fbpA* also stimulates cytokines that promote Th1 cell differentiation and propagation. (Layre, 2020)

In the table (4-21) revealed a significant positive correlation between *fbpA* gene expression and IL-6 levels in both sensitive TB and MDR-TB. The Pearson correlation coefficient for sensitive TB *fbpA* and IL-6 was (r= 0.46, P-value= 0.023), while for MDR-TB *fbpA* and IL-6 was (r= 0.514, P-value= 0.01). This results agreed by A study about the immunogenicity of *fbpA* in M.tb, A study done by Artama *et al.* (2020) explored the potential of two antigenic proteins from M.tb, *fbpA* and *fbpB* as subunit vaccine candidates for TB. They cloned and expressed these genes from clinical isolates of MDR-TB and predicted their T-cell epitopes. They identified seven specific T-cell epitopes for both genes, and twenty-three and sixteen specific T-cell epitopes for *fbpA* and *fbpB* genes, respectively, they concluded that *fbpA* and *fbpB*, which encode Ag85A and Ag85B proteins have epitopes that can stimulate T-cell responses and modulate the pro-inflammatory and anti-inflammatory cytokines. However, Copenhaver *et al*. (2004) disagreed with the results of the research by making a study in mice, showed the role of FbpA protein from M.tb in immunity and survival. They disrupted the *fbpA* genes that encode the 85A protein in MTB, and tested the mutants for their ability to survive and immunize mice. They found that the mutant without 85A (Delta*fbpA*) was weakened in mice after intravenous infection. They restored the *fbpA* gene in Delta*fbpA* and its ability to grow in the lungs of mice was recovered. The Delta*fbpA* mutant triggered a stronger IL-6 (165.2 ng/ml ± 22.3) than wild type MTB (88.0 ng/ml ± 80.0) (P-value= 0.0198).

pknF which is a serine/threonine kinase, is a critical player in the pathogenicity of M.tb (Pal *et al.,* 2022). It is involved in the regulation of cell wall biosynthesis and has been shown to interact with *Rv1747* ("a

conserved trans-membrane ATP-binding protein ABC transporter") in a phosphorylation-dependent manner (Hui, 2021). The absence of *Rv1747* results in a reduced growth level in macrophages (Li, 2021).

The investigation analyzed the association between *pknF* gene expression and cytokine levels did not demonstrate a statistically significant relationship between *pknF* and IL-4 and IL-6, with P-values greater than (0.05) for both correlations as in the table (4-22). This indicates that in this study, the expression of the *pknF* gene was independent of circulating IL-4 and IL-6 concentrations. Bonne Køhler *et al.* (2020) agreed with the current results by showed that *pknF*'s interact more with other proteins that are critical for the growth and survival of the bacterium within the host. Also Narayan *et al*. (2007) reported that the *pknF* is one of the Serine/Threonine Protein Kinases (STPKs) plays important roles in regulating various physiological processes including stress response, cell cycle regulation, and development.

CONCLUSIONS AND
RECOMMENDATIONS

7-1 Conclusions:

The study revealed several important aspects of M.tb pathogenicity and its interaction with the host's immune system, with a special focus on granuloma formation as a crucial mechanism that shapes the dynamic between M.tb and the host. The key findings of this study are summarized as follows:

1. The gene expression levels of *whiB3*, *whiB7*, and *fbpA* were significantly higher in MDR-TB patients than in sensitive TB patients, indicating that these factors are associated with M.tb drug resistance and virulence.
2. *whiB3* and *whiB7* correlated positively with IL-10 levels in MDR-TB patients, suggesting that they modulate the immune response by influencing the balance between pro- and anti-inflammatory cytokines.
3. *FbpA* was associated with IL-6 levels, indicating that it stimulated the Th1 cytokine response and CTL activity, which are essential for controlling MTB infection.
4. *pknF* showed no significant difference between the expression levels in MDR-TB and sensitive TB patients.
5. In this study, no significant correlation was found between *pknF* expression and IL-4 and IL-6 levels.
6. The cytokine levels among the four groups of the study (sensitive pulmonary TB, sensitive extra-pulmonary TB, TB patients during treatments , and MDR-TB patients) showed significant differences for all cytokines except for IL-12.

7-2 Recommendations:

Based on the findings of this thesis, some recommendations for future Research are:

1. To validate the results of this study using a larger sample size and different geographical regions to increase the generalizability and reliability of the findings.
2. To explore the molecular mechanisms and pathways involved in the regulation of gene expression and cytokine production of these genes using functional assays and knockout experiments.
3. Examine the influence of TB treatment on other cytokines and their function in infection and immunity, such as TNF-alpha, IL-17, and IL-22 in both groups.
4. Increase the number of TB strains to compare and validate the correlations between cytokine levels and strain types.
5. Extend the scope of genes to study and include other genes that involved in TB pathogenesis and immunity such as Early Secreted Antigenic Target-6 (ESAT-6), Culture Filtrate Protein-10 (CFP-10), and Phenolic Glycolipid-1 (PGL-1).

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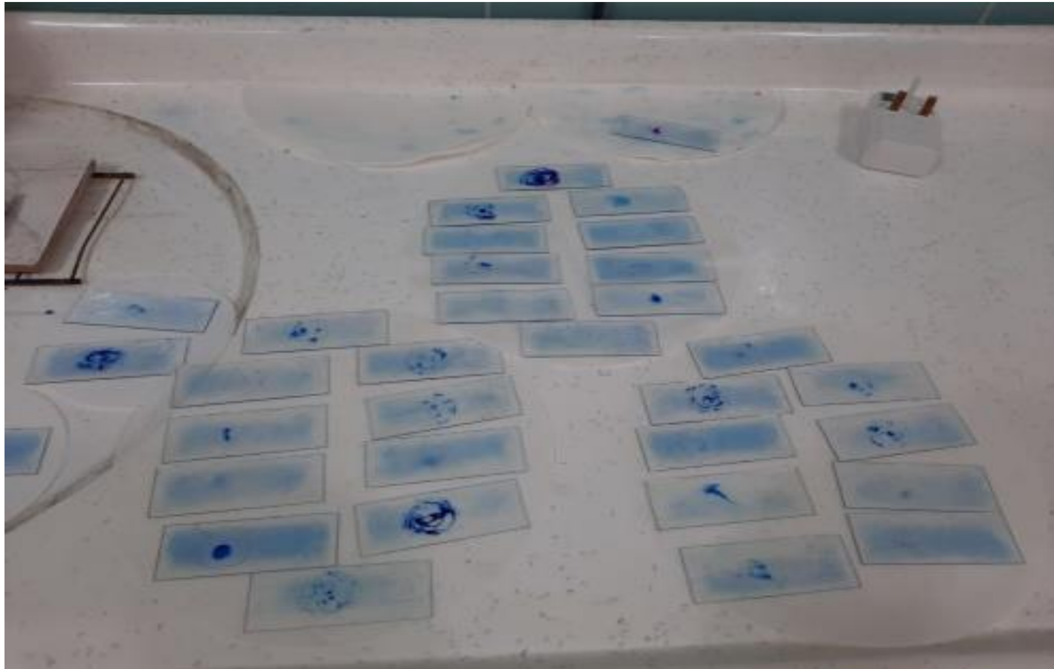
APPENDIXES

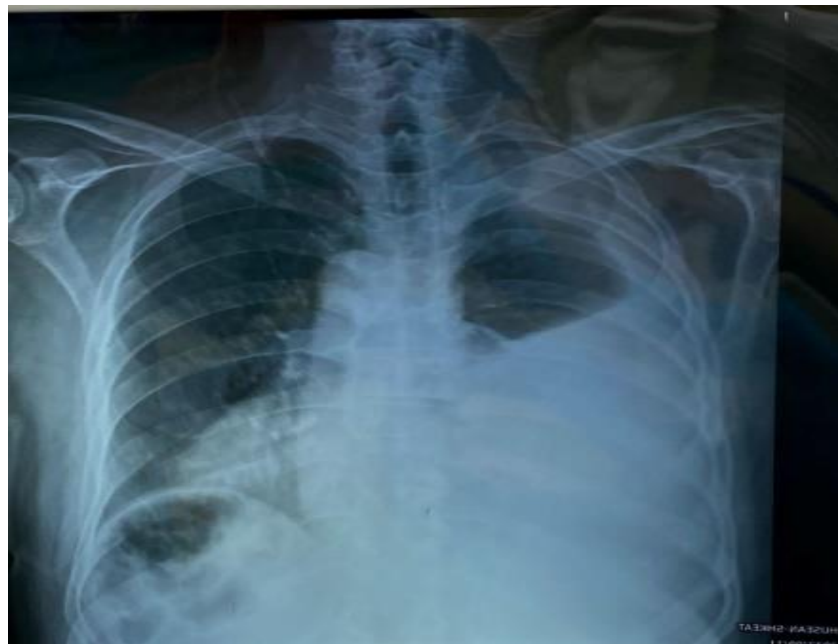


Appendix (1): Sputum smear slides of acid fast stain for the detection of M.tb

Appendix (2): Cups of sputum collected from the patients for the diagnosis of M.tb.

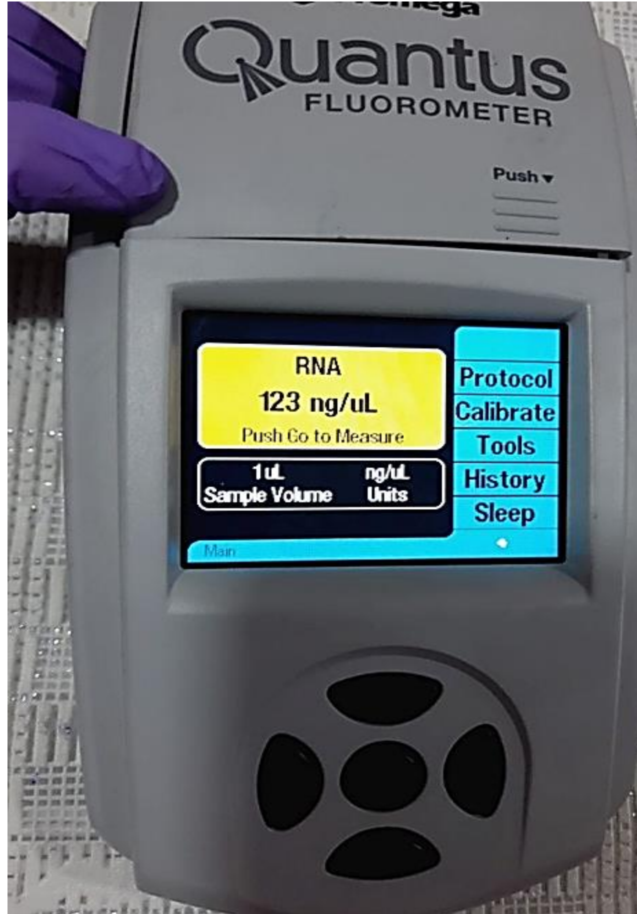
ت	اسم المريض	العمر	الجنس	العنوان	العمل	تاريخ التشخيص	نوع التدرن	مصاب بالتدرن		ملقح BCG		ملقح كورونا		مصاب بكورونا		ملاحظات
								نعم	لا	نعم	لا	نعم	لا	نعم	لا	

Appendix (3): A Questionnaire Sheet patients for tuberculosis infection.



Appendix (4): A patient chest X-ray has TB infection in the lungs

The recorded concentration of Sensitive TB samples ranged from 126 to 340 ng/ul. And the range purity was ranged from 1.4 to 2.01 While MDR-TB were ranged from 204 to 382 ng/ul and the purity fluctuated from 1.66 to 2.3



Appendix (5): Sample of measuring RNA concentration and purity

الخلاصـــــة

الخلاصة:

يعتبر السل الرئوي (TB) مشكلة صحية عامة كبيرة لم يتم إيقافها بالعديد من الجهود. تم القيام بالكثير من العمل من قبل برامج مكافحة السل، ولكن مكافحة مرض التدرن أصبح أكثر صعوبة بسبب مشكلة السل المقاوم للأدوية المتعددة (MDR-TB)، والتي تحتاج إلى حلول جديدة للحد من تأثيرها. هناك حاجة إلى برامج مكافحة السل الجديدة التي يمكنها التعامل مع السل المقاوم بشكل أفضل في جميع أنحاء العالم، وايضا حاجة خاصة لأدوات تشخيص جديدة لا تستخدم البلغم ويمكنها التحقق من مدى فعالية العلاج حيث تعتبر هذه الاجراءات مفيدًا جدًا لهذه المشكلة.

ان الهدف من هذا البحث هو فحص العلاقة بين مستويات تعبير أربعة جينات وأربعة سايتوكينات في مجموعات TB المختلفة. هذه الجينات هي *whiB3*، *whiB7*، *pknF*، و *fbpA*، والتي تلعب دورًا في امراضية ومقاومة الدواء لبكتيريا التدرن، و العامل المسبب للسل. هذه السايتوكينات هي IL-4، IL-6، IL-10 ، و IL-12، تلعب دورًا في الاستجابة المناعية للمضيف ضد بكتيريا السل.

تم إجراء الدراسة على 120 عينة من مصل المشاركين الذين تم اختيارهم من مركز التدرن في محافظة ميسان، العراق, للفترة من 23 آب/أغسطس 2022 إلى 20 كانون الثاني/يناير 2023. تم تقسيمهم إلى اربع مجموعات وفقًا لحالتهم ونوع الاصابة بالتدرن: G1 (السل الرئوي النشط)، G2 (السل خارج الرئوي النشط)، G3 (المرحلة العلاجية)، و G4 (السل المقاوم للأدوية المتعددة) وكذلك مجموعة الاصحاء. تم قياس مستويات IL-4، IL-6، IL-10 ، و IL-12 في كل مجموعة بوساطة تحليل المناعة المرتبط بالانزيم (ELISA)، وقياس مستويات التعبير الجيني للأربعة جينات بوساطة تفاعل البوليميريز المتسلسل الاني (qPCR). وتم استخدام معامل ارتباط بيرسون لتقييم العلاقة بين مستويات التعبير الجيني ومستويات السايتوكينات. اعتبرت الاختلافات بين المجموعات ذات الدلالة الإحصائية عند قيم P أقل من 0.05.

كانت مستويات IL-4 و IL-6 و IL-10 في المصل مختلفة بشكل كبير بين المجموعات الاربعة (P <0.0001، P <0.0001، P <0.0001 على التوالي). كان لدى G2 أعلى مستوى من (IL-4 (905.36pg / mL، بينما كان لدى G3 أدنى مستوى (421.96 pg / mL). وكذلك أظهرت مستويات IL-12 عن عدم وجود اختلاف ذي دلالة إحصائية بين المجموعات P = 0.3362، حيث تراوحت من (15.54 pg / mL) في G4 إلى (31.02 pg / mL) في G3. بينما

كان لدى G1 أعلى مستوى (18.66 pg / mL). كان لدى G4 مستويات IL-10 أعلى بشكل ملحوظ في G4 من جميع المجموعات الأخرى (41.45 pg / mL)، بينما كانت مستويات G1 و G2 متقاربة (13.51 pg / mL, 13.32 pg / mL). كانت قيم التعبير الجيني لـT *fbpA* و *whiB3* و *whiB7* مختلفة بشكل كبير بين سلالات السل الحساسة و المقاومة $P < 0.005$. في التدرن الحساس للادوية، كانت تعابير *whiB3* و *whiB7* مرتبطة إيجابيًا بمستوى IL-10 (0.59 =r)(P = 0.0024), (r = 0.63)،(P = 0.001) على التوالي، بينما كانت تعبيرات *fbpA* مرتبطة إيجابيًا بـ IL-6 في السل الحساس وسلالات السل المقاومة للأدوية (r = 0.46، P = 0.02؛ r = 0.514، P = 0.01) على التوالي. في سلالات السل المقاومة للأدوية كان تعبير *whiB3* مرتبطًا سلبيًا بمستوى IL-12 (r= -0.61 ، P = 0.001)، ومرتبطًا إيجابيًا بمستوى r= IL-10 (0.76، P = 0.0001). كان تعبير *whiB7* مرتبطًا سلبيًا بمستوى IL-12 (r = -0.60، P = 0.001)، ومرتبطًا إيجابيًا بمستوى IL-10 (r = 0.49، P = 0.015).

تشير النتائج إلى مستويات تعبير جينية وأنماط سيتوكينات مختلفة في المرضى المصابين بالسل الحساس والمقاوم. حددت الدراسة مستويات تعبير أعلى لجين *fbpA* و *whiB3* و *whiB7* في سلالات التدرن المقاوم مقارنة بسلالات السل الحساسة. كشفت الدراسة أيضًا عن ارتباطات مهمة بين مستويات التعبير الجيني ومستويات السيتوكين في كلا مجموعتي السل ، مع بعض الجينات التي تنظم إنتاج السيتوكين وتؤثر على نتيجة المرض. قد يكون قياس مستويات مصل IL-4 و IL-6 و IL-10 وقيم التعبير الجيني لـ *whiB3* و *whiB7* و *fbpA* مفيدًا كعلامات حيوية لتمييز مجموعات TB المختلفة وبالتالي قد يساهم في إدارة مرض التدرن ومراقبة استجابات العلاج.

جمهورية العراق
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جامعة ميسان/ كلية العلوم
قسم علوم الحياة

العلاقة بين التعبير الجيني لبعض جينات الضراوة لبكتيريا المتفطره السلية ومستويات الانترلوكينات في مرضى التدرن

رسالة مقدمة

الى مجلس كلية العلوم/جامعة ميسان

وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل

سامح رياض فيصل

بكالوريوس علوم/علوم الحياة (2015)

بإشراف

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