INTRODUCTION

Tuberculosis is a common and in many cases lethal, infectious disease caused by mycobacteria usually mycobacterium tuberculosis. Tuberculosis typically attacks the lung, but can also affect other parts of the body. it is spread through the air when people have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air, most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which if left untreated kills more than 50% of those so infected. **(Konstantinos, 2010)**

An estimated 2 billion people, one-third of the global population, are infected with mycobacterium tuberculosis. Most people recover from primary TB infection without further evidence of the disease. The infection may stay inactive (dormant) for years. However, in some people it can reactivate. Most people who develop symptoms of TB infection first become infected in the past. In some cases the diseases become active within weeks after the primary infection. **(Calver et al, 2010)**

Tuberculosis is a leading cause of worldwide preventable morbidity and mortality from an infectious agent. A definite

diagnosis of tubercular pleural effusion can be difficult to make because of low sensitivity and / or specificity of noninvasive traditional diagnostic tools. **(Bhoumik et al., 2013)**

The QuantiFERON-TB Gold IN TUBE test is a whole-blood test for use as an aid in diagnosing Mycobacterium tuberculosis infection, including latent tuberculosis infection (LTBI) and tuberculosis (TB) disease. This test was approved by the U.S. Food and Drug Administration (FDA) in 2007. **(Gerald H et al, 2010)**

AIM OF THE WORK

The aim of this study to evaluate the value of Quantiferon Gold in-tube test in diagnosis of tuberculosis infection.

TUBERCULOSIS

I. The Mycobacterium tuberculosis complex:

Members of the Mycobacterium genus widely occur in natural ecosystems. Most members of mycobacteria are decomposing organic matter and due to the nitrogen binding activity they are useful and essential inhabitants of the soil and surface waters. Some Mycobacterium species in the course of evolution has become pathogenic. Among the pathogenic ones the most important are the members of Mycobacterium tuberculosis complex (MTBC), especially M. tuberculosis and M. bovis. There are significant differences in the virulence and the host specificity among the complex members (M. tuberculosis, M. bovis, M. bovis bacillus Calmette-Guerin BCG, M. africanum, M. microti, M. canettii, M. bovis subsp. caprae) ***(Metchock et al., 1999)***.

M. tuberculosis, M. africanum and M. canettii are primarily human pathogens ***(van Soolingen et al., 1999)***; M. microti mainly causes disease in rodents, although a few human cases have been reported as well. M. bovis can cause disease in both humans and animals (cattle, goats, elephants, deer, cats, seals, etc.) ***(Aranaz et al., 1999)***, however the infection is more frequent in animals. Bacillus Calmette-Guerin is an attenuated

vaccine strain, which can cause disease by shocking of the immune system ***(Calmette et al., 1926)***. M. tuberculosis and M. bovis are the best examples of the differences in host specificity. The number of human infections caused by M. bovis is much lower compared to the number caused by M. tuberculosis. In addition, it was observed that transmission of the disease among close contacts is significantly lower in case of the M. bovis infection than in case of M. tuberculosis ***(Iseman, 1996)***.

Although the phenotypic similarity is less than 65% among the members of the complex, there is about 99.9% genetic identity observed at deoxyribonucleic acid (DNA) level ***(Brosch et al., 2002)***. Thus, these organisms are more subspecies rather than individual species.

The disease caused by infection with Mycobacterium tuberculosis complex members is called tuberculosis. The most common form is pulmonary tuberculosis where at the site of infection the production of various mediators, the activation and accumulation of alveolar macrophages and lymphocytes can be observed, leading consequently to a helper T lymphocyte/ macrophage alveolitis ***(Schluger, 2001)***. The course and progression of disease is depending on interaction the pathogen of the various regulatory mechanisms for induction of cytokine production of the host. The cytokine release rate at the site of

infection in the lungs, the release rate of T1 helper and T2 helper type cytokines in broncho-alveolar lavage (BAL) cells (alveolar macrophages and lymphocytes) and in circulating mononuclear cells correlates with the severity of the disease ***(Somoskövi et al., 2000)***.

In contrast, the secondary immune response function and the expression of molecules (CD58, CD80, CD86 and HLA-DR) in signal transduction system has shown that the activity of alveolar macrophages is not dependent on severity of disease ***(Somoskövi et al., 2000)***. This indicates that the aggravation is not a consequence of an immune-paralysis. As the symptoms of tuberculosis are not specific, laboratories have a vital role in the diagnosis of tuberculosis, in the monitoring and determining of the effectiveness of therapy used as well as in preventing the transmission of disease.

The chest X-ray examination provides a good basis for diagnosis of suspected tuberculosis; however lot of lung diseases may show a similar picture on the radiograph. Therefore, the laboratory findings must be accurate and also readily available.

A. M. tuberculosis organism:

M. TB is an aerobic, gram-positive bacterium that by definition, does not contain a true outer membrane. In general,

mycobacteria are most commonly associated with their extremely hydrophobic, impermeable cell wall. These properties contribute to make drug delivery a difficult proposition, and confer an innate resistance to most drugs without the explicit evolution of antibiotic resistance genes ***(Dover et al., 2007)***.

The hydrophobic nature of the cell wall is conferred by the presence of mycolic acids, which are long chain α-branched-β-hydroxyl fatty acids containing upwards of 60 to 90 carbon atoms. The successes of isoniazid and ethambutol as inhibitors of cell wall biosynthesis underscore its viability as a drug target, and inhibiting other proteins involved in cell wall synthesis may provide further means to increase its permeability to other antibacterial drugs and increase their efficacy against M. tuberculosis ***(Dover et al., 2007)***.

B. The mycobacterial cell wall:

The compositional complexity of the mycobacterial cell wall (Figure 1) distinguishes Mycobacterium species from the majority of other prokaryotes. Classified as Gram-positive organisms, their envelopes do in fact share notable features with Gram-negative cell walls, such as an outer permeability barrier acting as a pseudo-outer membrane ***(Brennan and Nikaido, 1995; Minnikin, 1982)***. Much of the early structural definition

of the cell wall was conducted in the 1960s and 1970s ***(Lederer et al., 1975; Petit et al., 1969; Adam et al., 1969)***. Minnikin, in 1982, proposed the currently accepted structural model for the cell wall architecture that was later supported by McNeil and Brennan ***(McNeil and Brennan, 1991; Minnikin, 1982; Minnikin et al., 2002)*** and numerous further investigations ***(Besra et al., 1995; Brennan and Nikaido, 1995; Minnikin, 1991; Nikaido, 1993)***.

The structural features of the cell envelope can be divided into three major components;

1. The plasma membrane,
2. The mycolyl-arabinogalactan-peptidoglycan (mAGP) complex ***(Besra and Brennan, 1997)***, consisting of three covalently linked macromolecules, peptidoglycan (PG), arabinogalactan (AG) and hydrophobic mycolic acids which decorate the nonreducing terminus of the arabinan domains,
3. Lipoarabinomannan (LAM), lipomannan (LM), non-covalently bound lipids and glycolipids.

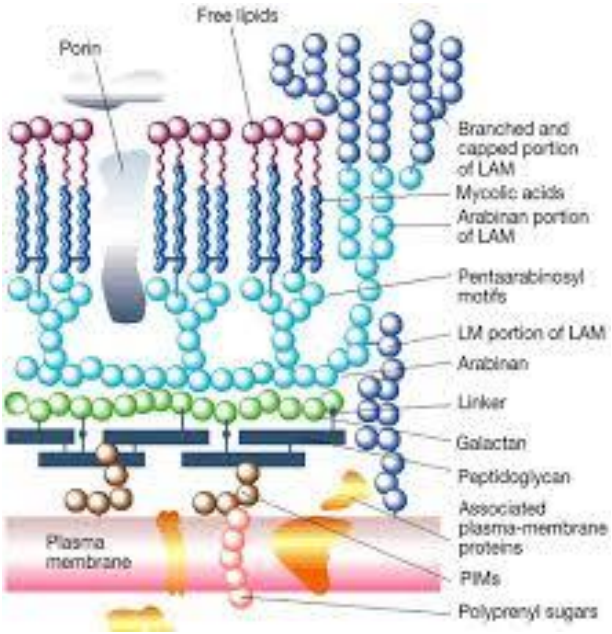

Fig. (): Mycobacterium cell wall structure.

1. The cell wall of M. tuberculosis as a drug target:

Mycobacterial diseases are especially problematic to treat owing to this unique cell wall architecture. This tightly packed, lipid rich envelope accounts for the inherent resistance of M. tuberculosis to various hydrophilic antimicrobials, as well as lipophilic molecules that have difficulty passing through this highly ordered mycolic acid layer. The cell wall is essential for growth and survival and cell wall inhibitors have been one of the most active agents of chemotherapy, and as such, the majority of front-line drugs target its biosynthesis ***(Zhang, 2005)***.

Drug regimens employ at least one cell wall biosynthetic inhibitor, for instance INH and ETH, which target mycolic acid biosynthesis or EMB which inhibits arabinan biosynthesis, taken in combination with other chemotherapeutic agents that have intracellular targets ***(Nikaido and Jarlier, 1991)***. However, due to the emergence of various drug resistant strains, there is a need for the discovery of novel drug targets and the development of active compounds against them ***(Heymann et al., 1999; Sreevatsan et al., 1997b; Telenti et al., 1997)***. In this regard, the biosynthetic machinery of mycobacterial cell wall represents an attractive target and numerous research studies have been and are being conducted, delving into the intricacies of the mycobacterial cell wall assembly ***(Bhatt et al., 2007b; Bhowruth et al., 2007; Brennan and Crick, 2007; Dover et al., 2008)***.

2. Plasma membrane:

The plasma membrane acts as a boundary between the cytosol and the periplasmic space, but it also harbours a number of substrates required by cell wall biosynthetic enzymes ***(Berg et al., 2007)***, thus playing a crucial role in the biogenesis of the cell wall. A group of glycosylphosphoprenols associated with the plasma membrane have been identified and operate as sugar

donor substrates for many of the TB glycosyltransferases. For instance, activated forms of D-ribofuranose ***(Wolucka et al., 1994; Wolucka and de Hoffmann, 1995)***, D-mannopyranose ***(Takayama and Goldman, 1970)*** and D-arabinofuranose ***(Wolucka et al., 1994)*** have been identified. Many of the involved glycosyltransferases are either embedded in the lipid bilayer as integral membrane proteins, or associated with the membrane surface by hydrophobic and/or electrostatic interactions. For instance, topology studies of the EmbCAB proteins, the putative arabinofuranosyltransferases revealed 13 transmembrane domains ***(Telenti et al., 1997)***. In addition, ***Alderwick et al. (2006b)*** recently discovered a further arabinofuranosyltransferase AfTA, which also contains integral transmembrane spanning domains. It is believed that glycosyltransferases involved in the biosynthesis of the arabinans of both AG and LAM are all integral membrane proteins and are therefore classified as members of the glycosyltransferase family C (GT-C) ***(Berg et al., 2007)***. With the exception of phosphatidylinositol mannosides (PIMs), which are constrained to Actinomycetales, the lipid composition of this biomembrane is similar to that of other prokaryotes, suggesting that the general properties of lipid bilayers are applicable for mycobacteria.

3. Structure and biosynthesis of peptidoglycan:

Peptidoglycan (PG) is a complex macromolecular structure situated on the outside of the plasma membrane of almost all eubacteria ***(Schleifer and Kandler, 1972; van Heijenoort, 2001a, 2001b)***. Its mesh-like arrangement confers rigidity to the cell, allowing it to withstand osmotic pressure maintaining cell integrity and cellular shape. Relatively little is known about M. tuberculosis PG synthesis, although it is generally assumed to be akin to that of E. coli ***(van Heijenoort, 2001a, 2001b)***, also being classified as A1γ according to the classification system of ***Schleifer and Kandler (1972)***.

Mycobacterial PG forms the backbone of the mAGP complex, composed of alternating N-acetylglucosamine (GlcNAc) and modified muramic acid (Mur) residues, linked in a β(1→4) configuration ***(Lederer et al., 1975)***. Unlike E. coli PG, the muramic acid residues in M. tuberculosis and M. smegmatis for instance, contain a mixture of N-acetyl and N-glycolyl derivatives, whereby the N-acetyl function has been oxidised to a N-glycolyl function to form MurNGly ***(Mahapatra et al., 2005a; Mahapatra et al., 2005b; Raymond et al., 2005)***.

These additional glycolyl containing residues result in extra hydrogen bonding, strengthening the mesh-like structure ***(Brennan and Nikaido, 1995)***, as well as possibly protecting the organism from lysozyme degradation. Tetrapeptide side chains consisting of L-alaninyl-D-isoglutaminyl-meso-diaminopimelyl-Dalanine ***(Petit et al., 1969)*** cross-link with identical short peptides of neighbouring glycan chains ***(van Heijenoort, 2007)***.

These cross-links include the expected meso-diaminopimelic acid (DAP) and D-alanine bond, common to most prokaryotes, but also a high degree of bonds between two residues of DAP ***(Ghuysen, 1968; Wietzerbin et al., 1974)***. The proportion of cross-linking in Mycobacterium species is 70-80%, significantly more so than E. coli, with only 50% ***(Vollmer and Holtje, 2004)***. An additional deviation from E. coli PG, is the use of the muramic acid residues as attachment sites for the galactan domain of the arabinogalactan, whereby carbon-6 of some of the muramic acid residues form a phosphodiester bond and are linked to the α-L-rhamnopyranose–(1→3)-α-D-GlcNAc(1→P) linker unit of AG ***(Hancock et al., 2002; McNeil et al., 1990)***.

4. Biosynthesis of the linker unit:

Mycobacterial viability rests heavily on the structural integrity of the cell wall, thus the attachment of which the AG proper is hinged to the PG layer is pivotal. ***Amar and Vilkas (1973)***, initially reported that AG is tethered to PG at intervals by a phosphodiester bond, supported by the presence of muramyl-6-phosphate in the cell wall preparations from several mycobacterial species ***(Kanetsuna, 1968; Liu and Gotschlich, 1967)***. The fundamental question of the chemical nature of this link wasn't answered until 20 years later when oligosaccharides containing galactofuranose (Galf) from the galactan domain were isolated along with rhamnose (Rha) residues ***(McNeil et al., 1990)***. The further discovery of the disaccharide L-Rhap-(1→3)-D-GlcNAc led to the conclusion that these constituents make up the linkage unit and the inference that the GlcNAc is directly attached to the 6-position of a proportion of the muramyl residues of PG ***(McNeil et al., 1990)***.

II. History:

While many may assume that infections by Mycobacterium tuberculosis (M. TB) and the associated disease is a recent phenomenon, the earliest known characterizations in

history actually date back to the 8000 BC, with bacilli having been isolated from mummies dating back to 700 BC ***(Herzog, 1998)***. Indeed, the symptoms of Tuberculosis (TB) have been described throughout history, and have been at least partially responsible for various plagues on multiple continents, although much of the recorded information revolves around the occurrences in Europe and the industrial United States ***(Daniel et al., 1994)***.

The person credited with first isolating and characterizing M. TB as the etiologic agent of TB is Dr. Robert Koch, who made the discovery in 1882 ***(Koch, 1882)***. Ultimately, Dr. Koch was awarded the Nobel Prize in Physiology in 1905 for his work, which laid the foundation for all future TB research.

During the time prior to the first use of antibiotics in the 1940s, there were few treatments and these produced varying levels of success. Pneumothorax4 and thoracoplasty procedures were performed on the lungs themselves in the attempt to improve function and breathing capacity for patients ***(Gaensler, 1982)***. However, the best overall treatment was to send the diseased to sanatoriums.