

INTRODUCTION

Tuberculosis (TB) is a serious global health problem, as about one third of the world's population has latent TB, which means people have been infected by TB bacteria but are not (yet) ill with disease and cannot transmit the disease. In 2011, 8.7 million people fell ill with TB and 1.4 million died from TB (*World Health Organization WHO, 2012*). Delay in diagnosis or in the start of the effective treatment results in poor prognosis and sequel in up to 25% of cases (*Haque, 2012*).

Egypt has intermediate level of incidence and mortality, the size of burden was a matter of concern, particularly in view of the fact that 74% of existed TB cases occur among socially and economically productive age groups of 15 to 54 years. The latest surveillance data in 2009 revealed that: a total of 18,000 tuberculosis cases existed in the country with a prevalence rate of 24/100,000 population, Estimated incidence rate of 19 and 8/100,000 for all forms and smear positive tuberculosis, respectively (*Hassanien, 2010*).

Analysis in patients with active TB showed anemia, leucocytosis, thrombocytosis, elevation in plasma fibrinogen, factor VIII, plasminogen activator inhibitor 1 (PAI-1) with depressed Antithrombin III (AT III) and protein C (PC) levels. Favorable therapeutic response may be identified by improvement of anemia, leucocytosis and thrombocytosis, decrease fibrinogen and factor VIII levels to normal levels and increase PC and AT III to normal levels (*Turken et al., 2002*).

These clinical reports emphasize that patients with severe pulmonary tuberculosis are at risk of developing thromboembolic events. Therefore, these complications should be investigated, especially in those who do not improve on therapy, who have other predisposing factors.

Deep vein thrombosis (DVT) is clinically observed in about 3–4% of patients with active TB. The real incidence is expected to be higher ($\geq 10\%$) because it is thought to be clinically unclear in some patients. It is possible that large amounts of interleukins (ILs) synthesized by the monocyte-macrophage system during active stage induce hepatic dysfunction and hemostatic abnormalities. Hemostatic changes (depressed AT III and PC levels) in pulmonary tuberculosis (PTB) may favour the development of hypercoagulable states (*Turken et al., 2002*).

Protein C (PC) is a vitamin K–dependent serine protease that is synthesized as a single polypeptide chain of 461 amino acids and is a natural anticoagulant protein. Whereas synthesis predominantly occurs in the liver, the PC has also been identified in the epididymus, kidney, lung, brain, and male reproductive tissue. The function of a PC as an anticoagulant is manifested by its ability to inactivate 2 important cofactors of the coagulation cascade, factor (F) V/Va and FVIII/VIIIa. These events are enhanced by the presence of Ca^{2+} , phospholipids, and cofactor protein S. Other functions of a PC in hemostasis are in keeping with its role of maintaining a fluid

state of blood. By virtue of the ability of a PC to downregulate thrombin, the activation of thrombin activatable fibrinolytic inhibitor (TAFI) is also suppressed, thus indirectly promoting fibrinolysis. Fibrinolysis is also stimulated by another activity of a PC, its ability to inhibit plasminogen activator inhibitor-1 (PAI-1). Both inherited and acquired; single and compound, and heterozygous or homozygous deficiencies of PC in humans have been described, and occur at numerous loci in the protein. Symptomatic heterozygous deficiencies can result in deep vein thrombosis and pulmonary embolism (*Danese et al., 2010*).

Antithrombin (AT, formerly AT-III) is a 58-kD glycoprotein that functions as a potent natural anticoagulant and is estimated to provide 80% of the inhibitory activity against thrombin (*Maclea and Tait, 2007*). It is synthesized in the liver and circulates in the plasma. Antithrombin III is a serine protease inhibitor (serpin) that inactivates many enzymes in the coagulation cascade, though thrombin and factor Xa are its primary targets (*Bauer, 2002; Roemisch et al., 2007*). In contrast to some direct thrombin inhibitors, which reversibly and transiently block thrombin activity, AT III inhibition of thrombin is irreversible (*Tanaka and Levy, 2007*).

AIM OF THE WORK

This work aims to assess the plasma level of protein C and Antithrombin III in patients with pulmonary tuberculosis (both active and latent) and correlate their levels with patients' coagulation state.

TUBERCULOSIS

I- DEFINITION:

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), which most commonly affects the lungs. It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease (*WHO, 2015*).

It is a common worldwide infection and a medical and social problem causing high mortality and morbidity, especially in developing countries (*Jeong and Lee, 2008*).

II- HISTORY:

Tuberculosis has a long history. It was present before the beginning of recorded history and has left its mark on human creativity, music, art, and literature; and has influenced the advance of biomedical sciences and healthcare. Its causative agent, *M. tuberculosis*, may have killed more persons than any other microbial pathogen. It was documented in Egypt, India, and China as early as 5000, 3300, and 2300 years ago, respectively (*Daniel, 2006*).

Typical skeletal abnormalities, including Pott's deformities, were found in Egyptian and Andean mummies and were also depicted in early Egyptian and pre-Colombian art (*Sotomayor et al., 2004*).

Pulmonary tuberculosis (PTB) in an Egyptian mummy was diagnosed and confirmed by polymerase chain reaction (PCR) and this was probably the first confirmed case of PTB using PCR in an ancient Egyptian mummy (*Nerlich et al., 1997*).

Only a few decades ago, TB was believed to be under control and decreasing in incidence, in both developed and developing countries. A number of scientists and physicians have contributed to the understanding of tuberculosis and have been honored on postage stamps by several countries around the world (*Shampo and Rosenow, 2009*).

III- ETIOLOGY:

There are five closely related mycobacteria in the *Mycobacterium tuberculosis* complex: *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, and *M. canneti*. All belong to the order of Actinomycetales and the family of Mycobacteriaceae. *M. tuberculosis* is the most important cause of TB in humans (*American Thoracic Society ATS, 2000*).

GENERAL CHARACTERISTICS:

The *M. tuberculosis* is aerobic, non-motile, and non-sporulated rod-shaped bacteria with two distinguishing characteristics: acid-fastness and slow growth (*Barrera, 2007*).

The rods are 2-4 µm in length and 0.2-0.5 µm in width. Therefore, the length of the microorganism is comparable to the

diameter of the nucleus of a lymphocyte. *M. tuberculosis* is an obligate aerobe, for this reason, in the classic case of TB, *M. tuberculosis* complexes are always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours, a physiological characteristic that may contribute to its virulence (Todar, 2008).

Unlike some fast growing mycobacteria and other actinomycetales, *M. tuberculosis* is rarely pleomorphic. It does not elongate into filaments, and does not branch in chains when observed in clinical specimens or culture. In the experimental macrophage infection, intracellular bacilli were described as being significantly elongated compared to broth-grown bacilli and, remarkably, to display bud-like structures (Chauhan et al., 2006).

IV- EPIDEMIOLOGY:

Although it is decreasing in incidence, TB remains one of the world's deadliest communicable diseases. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease. Of the estimated 9 million people who developed TB in 2013, more than half (56%) were in the South-East Asia and Western Pacific Regions.

A further one quarter was in the African Region, which also had the highest rates of cases and deaths relative to population. India and China alone accounted for 24% and 11%

of total cases, respectively. "About 60% of TB cases and deaths occur among men, but the burden of disease among women is also high. In 2013, an estimated 510,000 women died as a result of TB (*WHO, 2014*).

The association between poverty and TB is well recognized, and the highest rates of TB were found in the poorest section of the community (*Davis, 2000*).

Tuberculosis occurs more frequently among low-income people living in overcrowded areas and persons with little schooling. Poverty may result in poor nutrition which may be associated with alterations in immune function. On the other hand, poverty resulting in overcrowded living conditions, poor ventilation, and poor hygiene-habits is likely to increase the risk of transmission of TB (*Zaman, 2010*).

According to WHO, 2015:

- Tuberculosis is second only to Human immunodeficiency virus (HIV) as the greatest killer worldwide due to a single infectious agent.
- Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top 5 causes of death for women aged 15 to 44.

- In 2013, an estimated 550,000 children became ill with TB and 80,000 HIV-negative children died of TB.
- TB is a leading killer of HIV positive people causing one fourth of all HIV related deaths.
- Globally in 2013, an estimated 480 000 people developed multidrug resistant TB (MDR-TB).
- The estimated number of people falling ill with TB each year is declining, although very slowly, which means that the world is on track to achieve the Millennium Development Goal to reverse the spread of TB by 2015.
- The TB death rate dropped 45% between 1990 and 2013.
- An estimated 37 million lives were saved through TB diagnosis and treatment between 2000 and 2013.

In Egypt:

Incidence in 2013 was 13,000 cases and incidence rate was 16 per 100,000 populations. The notified new and relapse cases were 7876 cases (*WHO, 2014*).

V-MODE OF TRANSMISSION:

Tuberculosis is a disease that is almost exclusively transmitted by aerosolized droplets containing infectious *M. tuberculosis* (*Glickman and Jacobs, 2001*).

Droplet nuclei (1 to 5 mm in diameter that contain *M. tuberculosis* complex) are produced when persons with pulmonary or laryngeal tuberculosis cough, sneeze, speak, or sing. They also may be produced by aerosol treatments, sputum induction, aerosolization during bronchoscopy, and through manipulation of lesions or processing of tissue or secretions in the hospital or laboratory. Droplet nuclei, containing two to three *M. tuberculosis* organisms, are so small that air currents normally present in any indoor space can keep them airborne for long periods of time. Droplet nuclei are small enough to reach the alveoli within the lungs, where the organisms replicate.

A person with active but untreated TB infects approximately 10–15 other people per year (*Houben et al., 2006*).

Four factors determine the likelihood of transmission of *M. tuberculosis*:

- The number of organisms being expelled into the air.

- The concentration of organisms in the air determined by the volume of the space and its ventilation.
- The length of time an exposed person breathes the contaminated air.
- Presumably the immune status of the exposed individual. HIV infected persons and others with impaired cell mediated immunity are thought to be more likely to become infected with *M. tuberculosis* after exposure than persons with normal immunity

(ATS, 2000)

Risk Factors:

Anyone can catch TB but those at particular risk are those who have been exposed to TB bacteria, and those who are less able to fight latent infection. They include:

- Close contact of infectious cases.
- Those who have lived in/ travel to or receive visitors from places where TB is very common.
- Those who live in ethnic minority communities originating from places where TB is very common.
- Those with immune system weakened by HIV infection, immune suppressive therapy or other medical problems.

- The very young and the elderly, as their immune system are less robust.
- Those with chronic poor health (diabetes, end stage renal disease, cancer) and poor nutrition because of lifestyle problems such as homelessness, drug abuse or alcoholism.
- Those living in poor or crowded housing conditions, including those living hostels (**National Collaborating Centre for Chronic Conditions (NCC-CC), 2006**).
- Smoking more than 20 cigarettes a day increases the risk of TB by two to four times (*Davies et al., 2006*).
- Genetic predisposition:

Population-based studies have found an association between tuberculosis and some histocompatibility leucocyte antigens (HLA) alleles, as well as polymorphisms in the genes for natural resistance associated macrophage protein (NRAMP), the vitamin D receptor, and interleukin-1(IL-1) (*Bellamy, 2000*).

VI- PATHOGENESIS:

After inhalation, the droplet nucleus is carried down the bronchial tree and implants in a respiratory bronchiole or alveolus. Whether or not an inhaled tubercle bacillus establishes an infection in the lung depends on both the bacterial virulence and the inherent microbicidal ability of the alveolar macrophage that ingests it. If the bacillus is able to survive initial defenses, it can multiply within the alveolar

macrophage. The tubercle bacillus grows slowly, dividing approximately every 25 to 32 hour within the macrophage. *M. tuberculosis* has no known endotoxins or exotoxins; therefore, there is no immediate host response to infection. The organisms grow for 2 to 12 wk, until they reach 10^3 to 10^4 in number, which is sufficient to elicit a cellular immune response that can be detected by a reaction to the tuberculin skin test (TST).

Before the development of cellular immunity, tubercle bacilli spread via the lymphatics to the hilar lymph nodes and thence through the bloodstream to more distant sites (*ATS, 2000*).

Inhaled mycobacteria are phagocytized by alveolar macrophages, which interact with T lymphocytes, resulting in differentiation of macrophages into epithelioid histiocytes (Figure 1) (*Houben et al., 2006*).

Epithelioid histiocytes and lymphocyte aggregate into small clusters, resulting in granulomas. In the granuloma, CD4-T lymphocytes (effector T-cell) secrete cytokines, such as interferon- γ (IFN- γ), which activate macrophages to destroy the bacteria with which they are infected. CD8-T lymphocytes (cytotoxic T-cell) can also directly kill infected cells (*Kaufmann, 2002*).

For the majority of individuals with normal immune function, proliferation of *M. tuberculosis* is arrested once cell-

mediated immunity develops, even though small numbers of viable bacilli may remain within the granuloma. Although a primary complex can sometimes be seen on chest radiograph, the majority of PTB infections are clinically and radiographically inapparent. Most commonly, a positive TST result is the only indication that infection with *M. tuberculosis* has taken place. Individuals with latent tuberculosis infection but not active disease are not infectious and thus cannot transmit the organism. It is estimated that approximately 10% of individuals who acquire tuberculosis infection and are not given preventive therapy will develop active tuberculosis. The risk is highest in the first 2 years after infection, when half the cases will occur (ATS, 2000).

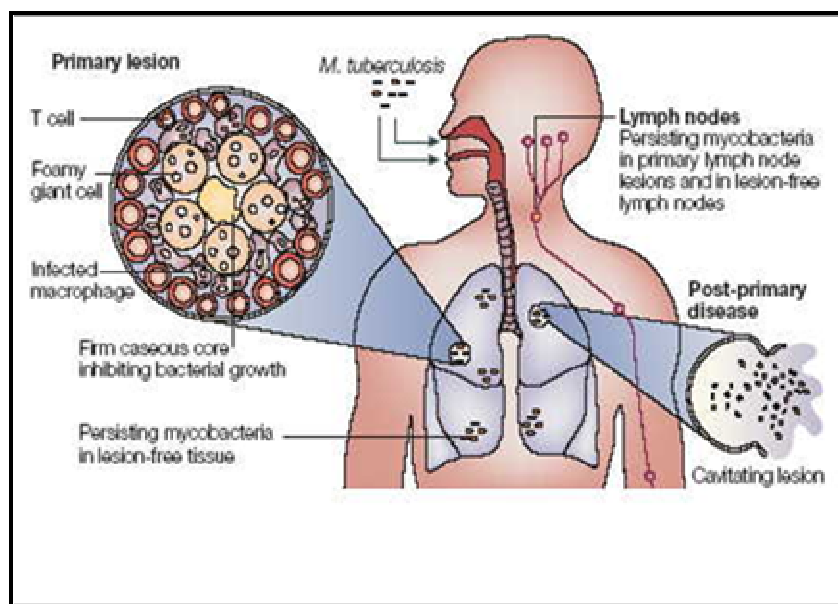


Fig. (1): Pathogenesis of tuberculosis (Houben et al., 2006).

- **Pathogenesis of Hypercoagulable State in TB:**

A number of studies have reported the occurrence of thrombotic complications in TB patients, particularly disseminated intravascular coagulation (DIC) and deep vein thrombosis (DVT) (*El Fekih et al., 2009*).

However, it is unclear how tuberculosis infection causes thrombotic complications in some patients as mycobacteria are not known to produce endotoxins or exotoxins that are known to initiate the clotting cascade (*Kothari et al., 2012*). Several experimental studies have shown that proinflammatory cytokines (IL-1, IL-6, tumor necrosis factor- α (TNF- α)) are produced during the acute phase of *M.tuberculosis* infection, either by peripheral blood mononuclear cells, or by lung, lymph node, and spleen macrophages interacting with mycobacterial products. These cytokines induce the production of various acute inflammation phase proteins and coagulation factors by hepatocytes, resulting in a hypercoagulable state (*Dentan et al., 2014*).

It has also been shown that, in vitro, *M. tuberculosis* induces the expression of a tissue factor (TF) in monocytes-macrophages, that is, a primary activator of the clotting cascade (*Kothari et al., 2012*).

Coagulation and inflammation share a bidirectional relation playing crucial roles in host defense. Proinflammatory cytokines produced in infections can induce TF expression on monocytes and endothelial cells.