Tuberculosis Susceptibility with Interleukin-10 and Interferon Gamma Genes

Thesis

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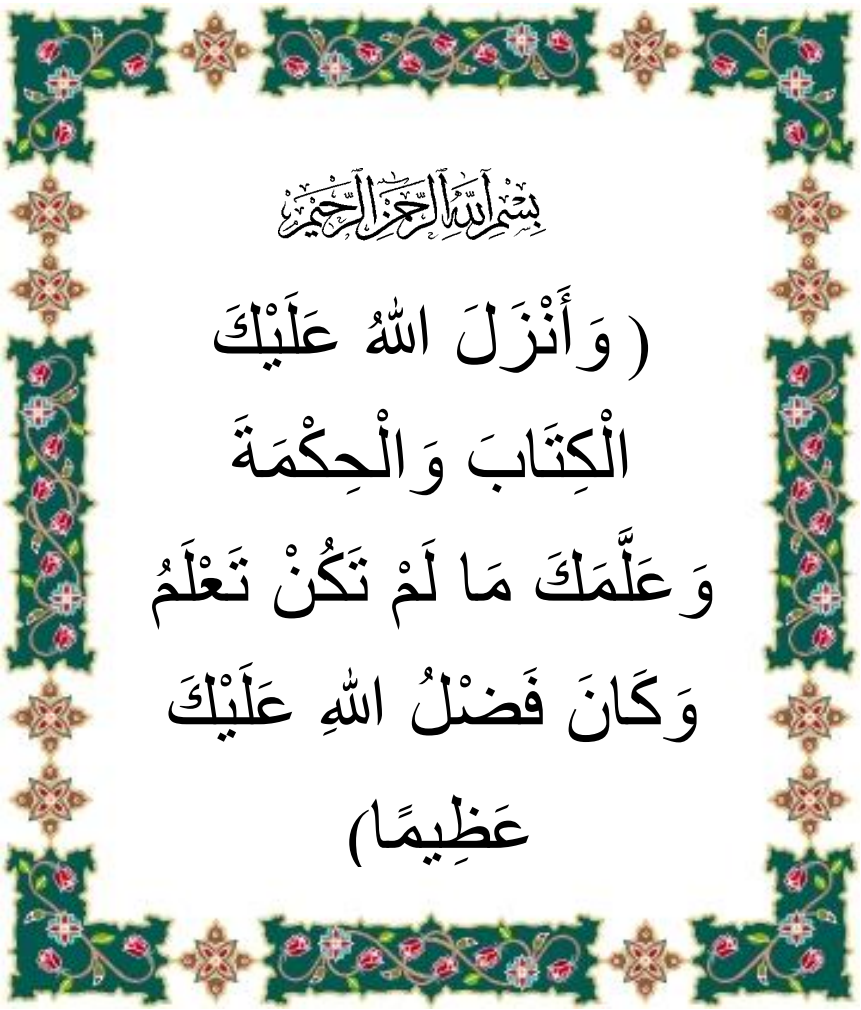
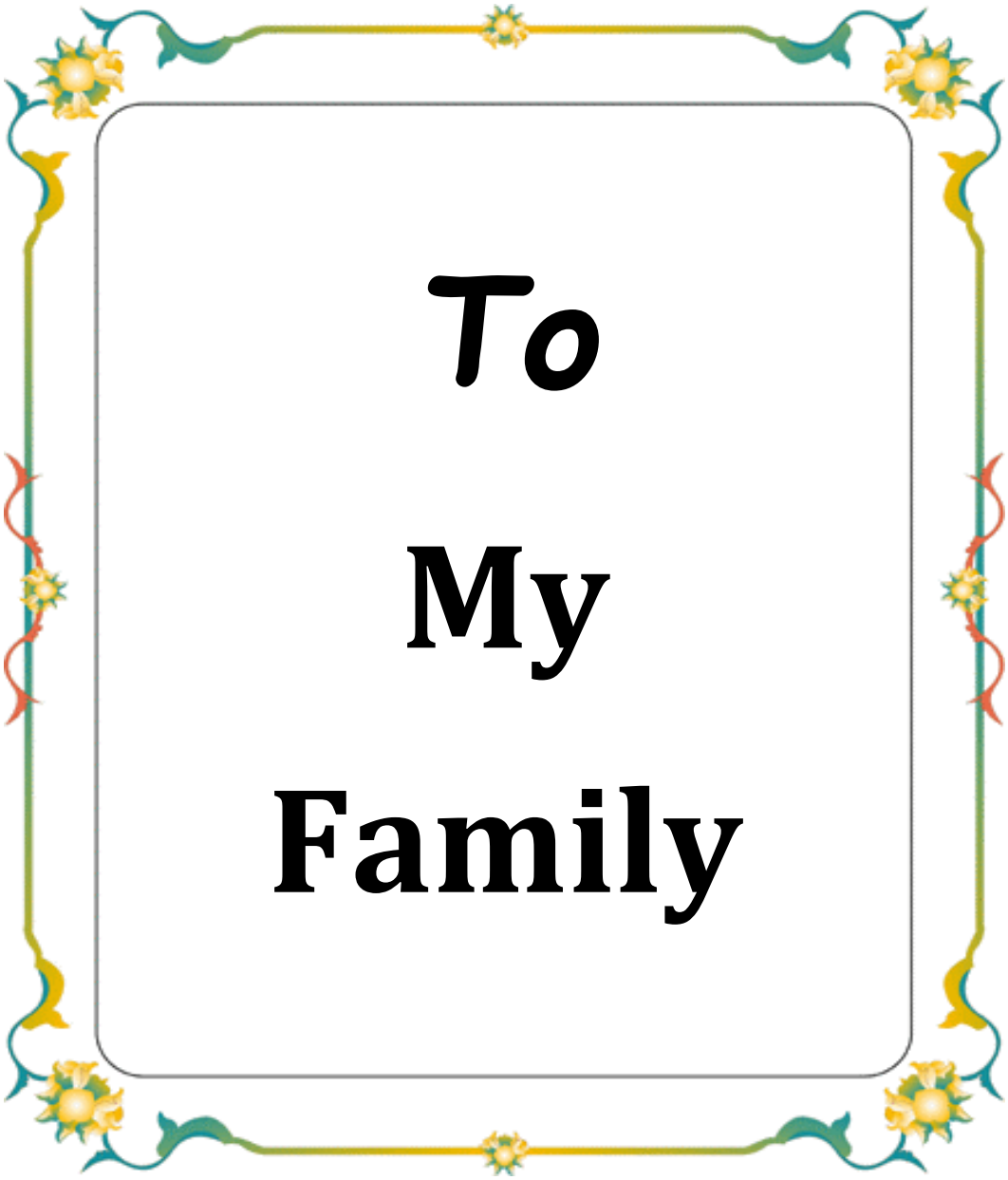

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
(وَأَنْزَلَ اللهُ عَلَيْكَ
الْكِتَابَ وَالْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ
وَكَانَ فَضْلُ اللهِ عَلَيْكَ
عَظِيمًا)


To
My
Family

Acknowledgment

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I must stress on my profound gratitude and honor on working under the supervision of **Prof./ Khaled Hussein Taman**, *Professor of Medical Childhood Studies Department, Dean of Institute of Childhood Studies. Ain Shams University. Special thanks for his gracious, precise Supervision aim at achieving better scientific levels, generous help and continuous encouragement throughout the whole research. I owe him more than express.*

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Manal Kishk

ABSTRACT

Tuberculosis is a high burden disease, especially in developing countries. According to the WHO, in 2006, there were 9.2 million new TB cases, 86% being in Asia and Africa. The reported percentage of TB cases occurring in children varies from 3% to more than 25%. It is now clear that susceptibility to virulent intracellular pathogens, like TB, is influenced by host genetic background as well, and it is supposed that efficient activation of cellular immune response specifically IFN-γ may play a key role in protection and control of TB infection. IL-10 is a multifunctional cytokine first described as cytokine synthesis inhibitory factor, which inhibits IFN-γ cytokine production. **Accordingly:** This study was designed to assess the relationship between IFN-γ and IL-10 gene polymorphism and susceptibility to TB with special emphasizes on their possible role in affecting clinical status. **Subjects and Methods:** This study was conducted on 60 children, 40 tuberculous patients and 20 healthy controls with matched age and sex. Molecular identification of IL-10 and IFN-γ genes polymorphism was carried out using Allelic discrimination by real time PCR. **Results:** This study revealed that G allele frequency of IL-10 (A-1082G) was higher in TB patients than of the controls with lower frequency of AA genotypes in patients group than of controls, also A allele frequency was higher in TB controls than those of patients. Mean while, IFN-γ (T+874A) AA genotype was higher in patients group than controls. On the other hand, TT genotype frequency was lower in patients group than controls. Moreover, significant association was observed in the frequencies of IL-10 (A-1082G) genotypes in IFN-γ (T+874A). Highly significant association was found between IL-10 (A-1082G) and IFN-γ (T+874A) expression in patients and controls. **Conclusion:** IL-10 (A-1082G) and IFN-γ (T+874A) genes polymorphism might be crucial for protective immuneresponses and may serve as biomarkers of protection or susceptibility of TB.

Introduction

Tuberculosis (TB) is a world wide pandemic and constitutes a major health concern and had been considered a global crisis. TB is disease of poverty affecting adults in their most productive years. It is a contagious disease, spreads through the air. Person with active TB infects on average 10 to 15 people every year. Despite progress in detection and therapy of TB, emergence of multidrug resistance (MDR-TB) is considered a major public health challenge. 450,000 new MDR-TB cases are estimated to occur every year ***(WHO, 2007).***

About 9 million people develop TB annually, of whom 2 million die. The reported percentage of TB cases occurring in children varies from 3% to more than 25% ***(WHO, 2006).*** In 2007, WHO report 2007, Global TB. control, estimates the prevalence rate of TB in Egypt was 32/100.000, TB, mortality rate was 3/100,000 and case detection rate 60% which still below WHO requirement.

At present it is impossible to predict in whom the disease' will develop and progress through several stages from mild to sever. ***Hill et al. (2001) and Casanova (2002) and Oral et al. (2006)***, reported that host genetic factors play significant roles in susceptibility to TB. Therefore, the identification of host genes responsible for susceptibility

and resistance to TB should provide a significant contribution for understanding of the pathogenesis at molecular level and may open the possibility for a new strategies of therapeutic intervention and prophylaxis ***(Bellamy et al., 2003 and Selvaraj et al., 2008).***

Cytokines produced at the site of disease after interactions between T-lymphocytes and infected macrophages are essential for the pathogenesis of TB, ***(Sher et al., 1992 and Henao et al., 2005).*** The course of TB infection is regulated by two distinct T cell cytokine patterns. T helper 1 (Th1) cytokines, Interleukin-2 (IL-2) and Interferon-gamma (INF-γ), are associated with resistance to infection, whereas Th2 cytokines, interleukin-4 (IL-4) and interleukin-13 (IL-13), are associated with progressive disease ***(Daniel et al., 1996).*** In addition, lnterleukine-10 (IL-10), one of the T regulatory cytokines, seem to play a pivotal role during the chronic/latent stage of pulmonary TB, with increased production playing a potentially central role in promoting reactivation of TB. ***(Akdis et al., 2000).*** Furthermore, ***TSO et al. (2005) and Yang et al. (2007)*** stated that individuals with low IFN-γ producing genotypes had higher risk in developing TB.

Of fundamental immunologic importance that influence the nature of cytokine response, such as polymorphisms of cytokine genes. Polymorphisms in several cytokine genes have been described and

demonstrated to influence gene transcription, leading to interindividual variations cytokine production ***(Bid-Well et al., 2001 and Ding et al., 2008).*** Cytokine gene polymorphisms have been shown to be involved in the susceptibility, severity and clinical outcome of several Diseases including infectious one ***(Haukim et al., 2002 and Pacheco et al., 2008)***.

Aim of the Work

This study was designed to assess relation ship between gene polymorphism for interferon gamma (IFN-γ) and lnterleukin-10 (IL-10) and susceptibility to tuberculosis with special emphasizes on their possible role in affecting clinical status.

Review of Literature

History of tuberculosis:

TB is an ancient disease that probably first appeared more than million years ago in soil and water and gradually adapted to animal hosts ***(Hass et al., 1995)***.

TB was documented in Egypt, India, and China as early as 5.000, 3.300, and 2.300 years ago, respectively. Typical skeletal abnormalities, including Pott's deformities, were found in Egyptian and Indian mummies and were also depicted in early Egyptian and pre-colombina art ***(Daniel, 2006)***.

The World Health Organization (WHO) has designated March 24th of each years as "World TB day" to mark the anniversary of 'Robert Koch's discovery in 1882 of the causes of TB ***(Cohn et al., 1997)***.

Epidemiology:

Global burden of tuberculosis:

TB remains one of the deadliest diseases in the world WHO estimates that nearly one-third of the global population, i.e. two billion people, is infected with Mycobacterium tuberculosis (M.TB) and at risk of developing the disease ***(WHO, 2006a)***.

TB accounts for 2.5% of the global burden of disease and is the commonest cause of death in young women. TB currently holds the seventh place in the global ranking of causes of death ***(Smith, 2004 and Dye, 2006)***.

Effective drugs to treat and cure the disease have been available for more than 50 years, yet every 15 seconds, someone in the world dies from TB. Even more alarming: a person is newly infected with TB every second of every day. Left untreated, a person with active TB will infect an average of 10 to 15 other people every year ***(Dye, 2005)***.

TB hinders socioeconomic development: 75% of people with TB are within the economically productive age group of 15-54 years. 95% of all cases and 99% of deaths occur in developing countries ***(Dye, 2006 and WHO, 2006a).***

Tuberculosis in Egypt:

In terms of incidence of TB. Egypt is ranked among the mid-level incidence countries. With estimated incidence 25-29 per 100.000 population. TB in Egypt is considered an important public health problem. The table below shows the recent data (as of 17 March 2008) according to the reports of WHO ***(WHO, 2008)***.

Table (1): Tuberculosis in Egypt

	2006
DOTS population coverage (%) → total	100
New and relapse cases → total	10.046
New and relapse cases (per 100.000 population) → total	14
New smear-positive cases (per 100.000 population) → total	6
New smear-positive cases → total	4.745
Whole country new smear-positive cases detection rate (%) → total	59
DOTS all new cases detection rate (%) → total	59
DOTS new smear-positive case detection rate (%) → total	59

(World Health Organization, 2008)

Tuberculosis in children:

It has been estimated that 3.1 million children under 15 years of age are infected with TB worldwide. According to the WHO, children with TB represent 10% of all TB cases ***(Dye, 1999 and WHO, 2006)***.

TB equally affects children of both genders, but an increased risk of mortality exists at the extremes of age. Therefore, young children and specially newborns are at high risk when they are exposed to a contagious source ***(Dye, 1999)***.

Children at high risk of tuberculosis infection:

Factors that may increase the risk of TB in children are poverty, with resultant poor nutrition, overcrowded

living conditions, and inadequate medical care children exposed to adults with increased risk of TB infection are also at increased risk of infection. The more prolonged and closer the exposure to the infectious case, the greater the risk of infection ***(Rieder, 2002)***.

Tuberculosis and (HIV):

HIV and TB form a lethal combination, each speeding the other's progress. HIV infection is a potent risk factor for TB. Not only does HIV increase the risk of reactivating latent TB infection, it also increases the risk of rapid TB progression soon after infection or reinfection. Persons infected by tubercle bacilli have about 10% chance of developing TB during the remainder of their lives, thus have a less than 0.5% chance of developing overt disease annually. By contrast, an HIV positive person already infected by the tubercle bacillus has an 8% chance of developing overt disease annually, or up to 50% during the remainder of their relatively short life span ***(Corbette, 2003; Aaron, 2004 and WHO, 2006b)***.

Etiology of T.B.:

Description and nature of the agent:

Genus mycobacterium is slender, rod shaped bacteria of the actinomycete family ***(Tanya and Stoker, 1998)***. The

genetic name mycobacterium (fungus bacterium) was proposed by ***Bahaman and Neuman (1869)***.

The most characteristic feature is their complex cell envelope containing high percentage of lipids which include the large branched mycolic acids, this envelope makes the bacteria resistant to leakage and relatively impermeable to antibiotics and is responsible for the acid fast staining properties used to identify the organisms ***(Clark-Curtiss, 1990)***.

TB of man and animals is caused by group of very closely related species forming the M.TB complex; these are:

1. M.Tuberculosis, the human tubercle bacillus.
2. M.bovis, the bovine tubercle bacillus.
3. M.africanum.

Features of the organism:

The characteristic features of the tubercle bacillus include:

(1) Its slow growth, (2) dormancy, (3), complex cell envelope (4) intracellular pathogenesis and (5) genetic homogencity ***(Wheeler and Ratledge, 1994)***. The generation time of M.TB in synthetic medium or infected animals is typically more than 24 hours. This contributes to the nature of the disease, imposes