

Investigating Healthy Egyptian Infants for BCG vaccine Induced Immunoprotection against TB

Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

لسبب انك لا تعلم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

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List of Abbreviations

APCs	Antigen-presenting cells
ART	Anti-retroviral therapy
BCG	Bacille Calmette–Guérin
CBC	Complete blood count
CD	Cluster of differentiation
CFP-10	Culture filtrate protein -10
CMF-PBS	Calcium and Magnesium Free Phosphate Buffered Saline
DCs	Dendritic cells
DTH	delayed type hypersensitivity
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immuno assay
ELISPOT	Enzyme-linked immunospot assay
EPI	Expanded Programme of Immunization
ESAT-6	Early secretory antigenic target-6
FACS	Fluorescence-activated cell sorter
FBS	Fetal bovine serum
GAVI	Global Alliance for Vaccines and Immunization
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
ID	intradermally
IFN- γ	Interferon gamma
IL	Interleukin
INH	Isoniazid
IQR	Inter quartile ranges
MDR	Multidrug resistance
MNC	Mononuclear Cell
MOH	Ministry of health
MP	multiple percutaneous puncture
Mtb	Mycobacterium tuberculosis
NHP	Non-human primate
NK	Natural Killer
NO	Nitric oxide
NTP	Natural TB control program
OD	Optical density
<i>p</i>	Probability
PBMC	peripheral blood mononuclear cells
PBS	Phosphate-Buffered Saline

PHA	Phyto-hem-agglutinin
PPD	Purified protein derivatives
RNI	Reactive nitrogen intermediates
ROI	Reactive oxygen intermediates
RT-PCR	Reverse transcription-polymerase chain reaction
SCID	Severe combined immunodeficient
SD	Standard deviation
TB	Tuberculosis
TCR	T cell receptors
Th	T helper
Th1	T helper-1
TNF	Tumor necrosis factor
TST	Tuberculin skin test
TU	Tuberculin units
$\gamma\delta$ cells	Gamma delta cells

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Introduction

Tuberculosis (TB) is considered to be responsible for 2 million deaths every year despite being a treatable airborne infectious disease. "Consumption" and "Phthisis" were terms historically used to describe TB, known also as the white plague, which was responsible for one in four deaths in the 19th century. Due to its infectious nature, chronic progression and long treatment, TB is a great burden for society. Moreover the emergence of multi-drug resistant TB and the current associated human immunodeficiency virus (HIV) epidemic has led to even greater concern. Treating and preventing TB have become a permanent challenge since the ancient times. Bacille Calmette-Guérin (BCG) is the only vaccine available today in practice and has been used for more than 90 years with wide safety range records. However, its efficacy remains controversial. No universal BCG vaccination policy exists, with some countries merely recommending its use and others that have implemented BCG vaccination in routine immunization programs (*Luca and Mihaescu, 2013*).

Over the past 50 years, the *Mycobacterium bovis* BCG vaccine against tuberculosis has maintained its position as the world's most widely used vaccine, despite showing

highly variable efficacy (0-80%) in different trials (*Andersen and Doherty, 2005*). The vast majority of BCG vaccinations given annually worldwide are from 4 main strains: Glaxo strain 1077, Danish strain 1331, French Pasteur strain 1173 P2 and the Tokyo strain 172 (*WHO/Supplementary information on vaccine safety, 2004*). The different BCG strains have been shown to differ in the percentage of viable bacteria at the time of vaccination, ability of the bacteria to survive in the host, and production of antigens. These differences hypothetically could make a difference to the efficacy of an individual vaccine strain compared to another (*Behr, 2002*).

In Egypt, TB is addressed and handled as a health problem affecting large sectors in the society, especially the poor and the vulnerable. In 2013, prevalence rate of TB was 27 per 100 000 populations (*WHO/Tuberculosis country profile, 2015*).

Aim of Work

This study was designed to investigate the rate of immune- protection against TB among a group of healthy BCG vaccinated infants in order to assess the efficacy of protection induced by the currently used vaccine among this age group in our country to evaluate the efficacy of this preventive measure.

Review of literature

Mycobacterial tuberculosis

Tuberculosis ranks as the second leading cause of death from a single infectious agent, after the human immunodeficiency virus (HIV). In 2014, there were an estimated 9.6 million new TB cases: 5.4 million among men, 3.2 million among women and 1.0 million among children. There were also 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people), of which approximately 890 000 were men, 480 000 were women and 140 000 were children (*WHO/Global tuberculosis report, 2015*).

TB is strongly associated with poverty, 95% of all TB deaths occurring in the developing world, and the disease mostly affects young adults in the prime of their lives with consequent high socio-economic impact (*WHO, 2011*).

Tuberculosis is an infectious disease primarily affecting the lungs, which in the majority of cases is caused in humans by infection with the intracellular bacterial agent, *Mycobacterium tuberculosis* (Mtb) (*North and Jung, 2004*). The majority of individuals infected with Mtb

appear to adequately control infection, this termed latent TB; which is defined as previous exposure of an individual to Mtb (determined by evidence of immunological sensitization to Mtb antigens) but with no demonstration of TB symptoms. It is estimated that one in ten people infected with Mtb will however develop the active disease during their lifetime, likely due to failure or breakdown of the appropriate host immune response, or predisposing factors including host genetics, immunosuppression and diabetes (*Nunn et al, 2005*).

Latent Mtb infection also poses iatrogenic clinical challenges, due to increases in TB reactivation following administration of biologicals, such as tumor necrosis factor-alpha (TNF α), interleukins(IL)-12, IL-23 blockers in treating inflammatory diseases like rheumatoid arthritis, Crohn's disease and psoriasis (*Ottenhoff and Kaufmann, 2012*).

Tuberculosis can be treated with chemotherapy. However, the treatment is lengthy, requiring a combination of at least four drugs for six months. This long drawn out and complicated treatment schedule often affects compliance, thus resulting in development of resistant strains. In several countries, single resistant strains are

responsible for more than 25% of all new cases of tuberculosis. Although single resistance does not complicate drug treatment significantly, once a Mtb. strain has developed resistance to one drug, development of multidrug resistance (MDR) is greatly increased (*Kaufmann, 2004*).

In 2010, the estimated number of prevalent TB cases in the WHO Eastern-Mediterranean Region was 1,000,000 (670,000–1 500,000). The estimated number of incident TB cases in 2010 was 650 000 (580,000–730,000), accounting for 7% of the global TB burden. Nine countries contribute 95% of the TB burden in the Region in 2010. These are Pakistan, Afghanistan, Sudan, Morocco, Somalia, Iraq, Egypt, Islamic Republic of Iran and Yemen (*WHO/ Epidemiological situation, 2011*).

In Egypt, TB is considered the third most important public health problem after schistosomiasis and hepatitis C (*Saad-Hussein and Mohammed, 2014*).

Incidence of tuberculosis in Egypt 2013 was 16 per 100,000; incidence of tuberculosis is the estimated number of new and relapse tuberculosis cases arising in a given year, expressed as the rate per 100,000 populations, and all

forms of TB are included (table 1) (*WHO/ Tuberculosis country profile, 2015*).

Table1: Epidemiological situation of TB burden in EGYPT (2013) (*WHO/ Tuberculosis country profile, 2015*).

Estimates of TB burden * 2013	Number (thousands)	Rate (per 100 000 population)
Mortality (excludes HIV+TB)	0.55 (0.49–0.62)	0.67 (0.6–0.75)
Mortality (HIV+TB only)	0.034 (0.013–0.064)	0.04 (0.02–0.08)
Prevalence (includes HIV+TB)	22 (12–36)	27 (14–44)
Incidence (includes HIV+TB)	13 (12–15)	16 (15–18)
Incidence (HIV+TB only)	0.097 (0.017–0.18)	0.12 (0.02–0.22)
Case detection, all forms (%)	59 (53–65)	

Numbers are presented as mean and range between the brackets.

➤ ***Causative organism***

TB is an airborne disease caused by the bacterium *Mtb* (Figure 1). *Mtb* and seven very closely related mycobacterial species (*M. bovis*, *M. africanum*, *M. microti*, *M. caprae*, *M. pinnipedii*, *M. canetti* and *M. mungi*) together comprise what is known as the *M. tuberculosis* complex. Most, but not all, of these species have been found to cause disease in humans. *Mtb* is carried in airborne particles, called droplet nuclei, of 1–5 microns in