Introduction

BCG vaccines derived from the original Calmette-Guerin culture of M.bovis. BCG vaccination is administered in infancy in most countries with the aim of providing protection against mycobacterial infections such as tuberculosis and leprosy ***(WHO, 2007).***

There has been debate about possible non- specific effects of BCG vaccination. Observational studies from low income countries (Guinea –Bissau, Bangladesh, Papua New Guinea and Malawi) have indicated that BCG may have a beneficial effect on all children mortality ***(Breiman et al., 2004 & Roth et al., 2005).***

Since early decades of BCG vaccine use, a positive tuberculin test was used as a marker of vaccine "take". Subsequent animal and human studies have shown that tuberculin reactivity after BCG vaccination is highly variable. The type of BCG vaccine, the number of doses of vaccine, time lapsed since BCG vaccination, age at BG vaccination, genetics of the host, exposure to NTM, and exposure to tuberculin can all influence tuberculin reactivity after BCG vaccination ***(Wilson, 2000).***

Although delayed –type hypersensitivity (DHT) and protection against TB may emerge in parallel after BCG vaccination or after infection with Mycobacterium Tuberculosis, they reflect different parts of the immune response. Cell mediated immunity and DTH are both immunologic processes initiated by T lymphocytes; however the specific cells and types of cytokines involved and pathological consequences differ: Cell mediated immunity produces local activation of macrophages in tissue; while DTH is associated with tissue damage, including caseation, at sites of tissue infection ***(Wilson, 2000)***.

In the last five years there has been renewed interest in the biological effects of Vitamin D on tuberculosis due to the growing evidence of the immunomodulatory properties of Vitamin D. Vitamin D alone has no direct anti-mycobacterial action, but its active metabolite 1 α25 (OH) D modulates the host response to M. tuberculosis infection ***(Martineau et al., 2007).***

Vitamin D has also been shown to have an immune-regulatory role in vitro. It has been shown to inhibit differentiation and maturation of dendritic cells and to act on T lymphocytes to inhibit T cell activation and proliferation resulting in altered cytokine expression. It has recently been shown to be important in controlling T cell receptor signaling ***(Van Etten et al., 2008)***.

Vitamin D deficiency is now recognized as widespread and is more common in TB patients than controls ***(Wejse et al., 2007)***. Patients with tuberculosis have, on average, lower serum concentrations of 25 (OH) D than healthy controls ***(Friis et al., 2008)***.

Aim of The Work

This cross sectional study objective is to determine if there was an association between serum 25 (OH) D concentrations and post – BCG vaccination status tested by tuberculin test (Mantoux) in infants.

Tuberculosis

Introduction and history:

Tuberculosis has claimed its victims throughout much of known human history. It reached epidemic proportions in Europe and North America during the 18th and 19th centuries, earning the sobriquet, "Captain Among these Men of Death." Then it began to decline. Understanding of the pathogenesis of tuberculosis began with the work of Théophile Laennec at the beginning of the 19th century and was further advanced by the demonstration of the transmissibility of ***Mycobacterium tuberculosis*** infection by Jean-Antoine Villemin in 1865 and the identification of the tubercle bacillus as the etiologic agent by Robert Koch in 1882 ***(Daniel, 2006).***

Clemens von Pirquet developed the tuberculin skin test in 1907 and 3 years later used it to demonstrate latent tuberculous infection in asymptomatic children. In the late 19th and early 20th centuries sanatoria developed for the treatment of patients with tuberculosis. The rest provided there was supplemented with pulmonary collapse procedures designed to rest infected parts of lungs and to close cavities. BCG vaccination was widely employed following World War I. The modern era of tuberculosis treatment and control was heralded by the discovery of streptomycin in 1944 and isoniazid in 1952 ***(Daniel, 2006).***

The rising of the HIV epidemic at the beginning of the 1980s has refueled tuberculosis spread in Africa and Asia and contributed to the expansion of drug-resistant tuberculosis worldwide making the development of new drugs and drug regimens for ambulatory treatment a top priority. The field of immunological diagnosis of TB infection, dominated since the early 1900s by the intradermal tuberculin reaction has been put back in motion by the discovery of M. tuberculosis-specific

proteins and peptides, now employed in blood tests of high sensitivity and specificity for the diagnosis of latent TB which may help with the identification of contacts at higher risk of active disease and the eradication of epidemic cases ***(Saltini, 2006).***

It is clear that the existing strategies of mass immunization with BCG, effective case detection and combination chemotherapy are insufficient to control the TB epidemic. The Stop TB Strategy, introduced in 2006, is broader in approach, with the express targets of halting and reversing TB incidence by 2015 and halving TB prevalence and deaths by 2015 compared with 1990 ***(Dye and Williams, 2010).***

Aetiology:

Tuberculosis is an infection caused by the rod-shaped, non–spore-forming, aerobic bacterium Mycobacterium tuberculosis ***(Porth, 2002).***

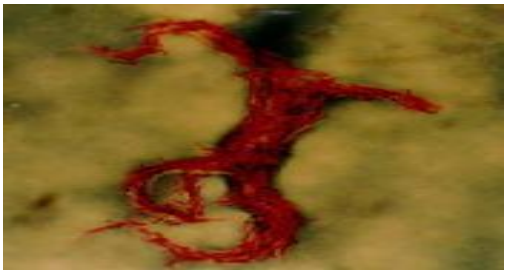

Fig (1) : Acid-fast stain of mycobacteria *(Costello et al., 1996).*

The tubercle bacilli are non-spore forming, non-motile, pleomorphic, weakly gram-positive curved rods 2-4 μm long. They may appear beaded or clumped in stained clinical specimens or culture media. They are obligate aerobes that grow in synthetic media containing glycerol as the carbon source (e.g. Loewenstein Jensen culture media). These mycobacteria grow best at 37- 41°c, produce niacin, and lack

pigmentation. A lipid-rich cell wall accounts for resistance to the bactericidal actions of antibody and complement. A hallmark of all mycobacteria is acid fastness (the capacity to form stable mycolate complexes with arylmethane dyes such as crystal violet, carbofuchsin, auramine, and rhodamine). Once stained they resist decoloration with ethanol and hydrochloric or other acids ***(Starke, 2001).***

Mycobacteria typically measure 0.5 μm by 3 μm, are classified as acid-fast bacilli, and have a unique cell wall structure crucial to their survival. The well-developed cell wall contains a considerable amount of a fatty acid, mycolic acid, covalently attached to the underlying peptidoglycan-bound polysaccharide arabinogalactan, providing an extraordinary lipid barrier. This barrier is responsible for many of the medically challenging physiological characteristics of tuberculosis, including resistance to antibiotics and host defense mechanisms. The composition and quantity of the cell wall components affect the bacteria's virulence and growth rate ***(Lee et al., 2005).***

The peptidoglycan polymer confers cell wall rigidity and is just external to the bacterial cell membrane, another contributor to the permeability barrier of mycobacteria. Another important component of the cell wall is lipoarabino-mannan, a carbohydrate structural antigen on the outside of the organism that is immunogenic and facilitates the survival of mycobacteria within macrophages ***(Knechel, 2009).***

The cell wall is key to the survival of mycobacteria, and a more complete understanding of the biosynthetic pathways and gene functions and the development of antibiotics to prevent formation of the cell wall are areas of great interest ***(Knechel, 2009).***

Epidemiology:

Incidence:

Mycobacterium tuberculosis is one of the most successful bacterial parasites of humans, infecting over one-third of the population of the world as latent infection without clinical manifestations. Over 8.8 million new cases and nearly 2 million deaths by tuberculosis (TB) occur annually. TB poses a significant health threat to the world population. The goal of this symposium is to open new avenues for combating tuberculosis ***(Okada and Kobayashi, 2007).***

The majority of cases occurred in 22 high burden countries, where a combination of high transmission rates and a large proportion of the population under the age of 15 years mean children account for up to 25-40% of cases, with incidence rates for paediatric TB ranging from 60-600 per 100, 000 per year. Rates of childhood TB have also been reported in Eastern Europe in the wake of the explosive TB epidemic which followed the break up of the Soviet Union ***(Walls and Shingadia, 2007).***

Prevalence :

Until recently under the WHO Directly Observed Treatment, Short Course(DOTS) strategy only smear positive cases have been reported for children, yet smears are seldom performed in many high burden settings and most disease in children is smear negative. Disease burden estimates, derived using estimates of the proportion of cases that are smear positive by age, suggest that children accounted for nearly 900, 000 (11 %) cases globally in 2000***(Nelson and Wells, 2004).***

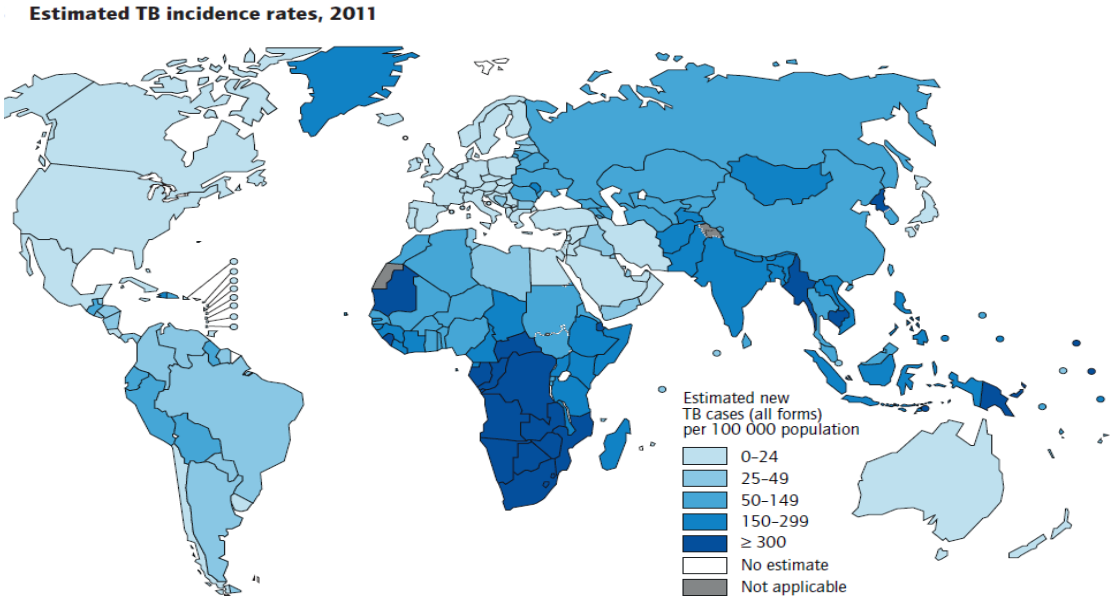

Fig (2) :Estimated incidence and distribution of TB in the world, 2011 *(WHO, 2012).*

Tuberculosis in Egypt:

TB remains a great public health concern in Egypt; however, little information on the prevailing genotypes of the tubercle bacilli and their spread is available. In a country where TB is endemic, it is critical to identify the genotypes of the predominant strains in order to study the transmission patterns and the epidemiological features of the disease. Cooksy et al study is a first attempt to provide insight into the population structure of *M. tuberculosis* in Egypt ***(Cooksey et al., 2002).***

Race / Ethnicity Distribution:

Minority population have been disproportionally burdened with tuberculosis. Socio-economic status is probably the most important factor explaining the difference in white and non-white population ***(Fishman, 2002).***

Based on 1990 CDC data, case rates were 10 times higher for Asians and Pacific Islanders; 8 times higher for non-Hispanic blacks; and 5 times higher for Hispanics, Native Americans, and Native Alaskans, as compared to non-Hispanic whites. However, race may not be an independent risk factor; Risk is best defined on basis of social, economic, and medical factors ***(Khan et al., 2002).***

Sex:

Among adults, two thirds of cases occur in males, but there is no significant difference in gender in childhood ***(Munoz and Starke, 2004).***

Age:

In white populations in the United States, tuberculosis rates are highest among the elderly who acquired infection decades ago. In contrast, among non-white populations, tuberculosis is most common in young adults and children < 5 years of age. The age range of 5-14 years is often called the " favored age " because in all human populations this group has the lowest rate of TB disease ***(Munoz and Starke, 2004).***

In elderly, most tuberculosis is not a result of newly acquired infection but rather of reactivation of latent infection acquired earlier in life while tuberculosis in infants and children serves as sentinel event of recent infection, primary tuberculosis may develop as many as 60% of untreated infected infants ***(Mandell et al., 2000 &Fishman, 2002).***

Most children infected with *M.tuberculosis* in their home by someone close to them, but outbreaks of childhood tuberculosis also occur in elementary and high schools, nursery schools, day-care centers, school buses, and sports teams ***(Munoz and Starke, 2004).***

HIV-infected adults with tuberculosis can transmit *M.tuberculosis* to children and children with HIV infection are at increased risk of developing tuberculosis ***(Mukadi et al., 1997 & Hopwell and Chaisson, 2000).***

Environmental and Socio- economic:

Social factors, community TB prevalence and age determine where exposure is most likely to occur and may vary between communities ***(Nelson and Wells, 2004).***

A household source is most commonly implicated for young children; older children are increasingly likely to be infected outside the household. Poverty, poor housing, urban environments and overcrowding are all associated with increased transmission ***(Nakaoka et al., 2006).***

Nutrition:

Several observational studies from adults and children show an association between malnutrition and TB, although proving the direction of a causal link is challenging as TB in itself causes wasting. Diagnosis is further complicated by frequently false negative TST in malnutrition, reverting to positivity only once nutrition has improved. Nevertheless these observational data, coupled with experimental animal data and impaired CMI observed in malnutrition, support its role as a risk factor for childhood TB ***(Cegielski and McMurray, 2004).***

Mortality & Morbidity:

The case-fatality rate for TB was 50 % for untreated patients before the advent of antibiotic therapy. Deaths worldwide are estimated at 3 million per year. Multidrug-resistant tuberculosis (MDR-TB) cases have a higher mortality rate. Patients with underlying diseases predisposing to active TB also have higher mortality rate. Mortality of untreated

congenital TB is 50% ***(Lobato et al., 2000 & ATS/CDC, 2003).***

Disease in HIV infected children:

The impact of the Human Immunodeficiency Virus (HIV) epidemic on the burden of childhood TB has been less well characterised than for adults. However the observed shift in disease burden to younger adults it has caused suggests that children are at particularly high risk of exposure as well as disease ***(Graham et al., 2001).***

Nevertheless an increased TB incidence and poorer outcome have been observed among HIV infected children in a variety of settings including an estimated 20 fold increased TB incidence associated with HIV infection in a study from South Africa. Methodological constraints in some studies may explain why this has not been a universal finding ***(Graham et al., 2001).***

Transmission:

As in adults, infection with Mycobacterium tuberculosis (MTB) usually occurs by inhalation of tubercle bacilli in aerosolised respiratory droplets derived from an infectious case of pulmonary TB. Risk of infection is therefore dependent on the probability, duration and proximity of exposure to an infectious case, and on the infectiousness of the source ***(Marais et al., 2004).***

Transmission within a community is measured by the Annual Risk of Infection (ARI).Infection rates rise with increased exposure in toddlers, around the ages of school entry and with increased social mobility in late teens and early adulthood. ARI is traditionally estimated using childhood tuberculin surveys, although this has limitations due to the poor specificity of the tuberculin skin test (TST), particularly

where Bacille Calmette Guerin (BCG) vaccine is given at birth and non-tuberculous mycobacteria (NTM) are endemic ***(Rieder, 2005).***

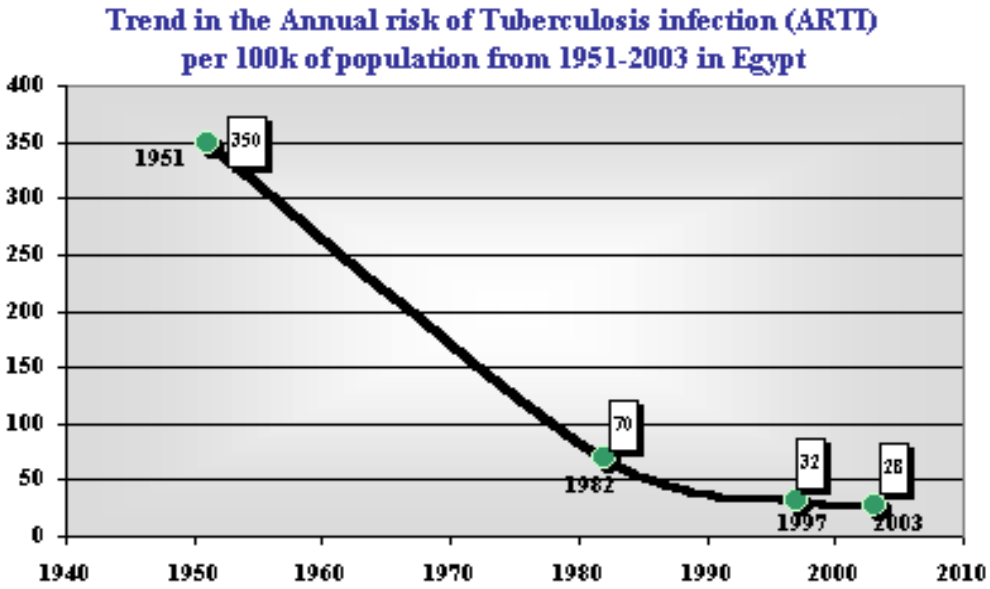

Fig (3): The trend in the annual risk of tuberculosis infection per 100k of population from 1951-2003 in Egypt *(NTCP, 2003)*

Pathogenesis:

Differences in the pathophysiology and clinical presentation of TB in children make diagnosis more challenging than in adults, and definitions of latent infection and disease are less clear cut. Nevertheless, following infection several factors appear to influence the balance of risk between latent TB infection (LTBI) or progression to active disease, including age and nutritional, vaccination and immune status ***(Guwatudde et al., 2003).***

Factors determine the likelihood of transmission of *M.tuberculosis* are the number of organisms being expelled into the air, the volume of the surrounding space and its ventilation, the exposure to ultraviolet light, the length of time

an exposed person breaths the contaminated air, and the immune status of the exposed person ***(Porche, 1999 & ATS, 2000).***

Once inhaled, the infectious droplets settle throughout the airways. The majority of the bacilli are trapped in the upper parts of the airways where the mucus-secreting goblet cells exist. The mucus produced catches foreign substances, and the cilia on the surface of the cells constantly beat the mucus and its entrapped particles upward for removal. This system provides the body with an initial physical defense that prevents infection in most persons exposed to tuberculosis ***(Frieder et al., 2003).***

Bacteria in droplets that bypass the mucociliary system and reach the alveoli are quickly surrounded and engulfed by alveolar macrophages, the most abundant immune effector cells present in alveolar spaces. These macrophages, the next line of host defense, are part of the innate immune system and provide an opportunity for the body to destroy the invading mycobacteria and prevent infection ***(Van Crevel et al., 2002).***

Cells involved in TB infection:

Macrophages are readily available phagocytic cells that combat many pathogens without requiring previous exposure to the pathogens. Several mechanisms and macrophage receptors are involved in uptake of the mycobacteria. The mycobacterial lipoarabinomannan is a key ligand for a macrophage receptor ***(Nicod, 2007).***

The complement protein C3 binds to the cell wall and enhances recognition of the mycobacteria by macrophages. Opsonization by C3 is rapid, even in the air spaces of a host with no previous exposure to M tuberculosis. The subsequent phagocytosis by macrophages initiates a cascade of events that results in either successful control of the infection, followed by

latent tuberculosis, or progression to active disease, called primary progressive tuberculosis ***(Frieder et al., 2003).***

The outcome is essentially determined by the quality of the host defenses and the balance that occurs between host defenses and the invading mycobacteria ***(Van Crevel et al., 2002).***

From the first exposure to Mycobacterium tuberculosis, a series of immune responses are triggered that define the course of the infection. However, this host defence response is not uniform in exposed people. In the vast majority of humans no disease develops at any time. Furthermore, a spectrum of possible clinical manifestations may occur at any stage of life in those patients who cannot control the infection ***(Lopez et al., 2003).***

The host immune response against mycobacterial infection has been explored. In mice and humans, infection is mainly controlled by macrophage activation induced through Th1 type cytokines. Interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) have a central role in this process by inducing macrophage activation and inducible isoform of nitric oxide synthetase (iNOS) expression. The NO produced in this process is essential − at least for mice − to kill intracellular mycobacteria ***(MacMicking et al., 1997).***

This protective activity fails if there is a marked release of Th2 type cytokines. The interplay of cytokines is depicted clearly in a BALB/c model of pulmonary tuberculosis following intratracheal infection.In this model, an initial phase is dominated by high production of Th1 cell cytokines that, together with high levels of TNF-α and iNOS, temporarily control the infection. Granulomas develop in this phase. Three weeks after infection the expression of Th1 cell cytokines, TNF-α and iNOS start to decline. Gradually, pneumonic areas prevail over granulomas. Pneumonia, in co-existence with a

high burden of bacteria, causes death ***(Hernandez –Pando et al., 2001).***

The alveolar macrophage is the first line of defense in the innate immune response to TB and plays a critical role in amplifying the response to infection. Studies in the animal and human host have consistently demonstrated reduced microbial killing, and diminished monocyte recruitment to the site of infection in infants compared to adults. Thus impairment of innate pulmonary defenses in the neonate and infant may allow mycobacteria to overwhelm the effects of the innate immune system prior to the initiation of an antigen-specific immune response ***(Upham et al., 2006).***

Antigen presentation by dendritic cells (DC), the major antigen-presenting cell (APC) in the lung, and the efficiency with which naïve T cells respond to antigen, also appears less effective in infants and may contribute to the delay in initiating an appropriate antigen-specific response, resulting in development of active disease. Blood derived DCs are functionally immature at birth relative to adult DCs and continue to express a less differentiated phenotype throughout early childhood ***(Upham et al., 2002).***

For persons with intact cell-mediated immunity, the next defensive step is formation of granulomas around the M tuberculosis organisms These nodular-type lesions form from an accumulation of activated T lymphocytes and macrophages, which creates a micro- environment that limits replication and the spread of the mycobacteria. This environment destroys macrophages and produces early solid necrosis at the center of the lesion; however, the bacilli are able to adapt to survive ***(Dheda et al., 2005).***