Original article

Immunological efficacy of Bacille Calmette-Guérin vaccination in Egyptian children: case series

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Abstract

Bacille Calmette-Guérin (BCG) vaccine is one of the most widely used vaccines in children. In Egypt, it is a part of the national compulsory childhood immunization program. The most controversial aspect of BCG is the variable efficacy found in different studies. This study was to evaluate the efficacy status of the available BCG vaccine in Egypt within the last 10 years (BCG-Copenhagen). The pilot cross sectional study included 597 Egyptian children randomly selected. Their ages ranged from 6 months to 10 years old (mean_5 years, median: 3 years). All were assessed for history of BCG vaccine intake (primary at infancy and/or secondary at school age) and examined for the presence BCG scar. A group of the vaccinated children (62 children with BCG scar and 69 children without BCG scar) were further assessed with tuberculin skin test (TST). Prevalence of BCG vaccine intake in the studied children was 86.9% (519/597). Efficacy in term of BCG scar after vaccination was 66.6% (346/519). However, efficacy in term of post BCG vaccination tuberculin sensitization was only 3.8% (5/131). BCG vaccination program in Egypt seems to be widely prevalent; however, the immunological efficacy of the available strain is questionable.

Keywords: BCG vaccine; efficacy; tuberculin skin test; Egypt; children

INTRODUCTION

The Egyptian Ministry of Health and Population (MOHP) has established the National Tuberculosis Control Programme (NTP) in 1979[1]. It is a detailed plan of action for effective TB control; including immunization with Bacille Calmette-Guérin vaccine (BCG); case finding and treatment; health education; and, surveillance of the disease in the community [2].

The Bacille Calmette-Guérin (BCG) vaccine has existed for 80 years and is one of the most widely used of all current vaccines[3]. It is compulsory in 64 countries[1] including Egypt[4]. The most controversial aspect of BCG is the variable efficacy found in different clinical trials. Clinical trials conducted in the UK have consistently shown a protective effect of 60 to 80%, but trials conducted elsewhere have shown no protective effect, and efficacy appears to fall the closer one gets to the equator[5].

BCG induced tuberculin sensitivity is a quantitative characteristic and has been used to compare vaccine efficacy. It has been also suggested that the protection which some BCG vaccines could confer against the development of TB in childhood, might be indirectly reflected by the subsequent development of BCG immune response[6].

The BCG vaccines that are currently in use are produced at several (seven?) sites throughout the world. These vaccines are not identical. To what extent they differ in efficacy and safety in humans is not clear at present. Each BCG is now known by the location where it is produced. For example, we have BCG (Paris), BCG (Copenhagen), BCG (Tice)

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and BCG (Montreal) among others[7]. In Egypt, the available BCG vaccine within the past 10 years was (the Copenhagen BCG vaccine - Danish strain 1331 of Mycobacterium bovis).

The first objective of this pilot cross sectional study was to assess for the BCG vaccine prevalence in Egypt. Second objective was to assess its immunological efficacy in term of scar formation and BCG induced tuberculin sensitivity of the available BCG Copenhagen strain in Egypt within the past ten years.

MATERIALS AND METHODS

This study was done over 17 month's duration, form May 2006 till September 2007.

Study population

A sample size of 597 randomly selected Egyptian children was considered appropriate for a cross sectional pilot study (80% confidence level of study power = 80%, calculated using Epi Info software for windows, version 3.3.2; Atlanta, CDCP; 2005) assuming that BCG coverage was estimated at 70% for possible drop-outs. The children were recruited while attending the out patient clinic of Pediatric Ain Shams University Hospital (Cairo, Egypt).

Their ages ranged from 6 months to 10 years old (mean: 5 years, median: 3 years). All were assessed for history of BCG vaccine intake (primary at infancy and/or secondary at school age) and examined for the presence BCG scar. For further testing with Tuberculin Skin Test (TST), 142 vaccinated children were randomly selected, but only 131 children had complete results. A sample size of 131 vaccinated children (62 children with BCG scar and 69 children without BCG scar) was considered appropriate for a cross sectional pilot study (80% confidence level of study power = 75%-calculated using Epi Info software for windows, version 3.3.2; Atlanta, CDCP; 2005) assuming that protective effect of available BCG vaccine is at least 60%, as it was estimated with same strain[7].

Exclusion criteria: Recent viral infections (e.g. measles, varicella), recent vaccination with live viral vaccines (within 6 weeks), malnutrition, bacterial infections (e.g. typhoid, leprosy, pertussis), immunosuppressive medications (e.g. corticosteroids), neonatal patient up to 6 months age, primary immunodeficiencies, diseases of lymphoid tissue (e.g. Hodgkin disease, lymphoma, leukaemia, sarcoidosis, HIV infection), low protein states and known tuberculous children (as all these criteria may induce a false negative tuberculin test).

Informed consent was obtained from the parents. This study was approved by the Research Ethics Committee of Ain Shams University Hospital.

Tuberculin Skin Test (TST)

A TST is the intradermal injection of a combination of mycobacterial antigens which elicit an immuneresponse (delayed-type hypersensitivity), represented by induration, which can be measured in millimetres. The TST using the Mantoux method is the standard method. Administering, reading and interpreting a tuberculin skin test were according to WHO/HTM/TB/2006 guidelines[2].

Tuberculin tests were carried out by one of the authors. All the children in the study were given 0.1 ml of TU (Vacsera PPD-S 5 tuberculin units/0.1ml with 5 mcg/0.1ml Tween 80 manufactured by the Egyptian BCG vaccine laboratory, Vacsera) intradermally on the volar surface of the left forearm by the Mantoux intradermal method of tuberculin skin testing.

Readings of the transverse diameter of induration were taken at 24 hr, 48 hr and 72 hr with a transparent plastic ruler using the ball point pen technique and recorded meticulously in mm by the same observer. A transverse diameter reading of $\geqslant$ 5 mm was designated as Tuberculin positive and $<$ 5 mm was taken as negative reading. The smallest induration size considered for assessment was 5 mm. The qualitative assessment of Koch (turgid) and Lister (non turgid) type of reactions was carried out according to the criteria of Stanford et al[8].

The reaction was designated Koch's type of response if any 3 of the following changes were recorded; 1. Hard induration. 2. Well delineated. 3. Painful 4. Skin changes - vesiculation or bullae, necrosis.

The reaction was designated Lister type of response based on the following criteria: 1. Soft induration. 2. Not well delineated. 3. Not painful.

Statistical Analysis

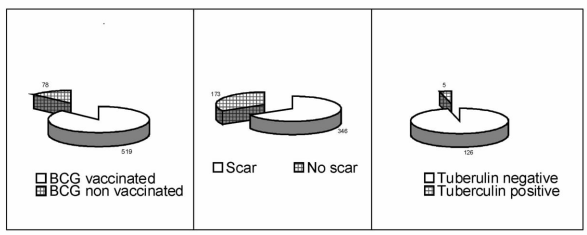
Descriptive data are expressed as means ± SD.

Categorical data were compared by 2 test analysis and expressed as *P* value, OR and 95% CI. Statistical significance was established at $P < 0.05$. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 11.5; SPSS Inc., Chicago, IL, USA; 2006).

RESULTS

During the survey, 597 children were randomly selected for assessment. Only 519 (86.9%) children documented the intake of BCG vaccine (either as primary vaccination at the first 40 days of life, and/or at their first year in school at the age 6 years). Of the 519 vaccinated children, 131 (25.2%) received the Mantoux Tuberculin Skin Test (TST). The age and sex distribution of these children is presented in (Table 1). The mean age of both boys and girls was 5 years (SD 3.3 years); 61% were males.

Of the 519 BCG vaccinated children included in the survey, 346 (66.6%) were considered to have a BCG scar (Figure 1). The mean age of children with an apparent BCG scar did not differ from that of children without a scar (*P* value 0.34).



(a) Prevalence of BCG vaccination. (b) Prevalence of BCG scar. (c) TST results after BCG vaccine.

Figure 1: Summary of the collected data

A group of the vaccinated children (62 children with BCG scar and 69 children without BCG scar) were further assessed with tuberculin skin test (TST). Post BCG vaccination tuberculin conversion was proved only in 3.8% (5/131) of the vaccinated children, with no significant difference regarding presence or absence of BCG scar (Table 2).

Table 1: Demographic Characteristics of the studied children

Variable	Number (%)
Total number of the included children	597(100%)
Age Range	6 months -10 years
Mean (+SD)	5 (+3.3) years
Median	3 years
6 months - 6 years	374 (62.6%)
More than 6 years	223 (37.4%)
Sex Male	363 (61%)
Female	234 (39%)
Residency * Cairo	517 (86.7%)
Giza	16 (2.5%)
Kaliobia	39 (6.5%)
Monofia	11 (1.8%)
Kafr El Sheikh	9 (1.5%)
Bohira	5 (0.8%)
BCG# vaccination	
Total (Primary and/or secondary)	519 (86.9%)
Primary at infancy	505 (84.7%)
Secondary at school	32 (5.3%)
BCG complications ¤	12/519 (2.3%)
BCG scar	346/519 (66.6%)
Positive Tuberculin skin test * *	5/131 (3.8%)
- with BCG scar	2/62 (3.2%)
-no BCG scar * * *	3/69 (4.3%)

* Egyptian governorate; # BCG = Bacille Calmette-Guérin; ¤ all were local skin reactions; * * Cut off value for positivity ≥ 5mm induration; * * * Two children of the 3 positive were highly positive (≥15mm) = Koch reaction, which points to latent Tuberculous infection.

Table 2: Data of tuberculin skin test

	Tuberculin positive	Tuberculin negative	Analysis
With BCG scar	2	60	$\chi 2 = 0.11$
			p = 0.55
			(Non Significant)
No BCG scar	3	66	* OR = 0.73
			95% CI (0.08-5.65)

* OR = odd ratio, 95% CI = 95% Confidence interval

DISCUSSION

However, there is abundant archaeological evidence that tuberculosis (TB) was prevalent in predynastic Egypt as early as 5500 years ago[9], the 1995-1997 tuberculin survey in Egypt has shown that the size of the tuberculosis problem was considerably smaller than it had been 45 years before[4]. Thanks to the WHO Expanded Programme on Immunization and the NTP which recommend BCG vaccination as soon as possible after birth in countries with a high TB prevalence-including Egypt [10]. This was evident by the wide prevalence of BCG use within Egyptian children (in our pilot survey was 86.9%).

Clinical Efficacy of BCG vaccine

The (clinical) efficacy of a vaccine is measured in terms of the percentage reduction in disease among vaccinated individuals that is attributable to vaccination [11].

In Egypt*, the clinical efficacy of BCG could be assessed with the Annual Risk of tuberculous Infection (ARTI) which represents the percentage of population that will be infected by tubercle bacilli every year. It has been estimated that one percent of ARI corresponds to 50 to 60 new smear-positive tuberculosis cases per 100,000 inhabitants per year.

After a nation wide tuberculin survey carried out in 1995-1997, the ARI is calculated at 0.32 percent. This means that new 32 TB cases will be discovered every year per 100000 individual of the Egyptian population.

In 2003, the incidence of TB Infection (ARTI) was calculated mathematically, in collaboration with WHO, at rate of 28 TB new cases per 100000 individual of population and modified in 2006 to 26 TB new cases per 100000 , 11 of them are smear positive pulmonary TB. In terms of incidence of tuberculosis, Egypt is now ranked among the mid-level incidence countries.

Reasons for variable BCG immunological efficacy

The reason for the variable efficacy of BCG in different countries is difficult to understand [12]. A number of possible reasons have been proposed but none have been proven.

Differences between BCG vaccines: It is well recognized that BCG vaccines differin various properties, both in the genetics of the mycobacterial strains and in thephysical properties of the vaccine preparations [13]. Several authors have thus explored whether these differences could explain the observed pattern of protection afforded by BCG vaccines[12,14,15].

A recent paper has suggested that efficacy declined with passage number of the seedsubstrain, interpreting this as evidence that manufacturers selected their strains toreduce lymphadenopathic reactions, and thereby compromised their efficacy [11]. However, the trend was confounded by geographic area (the higher passage strainswas tested at lower latitudes, where it is known that BCG vaccines perform less well) [3].

A recent review of BCG strain history and protective efficacy concluded that vaccine strain "is not a significant determinant of overall efficacy" [16].

Meanwhile, emphasis should especially be placed on the importance of the quality, transportation and preservation of the vaccine and on the technique of application [17], that may be responsible for the widely divergent results of prospective BCG trials in several countries, those have lead to current doubts about the efficacy of BCG vaccination in TB prevention.

Background frequency of exposure to tuberculosis; It has been hypothesised that in areas with high levels of environmental exposure to tuberculosis or mycobacteria other than members of the M. tubercu-

losis complex, every susceptible individual is already exposed prior to BCG, and that the natural immunizing effect then appears to wipe out any benefit of BCG[18]. In this population, BCG elicits only a transient immune response with a low frequency of mycobacterium-specific cells and no protective immunity against TB [14].

Genetic variation in populations; Difference in genetic make-up of different populations may explain the difference in efficacy. More directly, the fact that some BCG vaccine strains have been shown to perform well in some populations, but poorly in others, demonstrates that vaccine differences cannot explain all the variation: thus freeze dried Glaxo vaccine provided good protection in the UK, but none (against pulmonary tuberculosis) in Malawi[1].

Opposite to this; was the Birmingham BCG trial published in 1988. The trial was based in Birmingham, UK, and examined children born to families who originated from the Indian subcontinent (where vaccine efficacy had previously been shown to be zero). The trial showed a 64% protective effect, which is very similar to the figure derived from other UK trials, thus refuting the genetic variation hypothesis [3].

Interference by concurrent parasitic infection; another hypothesis is that simultaneous infection with parasites changes the immune response to BCG, making it less effective. A Th1 response is required for an effective immune response to tuberculous infection; one hypothesis is that concurrent infection with various parasites produces a simultaneous Th2-response which blunts the effect of BCG [13].

Tuberculin Skin Test for BCG efficacy

In this study, what was unexpected was the predominance of negative tuberculin reaction (96.2%) in the vaccinated children, whether they have a BCG scar or not. Mostly there was no evidence of reaction. That's why the authors themselves tried the TST on them and it had a positive reaction.

It is believed that immunological response to TST in BCG vaccinated children is related to what is called "two-pathway" theory. It could be either Koch type reactions which are related to hypersensitivity and Listeria type which is related to protective immunity of BCG vaccination the positive TST (Lister type) [19].

A possible relation between the turgid reaction (Koch) and tuberculosis disease was indicated. Koch type of reaction is more intense in inflammatory terms than the non turgid Listeria type. The greater degree of cutaneous reaction observed in Koch type-could mirror extensive destruction of tissue and spread of infection [20].

In our study; the rate of highly positive TST (Koch type of reaction) among the vaccinated children was 2/131 (1.5%). All of them had no scar of BCG. While 3/131 (2.3%) showed Listeria reaction to TST.

It is proved that none of the children with Listeria reaction progress to Koch type. Hence, the two variants appear to be independent in terms of formation and progression [21].

The state of activation and probably sensitization of local macrophages and lymphocytes influence the clinical picture of the disease. It is possible that after an adequate recruitment of sensitized cells at the tuberculin site, there may be cytokine mediated modulation of the release of mediators which may account for these variant forms [13].

While absence of either responses also could be explained by presence of neither; disease nor protection5, some authors have explained in the negative reactors, that BCG vaccination could have triggered protective (Lister type) rather than antagonistic (tuberculin or Koch type) reactions, which have been speculated to be the most protective [20].

Al-Kassimi et al [15] supported this "two- pathway" theory that BCG vaccination could trigger either protective (Lister type) or antagonistic (tuberculin or Koch type) reactions and that the most protective vaccines would have little tuberculin- sensitizing effect because the two pathways are competitive.

Further more, a genetically determined modulation of release of inflammatory mediators as a possible cause of variation in different individuals was considered by van Eden et al[22].

It seems unlikely that the impaired reaction to tuberculin in the vaccinated subjects had been caused by a defect of initial recognition of the antigen or an inability to retain this information or even by a failure of sensitized lymphocytes to react because the individual was malnourished or had a serious infection [23]. Instead, there is circumstantial evidence that even when given at birth, BCG achieves tuberculin conversion in a high proportion of

neonates [24], irrespective of race, ethnic origin or prematurity.

With proven clinical efficacy of BCG vaccine in Egypt, the prevalent negative BCG induced tuberculin sensitivity could be another novel face of the protective (Lister type) pathway reaction toward tuberculosis.

Future directions

New candidate of TB vaccines have to be evaluated against the existing Mycobacterium bovis BCG "gold standard" up till now [2]. It is therefore important to understand the type of immune responses elicited by BCG vaccination to enable comparisons with potential new candidates [19]. This step would be essential to fulfill the evolving goal proposed by Stop TB/WHO strategy by the year 2015 [3].

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