http://dx.doi.org/10.5272/jimab.2015212.788

Journal of IMAB
ISSN: 1312-773X
http://www.journal-imab-bg.org

OPEN ACCESS

Journal of IMAB - *Annual Proceeding (Scientific Papers)* 2015, vol. 21, issue 2

TUBERCULIN RESPONSE AND SCAR FORMATION IN BCG VACCINATED 7-YEARS OLD SCHOOLCHILDREN IN SOFIA, BULGARIA

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ABSTRACT

This study has been designed to evaluate safety and efficacy of BCG vaccine in school-entering children in terms of tuberculin response to PPD and scar formation as indicators for successful vaccination. 12 BCG lots were used to immunize 1054 tuberculin-negative children at age of 7 years. Local lesions were observed and recorded 1, 3 and 12 months after immunization, and tuberculin sensitivity was measured 3 months after BCG re-immunization. The mean diameter of local reaction was 5.58±1.63 mm at 1st month, 5.13±1.56 mm at 3rd month and 4.16±1.21 mm at 12th month respectively. Tuberculin conversion was 100% and the mean diameter of induration 3 months after BCG application was 10.53±1.66 mm. All of the results obtained pointed to the fact that Bulgarian BCG vaccine induces adequate tuberculin sensitivity to a low dose of PPD tuberculin without great local skin lesions and without occurrence of any untoward vaccination reactions. Very good consistency from batch to batch is observed and this is a proof that biological activity of the Bulgarian BCG strain is kept under continuous control.

Key words: BCG vaccine, Tuberculin sensitivity test, PPD, Delayed type of hypersensitivity

INTRODUCTION

It is estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis* and, in 1993 the World Health Organization declared tuberculosis a global emergency. Bacille Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis*, is the only vaccine available for the prevention of tuberculosis. More than three billion doses of BCG have been administered worldwide since 1921 and the global coverage in 2012 is estimated to be more than 90% (91.7%). According to the database available [1] 157 countries currently recommend BCG vaccination, in 64 of them the vaccination is compulsory [2]. A single BCG vaccination policy at or soon after birth is more often used, but in many countries, including Bulgaria, there is vaccination at birth and re-vaccination in PPD-negatives at school entry and adolescence.

The data about BCG protective efficacy are discrepant to a certain extent but it is unambiguously proven that the vaccine reduces the risk of disseminated forms of tuberculosis in early childhood, including miliary disease and tubercular meningitis. [3, 4]. The controversies concerning the efficacy of BCG vaccine are mostly based on unresolved issues in the quality assessment of the vaccine and lack of *in-vivo* and *in-vitro* correlates of the protection which BCG elicits. The biological activity of BCG vaccine is determined by three main characteristics: residual virulence, allergenic potency and immunogenicity. Residual virulence of BCG means the ability of the vaccine's cells to multiply and persist in-vivo to some extent. In order to be effective the vaccine strain must possesses an adequate residual virulence. The local lesion formed on the site of immunization is a sign of the vaccine's residual virulence and it depends on the vaccine strain, vaccine dose, age of immunized subject, technique of intradermal injection etc.

Allergenic potency is the ability of the BCG vaccine to induce delayed type of hypersensitivity (DTH) and it is demonstrated by tuberculin reactivity after BCG vaccination through tuberculin skin test (TST), introduced in 1910 by Mantoux. Indirectly, this is the most common measure of the effect of the BCG vaccine and its immunogenicity. That's why the information about the effect of vaccination on tuberculin reactivity should be reviewed carefully, because it provides important insights into the immunogenic activity and protective efficacy of the BCG vaccine.

As a sign of T-cell mediated immune response after mycobacterial infection a delayed type of hypersensitivity to Purified Protein Derivate (PPD) of tuberculin is developed. Clinically this process is visualized through positive tuberculin skin test. The same reaction is observed after BCG vaccination. Nowadays, due to a lot of limitation of diagnostic tools available, the tuberculin skin test remains the only routinely suitable method for the early screening of *M.tuberculosis* infection as well as the test for study the population structure in reference to evaluation of BCG vaccination, re-immunization needs and BCG efficacy.

As a part of national vaccine programme in Bulgaria, BCG vaccination is given to all newborn babies. Later, all negatives to 5 IU PPD tuberculin children – at 6-7 (entering school), 10-11 and 16-17 years of age are subject of revaccinations.

The aim of this study is to evaluate the safety and efficacy of BCG vaccine in one of the target population (7-years old children), observing local vaccine lesions and post-vaccine tuberculin sensitivity to 5 IU PPD Tuberculin and to comply the Requirements of the WHO [5] about the adequate control of BCG vaccine which includes clinical surveillance of the vaccine in man.

SUBJECTS AND METHODS

The study covers the period 2006-2010. Twelve BCG vaccine lots, manufactured by BB-NCIPD Ltd., were used to immunize tuberculin-negative children with pre-vaccination tuberculin reaction 0-5 mm at age of 7 years.

Totally 4786 children were tested. The pre-vaccination tuberculin test was carried out with 5IU PPD tuberculin (BB-NCIPD Ltd.). 1054 schoolchildren were PPD-negative and were re-vaccinated with BCG vaccine. The vaccine is applied intradermally. The immunizing dose is 0.05 mg/ 0.1 ml.

Local lesions were observed and recorded 1, 3 and 12 months after immunization, and tuberculin sensitivity – 3 months after immunization. All injections and readings were performed by one and the same qualified trained team.

For quantitative evaluation of the vaccination lesion the largest transverse diameter of the local lesion was measured. The type of tissue destruction (papule, vesicle, pustule, ulcer, crust /scab/ or scar) was judged and recorded. Vaccination lesions were examined at the time of the tuberculin injection. Special attention was given to the inspection of left and right axillary and cervical lymph nodes.

The tuberculin test was read 72 hours after 5 TU PPD application by measuring the largest transverse diameter of induration to the nearest millimeter, according to the recommendation of WHO.

Statistical analysis of the data was performed and frequencies and distribution for each variable category were determined for descriptive analysis.

RESULTS

The Mantoux test was carried out on totally 4786 children (7-years old). 1054 or 22.02% of them were tuberculin-negative. This percent corresponds to data previously reported [6, 7] and the level of post-vaccine allergy 7 years after birth confirms that Bulgarian BCG vaccine has a very good efficacy.

In our study 751 tuberculin-negatives were immunized during the period 2006 - 2009 with 8 different BCG vaccine lots, randomly selected (production of BB-NCIPD Ltd.) and the summarized results of local lesions during the first, third and twelfth month after vaccination are presented on Table 1.

Table 1. Size of the local lesions after BCG vaccination

No of Lots tested	No of vaccinated children	1 month		3 months		12 months	
		No of children examined	Local lesion mm±SD	No of children examined	Local lesion mm±SD	No of children examined	Local lesion mm±SD
8	751	681	5.58±1.63	620	5.13±1.56	493	4.16±1.21

One month after immunization 681 or 90.68% of vaccinated children were examined and all of them developed an indurated papule or pustule at the immunization site.

All of the lesions ulcerated and drained for a mean duration of 4.4 weeks. No significant differences were detected comparing the clinical reactogenicity associated with the eight BCG Lots tested. No temperatures of >38° C or other systemic symptoms were reported. At the 3rd month 620 or 82.55% of vaccinated children were examined. The mean diameter of scar was 5.13 ± 1.56 mm. All immunization sites were healed by 3 months after vaccination. At the end of follow-up (12 months after BCG immunization) the mean diameter of local lesion was 4.16±1.21 mm.

In 2010 another 4 BCG Lots, randomly selected, were tested on 303 tuberculin-negatives. The results of local lesions during the first, third and twelfth month after vaccination are presented on Table 2.

Table 2. Size of the local lesions after BCG vaccination: batch to batch consistency

Lots No.	No of vaccinated children	1 month		3 months		12 months	
		No of children examined	Local lesion mm±SD	No of children examined	Local lesion mm±SD	No of children examined	Local lesion mm±SD
601-1	68	68	5.01±1.52	62	4.59±1.38	62	3.77±1.05
610-2	102	93	5.08±1.25	92	5.10±1.12	86	4.47±1.10
632-1	89	86	5.32±1.34	83	5.34±1.45	80	4.47±1.43
632-2	44	38	6.15±1.77	41	5.82±1.58	38	4.36±1.09

The local response to an intradermal injection of BCG has typically developed along a common course of events. BCG vaccination of tuberculin negative children resulted in a local reaction with erythema and tenderness. In the second week, a small induration developed, followed by a softening process of the central area, which gradually turned into a yellow pustule and finally led to the formation of a crust. When this crust fell off, an ulcer appeared that slowly heals up to the third month. All immunization sites were healed by 3 months after vaccination. At the end

of follow-up (12 months after BCG immunization) the mean diameters of local lesions were between 3.77±1.05 mm and 4.47±1.43 mm.

From the table it can be seen that there is a very good consistency from batch to batch from the point of view of clinical surveillance of the Bulgarian BCG vaccine.

The results of tuberculin sensitivity in 751 BCG vaccinated children at the third month after vaccination are summarized on Table 3.

Table 3. Tuberculin sensitivity to 5IU PPD three months after BCG revaccination

No of Lots tested	No of vaccinated children	3 months		
		No of children examined	Tuberculin sensitivity mm±SD	No of cases with induration ≥5mm
8	751	620	10.53±1.66	620 (100%)

Each of tuberculin negatives responded to immunization three months after vaccination, i.e. tuberculin conversion is 100%. The mean diameter of induration was 10.53 mm ±1.66 which corresponds to the criteria for normal tuberculin allergy in Bulgaria [7]; namely 0-5 mm – negative reaction, 6-14 mm - normal allergy, and >14 mm – hyperergic reaction to tuberculin. All of the results available from this clinical surveillance pointed to the fact that Bulgarian BCG vaccine induce adequate tuberculin sensitivity to a low dose of PPD tuberculin (5 IU) without inducing great local skin lesions and almost without the occurrence of any untoward vaccination reactions.

The results from tuberculin sensitivity 3 months after applying another 4 BCG lots in 2010, are presented on Table 4.

Table 4. Tuberculin sensitivity to 5IU PPD three months after BCG revaccination: batch to batch consistency

Lots No	No of vaccinated children	3 months		
		No of children examined	Tuberculin sensitivity mm±SD	No of cases with induration ≥5mm
601-1	68	62	10.41±1.26	62 (100%)
610-2	102	92	7.08±3.78	92 (100%)
632-1	89	83	10.22±1.51	83 (100%)
632-2	44	41	10.48±1.36	41 (100%)

It should be mentioned the high percent of children reacted positively with induration ≥10 mm. For the BCG lots examined this percent varied between 71.08% and 88.04% which is an unambiguous proof for the allergenic potency of the Bulgarian BCG vaccine.

The statistical analysis of the results obtained shows normal distribution and homogeneity of the data which indicates a very good consistency of production.

DISCUSSION

Despite WHO efforts to standardize BCG vaccination by stabilization and lyophilization, considerable microbiologic and genetic differences still exist among BCG strains [8]. Also notable are discrepancies in numbers and proportions of viable and dead organisms according to dose. These differences could account for numerous variations in reactogenicity and immunogenicity of the vaccines. In our study we evaluate the safety and efficacy of BCG vaccine in first year schoolchildren, estimating local lesions and post-vaccine tuberculin sensitivity and adhering WHO requirements about the adequate control of BCG immunization by clinical surveillance of the vaccine in man.

The relatively low number of tuberculin negatives seven years after first vaccination at birth confirms the data from other studies on persistence of the immune response induced by BCG vaccination. According to R.E.Weir at al. who tested UK adolescents for interferon – gamma (INF-γ) response to different mycobacterial antigens, BCG vaccination in infancy and adolescence induces immunological memory to mycobacterial antigens that is still present and measurable for at least 14 years in the majority of vacinees [9].

The local skin reaction after intradermal injection of BCG vaccine depends mainly on the number of the vial cells inoculated. Of course, there are many other factors which can influence the vaccine reactogenicity. Since the local reaction is a sign for the vaccine's residual virulence, three main criteria are essential for its estimation: lasting of the reaction, size of the local lesion and smallest active dose which can induce lesion. Applying these criteria enables grading the virulence and attenuation of the BCG sub-strains. In humans the local reaction on the site of immunization is also indication for the vaccine's residual virulence. This reaction is manifested on different time after vaccination and can be expressed at different extent depending the level of post-vaccine tuberculin sensitivity, dose of the vaccine, vaccine strain used, age and sex of the vacinees, the competence of the vaccination team etc. The correlation between

BCG reactogenicity and vaccine-induced immunity is well described [10], but from the other hand, the high residual virulence can be associated with serious disseminated vaccine complications especially in immunocompromised individuals. Over the last several decades, attempts have been made to minimize the adverse reactions associated with BCG administration and maximize induction of DTH to PPD Tuberculin and BCG efficacy, respectively.

The development of local reaction in our trials is consistent with other reports demonstrating the kinetic of immune response and ulcer duration [11, 12]. Ulcerative drainage from vaccine lesions contains viable replicating BCG cells. Direct correlation between the duration of ulcer drainage (relative duration of BCG replication) and lymphoproliferative and INF-γ responses specific to mycobacteria after vaccination were detected. INF-γ is known to be a key cytokine in the induction of effector functions important for the control of intracellular pathogens, including mycobacteria causing human infections. The results from different studies [10] suggest that the optimal induction of protective mycobacterial immunity by BCG vaccination may require considerable in situ replication of the vaccine strain and the associated local tissue effects.

The direct effect of BCG vaccination is defined as prevention of tuberculosis in vaccinated persons and the indirect effect - as the reduction of tuberculosis in the population as a whole. The assumption that a positive skin test following vaccination is an indicator of BCG induced immunity against *M.tuberculosis* might not have been an absolutely correct, however, in the practice the most convenient way to estimate the immediate effect from the vaccine is to determine the delayed type of hypersensitivity in fixed time after immunization. Since BCG induced tuberculin sensitivity is a quantitative characteristic, the method is very convenient to be used to compare and study the vaccine efficacy.

In **conclusion**, the observation on field studies convinced that batch to batch variations and biological activity of the Bulgarian BCG strain have been kept under continuous control.

Both skin reactivity and tuberculin sensitivity did not differ significantly among the batches, as well as among the ampoules within one batch.

The critical characteristics of the vaccine, usually measured by in-process control and final specifications are consistently met for different production runs and this consistency is proved by clinical observations.

ACKNOWLEDGMENTS:

The author thanks the participating children, parents and the collaborating physicians and health authorities.

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Please cite this article as: Stefanova T. TUBERCULIN RESPONSE AND SCAR FORMATION IN BCG VACCINATED 7-YEARS OLD SCHOOLCHILDREN IN SOFIA, BULGARIA. *J of IMAB.* 2015 Apr-Jun;21(2):788-792. DOI: http://dx.doi.org/10.5272/jimab.2015212.788

Received: 12/03/2015; Published online: 10/06/2015

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