

## Number of live organisms in BCG vaccine and other related factors in tuberculin skin reaction

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### ABSTRACT

**Background:** With respect to the prevalence of tuberculosis in our country, BCG vaccination at birth seems to be an effective means of prevention. The present study was designed to evaluate the efficiency of BCG vaccination and factors influencing the tuberculin skin reaction.

**Materials and methods:** The inoculum of live BCG organisms in vaccinated infants was evaluated at birth and the correlation between the amount of CFU and PPD reaction was examined 3 months later. For this purpose, 854 newborn infants were included. PPD induration diameter was measured 72 hours after the tuberculin skin test.

**Results:** Results have revealed that most of the vaccine samples contained more than 3 million live organisms. Vaccines showing more than  $3 \times 10^6$  live organisms were associated with positive tuberculin skin test in more 93% of cases as compared with 69% positive induration of vaccines with less than  $3 \times 10^6$  live organisms. Breast-fed infants showed more positive reaction to tuberculin skin test.

**Conclusion:** The number of live organisms in vaccines can play an important role in the size of tuberculin skin reaction.

**Keywords:** *BCG vaccine, PPD, Tuberculin skin reaction, Children.*

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### INTRODUCTION

Tuberculosis (TB) entails a pivotal problem of global health. It is considered as a regional disease in our country and Bacillus Calmette-Guerin (BCG) vaccination has been shown to be an effective intervention (1). The routine screening methods to detect tuberculosis at an early stage have remained largely unchanged throughout this century. Tuberculin sensitivity continues to be used as a key indicator of tuberculosis infection. The

interpretation of tuberculin sensitivity in the light of previous vaccination (BCG) can pose a diagnostic dilemma (2,3). There are new data regarding tuberculin tests among children who received BCG at birth. This study examined the influence of CFU (colony-forming unit) of BCG on tuberculin reaction in 3-month old children.

### PATIENTS and METHODS

For this descriptive study, the inoculum of live BCG organisms in vaccinated infants was evaluated at birth while the correlation between the amount of CFU and PPD (purified-protein-

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derivative) reaction was examined 3 months later. For this purpose, 854 newborn infants were included. Children were breast- and/or bottle-fed.

The infants were inoculated by BCG vaccine (using French strain 1173 P2) that was produced in Pasteur Institute, Tehran, Iran. The viability of BCG vaccine was checked by inoculating BCG on the solid culture media and enumeration of the CFU resulted as recommended by WHO. The mantoux skin test given in the volar surface of the forearm using 0.1ml of 5 tuberculin units (TU) of PPD (produced by Pasteur Institute, Tehran, Iran) was used. Meanwhile, we controlled other factors, such as strain of BCG vaccine, storing, dosage and vaccination technique and method.

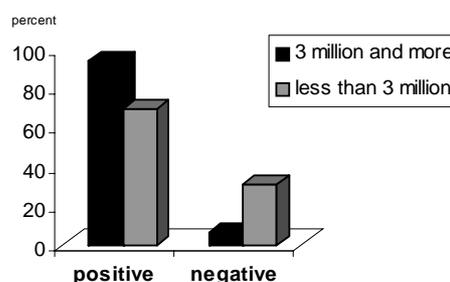
Seventy-two hours later, the maximum transverse diameter of induration using the palpation method was used to estimate tuberculin sensitivity. The presence or absence of a typical BCG scar was used as the indicator of BCG status. Data were analyzed by SPSS for Windows (version 10.5, USA) and chi-square test was used. The level of significance adopted for all tests was 5% ( $p < 0.05$ ).

## RESULTS

The study population included 441 (51.6%) boys and 413 (48.4%) girls. Totally, 91% of children showed positive reaction after BCG vaccination. Of 817 newborn infants weighted  $\geq 2500$  grams, 742 (90.8%) demonstrated positive reaction after 3 months, however, all the remaining infants (37 cases) weighted less than 2500gr showed positive reaction. Furthermore, 809 newborn infants had gestational age of at least 38 weeks among whom 734 (90.7%) cases had positive reaction. Like the previous group, all the remaining infants with gestational age of less than 38 weeks (45 cases), showed positive tuberculin skin reaction.

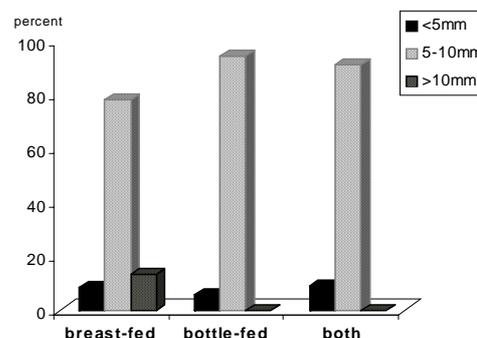
Most of the BCG vaccines were found to have more than  $3 \times 10^6$  live organisms (figure 1). Indeed,

vaccines showing more than  $3 \times 10^6$  live organisms were associated with positive tuberculin skin test in more 93% of cases as compared with 69% positive induration of vaccines with less than  $3 \times 10^6$  live organisms.



**Figure 1.** Tuberculin skin test and the number of live organisms in BCG vaccines.

Figure 2 presents the effect of nutrition on tuberculin skin test. Indurations of  $>10$ mm were only found among breast-fed infants.



**Figure 2.** Effects of nutrition on tuberculin skin test

## DISCUSSION

In the present study, factors such as newborn's gestational age, birth weight, feeding and the number of live organisms in BCG vaccine were considered, however, the dosage of the vaccine,

and its inoculation were excluded since all infants were treated similarly.

Breast-fed infants showed more positive reaction to tuberculin skin test, indeed, forty-seven breast-fed infants showed indurations of >10mm.

Totally, 776 infants were inoculated with more than 3 million live germs, whereas, 78 infants received less than 3 million live germs through vaccination. Among the former group, 93.4% had positive reaction while in the later group 69.2% showed positive reaction.

Tuberculosis is an endemic disease in our country, thus, BCG vaccination at birth is considered as the most important preventive tools. An appropriate BCG vaccine regimen may produce positive tuberculin reaction in 90% of the vaccinated candidates; however, the role of BCG vaccine and its related factors to control tuberculosis is a matter of controversy, nevertheless, it seems to be valuable (4,5). The efficiency of vaccine depends on its antigenic structure, live organism and dosage (6). If the live germ of vaccine reduces to half, the tuberculin skin reaction and its related scar (after inoculation of BCG in forearm of infants) will reduce to a limited measure (7). However, sometimes inoculation with BCG vaccine containing killed germs (killed during storing, production process, etc.) may induce scar but not positive tuberculin reaction (8). PPD reaction is far more sensitive than measuring the live germs of BCG. It indicates that immunization with killed bacilli is much harmless than live germs (9,10).

In the present study, 91% of the vaccinated newborn infants showed positive tuberculin skin reaction. That is in agreement with a study conducted in Chillie in which 94% were presented with positive skin test (10). Contrary, Grindlulis reported a 50%-response following the immunization at birth and in England (11) and Bener et al described 62% of candidates who responded to tuberculin skin test in UAE in 1994 (9).

In summary, tuberculin reaction following the BCG vaccination is under the influence of the number of live organisms in BCG vaccine, storage and injection techniques, dosage, and the reading's method of tuberculin reaction after 48-72 hours.

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