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Scar Formation and Tuberculin Skin Test Response After Bacillus Calmette-Guerin Vaccination: Does Prematurity or Low Birth Weight Have an Impact?

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Abstract

Objectives: The present study aimed at evaluating factors affecting scar formation and tuberculin skin test (TST) response in Bacillus Calmette-Guerin (BCG) vaccinated infants.

Methods: In the current study, 216 infants with gestational age (GA) of 26 to 40 weeks and birth weight of 730 g to 4590 g were included. The mean corrected age was 6.27 $\pm$ 3.79 weeks, and the mean weight was 4442.4 $\pm$ 1084.1 g (range 2100 - 7700 g) at the time of BCG vaccination. TST was applied at 8 to 16 weeks of vaccination. Factors affecting TST response and BCG scar formation were evaluated.

Results: A scar response to BCG vaccination was found in 60%, 49.4%, and 59.7% of the infants with GA < 32 weeks, 33 to 36 weeks, and $\geq$ 37 weeks, respectively. Of the male infants 65% produced a scar compared to the 45% of the female infants (P = 0.014). The mean weight at the time of vaccination was significantly higher in infants with scar development than in those without scars (6976.94 g vs. 6455.68, P = 0.002). A tuberculin reaction was detected in 57% of the infants (80%, 54%, and 50% according to birth weight > 2500 g, 1500 - 2500 g, and < 1500 g, respectively) (P = 0.213). TST response rate was 67%, 55%, and 47% according to GA $\geq$ 37 w, 33 - 36 w, and $\leq$ 32 w, respectively (P = 0,020). In the present study, 68.4% of the cases with TST of 5 - 10 mm, and 100% of the cases with TST > 10 mm developed scars $\geq$ 2 mm (r = 0.360; P = 0.001).

Conclusions: Prematurity or birth weight does not affect BCG scar formation. TST response is lower in preterm babies. The correlation between scar formation and TST response was too low to be interpreted as positive.

Keywords: Infant, Premature, Small for Gestational Age, BCG Vaccine, Vaccination, Tuberculin Test

1. Background

Bacille-Calmette-Guerin (BCG), a live attenuated vaccine that has been in use since 1921, is the most widely used vaccine worldwide (1, 2). BCG is efficacious against the most severe forms of tuberculosis (TB) such as tuberculous meningitis (73% protection) and miliary TB (77% protection) in children younger than 5 years (3, 4). It is one of the mandatory vaccines recommended by the ministry of health (MOH) in Turkey and had been administered to term newborns at birth until 1992. Ildırim et al. showed a significant difference in tuberculin responses when the vaccine was performed at birth; and at the 12th week (67% vs. 87%), the vaccination was moved to the third month after birth (5). Currently, the recommended schedule of BCG is eight weeks postnatal in Turkey. The usual response to administer BCG vaccine is the development of erythema or a papule at around 2 weeks, followed by an ulcer and healing with a scar at 6 weeks at the site of the injection. Although the size of reaction after vaccination is not generally thought to influence the degree of protection offered by BCG, the presence of the BCG scar has been used as a criterion to assess the uptake of vaccination (6, 7).

Preterm infants are at increased risk of disease and hospitalization from a number of vaccine-preventable diseases. However, they have immunologic immaturities that may affect vaccine responses. Tuberculin skin test (TST) is currently the most commonly used test for evaluation of cell-mediated immune response to Mycobacterium tuberculosis (8). Many studies suggest that preterm infants take up their BCG vaccination as efficiently as term neonates (9-11). However, there are conflicting results (12) in this regard. We aimed at evaluating the factors affecting TST response

and BCG scar formation in a large cohort of vaccinated infants including very low birth weight babies and preterms < 32 weeks gestation.

2. Methods

2.1. Participants

The present study was conducted at Bakirkoy Maternity and Children's hospital during a 6- month period in 2007. This cohort included infants born at this hospital and given BCG vaccine. Ballard score was used for gestational measurement (13). The corrected age was calculated according to the "postnatal age (PNA) (week) - (40-gestational week)" formula. Lubchenko intrauterine growth curves were used to assess intrauterine growth (14). Patients with congenital anomalies, severe sepsis, skin lesions on the left forearm, and those treated with steroids or intravenous immunoglobulin were excluded.

2.2. BCG Vaccination

All infants were vaccinated with BCG according to the recommendations of Turkish Ministry of Health (15). The weight, PNA, birth weight according to gestational age (GA), and the corrected age at the time of vaccination were noted. Following BCG vaccination, cases were scheduled for hospital follow-up at 8 to 16 weeks.

2.3. TST

Cell- mediated immune response to BCG was assessed using Mantoux test (16) at 8 to 10 weeks after BCG vaccination. Five tuberculin units (TU) and 0.1 mL purified protein derivative (PPD) were used. The Tween 80 preparation produced by BB-NCIPD Ltd was used as the PPD solution. The test solution was administered intradermally to the middle 1/3 inner surface of the skin of the forearm. Induration after 48 to 72 hours was measured. The cases were divided into 3 groups according to the induration size as < 5 mm, 5 - 10 mm and > 10 mm. TST reactivity was defined as an induration of 5 mm or higher. Infants with TST > 10 mm induration were further evaluated for TB by contact screening, physical examination, and chest X-ray.

2.4. Scar Formation

Scar formation was evaluated at 8 to16 weeks of BCG vaccination. The transverse diameter of BCG scar was measured. A visible scar was defined as a scar size equal to or larger than 2 mm in diameter.

2.5. Statistical Methods

The SPSS (statistical package for social sciences) 2007 for Windows 15.0 software was used for statistical analyses of the obtained data. Comparison of weight at the time of vaccination (g) and postnatal age (weeks) among infants with scar size $\leq$ 2 mm was done using Student's t test. Weight at the time of TST and PNA according to TST induration were comapred using oneway ANOVA test. The Chi-square test was used to determine the association between BCG scar and TST. The effects of gender, GA, birth weight, and birth weight according to GA on BCG scar formation and TST induration were also examined using Chi-square test. Karl Pearson correlation coefficient (r) was obtained between scar size and TST reaction size.

The results were evaluated with a confidence interval of 95% and at a significance level of $P < 0.05$.

The study was initiated after obtaining consent from the ethical committee of the hospital (Number: 134). The aims of the study were explained to the parents of children, and written informed consent was obtained.

3. Results

Among the 216 infants, the GA was $\leq$ 32 weeks in 65 infants, 33 to 36 weeks in 79, and 37 to 40 weeks in 72. The mean birth weight was 2099 g (730 - 4590 g). Of the total infants, 25% were preterm infants under 1500 g, 49% weighted between 1500 and 2500 g, and 26% were over 2500 g. Of the infants, 46 (21.3%) had growth retardation based on GA (SGA) (Table 1).

PNA of the infants ranged from 8 to 30 weeks with a mean of 11.26 weeks at the time of BCG vaccination. The mean corrected age was 6.27 weeks, and the mean weight was 4442 g (range 2100 - 7700 g). BCG scar formation was detected in 121 (56%) infants. GA, birth weight, weight appropriate for GA, type of delivery, PNA or corrected age at the time of BCG vaccination were not significantly associated with BCG scar formation. However, a significant relationship was found between scar formation and weight at the time of vaccination. Mean weight was 6976 g and 6450 g in infants with scar size $\geq$ 2 mm and < 2 mm, respectively ($P = 0.002$). Of the male infants, 65% developed a scar $\geq$ 2 mm compared to 48% of the female infants ($P = 0.014$).

Mean PNA and corrected age at the time of TST evaluation were 24 (range 16 - 40) and 19 (range 9 to 34) weeks. Mean weight was 6747 g (range 3800-9700 g). Tuberculin response rate was 57% (43.1% showed a TST induration of < 5 mm, 52.8% 5-10 mm and 4.2% >10 mm). None of the infants with a TST induration of > 10 mm was detected to have TB by clinical, radiological, or microbiological evaluation. Gender, type of delivery, weight appropriate for GA,

Table 1. Demographic Characteristics of BCG Vaccinated Infants

Characteristics	No. (%)
Gender	
Male	116 (53.7)
Female	100 (46.3)
Gestational age, w	
≤ 32	65 (30.1)
33 - 36	79 (36.6)
≥ 37	72 (33.3)
Birthweight, g	
< 1500	54 (25.0)
1500 - 2500	106 (49.1)
≥ 2500	56 (25.9)
Birth weight according to gestational age	
SGA	46 (21.3)
AGA	162 (75.0)
LGA	8 (3.7)

Abbreviations: AGA, appropriate for gestational age; LGA, large for gestational age; SGA, small for gestational age.

PNA, corrected age, and weight at the time of TST did not have a significant effect on TST response. TST response rate was 67%, 55%, and 47% according to GA ≥ 37 w, 33 - 36 w, and ≤ 32 w, respectively (P = 0.020). TST response rate was 80%, 54%, and 50% according to birth weight > 2500 g, 1500 - 2500, and < 1500 g, respectively (P = 0.213) (Table 2).

Of the infants, 68.4% with TST induration of 5 - 10 mm and 100% with TST > 10 mm developed scars ≥ 2 mm (r = 0.360, P = 0.00).

4. Discussion

Although the size of a TST reaction does not predict protection against TB disease (17), tuberculin response is usually used to assess the infant's response (8). We found that GA was a significant factor in development of TST response. Only 50% of the preterms with GA ≤ 32 weeks developed a TST response following BCG vaccination. No significant difference was detected between birth weight and TST response. Negrete-Esqueda and Vargas-Origel compared tuberculin conversion in 42 preterm infants born older than 30 gestational week and 42 term infants. The TST response rate was similar in both groups (81% vs. 86%) (11). Okan et al. evaluated 35 infants born at less than 35 weeks gestation and detected a TST response in 20 (57%) of them, which was similar to our results (18). Sedaghatian and Kardouni vaccinated 70 preterm infants (22 with a GA of < 32 weeks). The TST response rate was 18% and 38% in babies with GA < 32 weeks and 33 to 36 weeks, respectively. The authors suggested these low tuberculin conversion rates might have occurred because they did not exclude babies having respiratory distress syndrome, ventilatory support, jaundice, or apnea (12). In a second study by the same group, it was concluded that birthweight was significantly associated with tuberculin skin test reaction (19). No impairment of TST response was detected in babies with intrauterine growth retardation (20).

Tuberculin sensitivity appears 3 to 12 weeks after contact with antigens of tuberculosis bacilli. Many researchers performed TSTs 10 to 12 weeks after BCG (6, 9, 21). Soares et al. measured the magnitude and kinetics of the BCG-induced response using flow cytometry to quantify CD4+ T cells, expressing IFN-γ, TNF-α, IL-2, and/or IL-17.They showed that the BCG-specific CD4+ T-cell response peaked at 6 to 10 weeks after vaccination (22). The cell-mediated immune response to BCG was assessed using the Mantoux test and the lymphocyte migration inhibition test (LMIT) 6 to 8 weeks after BCG vaccination (9). In our study, TST response was evaluated 8 to 10 weeks after BCG vaccination according to the feasibility of infants. In a similar study among preterm infants in Turkey, TSTs were conducted 8 to 16 weeks after BCG vaccination (18).

The second most common measure of the effect of BCG vaccination is scar formation. Following BCG vaccination, a scar (measuring 2 - 8 mm) develops at the vaccination site in 86% to 96.4% of the vaccinees at 12 weeks, however, around 10% to 15% of them may not develop the scar (6). We evaluated scar development 8 to 16 weeks after BCG vaccination. In a study conducted in Peru, scar formation was assessed biweekly during the first 6 months. The scar size increased steadily during 7.5 weeks after vaccination and was then stabilized. A "visible scar" was defined as a scar measuring 2 or more millimeters. Scar failure rate was 1.4% (23).

We found that 56% of the infants developed scars ≥ 2 mm. No significant difference was detected in scar formation among the term and preterm babies. Birth weight did not affect scar development. However, the only factors affecting scar response were weight at the time of vaccination and gender. Dhanawade et al. reported that 64 out of 70 term infants exhibited a visible scar after 12 weeks of vaccination representing a scar failure rate of 8.6% (24). In preterms, BCG scar development was reported to be 69% and 90% (9, 12). Kaur et al. evaluated infants who were given BCG vaccine within 7 days after birth in the 3-month BCG scar response. They reported a positive scar response in 45.4% and 50% of the infants born under and over 2500 g, respectively, but the difference was not significant (25). Roth et al. detected no difference between low birth weight

Table 2. Characteristics of the Infants in Relation to TST Induration Following BCG Vaccination

	TST, mm			P Value
	< 5	5 - 10	> 10	
Gender				0.866
Female	48 (41.4)	63 (54.3)	5 (4.3)	
Male	45 (45.0)	51 (51.0)	4 (4.0)	
Birthweight, g				0.213
< 1500	27 (50.0)	26 (48.1)	1 (1.9)	
1500 - 2500	46 (43.4)	57 (53.8)	3 (2.8)	
> 2500	20 (35.7)	31 (55.4)	5 (8.9)	
Gestationalage, w				0.020
$\leq$ 32	34 (52.3)	30 (46.2)	1 (1.5)	
33 - 36	35 (44.3)	43 (54.4)	1 (1.3)	
$\geq$ 37	24 (33.3)	41 (56.9)	7 (9.7)	
Birth weight according to gestational age				
AGA	76 (46.9)	78 (48.1)	8 (4.9)	
SGA	15 (32.6)	30 (65.2)	1 (2.2)	
LGA	2 (25.0)	6 (75.0)	0 (0)	0.193

Abbreviations: AGA, Appropriate for Gestational Age; LGA, Large for Gestational Age; SGA, Small for Gestational Age.

and normal birth weight children in TST or BCG scarring (26).

The correlation between BCG scar and TST is controversial. Children with a BCG scar or a positive tuberculin skin test reaction had a better survival rate than children who had no measurable reaction (27). In the present study, 63.4% of the babies with negative TST failed to develop a visible scar. However, the correlation was very weak to be interpreted as positive. This relationship might have been due to the large sample size. A good correlation between scar positivity and tuberculin conversion was reported among term infants (18). Mallol et al. reported no difference among infants with and without scar formation in terms of TST response (28). Rani et al. tested cell- mediated immunity in 655 BCG vaccinated babies and found that in vitro leukocyte migration inhibition levels against PPD were similar regardless of scar formation. They concluded that failure of formation of a BCG scar may not necessarily imply failure of immunization (6). Recently, a strong association has been found between IL-5 or IL-13 responses to PPD soon after BCG vaccination and scar formation at 4 years. The authors commented that assessing scar size reflects an early Th2 response induced by BCG rather than generally accepted Th1 responses (29).

Our study included babies with a wide range of gestational age (26 - 40 weeks) and birth weight (730 - 4590 g). Thus, we were able to check scar formation and TST response in low birth weight infants and very small preterms. Prematurity or birth weight did not have an effect on BCG scar formation. On the other hand, one half of the preterm infants $\leq$ 32 weeks and infants < 1500 g did not produce a TST response. However, babies small for their gestational age produced efficient TST responses. These results should be confirmed by further studies.

4.1. Conclusions

Although TST is not completely consistent in the protection provided by BCG vaccine against TB, we could conclude that GA had a significant effect on TST response in this cohort of infants. BCG scar formation was related to weight at vaccination rather than prematurity. The correlation between scar formation and TST response was very low to be interpreted as positive.

References

1. WHO . Basic Immunization Schedules and Strategies. Global Programme for Vaccines and Immunization/EPI/WHO Immunization policy. ; 1996.
2. Clarke A, Rudd P. Neonatal BCG immunisation. *Arch Dis Child.* 1992;**67**(4):473-4. [PubMed: 1580673].
3. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis.

Meta-analysis of the published literature. *JAMA.* 1994;**271**(9):698–702. [PubMed: 8309034].
4. Thilothammal N, Krishnamurthy PV, Runyan DK, Banu K. Does BCG vaccine prevent tuberculous meningitis?. *Arch Dis Child.* 1996;**74**(2):144–7. [PubMed: 8660078].
5. Ildirim I, Sapan N, Cavusoglu B. Comparison of BCG vaccination at birth and at third month of life. *Arch Dis Child.* 1992;**67**(1):80–2. [PubMed: 1739343].
6. Rani SH, Vijayalakshmi V, Sunil K, Lakshmi KA, Suman LG, Murthy KJ. Cell mediated immunity in children with scar-failure following BCG vaccination. *Indian Pediatr.* 1998;**35**(2):123–7. [PubMed: 9707854].
7. EPI . Expanded programme on immunization. Programme review. *Wkly Epidemiol Rec.* 1994;**69**(12):87–90. [PubMed: 8003403].
8. WHO . Global Tuberculosis Report 2013 Geneva: World Health Organization; 2014. Available from: http://www.who.int/tb/publications/global_report/en/.
9. Thayyil-Sudhan S, Kumar A, Singh M, Paul VK, Deorari AK. Safety and effectiveness of BCG vaccination in preterm babies. *Arch Dis Child Fetal Neonatal Ed.* 1999;**81**(1):F64–6. [PubMed: 10375366].
10. Camargos P, Ribeiro Y, Teixeira A, Menezes L. Tuberculin skin reactivity after neonatal BCG vaccination in preterm infants in Minas Gerais, Brazil, 2001-2002. *Rev Panam Salud Publica.* 2006;**19**(6):403–7. [PubMed: 16968595].
11. Negrete-Esqueda L, Vargas-Origel A. Response to Bacillus Calmette-Guerin vaccine in full-term and preterm infants. *Am J Perinatol.* 2007;**24**(3):183–9. doi: 10.1055/s-2007-970080. [PubMed: 17372860].
12. Sedaghatian MR, Kardouni K. Tuberculin response in preterm infants after BCG vaccination at birth. *Arch Dis Child.* 1993;**69**(3 Spec No):309–11. [PubMed: 8215572].
13. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. *J Pediatr.* 1991;**119**(3):417–23. [PubMed: 1880657].
14. Battaglia FC, Lubchenco LO. A practical classification of newborn infants by weight and gestational age. *J Pediatr.* 1967;**71**(2):159–63. [PubMed: 6029463].
15. Ministry of Health General Directorate of Primary Care . Expanded Immunization Program. Circular : 1-83. (No: B10OTSH0110005). ; 2006.
16. Court D. "Patch Test" and Mantoux Test in Childhood. *Br Med J.* 1939;**1**(4085):824–836 2. [PubMed: 20782291].
17. al-Kassimi FA, al-Hajjaj MS, al-Orainey IO, Bamgboye EA. Does the protective effect of neonatal BCG correlate with vaccine-induced tuberculin reaction?. *Am J Respir Crit Care Med.* 1995;**152**(5 Pt 1):1575–8. doi: 10.1164/ajrccm.152.5.7582297. [PubMed: 7582297].
18. Okan F, Karagoz S, Nuhoglu A. Bacillus Calmette-Guerin vaccination in preterm infants. *Int J Tuberc Lung Dis.* 2006;**10**(12):1337–41. [PubMed: 17167949].
19. Sedaghatian MR, Hashem F, Moshaddeque Hossain M. Bacille Calmette Guerin vaccination in pre-term infants. *Int J Tuberc Lung Dis.* 1998;**2**(8):679–82. [PubMed: 9712284].
20. Ferreira AA, Bunn-Moreno MM, Sant'Anna CC, Ferreira MF. BCG vaccination in low birth weight newborns: analysis of lymphocyte proliferation, IL-2 generation and intradermal reaction to PPD. *Tuber Lung Dis.* 1996;**77**(5):476–81. [PubMed: 8959154].
21. Aggarwal A, Dutta AK. Timing and dose of BCG vaccination in infants as assessed by postvaccination tuberculin sensitivity. *Indian Pediatr.* 1995;**32**(6):635–9. [PubMed: 8613331].
22. Soares AP, Kwong Chung CK, Choice T, Hughes EJ, Jacobs G, van Rensburg EJ, et al. Longitudinal changes in CD4(+) T-cell memory responses induced by BCG vaccination of newborns. *J Infect Dis.* 2013;**207**(7):1084–94. doi: 10.1093/infdis/jis941. [PubMed: 23293360].
23. Santiago EM, Lawson E, Gillenwater K, Kalangi S, Lescano AG, Du Quella G, et al. A prospective study of bacillus Calmette-Guerin scar formation and tuberculin skin test reactivity in infants in Lima, Peru. *Pediatrics.* 2003;**112**(4):e298. [PubMed: 14523215].
24. Dhanawade SS, Kumbhar SG, Gore AD, Patil VN. Scar formation and tuberculin conversion following BCG vaccination in infants: A prospective cohort study. *J Family Med Prim Care.* 2015;**4**(3):384–7. doi: 10.4103/2249-4863.161327. [PubMed: 26288778].
25. Kaur S, Faridi MM, Agarwal KN. BCG vaccination reaction in low birth weight infants. *Indian J Med Res.* 2002;**116**:64–9. [PubMed: 12592992].
26. Roth A, Jensen H, Garly ML, Djana Q, Martins CL, Sodemann M, et al. Low birth weight infants and Calmette-Guerin bacillus vaccination at birth: community study from Guinea-Bissau. *Pediatr Infect Dis J.* 2004;**23**(6):544–50. [PubMed: 15194836].
27. Garly ML, Martins CL, Bale C, Balde MA, Hedegaard KL, Gustafson P, et al. BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa. A non-specific beneficial effect of BCG?. *Vaccine.* 2003;**21**(21-22):2782–90. [PubMed: 12798618].
28. Mallol J, Girardi G, Quezada A, Montenegro C, Espinoza P. [Tuberculin reaction in healthy infants vaccinated with BCG at birth]. *Rev Chil Pediatr.* 1990;**61**(5):252–7. [PubMed: 2089492].
29. Djuardi Y, Sartono E, Wibowo H, Supali T, Yazdanbakhsh M. A longitudinal study of BCG vaccination in early childhood: the development of innate and adaptive immune responses. *PLoS One.* 2010;**5**(11):e14066. doi: 10.1371/journal.pone.0014066. [PubMed: 21124909].