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Research Article

Study of Tuberculin Skin Test Conversion and Bacillus Calmette-Guérin (BCG) Vaccine Scar in Infants Given BCG at Birth

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Abstract: Tuberculosis remains a major public health problem in developing countries including India. Although investigations pertaining to TB vaccines are resurging, immunization against TB is limited to the BCG vaccine. Because sputum production and the Paucibacillary status in children remain the limiting factors to use the gold standard of microscopy for AFB for diagnosis of Tuberculosis. Aim of the study was to evaluate the Tuberculin test conversion with the progression of the BCG vaccination given at birth into scar at 3 month of age. This Prospective study was conducted over a period of 2 years on 100 Newborns. All newborns received 0.1ml of the BCG vaccine as per standard protocol on BCG administration. The infants were reviewed at 6 weeks and 3 months of age for Tuberculin testing. The presence of BCG scar was noted and correlated with tuberculin sensitivity. The study showed Tuberculin skin test reactivity in all 100 infants at 6 weeks but did not show positive conversion and at 3 months Tuberculin test conversion was 54%. The most common BCG reaction observed at 6 weeks following BCG vaccination was an ulcer. There was a positive correlation between birthweight and rate of tuberculin reaction i.e. with increasing birth weight the rate of Tuberculin reaction was also increasing. This study shows that Tuberculin skin test is a good screening test for BCG vaccination status and correlated with scar of BCG.

Keywords: BCG vaccination, Tuberculin skin test, BCG reactions, Birth weight

INTRODUCTION

Tuberculosis (TB) is a common and fatal infectious disease caused by various strains of Mycobacterium; usually *M. tuberculosis* in humans [1]. Most tubercular infections in humans result in an asymptomatic latent infection and about one in ten latent infections eventually progresses to active disease, which, if left untreated kills more than 50% of its victims [2]. The WHO estimates that one third of the world's population is infected with M. Tuberculosis, with the highest prevalence of Tuberculosis being in Asia [3,4]. Developing countries carry the highest risk with more than 90% of cases and more than 95% of the deaths. Children less than 5 years are most affected with a sharp decline until the mid- teens. The lifetime risk of developing the disease after the infection is 43% in infants, 24% in children between 1 to 5 years and 15% in adolescents, compared to immunocompetent adults who have a lifetime risk of 5 % to 10% [5]. Tuberculosis remains an important cause of mortality and morbidity Worldwide [6,7]. India accounts for one fifth of the global incidence of TB cases. Each year nearly 2 million people in India develop TB of which around 0.87 million are infectious cases. In India nearly 3-4 million children have tuberculosis and another 94 million are at risk for this disease. The annual infection rate is about 3% [8,9]. The diagnosis of TB remains fraught with uncertainties, though the gold standard is to demonstrate the presence of Mycobacteria in some body sample commonly sputum or by culture techniques. However in a paucibacillary situation like in Paediatric TB, the above remains a difficult possibility and hence certain indirect tests are taken as surrogate of existence of the disease. One of the commonly used tests in this is Tuberculin test and the various modifications of the same. These tests though not specific are said to be sensitive but have their limitations and need intelligent interpretation of the test in a given situation. BCG administration also interferes with the result of the tests and the present study is undertaken to evaluate the level of interference.

OBJECTIVE

To evaluate tuberculin test conversion in relation to bacillus calmette-guérin (BCG) vaccine scar in infants.

MATERIALS AND METHODS

This Prospective study was conducted at a tertiary care hospital over a period of 2 years. 100 Term newborns fulfilling inclusion criteria were included in the study after obtaining informed consent from the parents and followed up till 3 months of age. Newborns with a) significant neonatal illness needing intensive care b) history of TB contact in the family c) born to HIV positive mother were excluded from the study. All newborns received 0.1ml of the BCG vaccine, which was stored at 2-4°C.The vaccine was administered intradermal route over left deltoid region in the first week of life, according to standard vaccination schedule given at our hospital after cleaning with saline by the investigator as per standard protocol on BCG administration. The infants were reviewed at 6 weeks and 3 months of age. At these reviews Tuberculin testing was done using the purified protein derivative (PPD). Manufacturers claim that the source material is calibrated against Batch RT 23. It was diluted with a special buffer containing Tween 80 as a stabilizer. The strength of PPD used was 1 tuberculin units (TU) per 0.1 ml. The PPD solution was drawn in a sterile tuberculin syringe. The skin of volar aspect of left forearm was cleaned and then stretched with one hand. The solution was injected intradermally raising a bleb of 6-8 mm diameter. The reading was taken 72 hours after the injection. Induration was measured by the Pen method and recorded in millimetres using non-stretchable measuring tape. The presence of BCG scar was noted and correlated with tuberculin sensitivity. At 3rd month visit the BCG scar was also evaluated in terms of presence, size and any other characteristics.

Statistical Methods

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Chi-square/ Fisher Exact test and Karl Pearsons Correlation Coefficient (r = 0.28689) method has been used to find the significance of study parameters on categorical scale between two or more groups. 95% Confidence Interval has been computed to find the significant features. Paired proportion test has been used to find the significance of Size (mm) at 6 weeks and 3 months.

RESULTS

Total 100 newborns included, female (53%) more than male(43%).Majority (72%) of babies were weighing between 2.5 to 3 kilograms[Table.1].

Most (90 %) babies born at Gestational age of 37-38 weeks [Table.2].

Majority of babies received BCG vaccine within 48 hours of birth. 89% of babies showed a wheel of >5mm at the site of vaccination [Table.3].

It was observed that most of the babies (88%) were exclusively breast fed at 6 weeks of life. All babies showed reaction at 6 weeks of age, 91% in the form of an ulcer , 6% papule and 3% of them had an abscess[Table 4].

Exclusively breastfed babies showed good weight gain than top feed babies at 10 weeks of age[Table.5].

Newborns with BCG reaction of a papule, 3 out of 6 had positive TST reaction.2 out of 3 with abscess as BCG reaction had positive TST reaction and 76 out of 91 who had Ulcer as BCG reaction showed TST reaction positive[Table6].

All the newborns who were on top feeds did not show a tuberculin conversion after giving BCG vaccination at birth and 54 out of 88 who were on EBF showed positive TST reaction[Table.7].

Tuberculin skin test done at 6 weeks had reaction <5mm in all the babies as a result of delayed hypersensitivity reaction which usually takes more than 6 weeks for the response to come [Table.8]**.**

The Tuberculin skin test reaction was significantly positive (> 5mm) in 54% of the study newborns and in remaining 46% the reaction was negative (< 5mm). It was observed that majority of the newborns had TST conversion at 3 months of age after giving BCG vaccination[Table.9].

It was noticed that TST conversion was more in females (53.7%) than males (46.3%). TST induration was proportionately more with increasing birth weight[Table 10].

No significant difference in TST reaction with gestational ages of the term babies[Table.11].

The overall tuberculin conversion rate after 6 weeks was Nil and at 3 months was 54%. We observed that there is significant TST conversion at 3 months of age.

Table-1: Distribution of cases according to Birth weight (n=100)

Birth weight (kg)	Number of infants	%
2.50-3.00	72	72.0
3.0-3.50	20	20.0
>3.50	8	8.0

Table-2: Distribution of cases according to Gestational age.

Gestational age (weeks)	Number of infants	%
37-38	90	90.0
39-40	10	10.0

Table3: Distribution of cases according to day of BCG vaccination at birth and Size of the wheal.

Age at BCG vaccination	Number of infants (n=100)	%
0 days	12	12.0
1-2 days	38	38.0
3-4 days	26	26.0
5-6 days	24	24.0
Size of wheal (mm)		
<5mm	11	11.0
≥5 mm	89	89.0

Table-4: BCG reactions at 6 weeks following vaccination

Type of Reaction	Number of infants (n=100)	%	95%CI
Ulcer	91	91.0	83.77-95.19
Papule	6	6.0	2.78-12.48
Abscess	3	3.0	1.03-8.45

Table-5:.Distribution of cases at 10 weeks according to weight and type of feeding.

Weight(kg) at 10 weeks	EBF(n=88)	Top Feeds(n=12)
4.0-5.0	7	11
5.1-6.0	71	1
>6.0	10	0.0

Table-6:Correlation of vaccination site lesions and PPD skin at 3 months

Type of reaction	Number of infants (n=100)	Tuberculin test (2): Size mm	
		≤5.0	>5.0
Papule	6	3(50%)	3(50%)
Abscess	3	1(33.3%)	2(66.7%)
Ulcer	91	15(16.4%)	76(83.5%)

Table7: Distribution of cases according to feeding and TST conversion at 3 months

Type of Feed	Number of infants (n=100)	Tuberculin test (2): Size mm	
		≤5.0	>5.0
EBF	88	34	54
Top feeds	12	12	0

Table-8:Tuberculin skin test conversion at 6 weeks after BCG vaccination.

Tuberculin test (1)	**Number of infants (n=100)**	**%**	**95%CI**
Reaction after 72hrs			
Yes	100	100.0	96.30-100.0
No	0	0.0	-
Size (mm)			
≤5.0	100	100.0	96.30-100.0
>5.0	0	0.0	

Table-9:Tuberculin skin test conversion at 3 months following BCG Vaccination

Tuberculin test (2)	**Number of infants (n=100)**	**%**	**95%CI**
Reaction after 72hrs			
Yes	100	100.0	96.30-100.0
No	0	0.0	-
Size (mm)			
≤5.0	46	46.0	36.56-55.74
>5.0	54	54.0	44.26-63.44

Table-10:.Birth weight wise distribution of cases with TST conversion at 3 months of age.

Birth weight (kg)	**Number of infants (n=100)**	**Tuberculin test (2): Size mm**		
		≤5.0	**>5.0**	P value0.0001
2.50-3.00	72	36(50%)	36(50%)	
3.0-3.50	20	9(45%)	11(55%)	
>3.50	8	1(12.5%)	7(87.5%)	

Table-11: Gestational age wise distribution of cases with TST conversion at 3 months of age.

Gestational age (weeks)	**Number of infants (n=100)**	**Tuberculin test (2): Size mm**	
		≤5.0	**>5.0**
37-38	90	38(42.3%)	52(53.7%)
38-39	10	8(80%)	2(20%)

Table-12:Evaluation of Tuberculin test (s) Size at Six weeks and 3 months.

Tuberculin test: Size (mm)	**At 6 weeks (n=100)**	**At 3 months (n=100)**
≤5.0	100	46
>5.0	0	54
Inference	54% of cases, Tuberculin test Size >5 mm at 3 months with Z=10.834; P<0.001**	

DISCUSSION

The present study was undertaken to find the effect of BCG on the tuberculin skin test in the period of infancy since the effect of BCG quite often affects the Tuberculin test reading in clinical situations. The neonates included in the study group were term babies appropriate for weight so that no other confounding variables due to low birth weight or prematurity were included since these factors do have their bearing on the T cell mediated response on which the Tuberculin test depends on. Though all the study population were appropriate for age groups, they were further grouped into three groups (Table-1) to see if the groups do differ in their response to the tuberculin test. Majority of the babies weighed between 2.5 to 3.0 kg as is expected in a rural population coming from lower socio economic status as reported by various authors like M.M.A Faridi [10] et al and Sharifi [11] et al. The groups were also divided into two groups based on gestation age to see if they do differ in their response to the BCG vaccination

(Table 2). Though T cell differentiation starts as early as 20 weeks of gestation, the maturation of T cell function occurs over a period of time. Though at birth it is competent enough to mount a response in the new born period, its maturation progresses in the first year of life [12]. The ability to mount the response depends on the time of administration, type of antigen, route of administration and the dose of the antigen particularly for Cell mediated immunity. To see the effect of the day of administration and also the efficacy of the same on the tuberculin reaction, the study groups were also studied for the effect due to day of administration and the size of wheal generated while administration (Table-3). Breast feeding has a lot of significant beneficial effects for the neonate. One of the remarkable and child protection effects is its anti-infective properties which are either non specific or specific mediated through its humeral effect or by its effect on the cell mediated immunity [13]. In the present study we also evaluated the effect of breastfeeding on tuberculin conversion which would act as a surrogate marker of cell mediated immunity. The hospital being a baby friendly hospital where exclusive breast feeding is the norm, this group constituted a majority of the study population even as late as 3 months of age (Table 7).The effectiveness of BCG vaccination is clinically assessed by the response it produces weeks after administration and its effect on tuberculin test though for determining its functional efficacy it would require far more advanced tests like lymphocyte migration inhibition test etc. In the present study at the 6 week follow up all the cases had manifested with response at site of administration(Table 4) the most common being a superficial ulcer. The tuberculin response is also dependant on the nutrition status of the infant. To study this the weight gain by the study population at 10 weeks based on the type of feed was also studied (Table 5) in which breast fed children which not surprisingly was associated with better weight gain than top fed infants. The correlation of BCG change at the site of administration to tuberculin reaction was studied (Table.6). Ulceration/ abscess formation at site was associated with higher percentage of tuberculin reaction compared to papule formation which was statistically significant. Since tests for CMI are costly, this test probably confirms presence of scar which is fallout of the local reaction as an adequate indicator of CMI in that child. Similar correlation has also been established by other workers like Chung min shen et al [14]. Breast feeding as already reiterated is associated with significant protective effects involving both humeral and cell mediated immunity. The present study (table 7) also showed proof of the same. Of the 88 children who were exclusively breast fed 100% had positive response to tuberculin test with over 62% showing tuberculin conversion of > 5 mm at 3months of age. Though the 12 infants not on exclusive breast feed did show a tuberculin response, none of them had a response > 5 mm indicating higher level of reactogenicity in breast fed children which was statistically significant. The time for manifestation of CMI is also dependant on the time duration since exposure to the antigen. The time for onset of this could be as less as 4 weeks but is usually 10 to 12 weeks. In the present study the tuberculin response though present in 100 % of cases did not achieve significant level in any of the cases at 6 weeks but at 12 weeks over 54% of cases had a tuberculin conversion (Table12). This is similar to findings by Faridi et al[10] who noticed a higher level of conversion of 42.7% in their study at 12 weeks. Though no specific changes are expected in terms of response to CMI between the sexes the present study looked at the response of tuberculin conversion in male neonates versus female neonates. The tuberculin response in male neonates versus female neonates was almost similar and was not statistically significant. Sharifi et al[11] In their study also did not find any difference in response between the sexes in their study of over neonates. Nutrition has a very important bearing on the generation of CMI. Though malnutrition is often associated with poor immunity, and better nutrition is associated with good CMI. In the present study this correlation was amply proved. Infants with higher weight gain were associated with higher level of tuberculin responsiveness as compared to lower weight gains and this response was statistically significant(Table 10). This type of response has been noticed by other workers like M.M.A Faridi et al thereby emphasising the importance of better nutrition as an importance strategy of infection control against tuberculosis. Comparison of the response to tuberculin as per gestation age revealed interesting findings. Neonates with gestation of 37 -38 weeks were equivocal in tuberculin conversion. The group of 38-39 weeks had a larger percentage with response less than <5 mm (Table11). Other workers did not find any difference in response at the various gestation ages and may need a larger study to see if this difference is significant or not [10]. The tuberculin test at 6 weeks and when followed up at 12 weeks (table 12) showed higher response rates in terms of size of reaction though reaction rates were the same in both the groups. This phenomenon is explainable by the fact that CMItakes time to mature anytime from 6 weeks to 6 months as reflected in this study. However the boosting response of previous tuberculin reaction on the subsequent test cannot be discounted though it is more likely if it is done within a period of 4 weeks from the previous test unlike our study where the repeat test was done after a period of 6 weeks from the previous tests. Similar findings have also been reported by the study of M.M.A Faridi et al[10]. In no case was the response significant enough to cause a diagnostic dilemma of a positive test of > 10 mm and hence the relevance of tuberculin test in diagnosis of tuberculosis in children less than 3 years still is a valid tool in tuberculosis diagnosis. This study was able to demonstrate a significant correlation of Tuberculin response to BCG administration in a rural population of neonates who were largely exclusively breast fed proved utility and implications of using

tuberculin test in the diagnosis and screening for Tuberculosis.

CONCLUSIONS

- The tuberculin response is dependent on the nutrition status of the infant. Breast fed children have better weight gain than top fed infants.
- Infants with higher birth weight were associated with significantly higher level of tuberculin responsiveness as compared to lower birth weight.
- Ulceration, abscess formation at site is associated with significantly higher percentage of tuberculin reaction compared to papule formation.
- Tuberculin skin test takes more than 6 weeks for the response to come after giving BCG vaccination at birth.
- The tuberculin test at 6 weeks and when followed up at 12 weeks showed higher response rates in terms of size of reaction though reaction rates were the same in both the groups.

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