CELL MEDIATED IMMUNITY IN CHILDREN WITH SCAR-FAILURE FOLLOWING BCG VACCINATION

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Objective: *To find out the incidence of BCG-scar failure, in BCG vaccinated children and assess their in vitro cellular response.* ***Design:*** *Four year prospective cohort observational study.* ***Setting:*** *Immunization centers at: (a) State Tuberculosis Center; (b) Tuberculosis Association of Andhra Pradesh; and (c) Niloufer Hospital for Women and Children in Hyderabad.* ***Methods:*** *Healthy children brought to the immunization centers for BCG vaccination and were followed up till 6 months of age for scar failure. These 655 BCG vaccinated children were classified into three groups based on the age at vaccination: (i) 0 day-1 day; (ii) 2 days-30 days; and (Hi) 31 days-90 days. Of these children, in vitro leukocyte migration inhibition (LMI) levels against PHA/PPD were investigated in 228 of them.* ***Results:*** *Of the 655 children, 591 (90.2%) showed presence of scar. Out of the three groups, number of children belonging to the first group in whom the scar was absent, was highest. Of 591 children with scar, LMI was performed in 34,110 and 43 of them in the three different age groups, respectively out of whom 88.2%, 87.2% and 86% had positive response (> 20%) to PPD. Of 64 children who failed to develop a scar, LMI was performed in 17, 19 and 5 in three different age groups out of whom 88.2%, 94.7% and 80% had positive (> 20%) in vitro response to PPD.* ***Conclusion:*** *Scar failure may occur in 10% of BCG vaccinated and is more common with immunization within 48 hours of life. Failure of formation of BCG-scar at the site of BCG vaccination may not necessarily imply failure of immunization because majority of them do elicit positive in vitro LMI response.*

Key words: *BCG vaccination, Immune response, Leukocyte migration inhibition test.*

BACILLUS Calmette Guerin (BCG) vaccine has been extensively used since 1928 for inducing specific immunity against tuberculosis and has been in use in India for about five decades. It was included in the Expanded Programme Immunization (EPI) and is currently restricted to infants, under the Universal Immunization Program (UIP)(1).

Since its inception, the efficacy of BCG vaccine has been controversial despite several trials carried out in different countries. In most studies, differentiation between vaccinated and unvaccinated subjects in the study population was made on the basis of presence or absence of scar(l-4). Thus, inspection and counting of post BCG vaccination scar at the site of vaccination is thought to act as an important index in BCG vaccination programs(2) implying the crucial role of scar formation in the evaluation of uptake of the vaccine and effectiveness of the program.

Leukocyte migration inhibition factor (LIF) is one of the lymphokines secreted by sensitized lymphocytes on antigenic stimulation. This lymphokine is tested *in vitro* by leukocyte migration inhibition test (LMIT).

The aims of this study were to estimate the incidence of failure of formation of BCG scar and to evaluate the *in vitro* response by leukocyte migration inhibition test.

Subjects and Methods

Children who were brought to the immunization centers of *(a)* State Tuberculosis Centre, *(b)* Tuberculosis Association of Andhra Pradesh and *(b)* Niloufer Hospital for Women and Children in Hyderabad were included in the study during the period 1990-1994. Children were immunized with BCG by trained and skilled vaccinators. While a single vaccinator vaccinated all the children in the first two centers, in the last center two persons were involved. The same dose (0.05 ml) was used for immunization of BCG in three centers. It was administered on the deltoid region of the left arm.

Children (n=655) vaccinated either at birth or within three months of age were included and were classified accordingly, namely, (z) 0 day-lday; (n) 2 days-30days; *(Hi)* 31 days-90 days. After an informed consent, parents were given a reference card: a detailed history of the child including date of birth, date of BCG vaccination, sex, skin fold thickness and general health status were also recorded. Children who were malnourished or those having infection requiring treatment with antibiotics or children with a history of exposure to a case of tuberculosis were excluded. Although parents were given a follow up date 3 months after vaccination, blood sample was collected from children brought between 3 to 6 months after BCG vaccine was administered.

Leukocyte migration inhibition (LMI) test was performed to assess the *in vitro* cell mediated immune (CMI) response by the capillary method. Phytohemagglutinin (PHA) was used as a general mitogen and purified protein derivative (PPD-Span diagnostics) was utilized as a specific antigen. The per cent migration inhibition was calculated as

$$100 \quad \frac{\text{(Area of migration with antigen)}}{\text{(Area of migration with out antigen)}} \times 100$$

and > 20% was considered to be positive. The LMI test was carried out in 228 children. Statistical analysis was done by Chi-Square test.

Result

Of 655 BCG vaccinated children, 591 (90.2%) showed presence of scar. Out of 591 scarpositive children, 34 (65.3%), 270 (88.6%) and 287 (95.8%) belonged to three different age groups, respectively *(Table I);* while out of 64 children who failed to develop BCG-scar 18 (34.7%), 33 (11.4%) and 13 (4.2%) belonged to these age groups, respectively. Thus scar-failure was higher if BCG vaccination was given at birth ($p < 0.05$).

Among 187 BCG-scar positive children tested for LMI; 30/34 (88.2%), 96/110 (87.2%), and 37/43 (86%) had positive LMI levels to PPD in three age groups respectively *(Table II)*. Within 41 scar-negative group children tested for LMI, 15/17 (88.2%) 18/19 (94.7%), and 4/5 (80%) had positive (> 20%) LMI levels to PPD in three different age groups *(Table II)*. There were no significant differences ($p > 0.05$) among children with positive LMI between the scar-positive and scar-negative groups.

TABLE I–*Scar Prevalence in Children Vaccinated with BCG at Different Ages.*

Scar Status	Age at vaccination		
(n=655)	0 day-1 day	2 days-30 days	31 days-90 days
Scar absent (n=64)	18 (34.7%)	33 (11.4%)	13 (4.2%)
Scar Present (n=591)	34 (65.3%)	270 (88.6%)	287 (95.8%)

p <0.05 between scar and age at vaccination.

TABLE II–*LMI Positivity in Relation to Scar Status.*

Scar Status	Age at vaccination		
(n=228)	0 day-1 day	2 days-30 days	31 days-90 days
Scar absent (n=41)	15/17 (88.2%)	18/19 (94.7%)	4/5 (80%)
Scar Present (n=187)	30/34 (88.2%)	96/110 (87.2%)	37/43 (86%)

Discussion

The aim of BCG vaccination is to induce protective immunity against tuberculosis. A correct intradermal injection of a potent vaccine gives rise to a local superficial ulcer after about 6 weeks and after healing it leaves a permanent round scar, typically 2-8 mm in diameter(5). In this study, immunization was carried out by trained and skilled vaccinators.

Majority of the children (90.2%) developed scar after BCG vaccination in the present study. A similar pattern has been observed in other studies as well (86% to 96.4%)(3,5-9). This implies that the formation of scar at the site of BCG vaccination is a usual phenomenon. Absence of scar at the site of vaccination in the remaining children is a cause for concern.

The chances of not developing a scar were higher when the children were vaccinated at birth compared to the other groups of children in the present study. In one report, 9.2% of children who received BCG in neonatal period and 4.2% of those vaccinated in the postnatal had scar failure(8). In another study, 25% of children in whom BCG was administered at birth did not develop scar(10). It has been suggested that an appreciable proportion of infant BCG vaccinations probably do not leave a permanent scar(5).

The relatively low incidence of scar-formation among children who received BCG immediately after birth, could be attributed to lack of maturation of immunocompetent cells or due to some perinatal phenomenon(10). Conversely, the comparatively higher incidence of scar-formation in the children vaccinated at a later age may be due to higher post vaccination allergy. There is no reason for concern if failure of scar-formation is due to lack of post-vaccination allergy in the former group.

The *in vitro* cellular responses evaluated in the present study were comparable in

the three groups of children, although their age at vaccination differed. In other words those vaccinated at birth were as capable of eliciting an *in vitro* response as the children vaccinated later, an observation also made in an earlier study(11).

In an earlier series, no difference in the incidence of PPD response in scar-negative and scar-positive children was observed(12). Sedaghation *et al.* also found that there were no significant differences between the rate of scarring and tuberculin conversion (in the infants born before or after 32 weeks gestation) (6), although another report from the same group in preterm infants was contradictory(13). In another report, a close relationship was shown between tuberculin reactivity and scar-formation after BCG vaccination(14).

In the present study, most of the scar-negative children (87.6%) showed a positive (> 20%) *in vitro* response to PPD, implying that inspite of scar-failure, PPD sensitized lymphocytes are present in them. This is perhaps akin to earlier observations that an absent skin test response to PPD does not necessarily mean absent cell mediated immunity *(in vitro)* (4,6,15).

In both the groups of children those with and without a BCG-scar, an almost equal number had negative *in vitro* response to PPD. This phenomenon was also observed in an earlier study where a positive *in vitro* immune response to PPD was not universal(16). A similar observation was made by others(17).

It is evident from the results of the present study that in majority of BCG vaccinated normal children, a reaction occurs at the site of BCG vaccination resulting in the formation of a scar. Absence of this scar may however, not mean that the child is not benefited from the vaccination because majority of them do elicit a positive (> 20%) *in vitro* cellular response. Further work is required to evaluate why BCG scar-failure after vaccination in children is common when vaccinated within 48 hours of birth.

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