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ORIGINAL ARTICLE

TO ESTIMATE SERUM ADA LEVELS IN BCG VACCINATED CHILDREN

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ABSTRACT: BACKGROUND: Tuberculosis is an important cause of morbidity and mortality in both adults and children, especially in developing countries. For prevention of childhood tuberculosis, BCG vaccination is advocated. Protection is attained 4-6 weeks after BCG vaccination and is mainly due to cell mediated immunity. After BCG vaccination almost 12 to 15% of neonates do not develop scar but have positive cell mediated immune response. ADA estimation is simple and inexpensive method to assess the cell mediated immunity. **OBJECTIVE:** To estimate serum ADA levels in children with and without BCG scar, after receiving BCG vaccination. **MATERIAL AND METHODS:** This prospective observational study was conducted at a tertiary care hospital for a period of 2 years. Babies in post natal ward and infants up to the age of 12 weeks attending well baby clinic for BCG vaccination were included in the study. Serum ADA levels were estimated before BCG vaccination and 12- 14 weeks after the vaccination. ADA levels were estimated by colorimetric method. Presence or absence of BCG scar was noted at 12-14 weeks of age. **RESULTS:** A total of 75 babies followed up, of which only 60 babies noted to have scar and in rest 15 babies there was no scar noticed. Twenty unvaccinated babies at 12 weeks of age were included as controls. The Mean ADA levels are significantly elevated after BCG vaccination (34.12 ± 3.28 U/L) in comparison to levels before vaccination (12.55± 2.64 U/L) with p value < 0.001. The mean ADA levels in unvaccinated babies at 12weeks was (17.25 ± 2.45 U/L) significantly (p value < 0.001) lower than the ADA levels (34.12 ± 3.28U/L) of vaccinated babies. There was no statistically significant difference in the mean ADA levels of children with (34.18 ± 3.52U/L) and without (31.22 ± 2.32U/L) scar after BCG vaccination (p value > 0. 06). **CONCLUSION:** After BCG vaccination, there is increase in serum ADA levels indicating adequate immunity. Increase in ADA levels in children without scar after BCG vaccination may indicate the probability of adequate immunity.

KEYWORDS: BCG Vaccine, ADA Levels, Scar.

INTRODUCTION: Tuberculosis (TB) is an important cause of morbidity and mortality in both adults and children, especially in developing countries. It is the first infectious disease to be declared as global health emergency in 1993.[1] In 2000, 8.3 million incident cases of TB were reported, an estimated 11 percent were children and the reported proportion of TB occurring in children ranged from 3-25 percent.[2] For prevention of childhood tuberculosis, BCG vaccination is advocated. Protection is attained 4-6 weeks after BCG vaccination and is mainly due to cell mediated immunity. BCG vaccination site forms a small hypo pigmented scar 5-7 mm in size by 10-12 weeks.[3] It has been seen by invitro estimation of Cell Mediated Immune Response (CMIR) after BCG vaccination that almost 12 to 15% of neonates do not develop scar but have positive CMIR.[4] 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.[5]

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Various methods are there to assess cell mediated immune response (CMIR) like lymphocytic migration inhibition test, phytohemagglutination inhibition test, Adenosine De Aminase (ADA) estimation. ADA is simple and inexpensive unlike other sophisticated and expensive ones.[6] Adenosine deaminase is an enzyme secreted by activated T lymphocytes and macrophages and is raised when cellular immunity is stimulated.[6]

Since BCG vaccination is known to stimulate cell mediated immunity, which in turn would raise the ADA levels, it was suggested that ADA levels could indicate successful BCG vaccination. There is paucity of studies in this direction. Hence a study was conducted to determine the relation between BCG vaccination and serum ADA levels.

OBJECTIVE: To estimate serum ADA levels in children with and without BCG scar, after receiving BCG vaccination.

MATERIAL AND METHODS: This prospective observational study was conducted at a tertiary care hospital for a period of 2 years (November 2009 to October 2011). Babies in post natal ward and infants up to the age of 12 weeks attending well baby clinic for BCG vaccination were included in the study. Babies with sepsis, severe birth asphyxia, intrauterine infections, maternal tuberculosis and sick babies were excluded from the study. All the babies fulfilling the inclusion criteria were subjected to detailed history and thorough physical examination after obtaining informed consent from the parents. Venous blood for ADA levels were collected in labelled test tubes from these children before BCG vaccination. These children were followed up in well baby clinic at 12- 14 weeks and noted for presence or absence of BCG scar. A second blood sample for ADA levels was collected during this follow up. Blood sample from unvaccinated children at the age of 12 weeks were also taken, which served as a control group. ADA levels were estimated by colorimetric method.

RESULTS: A total of 100 babies were included in the study and received BCG vaccination, 25 babies lost the follow up. Of the follow up 75 babies total 60 babies noted to have scar and in rest 15 babies there was no scar noticed. There were 20 unvaccinated babies noted during follow up at 12 weeks of age and were included as controls after collecting blood samples for ADA levels. Out of 75 children followed up 44 (58.66%) were male and 31 (41.33%) were female. Out of 20 controls 12 (60%) were male and 8 (40%) were female.

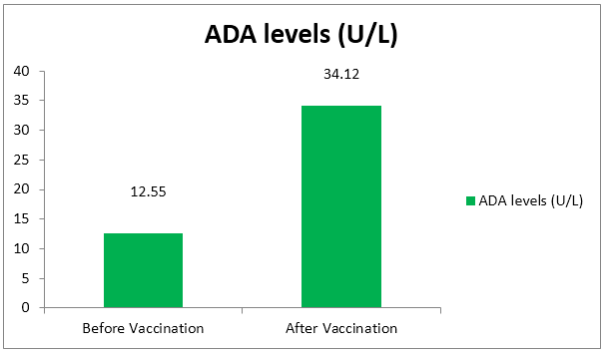

Fig. 1: Comparison of ADA levels of babies before and after BCG vaccination

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The Mean ADA levels at the age of 12 weeks in babies who were vaccinated at birth were 34.12±3.28 U/L and mean ADA levels before BCG vaccination was 12.55±2.64 U/L. This shows significant rise in mean ADA levels by 12weeks following BCG vaccination at birth. This difference is statistically significant with p value < 0.001.

	Vaccinated n=75	Unvaccinated n=20	Statistical significance
ADA levels (U/L) Mean±SD	34.12±3.28	17.25±2.45	P value < 0.001

Table 1: ADA levels in vaccinated and unvaccinated babies at 12 weeks of age

The mean ADA levels of unvaccinated babies at 12weeks was 17.25 ± 2.45 U/L, which is significantly (p value < 0.001) lower than the ADA levels (34.12 ± 3.28U/L) of vaccinated babies at 12 weeks of age.

	Number of babies	Serum ADA levels (U/L) Mean±SD
With scar	60	34.18±3.52
Without scar	15	31.22±2.32

Table 2: ADA levels in babies with scar and without scar after vaccination

Out of the 75 children vaccinated at birth and followed up at 12 weeks of age, 15 babies i.e.20% of children showed no evidence of scar, whereas 60 babies i.e.80% developed scar after BCG vaccination. There was no statistically significant difference in the mean ADA levels of children with (34.18 ± 3.52U/L) and without (31.22 ± 2.32U/L) scar after BCG vaccination (p value > 0.06).

	Vaccinated babies without scar n=15	Unvaccinated Babies n=20	Statistical Significance
ADA levels (U/L) Mean±SD	31.22±2.33	17.25±2.45	P value <0.001

Table 3: ADA levels in scar negative BCG vaccinated children and unvaccinated children at 12 weeks of age

The mean ADA levels (31.22 ± 2.33 U/L) of children without scar after BCG vaccination were statistically higher than ADA levels (17.25±2.45) of unvaccinated children at 12 weeks (p value <0.001).

DISCUSSION: The results of the present study showed significantly higher ADA levels in those children who were vaccinated with BCG. Search of the literature shows paucity of studies in this direction. However we could find a study done by S. Thora et al.[6] from Indore in 1992 which is similar with some minor difference from our study showing the relation between ADA levels and BCG vaccination.

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Study	Serum ADA levels (U/L)		Statistical Significance
	At birth	Post BCG Vaccination	
S. Thora et al	6.69±1.80	13.83±3.87	P <0.001
Present Study	12.55±2.64	34.12±3.28	P<0.001

Table 4

The study by S. Thora et al showed that in 100 normal babies at birth before vaccination, the mean ADA levels were 6.69±1.807 U/L and a level of 13.83±3.870 U/L after vaccination showing an elevation of 7 U/L which indicates significant two fold rise in ADA levels ($p < 0.001$). In present study, mean ADA levels were 12.55±2.64 U/L at birth compared to 6.69±1.807 U/L in the study done by Thora et al. In present study, mean ADA levels after vaccination were 34.12±3.28 U/L when compared to the study done by S. Thora et al which showed mean ADA levels of 13.83±3.870 U/L after vaccination. Even though, there is a difference in ADA levels between both studies at birth, there is an increase in ADA levels after giving BCG vaccination in both studies. The increase in ADA levels from 6.69±1.807 U/L to 13.83±3.870 U/L in study done by Thora et al is about 2 fold increase. Compared to our study whose ADA levels increased from 12.55±2.64 U/L to 34.12±3.28 U/L indicating almost 3 fold increase in ADA levels.

However the ADA levels were measured 6 weeks after vaccination in Thora et al study, compared to 12 weeks after vaccination in the present study. As the present study is conducted to know the significance of presence of BCG scar, the ADA levels were done at 12 weeks after vaccination. The time of sampling of blood for estimation of ADA levels in post vaccinated children may explain to certain extent the difference in observed ADA levels between the two studies. The mean ADA levels at birth in the study group are different from mean ADA levels at birth from other studies, which may be due to the difference in the population and laboratory studied.

In the present study, the mean ADA levels (34.12±3.28 U/L), in post vaccinated children at the age of 12 weeks are higher compared to mean ADA levels (12.55±2.64 U/L) in children before vaccination at birth. The mean ADA levels (12.55±2.64U/L) at birth may not be comparable with mean ADA levels (17.25±2.45 U/L) in unvaccinated children at 12 weeks of age due to several factors which may affect ADA levels like infection and developmental process. Hence the mean ADA levels (34.12±3.28 U/L) in children who are at 12 weeks of age and vaccinated at birth are compared with mean ADA levels (17.25±2.45 U/L) of children at the age of 12 weeks who were not vaccinated (Fig. 2).

The mean ADA levels (34.12±3.28 U/L) in children at 12 weeks of age who were vaccinated at birth were significantly higher ($p<0.001$) when compared to children who were not vaccinated at all 17.25±s2.45 U/L, indicating significantly adequate immunity in vaccinated group compared to unvaccinated group.

Our study showed 15 children i.e., 20% did not develop scar after BCG vaccination, compared to studies done by chinnab asavaiah et al,[7] in which the children who did not developed scar were 25%. Studies done by Thora et al,[6] Surekharani et al,[5] Vijayalaxmi et al,[8] the children who did not develop scar were 8.95%, 9% and 10% respectively showing wide variation in percentage of children developing scar.

Absence of scar in these children who are vaccinated will not give a clear picture of success or failure of BCG vaccination. Hence mean ADA levels of children with and without scar were compared. The mean ADA levels in children with scar (34.18 ± 3.52 U/L) are comparable with mean ADA levels of children who were vaccinated but did not develop scar (31.22 ± 2.32 U/L) ($p > 0.06$). This indicates the probability of adequate immunity not only in those with scar formation but also in those children without scar formation. As the mean ADA levels at birth in the study group are different from mean ADA levels at birth from other studies, to have appropriate comparison, an age appropriate control group is taken at the age of 12 weeks who were not vaccinated and ADA levels were estimated for comparison with ADA levels of those at 12 weeks of age who were vaccinated at birth.

The mean ADA levels (31.22 ± 2.32 U/L) in scar negative vaccinated children at the age of 12 weeks were significantly higher than the mean ADA levels (17.25±2.45) in children who were not vaccinated at the age of 12 weeks. This supports the above assumption that the probability of adequate immunity in those children without scar formation after BCG vaccination.

CONCLUSION: After BCG vaccination, there is increase in serum ADA levels indicating adequate immunity. Increase in ADA levels in children without scar after BCG vaccination may indicate the probability of adequate immunity. Absence of BCG scar may not indicate unsuccessful BCG vaccination.

BIBLIOGRAPHY:

1. Md. Khurshid Alam Hyder, Nani Nair, Tahmeed Ahmed. Global Epidemiology of Pediatric Tuberculosis. In: Seth V, Kabra SK. Essentials of Tuberculosis. 4thed. New Delhi: Jaypee Brothers Medical Publishers (p) Ltd; 2011; p 11-18.
2. Global Tuberculosis Control Report. 2009. Available from: URL http: //WWW. Who. int/tb/Publications/Global/2009/pdf/fullreport. pdf. Accessed November 26, 2009.
3. Kabra SK, Seth V. BCG Vaccination. In: Seth V, Kabra SK editors. Essentials of tuberculosis in Children. 4thed. New Delhi: Jaypee Brothers Medical Publishers (p) Ltd; 2011; p 555-588.
4. Seth V, Kukerja N, Sundram KR. Wanning of Cell Mediated Immune Response in pre-school children given BCG at birth. Indian J Med Res. 1982; 74: 710-715.
5. Surekha Rani H, Vijayalaxmi V, Sunil kumar, Lakshmi kiran A, Suman Latha G, Murthy KJR. Cell Mediated Immunity In Children with Scar Failure Following BCG Vaccination. Indian pediatr. 1998; 35: 123-127.
6. Thora S, Rajasekaran P, Chaparwal BC. Serum Adenosine Deaminase Estimation in Relation to BCG Vaccination. Indian Pediatr. 1995; 32: 1087-1088.
7. Channabasavaiah R, Murali Mohan V, Suryanarayana HV, Krishnamurthy MS, Shashidhara AN. Waning of BCG Scar and its Implications. Ind J Tub. 1993 July; 40 (3): 137-44.
8. Vijayalaxmi V, Devi PS, Murthy KJ, Rao DV, Jain SN. Cell Mediated Immune Responses in BCG Vaccinated Children. Indian Pediatrics. 1993 Jul; 30 (7): 899-903.

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