STUDY OF BCG SCAR AND SERUM ADA LEVELS IN INFANTS

Harishchandra Venkata Yanamandala[1]

[1]*Associate Professor, Department of Paediatrics, Gitam Institute of Medical Sciences and Research, Rushikonda, Visakhapatnam.*

ABSTRACT

BACKGROUND

In developing countries, in both adults and children, tuberculosis is an important cause of morbidity and mortality. In 1993, it is declared as the first infectious disease by global health emergency.[1] According to WHO report globally, there were an estimated 9.27 million ancient cases of TB in 2009. The cases reported were 8.3 million, the children covered an estimated percentage of 11 and it ranged from 3-25 percent.[2] BCG vaccination was advocated for prevention of tuberculosis in children.

The aim of the study is to estimate serum ADA levels in newborns before BCG vaccination, serum ADA levels in children with and without BCG scar, after receiving BCG vaccination, serum ADA levels in children without BCG vaccination and to find significance of serum ADA levels in BCG vaccinated children by comparing the above groups.

MATERIALS AND METHODS

This study was conducted at the Department of Paediatrics, Gitam Institute of Medical Sciences and Research Institute, October 2015 to September 2016. Babies who were in postnatal ward and infants of age of 12 weeks attending for BCG vaccination were included in the study. The total numbers of infants studied were 150.

RESULTS

In our study, out of 120 children included in the study before BCG vaccination comprising group-1, 61% were males and 39% were females. Out of 120 children received BCG vaccination, only 100 came for follow up comprising group-2, of which 67 (67%) were males and 33 (33%) were females. 15 children who did not receive BCG vaccination at 12 weeks of age were included in group 3 out of which 11 (73.33%) were males and 4 (26.67%) were females. Mean ADA levels at the age of 12 weeks in group-2 who were vaccinated at birth were 30.89 ± 5.27 U/L compared to mean ADA levels at the age of 12 weeks in group-3, which was 15.47 ± 1.85 U/L. This shows significant rise in mean ADA levels at 12 weeks of age in those who were vaccinated at birth comprising group-2 compared to their mean ADA levels at birth before vaccination comprising group-1. This difference is statistically significant with p value <0.001. On comparison of ADA levels in vaccinated and unvaccinated babies at 12 weeks of age is statistically significant with p value <0.001. On comparison of ADA levels in babies with scar and without scar after vaccination, there is no statistically significant difference (p value >0.05). ADA levels in scar negative BCG vaccinated and unvaccinated children at 12 weeks of age difference is statistically significant with p value <0.001.

CONCLUSION

There is increase in serum ADA levels in after BCG vaccination administration and even in without scar there by indicating adequate immunity.

KEYWORDS

ADA, BCG Scar, CMIR.

HOW TO CITE THIS ARTICLE: Yanamandala HV. Study of BCG scar and serum ADA levels in infants. J. Evid. Based Med. Healthc. 2017; 4(88), 5172-5175. DOI: 10.18410/jebmh/2017/1033

BACKGROUND

In developing countries, in both adults and children, tuberculosis is an important cause of morbidity and mortality. In 1993, it is declared as the first infectious disease by global health emergency.[1] According to WHO report globally, there were an estimated 9.27 million ancient cases of TB in 2009. The cases reported were 8.3 million, the children covered an estimated percentage of 11 and it ranged from 3-25 percent.[2] BCG vaccination was advocated for prevention of tuberculosis in children. After BCG vaccination, protection is attained 4-6 weeks, which is due to cell-mediated immunity. When 0.1 mL of the vaccine is injected intradermally, it raises a wheal of 5-8 mm in diameter over the injection site. By the 3rd or 4th week post-vaccination, an induration is felt at the vaccination site, which becomes a lump of 6-10 mm by the 6th week. Healing is usually completed by 10-12 weeks and the site is marked by a small hypopigmented scar 5-7 mm in size. The in vitro estimation of Cell-Mediated Immune Response (CMIR) post-BCG vaccination that almost 12 to 15% of neonates do not develop scar, but have positive CMIR was seen by Seth et al. Children who received BCG immediately after birth have

Financial or Other, Competing Interest: None.
Submission 12-10-2017, Peer Review 17-10-2017,
Acceptance 29-10-2017, Published 03-11-2017.
Corresponding Author:
Dr. Harishchandra Venkata Yanamandala,
Flat No. 206, Dr. Staff Quarters,
Gitam University Campus, Rushikonda,
Visakhapatnam-530045, Andhra Pradesh.
E-mail: harivy1971@gmail.com
DOI: 10.18410/jebmh/2017/1033

shown the relatively low incidence of scar formation, which could be attributed to lack of maturation of immunocompetent cells or due to phenomenon perinatally.[3] To assess Cell-Mediated Immune Response (CMIR) like lymphocytic migration inhibition test, phytohemagglutinin inhibition test, Adenosine Deaminase (ADA) estimation, various methods are there. Unlike other sophisticated and expensive ones, ADA is simple and inexpensive.[4] The enzyme of the purine salvage pathway, which is secreted by activated T lymphocytes and macrophages and it is raised when cellular immunity is stimulated is adenosine deaminase.[4] It was suggested that ADA levels could trigger BCG vaccination successfully, since BCG vaccination is known to stimulate cell-mediated immunity, which in turn would raise the ADA levels. This study was conducted to determine the relation between BCG vaccination and serum ADA levels.

MATERIALS AND METHODS

This study was conducted at the Department of Paediatrics, Gitam Institute of Medical Sciences and Research Institute, October 2015 to September 2016. Babies who were in postnatal ward and infants of age of 12 weeks attending for BCG vaccination were included in the study. The total numbers of infants studied were 150.

Inclusion Criteria

Neonates in postnatal ward and infants up to the age of 12 weeks for BCG vaccination. Babies who are not BCG vaccinated till 12 weeks of age.

Exclusion Criteria

Babies with intrauterine infections, sepsis, severe birth asphyxia, maternal tuberculosis and sick babies due to other causes. Parents of those babies who were in postnatal ward and infants up to the age of 12 weeks for BCG vaccination were counselled. All the children in the study group were subjected to detailed history and thorough physical examination as per the proforma. Venous blood were collected from all the subjects in labeled test tube for determination of ADA levels of these children. These babies were then given BCG vaccination. These children were followed up in outpatient when they came for subsequent vaccinations at 6, 10 and 14 weeks. Another blood sample from these children was taken for ADA levels when they came for follow up at 11-15 weeks. At this time, the presence or absence of BCG scar was noted. A control group was considered and blood sample was collected from unvaccinated children at age of 12 weeks. All babies included in the study are grouped based on their vaccination status and presence or absence of scar in vaccinated children. All the newborn babies before BCG vaccination are included in group-1. The above infants who came for follow up at 12 weeks of age are included in group-2. Those children who were vaccinated and developed scar at 12 weeks of age are included in group-2S. Those children who were vaccinated and without scar are included in group-2SA. The babies who are not vaccinated till 12 weeks of age are included in group-3. Total number of babies studied were 150. Children in group-1 were 120. Children lost for follow up after BCG vaccination were 30. Children in group-2 were 100. Children in group 2S (with scar) were 50. Children in group 2SA (without scar) were 35. Children in group-3 were 15.

RESULTS

Out of 120 children included in the study before BCG vaccination comprising group-1, 61% were males and 39% were females. Out of 120 children received BCG vaccination, only 100 came for follow up comprising group-2, of which 67 (67%) were males and 33 (33%) were females. 15 children who did not receive BCG vaccination at 12 weeks of age were included in group 3, out of which, 11 (73.33%) were males and 4 (26.67%) were females. Mean ADA levels at the age of 12 weeks in group-2 who were vaccinated at birth were 30.89 ± 5.27 U/L compared to mean ADA levels at the age of 12 weeks in group-3, which was 15.47 ± 1.85 U/L. This shows significant rise in mean ADA levels at 12 weeks of age in those who were vaccinated at birth comprising group-2 compared to their mean ADA levels at birth before vaccination comprising group-1. This difference is statistically significant with p value <0.001.

	Vaccinated (Group-2); n=100	**Unvaccinated (Group-3); n=15**	**P value**
ADA levels (U/L) (mean ± SD)	30.89 ± 5.27	15.47 ± 1.85	<0.001

Table 1. Comparison of ADA Levels in Vaccinated and Unvaccinated Babies at 12 Weeks of Age

On comparison of ADA levels in vaccinated and unvaccinated babies at 12 weeks of age is statistically significant with 'p' value <0.001.

Group-2	**Number of Babies**	**Serum ADA Levels (u/L) mean ± SD**
With scar (group-2S)	50	30.15 ± 8.99
Without scar after vaccination (group-2SA)	35	29.17 ± 7.41

Table 2. Comparison of ADA Levels in Babies with Scar and without Scar after Vaccination

On comparison of ADA levels in babies with scar and without scar after vaccination, there is no statistically significant difference (p value >0.05).

	Vaccinated without Scar (Group-2SA) (n=35)	**Not vaccinated at 12 weeks Age (Group-3) (n=15)**	**Statistical Significance**
ADA levels (U/L) (mean ± SD)	29.17 ± 7.41	15.47 ± 1.85	<0.001

Table 3. Comparison of ADA Levels in Scar Negative BCG Vaccinated and Unvaccinated Children at 12 Weeks of Age

ADA levels in scar negative BCG vaccinated and unvaccinated children at 12 weeks of age, difference is statistically significant with p value <0.001.

DISCUSSION

In our study, out of 120 children included in the study before BCG vaccination comprising group-1, 61% were males and 39% were females. Out of 120 children received BCG vaccination, only 100 came for follow up comprising group-2, of which, 67 (67%) were males and 33 (33%) were females. 15 children who did not receive BCG vaccination at 12 weeks of age were included in group-3, out of which, 11 (73.33%) were males and 4 (26.67%) were females. Mean ADA levels at the age of 12 weeks in group-2 who were vaccinated at birth were 30.89 ± 5.27 U/L compared to mean ADA levels at the age of 12 weeks in group-3, which was 15.47 ± 1.85 U/L. This shows significant rise in mean ADA levels at 12 weeks of age in those who were vaccinated at birth comprising group-2 compared to their mean ADA levels at birth before vaccination comprising group-1. This difference is statistically significant with p value <0.001. On comparison of ADA levels in vaccinated and unvaccinated babies at 12 weeks of age is statistically significant with p value <0.001. On comparison of ADA levels in babies with scar and without scar after vaccination, there is no statistically significant difference (p value >0.05). ADA levels in scar negative BCG vaccinated and unvaccinated children at 12 weeks of age, difference is statistically significant with p value <0.001. In a study conducted by Surekha Rani et al,[5] their objective was to find out the incidence of BCG scar failure in BCG vaccinated children and assess their in vitro cellular response. It was a 4-year prospective cohort observational study. It was conducted in immunisation centers at state tuberculosis center, tuberculosis association of Andhra Pradesh, Niloufer Hospital for women and children in Hyderabad. Healthy children brought to the immunisation centers for BCG vaccination and were followed up till 6 months of age for scar failure. These 655 BCG vaccinated children were classified into 3 groups based on the age at vaccination- (i) 0 day-1 day; (ii) 2 days-30 days; and (iii) 31 days-90 days. Of these children, in vitro Leucocyte Migration Inhibition (LMI) levels against PHA/PPD were investigated in 228 of them. Of the 655 children, 591 (90.2%) showed presence of scar. Out of the 3 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 3 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 3 different age groups, out of whom, 88.2%, 94.7% and 80% had positive (>20%) in vitro response to PPD. Scar failure may occur in 10% of BCG vaccinated and is more common with immunisation within 48 hours of life. Failure of formation of BCG-scar at the site of BCG vaccination may not necessarily imply failure of immunisation, because majority of them do elicit positive in vitro LMI response. V.K. Chadha et al[6] conducted a study, the tuberculin sensitivity patterns among BCG vaccinated and unvaccinated children and possibility of estimating annual risk of tuberculosis infection (ARI) from among BCG vaccinated children, a total of 11,132 children (5.107 aged 0-4 years and 6,025 aged 5-9 years) were tested using ITU of PPD RT23 with Tween 80. Children with BCG scar comprised 68.2% of the test-read population aged 0-4 years and 47.5% of those aged 5-9 years. Overall, 68.9% of the children with BCG scar had either no test reaction or reaction <10 mm in size. In the 0-4 year age group, a bimodal distribution of reaction sizes was observed among the unvaccinated children, but not so in the vaccinated children. In the 5-9 year age group, the distributions were bimodal in both the vaccinated as well as unvaccinated children. The mode on the right side of the distribution was at 25 mm in the vaccinated as well as unvaccinated 5-9 years old children. Based on this mode, the prevalence of infection was estimated to be 8.08% and 8.6% among the unvaccinated and vaccinated children, respectively. The respective ARI rates were 1.12% and 1.19%. This study indicates that in the event of non-availability of a sufficient number of unvaccinated children for estimation of ARI, tuberculin surveys maybe conducted among the 5-9 year aged including vaccinated children. In Janine Jason et al study,[7] the World Health Organization recommends Mycobacterium bovis BCG vaccination in areas of high tuberculosis prevalence. BCG's clinical and immune effects, not necessarily Mycobacterium tuberculosis specific are unclear. BCG vaccine scarring often is used as a surrogate marker of vaccination or of effective vaccination. We evaluated BCG scarring status in relation to clinical findings and outcome in 700 hospitalised Malawians, of whom, 32 had M. tuberculosis Bloodstream Infections (BSI) (10 of whom had cellular immune studies done) and of whom 48 were infants <6 months old and therefore recently vaccinated (19 of whom had immune studies). In the patients >6 months old, scarring was not related to the presence of pulmonary symptoms (35 versus 30%), chronic cough, fever, mortality or M. tuberculosis BSI. In M. tuberculosis BSI patients, scarring was unrelated to mortality, vital signs or clinical symptoms, but those with scarring had higher proportions of memory and activated T cells and more type 2-skewed cytokine profiles. Infants with either BCG scarring (n10) or BCG lesional inflammation (n5) had no symptoms of sepsis, but 18 of 33 infants without BCG vaccination lesions did. Those with BCG lesions had localised infections more often than did those without BCG lesions. These infants also had lower median percentages of lymphocytes spontaneously making Interleukin-4 (IL-4) or Tumour Necrosis Factor Alpha (TNF-α) and lower ratios of T cells spontaneously making IL-4 to T cells making IL-6. Thus, we found that, in older patients, BCG vaccine scarring was not associated with M. tuberculosis-specific or nonspecific clinical protection. Those with M. tuberculosis BSI and scarring had immune findings suggesting previous M. tuberculosis antigen exposure and induction of a type 2 cytokine pattern with acute reexposure. It is unlikely that this type 2 pattern would be protective against

mycobacteria, which require a type 1 response for effective containment. In infants <6 months old, recent BCG vaccination was associated with a non-M. tuberculosis-specific anti-inflammatory cytokine profile that the vaccinated infants had a greater frequency of localised infections and lesser frequency of sepsis symptoms suggests that this post-vaccination cytokine pattern may provide some non-M tuberculosis-specific clinical benefits. Manjunatha Babu R et al[8] conducted a study to estimate serum ADA levels in children with and without BCG scar after receiving BCG vaccination. This prospective observational study was conducted at a tertiary care hospital for a period of 2 years. Babies in postnatal 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. 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 12 weeks was (17.25 ± 2.45 U/L) significantly (p value <0.001) lower than the ADA levels (34.12 ± 3.28 U/L) of vaccinated babies. There was no statistically significant difference in the mean ADA levels of children with (34.18 ± 3.52 U/L) and without (31.22 ± 2.32 U/L) scar after BCG vaccination (p value >0.06). 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.

CONCLUSION

There is increase in serum ADA levels in after BCG vaccination administration and even in without scar, thereby indicating adequate immunity.

REFERENCES

[1] Global Tuberculosis Control Report. 2009. http://www.who.int/tb/Publications/Global/2009/pdf/fullreport.pdf.

[2] Kabra SK, Seth V. BCG Vaccination. In: Seth V, Kabra SK, eds. Essentials of tuberculosis in children. 4th edn. New Delhi: Jaypee Brothers Medical Publishers (p) Ltd 2011:555-588.

[3] Palomino JC, Leão SC, Ritacco V. Tuberculosis 2007; from basic science to patient care. Amedeo Challenge 2007:409-410.

[4] Hyder MKA, Nair N, Ahmed T. Global epidemiology of pediatric tuberculosis. In: Seth V, Kabra SK, eds. Essentials of tuberculosis. 4th edn. New Delhi: Jaypee Brothers Medical Publishers (p) Ltd 2011:11-18.

[5] Rani HS, Vijayalaxmi V, Kumar S, et al. Cell mediated immunity in children with scar failure following BCG vaccination. Indian pediatrics 1998;35(2):123-127.

[6] Chadha VK, Jagannath PS, Suryanarayana HV. Tuberculin sensitivity in BCG vaccinated children and its implication for ARI estimation. International Journal of Tuberculosis 2000;47:139.

[7] Jason J, Archibald LK, Nwanyanwu OC, et al. Clinical and immune impact of mycobacterium Bovis BCG vaccination scarring. Infection and Immunity 2002;70(11):6188-6195.

[8] Babu MR, Reddy TV, Thomus SRJ, et al. To estimate serum ADA levels in BCG vaccinated children. J of Evolution of Med and Dent Sci 2015;4(31):5286-5291.