

*Full Length Research Paper*

# Determining the correlation between the scar size of Bacille Calmette-Guérin (BCG) vaccination and suffering from asthma in children, by assessing the level of T helper 1 / T helper 2

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The Bacille Calmette-Guérin (BCG) vaccine is used more than any other vaccines in the world, whereas a lot of studies have spoken about allergy and its relationship with the BCG vaccine. It is believed that many factors can influence this relationship and its symptoms, can be observed via the occurred scar following BCG vaccination. The aim of this study was to determine the relationship between the levels of T helper 1 (INF-gamma) / T helper 2 (IL-4, IL-13) in children suffering from Asthma and the scar size of BCG in them. In this case-control study, 100 children who have got scar, 60 of them suffered from asthma and 40 did not suffer from it, were studied. Subjects with no BCG scar are excluded from the study. The Chi-square test revealed that the frequency of cases with scar size larger than 5 mm, is 43% in Asthmatic patients and 70% in control group that the observed difference was statistically significant ( $P = 0.01$ ). T test also revealed that the average of INF-gamma is considerably lower in patients suffering from Asthma than the control group ( $6.95 \pm 3.83$  (pg/ml) in asthmatic group compared with  $10.75 \pm 6.98$  (pg/ml) in control group) ( $P = 0.001$ ) whereas the average of IL-4 ( $30.90 \pm 16.51$  (pg/ml) in asthmatic group compared with  $9.95 \pm 7.44$  (pg/ml) in control group) ( $p < 0.001$ ) and IL-13 ( $48.85 \pm 13.66$  (pg/ml) in asthmatic group compared with  $10.49 \pm 12.44$  (pg/ml) in control group) ( $p < 0.001$ ) is higher. Therefore, it was demonstrated that the average of the proportion of INF-gamma/IL-13 (0.3054 in asthmatic group compared with 1.9334 in control group) in patients suffering from asthma, is lower. The size of BCG scar in Asthmatic patients was, significantly, smaller than the control group. Moreover, the average of T helper 1 (INF-gamma) was lower and the average of T helper 2 (IL- 4, 13) was higher in asthmatic patients. Therefore, there is a correlation between the size of BCG scar and the levels of T helper 1 (INF-gamma) and T helper 2 (IL- 4, 13) with asthma; thus, there could also be a correlation between the scar size of BCG, T helper 1 and 2.

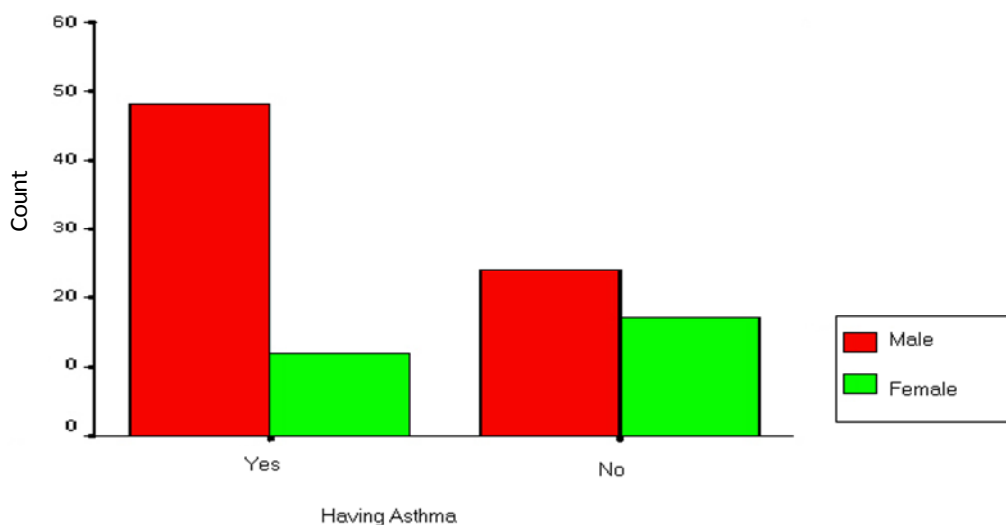
**Key words:** Bacille Calmette-Guérin (BCG), Vaccine, Asthma, BCG scar, T helper 1 and 2.

## INTRODUCTION

BCG (Bacille Calmette-Guérin) vaccine stimulates reticuloendothelial system, increases the phagocytic and cytotoxic activity of macrophages and aggravates dermal hyper sensitivity reaction. Injecting BCG to animals, causes

spleen hyperplasia and overweighting. Further more, it increases resistance to bacterial and viral infections, humoral immunity and Interferon production (Floyd et al., 2000; Sarinho et al., 2000). In addition, BCG vaccination causes the secretion of IL-4 (Interleukin 4), IL-13 (Interleukin 13) and TNF (Tumor Necrotizing Factor); Thus, it seems that in addition to BCG's main role, this vaccine plays a magnificent role in immunity induction (immunization) which is proved by the different levels of atopic involvements in

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**Figure 1.** The gender frequency in studied individuals, regarding the existence of asthma. The frequency of boys suffering from asthma was 48 out of 60 (80%) and the girls suffering from it, was 12 out of 60 (20%) in control group.

childhood period of the atopic patients (Vargas et al., 2004).

A lot of studies have investigated on the effect of infectious diseases on atopy and there are a lot of contradictory information about the relationship between atopic disorders (like asthma and allergy) and BCG vaccination (Rani et al., 1998).

There are evidences proving the superiority of T helper 2 to T helper 1, when the asthmatic patients come across different common allergens, this fact is believed to be due to the immune memory from the early childhood (Racila and Kline, 2005). On the other hand, the amount of Gama Interferon (INF-gamma) in children suffering from asthma or other atopic disorders is low (Martinez et al., 1995). However, the children are vaccinated with sub-cutaneous BCG in early hours of their lives which causes extreme increase in the production of cytokines derived from T helper 1 and also gama interferon (INF-gamma) in response to mycobacterial antigens (Obihara et al., 2006).

The purpose of this study was to investigate the correlation between asthma and the scar size of BCG by assessing the IL-4, IL-13, INF-gamma and T helper 1 / T helper 2 levels, simultaneously.

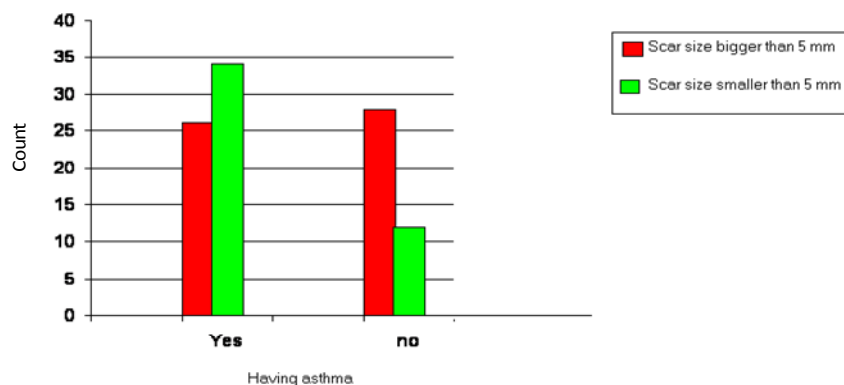
## PATIENTS AND METHODS

The age and gender of individuals, presence of cigarette smoking members in their family, having tuberculosis background and suffering from atopic disorders (asthma, allergic rhinitis and eczema) in their family were recorded via interviewing. The size of their BCG scar was measured by 2 individuals via a transparent ruler and then the average of the 2 measured sizes, was calculated. After that, the proportion of T helper 1 / T helper 2 was assessed by measuring the levels of INF-gamma, IL-13 and IL-4 in the blood samples of individuals, by ELISA Technique, using standard methods.

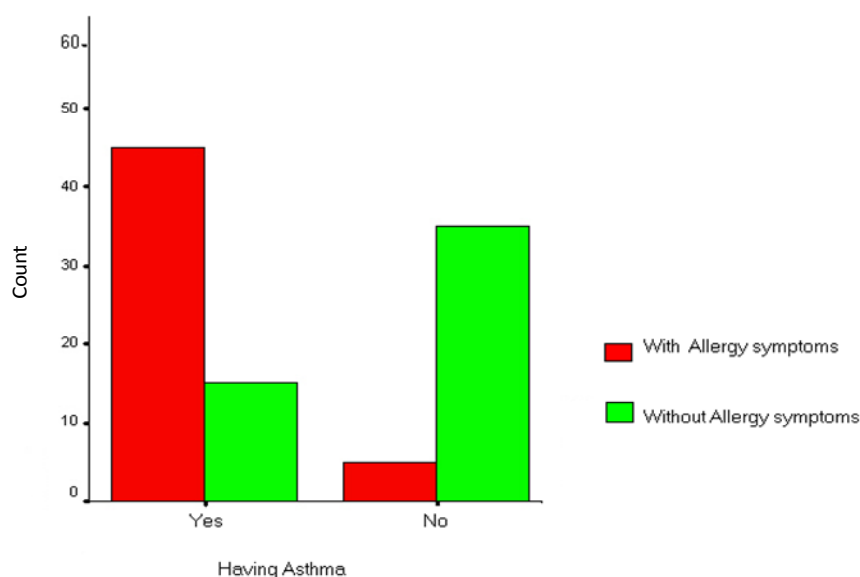
3 to 12 years old children, vaccinated with BCG were divided in 2 groups: (1) The case group (having the distinct scar and criteria of asthma), and (2) The control group (Children in the same age group, vaccinated with BCG, having the distinct scar but without asthma). The inclusion criteria were being vaccinated with BCG within the first hours of childbirth with the same strain of BCG vaccine (1173 P2), weighing more than 2500 g after childbirth (information obtained from the vaccine status document), without being immune suppressed by any drug or disease and the exclusion criterion was lacking distinct BCG scar. The case and control members were selected from the patients visiting the Allergy Clinic of Mofid Hospital. The method of sampling was continuous or non-sequential and the amount of samples was estimated to be 100 individuals (60 individuals for the case group and 40 individuals for the control group). Using a transparent millimetric ruler, two different observers, recorded the diameter of the BCG scar. The transverse and longitudinal diameters of the scar and after that their average were calculated. The result of measuring for each individual was categorized in two groups (< 5 mm or ≥ 5 mm), as being definite. Unless the two measures were the same, measuring would be done by a third person. The blood samples of the patients were taken for assessing the level of T helper 1 / T helper 2 by ELISA technique. The correlation between suffering from asthma and having a smaller BCG scar, was determined using odds ratio (with a confidence interval of 95%). Also, for determining the differences between various variables, the K square test and for achieving the final conclusion, SPSS programme were applied. In this study collecting information was based on observation, interviewing, physical examination and blood sampling examination.

## RESULTS

In this study, the number of boys suffering from asthma was 48 out of 60 (80%) and the number of girls suffering from it was 12 (20%) (Figure 1). The vaccinated children with the scar larger than 5 mm in the group of asthmatic patients, were 26 (43%) and in control group, 28 (70%); the observed difference was, statistically meaningful,



**Figure 2.** The correlation between the scar size of BCG and suffering from asthma. The frequency of cases with the scar size larger than 5 mm in asthmatic patients was 26 out of 60 (43%) and 28 out of 40 (70%) in control group; based on Chi-square test, the observed difference was statistically meaningful ( $p = 0.001$ ).



**Figure 3.** The frequency of allergy symptoms in studied individuals, regarding the existence of asthma. The frequency of allergy symptoms in asthmatic patients was 45 out of 60 (75%) and 5 out of 35 (12.5 %) in control group.

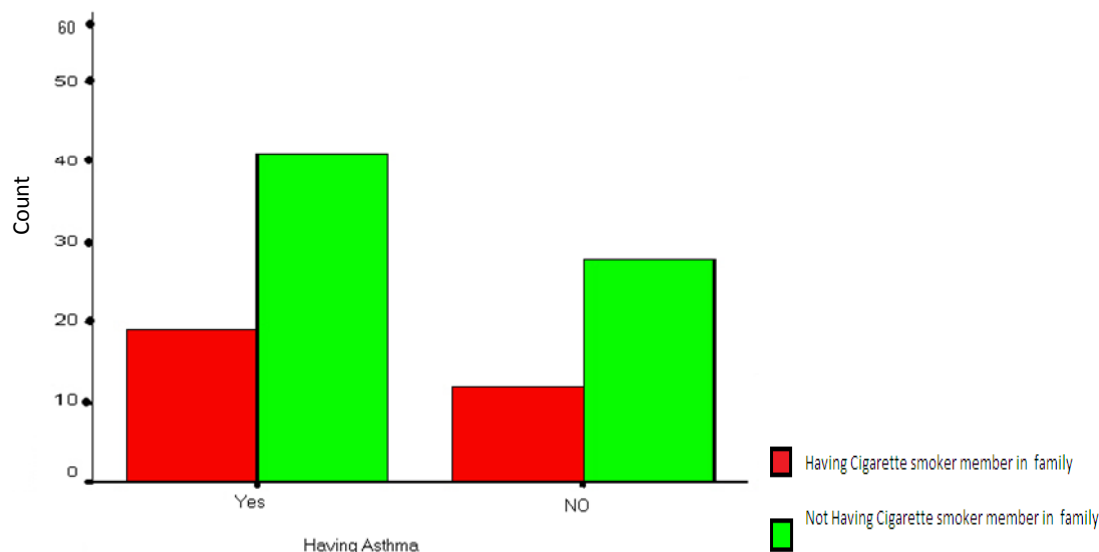
based on Chi-square test ( $p$ -value 0.001) (Figure 2). The frequency of cases having allergic disorders were 45 (75%) in asthmatic patients and 5 in control group (12.5%) (Figure 3). The frequency of having cigarette smoker members in the family of asthmatic patients, was 20 (33.5%) and 12 (30%) in control group (Figure 4). Measuring the secreted cytokines from T cells using T-test revealed that:

- The average of INF-gamma in the group of patients suffering from asthma was  $6.95 \pm 3.83$  and in control group was  $10.75 \pm 6.98$  (pg/ml) that this difference was statistically significant. ( $p$ -value = 0.001).
- The average of IL-4 in the group of patients suffering

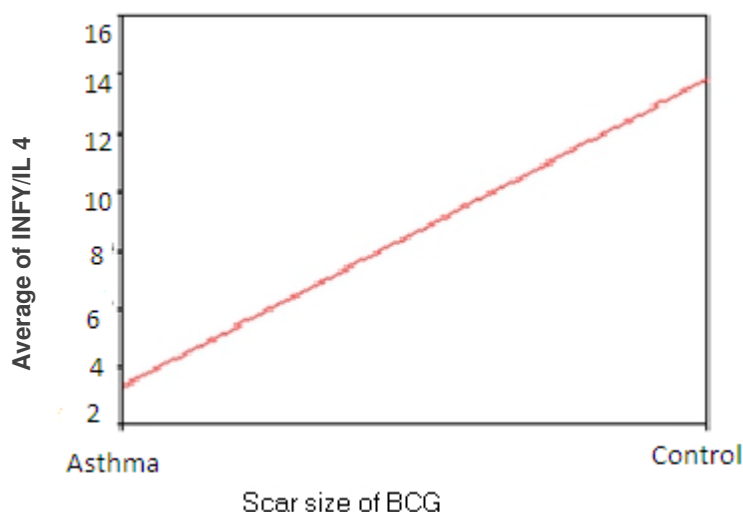
from asthma was  $30.90 \pm 16.51$  and in control group was  $9.95 \pm 7.44$ ; this difference was statistically significant ( $p < 0.001$ ).

- The average of IL-13 in the group of patients suffering from asthma was  $48.85 \pm 13.66$  and in control group was  $10.49 \pm 12.44$ ; this difference was statistically significant ( $p < 0.001$ ).

We demonstrated that there are correlations between: BCG scar size and asthma; the lower level of T helper 1 and asthma (Figure 5), the higher level of T helper 2 and asthma (Figure 6), T helper 1/T helper 2 proportion and asthma, and, finally, there is a correlation between T helper 1/T helper 2 proportion and BCG scar size (Figures 7 and 8).



**Figure 4.** The frequency of cigarette smoking members in the family of studied individuals, regarding the existence of asthma. The frequency of cigarette smokers in the family of asthmatic patients was 20 out of 60 (33.5%) and 12 out of 40 (30%) in control group.



**Figure 5.** The correlation between the average of INF-gamma / IL-4 and suffering from asthma.

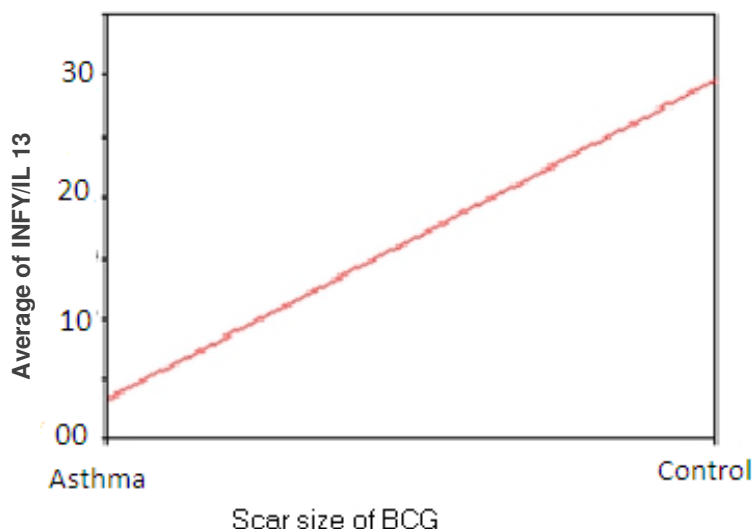
## DISCUSSION

In this study, we investigated that there is a correlation between the scar size of BCG and suffering from Asthma in children, by assessing the level of T helper 1 / T helper 2.

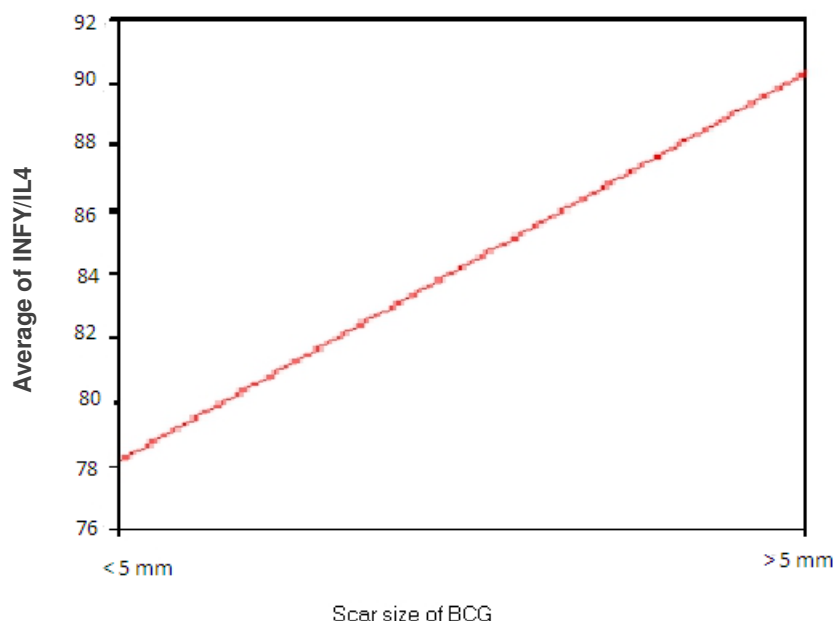
Liao et al. (1996) found that the size of BCG scar in asthmatic patients is small. According to this study, it was recommended that in asthmatic patients, the production of T helper 1 and therefore INF-gamma is different as well. Martignon et al. (2005) proved that BCG vaccination

in the early hours of child's life can increase the amount of T helper 1, so it prevents the occurrence of atopic symptoms. This fact was proved by another study performed on schoolchildren in Spain (García-Marcos et al., 2005). However, the smaller size of BCG scar children suffering from asthma shows that BCG vaccine cannot perfectly stimulate their Immune System and thus the proportion of T helper 1 / T helper 2 is low (Silverman, 1997).

Many other studies revealed that there is a significant correlation between the scar size of BCG and asthma.



**Figure 6.** The correlation between the average of INF-gamma / IL-13 and suffering from asthma.

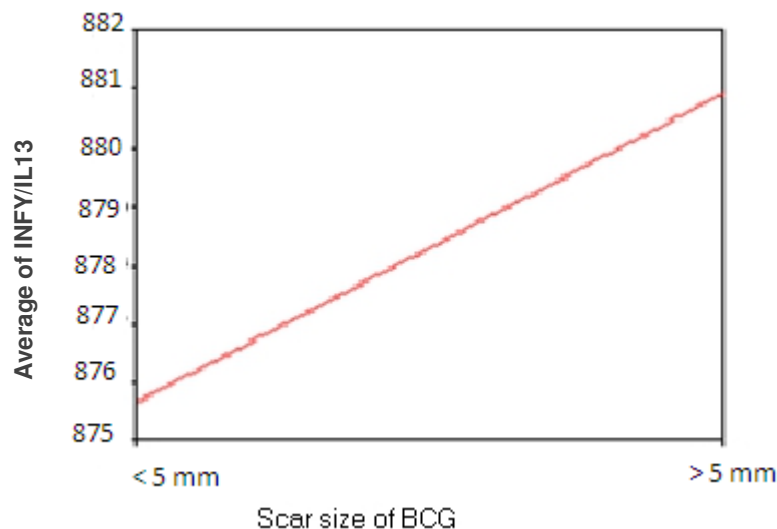


**Figure 7.** The correlation between the average of INF-gamma / IL-4 proportion and the scar size caused by BCG vaccine.

This means that the scar size is significantly smaller in asthmatic patients and is significantly bigger in control individuals. It seems that BCG plays its role by stimulating macrophages and increasing the antibody production and thus; the amount of immunity (Queiroz et al., 2004).

It should be noted that the amount of gamma interferon (INF-gamma) in patients suffering from asthma was lower

in our study. The same significant correlation (production of higher levels of IL-4 and 13 in asthmatic patients) was found for IL- 4 and 13, as well. The recent studies revealed that BCG vaccination and the response to it will result in an environment full of INF-gamma in the body; and thus, the higher level of INF-gamma will result in the suppression of T helper 2. Therefore the decrease happened in the amount of T helper 2 that will prevent



**Figure 8.** The correlation between the average of INF-gamma / IL-13 proportion and the scar size caused by BCG vaccine.

atopic disorders. Therefore the production of INF-gamma during T helper 1 response prevents the completion of T helper 2 cells. In asthmatic patients, the average of INF-gamma (T helper 1) is decreased and the average of IL-4 and 13 (T helper 2) is increased; on the other side, the response to BCG vaccine, which is determined by its scar, will result in the increase of T helper 1 (e.g. INF-gamma) and decrease of T helper 2 (e.g. IL-4 and 13) and thus, results in the decrease of asthmatic symptoms.

The scar size of BCG is as a result of T helper 1 reaction to the vaccine in the early hours of child's life. Thus, by measuring the scar size of BCG, one can assess the type of T helper 1 immune reaction in asthmatic patients versus non-asthmatics (Martignon et al., 2005).

Consistent with previous studies, there was a significant correlation between the size of BCG scar and asthma in this study as well (Martignon et al., 2005). The occurrence of scar depends on different factors such as the dosage and strain of vaccine, gender and age of individuals and the method of immunization (Agarwal et al., 1990); thus in this study, the different factors mentioned, were controlled. Generally, these results, in agreement with previous data, support the hypothesis that early vaccines could promote T helper 1 proliferation in response to the infectious agent it contains, which inhibits the enhancement of atopic manifestations (Martignon et al., 2005). In another study, the researcher found no protective effect of early BCG vaccination against atopy in school age, although tuberculin responses and allergic symptoms were inversely related (Annus et al., 2004).

Further studies are needed to confirm the phenomenon, such as: Studying the details of tuberculin test mechanism considering the amount of T helper 1 and T

helper 2, the correlation between BCG vaccine, T helper 1 and T helper 2 in asthmatic patients during childhood period and following children in 2 years considering the levels of T helper 1 and T helper 2 after BCG vaccination.

## Conclusion

The size of BCG scar is significantly smaller in individuals suffering from asthma and is larger in individuals who do not suffer from it and also, the average of T helper 1 (INF-gamma) is significantly lower and the average of T helper 2 (IL-4 and 13) is higher in asthmatic patients.

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