Reactivity of tuberculin among Bacillus Calmette-Guérin vaccinated school children, Kassala State, Sudan Fatima A. Khalid1. Elderidiry MM2, MM Mukhta3

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Abstract

Background Tuberculin skin test (TST) is a delayed hypersensitivity reaction of latent TB infected patients and BCG vaccinated individuals. It is recommended for screening of mycobacterium infection in many countries. Kassala state in eastern Sudan is known as the highest pulmonary TB endemic region in Sudan. Little data is available on the TST reactivity among school children in Kassala. This activity aimed to measure the prevalence of TST reactivity among school children in Kassala as indictor for exposure and response to BCG vaccination. Method Five tuberculin units of PPD were injected intradermally in the left forearm of 2600 children aged 5-15 years attending governmental basic schools in Kassala. BCG scar, child health and nutrition status were assessed before the test. The reactivity of TST was read for 2568 children after 48- 72 hours, interpreted at different cut-off level based on published guidelines. Data were analyzed using SPSS (V. 20.0) Results Tuberculin reactivity was measured to 2568 out of the 2600, 32 were absent during the measurement. Most of children 2100 (81.8%) had no reaction with TST (no induration). Four hundred and sixty eight (18.2%) had induration ranged from 1mm to 28 mm with mean 3.1mm ±3.29, among them, 17 cases had induration ranged from 10- 28mm. prevalence of BCG scar was 1688 (64.9%). TST reactivity was statistically associated with geographical localities, child gender, and age (P<0.001). High percentage of TST reactivity was recorded in females (65.8%) compared to males (34.1%). Furthermore, TST reactivity was increased with the age, among the strong positive reaction, 9/17 were in age 13-15 years. Also TST reactivity was statistically correlated with nutrition and socioeconomic status ((P<0.001). TST reactivity was not affected by BCG vaccine (P>0.05). Conclusion The study provides data on BCG vaccination coverage in Kassala state, the prevalence of non reactivity of TST. No effect of BCG vaccine on reactivity of TST, hence it can be used as the diagnostic tool for detection of LTBI in children. Different confounding factors were associated with TST reactivity including child age, gender and nutrition status, health and socioeconomic status.

Background

Globally, tuberculosis remains a major cause of morbidity and mortality, especially in developing countries. The disease is caused by mycobacterium tuberculosis. One third of the world populations were infected with MTB. However, only ~ 10% of individuals develop TB and ~90% of cases remain dormant (LTBI)(1). In 2017, TB globally caused an estimated 1.5 million deaths of which 230 000 (15.3%) occurred among children(2).

Neonatal BCG vaccine had been adopted for protection against severe and disseminated TB, leprosy and reduction of mortality(3,4). It has been used as routine in Sudan according to BCG immunization policy at birth or within a few days after birth(5). BCG vaccine scarring is often used as a surrogate marker of vaccination efficacy(6).However scar failure is a well-known phenomenon with various prevalence(7,8,9).

Despite advances of TB diagnosis and drugs for treatment in adults, childhood TB diagnosis lagged behind (10) due to paucibacillary disease and reduction the yield of conventional methods. Still the childhood diagnosis relies on chest x-ray and tuberculin skin test(2). TST reactivity can be complicated by
different factors including; previous BCG vaccination, Tuberculin units, nutrition status and infection by non tuberculous MTB\(^{(11)}\).

Kassala is a border state in Eastern Sudan neighboring Eritrea and Ethiopia where TB prevalence is relatively high. Administratively, Kassala is divided into eleven localities. It suffers from poverty and limited economic resources that contribute to the chronic vulnerability and increase the prevalence of infectious diseases including TB\(^{(12)}\). No data on pediatric TB in Kassala is available. This study aimed to assess the reactivity of tuberculin and related factors among school children as indicator of BCG vaccination rate and detection of latent TB.

**Methods**

The study was designed to enroll governmental basic school children (5-15 years old) from three localities in Kassala State (urban and rural), namely, Kassala, West Rural Kassala, Rural Kassala, during 2016-2018.

**Data collection and Tuberculin skin test**

Baseline information was collected by trained teachers aided by children's parents or guardians through structured questionnaire. The collected information includes: locality, child age, sex, BCG vaccination history, and socioeconomic status.

Children who had history of chronic disease, symptoms of infectious disease that might interfere with TST reactivity, allergic disease and incomplete questionnaire at the time of screening were excluded from the study.

Clinical examination was performed by medical practitioner to detect any sign of active TB, and then all children were nutritionally assessed by measuring weight, height,

BCG vaccine scar was checked for 2600 of children. TST was performed as described by manufacturer, briefly by injecting 0.1 ml containing five TU PPD (Tuberculin Diluted, India, Ref. 10LM005) intradermally in the left forearm after cleaning the skin by 70% ethanol. The induration was measured in millimeter after 48-72 hours using a transparent, flexible 15 cm ruler. The reactivity of TST was read for 2568 children using several ranges of induration size (0, 1-4, 5-9, 10-14, and \(\geq 15\) mm), and interpreted at different cut-off level (induration size \(\geq 5\) mm, \(\geq 10\) mm and \(\geq 15\) mm) based on published guidelines\(^{(13)}\).

**Statistical analysis**
Data entry and analysis was conducted using SPSS software version 20. Age was categorized as 5–9, 10–12 and 13-15 years, and TST reaction induration size was categorized at cutoff points of < 5 mm, ≥5 mm, ≥10 mm and ≥15 mm. The results for each variable were presented as frequencies and proportions.

**Results**

Of 3200 children, 2600 were enrolled in the study. Six hundred children were excluded due to incomplete data or absent at time of TST, while 23 suffered from chronic illness. The enrolled children were from three localities; Kassala 1019(39.2%), Rural Kassala1010 (38.8%) and Western Rural Kassala571 (22.0%). One thousand and hundred eighty three (45.5%) were males and 1417 (54.5%) were females with gender ratio of 1: 1.2. Their age ranged between 5-15 years (mean 10.4 ±2.4), 40.3% were in the age group of 10-12 years. The majority of the parents or guardians had low level of education, educated fathers and mothers represented by only 14.1% and 8.3% respectively. Most of mothers (94.7%) were housewives and 70.3% of fathers were daily worker. One thousand and four hundred sixteen (55.1%) had healthy weight. No suspected active TB cases were detected among children.

As mothers mentioned, all the children were vaccinated against TB. However, the card of vaccination was kept by (64.2%) of them. Presence of BCG scar as indicator of BCG coverage was found in1688 (64.9%) of children. It is statistically associated with geographical location and child health (P<0.01). High percentage (70.95%) of children with BCG scar was from Kassala, followed by western rural Kassala (68.47%) and Rural Kassala (56.83%), among healthy children, 843/2352 had no BCG scar. Table (1)

Tuberculin skin test, which is read for 2568 of children, showed high percentage (81.8%) of non reaction. Its reactivity was reported among 468 (18.2%), where the induration varied from 1mm to 28 mm (mean 3.19mm ±3.29); < 5mm 359 (14%), ≥ 5mm 92 (3.6%), ≥ 10mm 7 (0.3%) and the high positivity (≥ 15mm) was reported among 10 (0.4%) of students. TST reactivity was statistically associated with the localities, sex, age, child health and nutrition status (P< 0.001), and socioeconomic status; father education and occupation found to be positively affect the reactivity (P<0.05). However, BCG scar had no effect on TST reactivity (P>0.05). Kassala locality had high percentage of positive reaction (79.6%) compared with Western Rural Kassala, which had high negative reaction of TST (97.89%). Females represented high percentage of reactivity (65.96%) compared with males (34.03%). Moreover, increase the child age increasing the reactivity of TST. The nutrition status has positive impact on TST, contrary the child heath was negatively associated with TST reactivity.

**Discussion**

BCG vaccination continues to be used for protection of TB and reduction of mortality in childhood(4). The coverage and response of vaccination was examined by the presence of scar(14,15). Based on immunization program and mothers interview, all participants of this study were expected to be vaccinated against TB and had scar. However, the scar was present in 65% of children and absence in 35% of them. The evident vaccination is lower than the reported national coverage of BCG vaccination.
(92%) in Sudan\(^{16}\). In Ghana BCG scar was visible in 89.3% \(^{17}\) and in Nigeria the BCG scar was visible in 81.5% \(^{18}\). Absence of BCG scar was reported in Egypt\(^{19}\), Korea \(^{20}\) and West Africa \(^{21}\), Nigeria\(^{9}\).

Scar failure may be attributed to different factors including the strain of BCG used for vaccination as there are some strains of BCG produced by different manufacturers with no standardized method between them\(^{22}\), hence the strains may genetically differ\(^{23}\), and result in different reactivity and immune response that affect protection against TB \(^{22, 24}\). Potency of the vaccine may be affected by vaccinated technique \(^{25}\). Ineffective vaccine, leakage of vaccine fluid during vaccination should be considered for the failure of BCG scar development\(^{26}\). Furthermore, failure of BCG scar formation may be related to the child impairment or lack of maturation of immune system during the time of vaccination and gene mutation\(^{27}\).

Our study showed Strong positivity of TST $\geq 10$ mm in 17 (0.7%) of children indicating TB infection as reported in Taiwan\(^{28}\). Poor effect of BCG vaccine on tuberculin reactivity encourages the use of TST for estimation the prevalence of TB infection.

The majority of children (81.8%) did not have any reaction to tuberculin, despite having received BCG immunization soon after birth. Same finding was found in some studies \(^{29, 30, 31, 32, 33}\). Poor effect of BCG vaccine on tuberculin reactivity encourages the use of TST for estimation of prevalence of TB infection. Association of BCG scar and TST was reported\(^{21, 34, 35}\). High percentage of non reactivity may be attributed to different factors including tuberculin itself as no study found to assess its quality. Non reactivity of TST can be related to the strain of BCG used for vaccination and the time interval between vaccination and TST. Prevalence of positive tuberculin skin test reactions has been reported in many studies in different parts of the world\(^{30, 35}\).

The study approved the effect of child age, gender and localities on reactivity of TST, additionally nutrition and socioeconomic status. Our findings supported by \(^{14, 36, 37, 38}\) who explained the association of TST reactivity with localities, child age and nutrition status, as increase of age followed by increase of TST reactivity\(^{28, 39}\). Contrary other studies determined no association of TST reactivity with nutrition status \(^{16, 32}\). Low socioeconomic status reflects poor living condition and overcrowded \(^{18}\), as some family size exceeded 10 members. Furthermore, Kassala state has long suffered chronic poverty, lack of adequate access to basic services such as education and health care, besides high level of malnutrition\(^{40}\). Collectively these factors affect the nutrition status and exposed children to infectious diseases and therefore, response to TST \(^{18}\).

Moreover, Kassala state as other parts of Sudan had different ethnic groups that may be attributed to reactivity of TST as mentioned early\(^{16}\) and also the endemicity of the State of TB, viral diseases and recent viral vaccination.
Conclusion: The study provides data on BCG vaccination coverage in Kassala state, the prevalence of non reactivity of TST. No effect of BCG vaccine on reactivity of TST, hence it can be used as the diagnostic tool for detection of LTBI in children. Different confounding factors were associated with TST reactivity including child age, gender and nutrition status, health and socioeconomic status. More studies and revision of BCG vaccination policy are needed.

**Abbreviations**

BCG: Bacillus Calmette-Guérin; LTBI: latent tuberculosis infection; MTB: Mycobacterium tuberculosis; PPD: purified protein derivative; TB: tuberculosis; TST: Tuberculin Skin test

**Declarations**

**Ethics approval and consent to participate**

Ethical committee at Ministry of health, Kassala State was approved this study (2016), followed by permission from general director of Ministry of Education, Kassala State and principals of selected schools after clear explanation of the objectives. Verbal consent was obtained from children’s parents/guardians before interview, because most of parents were uneducated or had poor level of education.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Conflict of interest**

No conflict of interest

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**Author contribution**

Study design: All authors. Data collection, management and analysis were performed by FAK and MME. Analyzed data were revised by MMK. All authors wrote and revised the manuscript.

**Consent for publication**

Not applicable
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Tables

Table (1): Distribution of BCG scar according to the age, sex and localities of studied group

| Character     | Presence of BCG scar | Absence of BCG scar | Total   |
|---------------|----------------------|---------------------|---------|
| Sex           |                      |                     |         |
| Male          | 775                  | 408                 | 1183    |
| Female        | 913                  | 504                 | 1417    |
| Age           |                      |                     |         |
| 5-9 yrs       | 635                  | 344                 | 979     |
| 10-12 yrs     | 686                  | 363                 | 1049    |
| 13-15 yrs     | 367                  | 205                 | 572     |
| Localities    |                      |                     |         |
| Kassala       | 723                  | 296                 | 1019    |
| Rural Kassala | 391                  | 180                 | 571     |
| Western rural Kassala | 574 (56.8%) | 436                 | 1010    |

Table (2): Factors associated with TST reactivity
|                      | Not reactive | < 5 mm | ≥5mm  | ≥10mm | ≥15mm | Total |
|----------------------|--------------|--------|-------|-------|-------|-------|
| **Sex**              |              |        |       |       |       |       |
| Male                 | 1006         | 140    | 12    | 03    | 05    | 1166  |
| Female               | 1094         | 219    | 80    | 04    | 05    | 1402  |
| **Age**              |              |        |       |       |       |       |
| 5-9 yrs              | 833          | 110    | 24    | 0     | 02    | 969   |
| 10-12 yrs            | 841          | 152    | 39    | 03    | 03    | 1038  |
| 13-15 yrs            | 426          | 97     | 29    | 04    | 05    | 561   |
| **Locality**         |              |        |       |       |       |       |
| Kassala              | 616          | 266    | 92    | 06    | 08    | 988   |
| Western rural Kassala| 559          | 11     | 00    | 00    | 01    | 571   |
| Rural Kassala        | 925          | 82     | 00    | 01    | 01    | 1009  |
| **Vaccinated:**      |              |        |       |       |       |       |
| Presence of BCG scar | 1366         | 236    | 54    | 05    | 07    | 1668  |
| Absence of BCG scar  | 734          | 123    | 38    | 02    | 03    | 900   |
|                      | 2100         | 359    | 92    | 07    | 10    | 2568  |
| **Nutrition status** |              |        |       |       |       |       |
| Unhealthy weight     | 988          | 127    | 32    | 01    | 04    | 1152  |
| Healthy weight       | 1112         | 232    | 60    | 06    | 06    | 1416  |
| Total                | 2100         | 359    | 92    | 07    | 10    | 2568  |