

Screening of BCG Vaccine Efficacy among Healthy Vaccinated Adults in Khartoum, Sudan

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Abstract

Tuberculosis (TB) is a major global health problem. The BCG is an attenuated vaccine. Tuberculin test is used as a nonspecific TB vaccination indicator. This study was aimed to determine the efficacy of BCG vaccine through screening of healthy, vaccinated adults' subjects in Khartoum. A total of one hundred (n=100) healthy and TB symptoms free participants were screened by Mantoux test. The participants were involving 55 (55%) males and 45 (45%) female. All participants were over 20 years and most of them had a scar in their vaccination site. All participants were screened through injection of purified protein derivative PPD (only 0.1 mL) intra-dermally into their volar forearm then 48-72 post-injection their induration diameter was measured. The results showed that out one hundred (n=100) participants, only 39 (39%) were positive for Mantoux test (≥ 10 mm diameter), while 61 (61%) were negative (≥ 10 mm diameter). Among the 39 positives, 33 show reading between 10 mm to 15 mm and 6 of them show zone ≥ 15 mm. In the other hand 53 out of 61 tuberculin test negative participants showed no induration post PPD injection and the rest were shows reading zone between 5 to 9 mm. The result shows that among the 39 positive participants 23 (58.97%) were male while only 16 (41.03%) were female. The mean of zone reading among the positive participants is higher in male 13.96 ± 3.29 than female 13.81 ± 2.22 . The study concluded that more than half of the participants were negative for tuberculin test and this may be interpreted by either the vaccine was invalid or their cell-mediated immunity against TB is reduced. The discrepancy in the zone reading means between male and female may be related to some physiological difference. Further studies with more sample size and by using a more advanced technique (IFN γ) should be done to clarify the results.

Keywords Tuberculosis; BCG; Mantoux test; Cell mediated immunity

Introduction

Tuberculosis (TB) is a major global health problem. It causes ill-health among millions of people each year and ranks alongside the human immunodeficiency virus (HIV) as a leading cause of death worldwide [1]. Tuberculosis generally affects the lungs, but can also spread to other parts of the body including the renal system, central nervous system, gastrourinary system, lymphatic system, circulatory system, bones, joints and skin. Most infections do not have symptoms, in which case it is known as latent tuberculosis and 10% of latent infections progress to active disease which, if left untreated, kills about half of those infected [2]. A chronic cough with blood-containing sputum, fever, night sweats, and weight loss are the classic symptoms of active TB [2]. Further symptoms can occur later when the infection spread to other organs [3]. Spreading of Tuberculosis is occur through the air when people who have active TB in their lungs a cough, spit, speak, or sneeze [1,4]. People with latent TB do not spread the disease. The disease reactivation occurs more commonly among people with HIV/AIDS and in those who smoke. Diagnosis of active TB is based on chest X-rays, as well as microscopic examination and culture of body fluids besides polymerase chain reaction, while the diagnosis of latent TB based on the tuberculin skin test (TST) or blood tests [5]. In patients with drug-susceptible TB, a 6-month rifampicin-based regimen (2 months of isoniazid, rifampicin, pyrazinamide, and

ethambutol, followed by 4 months of isoniazid and rifampicin) should be used. MDR-TB (multidrug-resistant tuberculosis) is caused by bacteria that do not respond to the 2 most powerful first-line anti-TB drugs, isoniazid, and rifampicin. MDR-TB and rifampicin-resistant TB (RR-TB) are treatable using second-line treatment options which are limited with respect to availability and efficacy and require treatment of considerably longer duration of [6].

TB is caused by the infectious agent known as *M. tuberculosis* (MTB), this rod-shaped bacterium also called Koch's bacillus, was discovered by Dr. Robert Koch in 1882 [7]. MTB is a unique acid-fast bacterium. It is unique because it is high lipid and mycolic acid content of its cell wall. The physiology of *M. tuberculosis* is highly aerobic and requires high levels of oxygen [8].

TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 374 000 deaths among HIV-positive people. In 2016, 2.5 million people fell ill with TB in the African region, accounting for a quarter of new TB cases worldwide. An estimated 417,000 people died from the disease in the African region (1.7 million globally) in 2016. Over 25% of TB deaths occur in the African Region [9].

In Sudan, the tuberculosis-related mortality rate is estimated at 25.0 per 100 000 population. A total of 20,181 detected tuberculosis cases were reported in 2013, of which 5980 (30%) were new sputum smear-positive cases. The treatment success rate of new and relapsed cases

registered in 2012 was 75.0%. Drug-resistant tuberculosis is estimated at 1.9% among new cases and 20.0% among previously treated cases [10].

TB is the most unpardonable infectious disease and the most common one, which easily spread. Bacillus of Calmette-Guerin (BCG) is the only successful TB vaccine [11]. The BCG vaccine was developed over the period of 13 years from (1908-1921) its live vaccine derived from the strain of *Mycobacterium bovis*. That was attenuated by Calmette and Guerin at Pasteur Institute in Lille France. And it was first administered to a human in 1921. The BCG is usually given intramuscular to babies and children birth up to the age of 16, it's also sometimes given to adult up to the age of 35 years. But the vaccine does not work well in adults, the adults are often given skin test before vaccine. The rate of protective efficacy of BCG vaccine has been affected by the method, route of administration environment and characteristic of the population [6].

The standard dose of BCG vaccine is 0.05 mL of the reconstituted vaccine for infants aged 1 year. BCG vaccines must be administered by intradermal injection. Correct intradermal administration can be verified by bleb formation. BCG vaccine should be injected in a clean healthy area of skin. The vaccine should be given preference in the lateral aspect of the upper arm. There are no published data on efficacy/effectiveness and safety for other anatomic sites of administration. Among the many available BCG vaccine products, there is no preferred product for use, in any age or risk group.

About 95% of BCG vaccine recipients experience a reaction at the injection site characterized by a papule which may progress to become ulcerated, with healing after 2-5 months leaving a superficial scar. This is considered normal. Adverse events following immunization (AEFI) with BCG are dependent on a number of factors including the strain used in the vaccine, the number of viable bacilli in the batch, and variation in injection technique. Severe AEFI includes local reactions such as injection site abscess, severe ulceration or suppurative lymphadenitis usually caused by inadvertent injection of the vaccine sub-dermally. The advent of molecular tests has facilitated the identification of rare events, such as disseminated BCG disease that may occur between 1.56 and 4.29 cases per million dose.

A systematic review concluded that protection after primary infant BCG vaccination could last for up to 15 years in some populations. Longer duration of protection was found in persons who had a negative TST result prior to vaccination, and in those who had received neonatal BCG vaccination. However, protection was found to decline with time [12]. In a study in northern North America, long-term follow-up among adults who had been vaccinated neonatally with BCG found protection against all TB outcomes after 50–60 years. Data from a retrospective study in Norway also provided evidence of long duration of protection that declined after 20 years. The latter observation was confirmed by a recent observational study in England which found 20 years of protection against all TB outcomes in children vaccinated during school age, after which protection declined [13].

The efficacy of BCG remains to vary from 0%-80% [14] and it's 70%-80% effective against the most severe form of TB such as TB meningitis. It's less effective in preventing the form of TB that affect the lung but it's still considered important strategies in countries with high burden of tuberculosis because it's benefit to the infant but it's affecting the control of TB has been limited [15].

The immune response to mycobacterial infection is predominantly cellular. It is highly dependent upon gamma interferon (IFN- γ) production by macrophages and antigen-specific T cells.

The Mantoux Test (MT) is a classical delayed-type hypersensitivity (DTH) response to the intradermal injection of tuberculin purified protein derivative (PPD). It represents a cutaneous T cell-mediated memory recall immune response. The Mantoux test is also known as Tuberculin skin test has been the traditional method for detection of infection with tubercle bacilli (latent infection) [16] it was performed by using 5 TU (tuberculin unit) equivalent to 0.1 mL of tuberculin PPD RT23. The Mantoux test assesses the patient's response to a stimulus of purified protein derivative (PPD) 0.1 mL is injected intradermally into the volar forearm to produce a wheel of 6-10 mm diameter [17]. After 48-72 hours the induration is measured in millimeters at the point of injection and interpreted according to current guidelines. To get a reliable reading of the Mantoux skin test usually standardization of procedures, training, supervision, and practice are required. The results of mantoux test must be interpreted carefully. The person's medical risk factors determine the size of induration the result is positive (5 mm, 10 mm, or 15 mm) [18]. Montoux test is a sensitive but non-specific in the diagnosis of active tuberculosis. The interpretation of Montoux needs to be correlated to the patient's clinical context.

Montoux test has been also used for a long time as vaccination marker when there is no previous household contact with tuberculosis or history of infection so the positive reaction may be a useful signal of cell-mediated immunity against TB. This study was sought to describe the immune response to BCG vaccine among healthy, vaccinated Adults.

Materials and Methods

This study was a cross-sectional-hospital-based conducted in Khartoum state in ALSHAB Hospital, during the period of January to July 2018. A total of one hundred participants (n=100) were incorporated in this study. All participants were adult, healthy, vaccinated most of them had a scar in their vaccine injecting site. The participants were free of tuberculosis, HIV, renal disease, other mycobacterial infection also they are not injectable drug users or Mycobacteriology lab personnel and have no history of tuberculosis disease or TB household contact, so that the presence of zone may indicate the immunity against TB. All participants were screened by using the Montoux test.

Procedure of Montoux test

Montoux testing was performed using 5 TU (tuberculin unit) of tuberculin PPD RT23 through injection into the forearm. Results were read within 48 and 72 hours post injection and recorded as the transverse diameter (in millimeters) of palpable induration. History of BCG vaccination has been taken.

Interpretation of results

Once the mantoux test used for the diagnosis of latent tuberculosis the result should be interpreted carefully. In stat of no previous exposure to the TB infection and no immune system dysfunction, the vaccinated adult should be developed delayed-type hypersensitivity reaction resulting in induration zone reading more than 10 mm.

Quality control and of the results

PPD reagent which used in this test was checked for storage, stability and reconstituted before starting work.

Method used for data collection

Data was collected by using administrated questionnaire include the Gender and Age.

Data analysis

The data that collected from questionnaire and laboratory results were analyzed by SPSS version 15 computerized programs.

Results

A total of one hundred (n=100) healthy and TB symptoms free participants were screened by mantoux test. The participants were involving 55 (55%) males and 45 (45%) female (Table 1). The mean of induration zone diameter reading was 6.0 ± 6.74 (Table 2). The results showed that out of one hundred (n=100) participants screened, only 39 (39%) were positive for Mantoux test (show ≥ 10 mm induration diameter), while 61 (61%) were negative (show ≤ 10 mm induration diameter) (Figure 1). In addition, the result shows that among the 39 positive participants 23 (58.97%) were male while only 16 (41.03%)

were female (Table 3). Moreover, the result demonstrates that among the positive participants the mean of induration zone reading is higher in male 13.96 ± 3.29 than female 13.81 ± 2.22 (Figure 2). Furthermore, Among the 39 positive participants, 33 show induration diameter reading between 10 mm to 15 mm and 6 of them show zone reading more than 15 mm. In the other hand 53 out of 61 tuberculin test, negative participants showed no induration post PPD injection and the rest were shows reading zone between 5 to 9 mm (Figure 3).

Gender	Frequency	Percentage (%)
Male	55	55
Female	45	45
Total	100	100

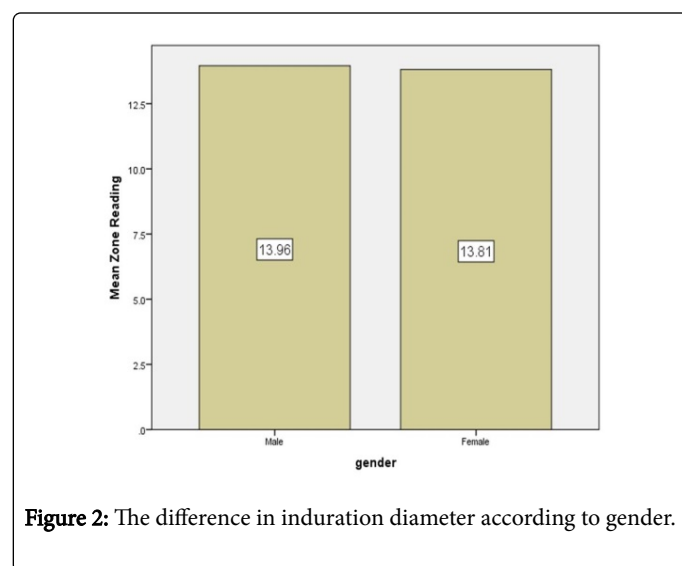
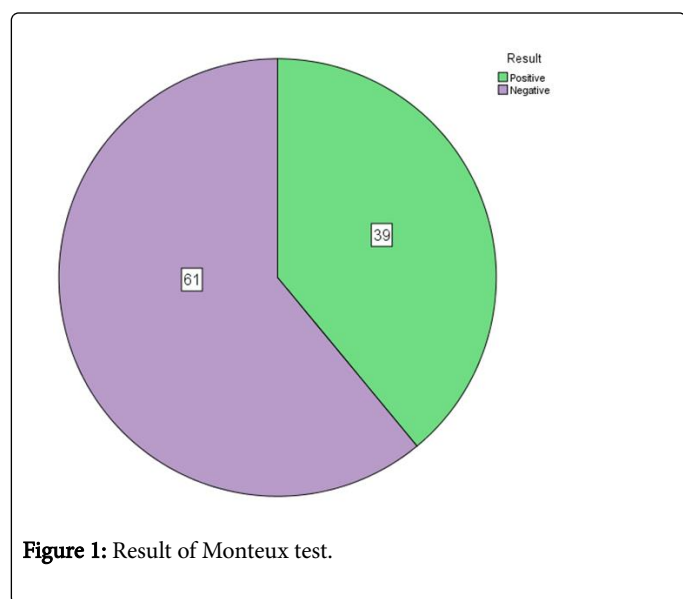
Table 1: Distribution of participants according to the gender.

Mean	Median	Mode	Maximum	Minimum	Standard dev.
6	3	0	20	0	6.742

Table 2: Mean median mode maximum and minimum of induration Zone reading.

		Result				Total
		Positive		Negative		
		Frequency	Percentage (%)	Frequency	Percentage (%)	
Gender	Male	23	58.97	32	52.46	55
	Female	16	41.03	29	47.54	45
Total		39	100	61	100	100

Table 3: Result of Mantoux test according to the gender.



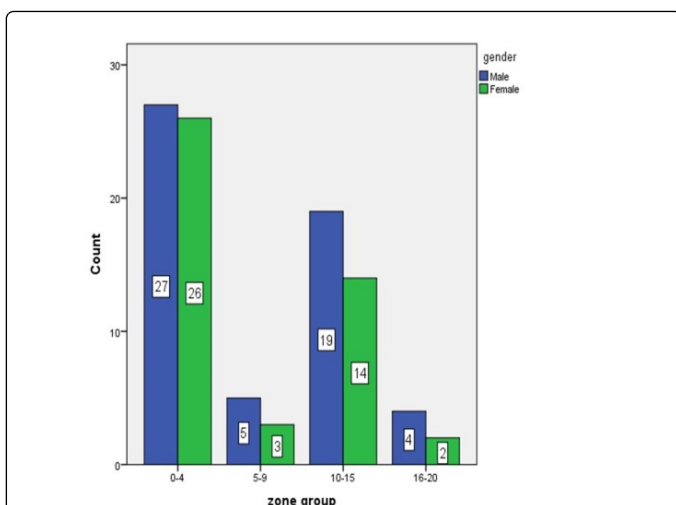


Figure 3: Zone reading group according to gender.

Discussion

The tuberculin skin test (TST) is used as a diagnostic tool to assess the latent infection with *M. tuberculosis*, it was also widely used as BCG vaccination indicator. But the interpretation of TST result for vaccinated adults remains controversial because the exposure to the TB antigen may give a false positive reaction for unvaccinated individuals. The present study was aimed to determine the immune response to BCG vaccine among healthy, vaccinated adults in Khartoum state by using mantoux test as a screening method. The results showed that out one hundred (n=100) participants screened, only 39 (39%) were positive for Mantoux test (show ≥ 10 mm induration diameter), while 61 (61%) were negative (≤ 10 mm induration diameter) and to avoid false positive and false negative result all participants were selected carefully, they were free of tuberculosis, HIV, renal disease, other mycobacterial infection also they are not injectable drug users or Mycobacteriology lab personnel and have no history of tuberculosis disease or household TB contact, so that the presence of zone and induration may be used as good indicator of the immunity against TB.

The result obtained by our study group may reflect relatively intermediate BCG efficacy rate but not absolute term because the absence of induration among vaccinated adults after mantoux test is not clear-cut for loss of cell-mediated immunity against TB, besides, the mantoux test is only screening approaches and give the only idea about the immunity of a vaccinated person. So, we need to use standard IFN γ measurement to clarify the result. The result shows that more than half of participants failed to develop induration post tuberculin test and this may be explained by either their improperly vaccinated or they take the invalid vaccine (improper vaccine storage). For those whom develop induration but they are still considered as negative (<10 mm diameter) the most accepted interpretation of their cases is that they may take subclinical dosage of vaccine or their immunity is decreased by the time and this finding agrees with some reports that the immunity against TB is deteriorated and affected by the age.

The result obtained by this study agreed with that obtained by Rosenthal et al. [19] and Smith et al. [20] whom reported that the protective efficacy ranging from zero to 75% and from zero to 80%

respectively. Our result disagrees with that obtained by Clemens et al. [21] whom indicated that the rates of protective efficacy ranged from 56% to 80% and this might be due to the difference in the technique that used in measuring the immunity, in addition, the difference in the demographic character and type of nutrient of participants may also play an important role. We found that among the positive participants the efficacy rate is higher in male than females. Moreover, the mean of the induration zone reading of male was also higher than female these differences may be explained by their different physiological feature and functions.

It is worth mentioning that our study group only do this work to give a picture about the efficacy of BCG vaccine among healthy vaccinated participants in Khartoum state and in order to clarify the picture we need to use an advanced technique like interferon gamma measurement.

Conclusion

We concluded that the efficacy rate of BCG vaccine is intermediate, male were more respond well to the vaccine than female, in addition, more than half of participants failed to develop any induration or zones. Further, studies with more sample size and using more advanced techniques (IFN γ measurement) should be done to clarify the results.

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