

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

Babbiker MTG<sup>1\*</sup>, Fatima MS<sup>2</sup>, Fairouz AM<sup>2</sup>, Rofida AY<sup>2</sup>, Safaa AM<sup>2</sup>, Sulafa RA<sup>2</sup> and Maysa IM<sup>2</sup>

<sup>1</sup>Department of Microbiology, Sudan University of Science and Technology, Sudan

<sup>2</sup>Department of Microbiology, Academy of health Science, Sudan.

Received July 26, 2018; Accepted August 16, 2018; Published November 29, 2018

### ABSTRACT

**Background:** Tuberculosis (TB) is a highly infectious disease that classified as a major global health problem and to protect against the infection two French scientists develop a live attenuated vaccine that called Bacillus of Calmette-Guerin. Before vaccination, sometime Tuberculin test is to indicate previous exposure to TB.

**Objective:** This study was aimed to determine the efficacy of BCG vaccine by a screening of healthy, vaccinated adult's subjects in Khartoum capital of Sudan.

**Method:** A total of one hundred (n=100) healthy participant were introduced in this study. The participants were involving 55 (55%) males and 45 (45%) female. In addition, they were over 20 years and most of them had a scar in their vaccination site. Only those whom TB symptoms free participants were included and screened by mantoux test. The mantoux test was done through injection of each participant by purified protein derivative PPD (only 0.1ml) intradermally into his volar forearm, then 48-72 post-injection the induration was observed and the diameter was measured.

**Results:** The results showed that out of one hundred (n=100) participants screened, only 39 (39%) were positive for Mantoux test ( $\geq 10$  mm diameter), while 61 (61%) were negative ( $\leq 10$  mm diameter). Among the 39 positives, 33 show reading between 10mm to 15mm and 6 of them show zone  $\geq 15$  mm. Among 61 tuberculin test, negative participants 53 were showed no induration post PPD injection and the rest were shows reading zone between 5 to 9 mm. Furthermore, 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  $13.96 \pm 3.29$  than female  $13.81 \pm 2.22$ .

**Conclusion:** 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 at the time of vaccination or their cell-mediated immunity against TB is reduced. Moreover, the discrepancy in the means of the zone reading 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 $\gamma$  measurement) 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, gastro urinary system, lymphatic system, circulatory system, bones, joints and skin [1]. Most infections do not have symptoms, in which case it is known as latent tuberculosis and 10% of latent infections progress to active

**Corresponding author:** Babbiker MTG, Department of Microbiology, Sudan University of Science and Technology, Sudan, E-mail: qorish456@gmail.com

**Citation:** Babbiker MTG, Fatima MS, Fairouz AM, Rofida AY, Safaa AM, et al. (2018) Screening of BCG Vaccine Efficacy among Healthy Vaccinated Adults in Khartoum, Sudan. Adv Vaccines Vaccin Res, 1(1): 9-15.

**Copyright:** ©2018 Babbiker MTG, Fatima MS, Fairouz AM, Rofida AY, Safaa AM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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 [1]. The disease reactivation occurs more commonly among people with HIV/AIDS and in those who smoke [1,4]. The only known reservoirs of *M. tuberculosis* are humans [5]. TB infection begins when the mycobacteria reach the alveolar air sacs of the lungs, where they invade and replicate within endosomes of alveolar macrophages [6]. About 90% of those infected with *M. tuberculosis* have asymptomatic, latent TB infections (sometimes called LTBI) with only a 10% lifetime chance that the latent infection will progress to overt, active tuberculous disease [7,8]. Tuberculosis of the lungs may also occur via the infection from the bloodstream. This is known as a Simon focuses and is typically found in the top of the lung. This hematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain and the bones. All parts of the body can be affected by the disease, though for unknown reasons it rarely affects the heart, skeletal muscles, pancreas or thyroid [9-11].

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 [12]. In patients with drug-susceptible TB, a 6 months 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 [13].

TB is caused by the infectious agent known as *Mycobacterium tuberculosis* (MTB), this rod-shaped bacterium also called Koch's bacillus, was discovered by Dr. Robert Koch in 1882 [14]. 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 [15].

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 [15]. 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 [16].

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 [17].

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 [18]. 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 administrated to a human in 1921 [13]. 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 [13]. The rate of protective efficacy of BCG vaccine has been affected by the method, route of administration environment and characteristic of the population [13].

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 [13].

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 doses [13].

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 [19]. 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 [20].

The efficacy of BCG remains to vary from 0%-80% [21]. Its 70%-80% effective against the most severe form of T.B such as T.B meningitis. It's less effective in preventing the form of T.B 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 T.B has been limited [22].

The immune response to mycobacterial infection is predominantly cellular [23]. It is highly dependent upon gamma interferon (IFN- $\gamma$ ) production by macrophages and antigen-specific T cells [24].

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) [23] it was performed by using 5 TU (tuberculin unit) equivalent to 0.1 ml of tuberculin PPD RT23 [24]. 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 [24]. After 48-72 h the induration is measured in millimetres at the point of injection and interpreted according to current guidelines [25]. To get a reliable reading of the Mantoux skin test usually standardization of procedures, training, supervision and practice are required [25]. 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) [25]. Mantoux test is a sensitive but non-specific in the diagnosis of active tuberculosis. The interpretation of Mantoux needs to be correlated to the patient's clinical context [25].

Mantoux 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 Mantoux test.

### The procedure of Mantoux test

Mantoux testing was performed using 5 TU (tuberculin unit) of tuberculin PPD RT23 through injection into the forearm. Results were read within 48 and 72 h post injection and recorded as the transverse diameter (in mm) 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.

### The method used for data collection

Data was collected by using administrated questionnaire include the gender and age.

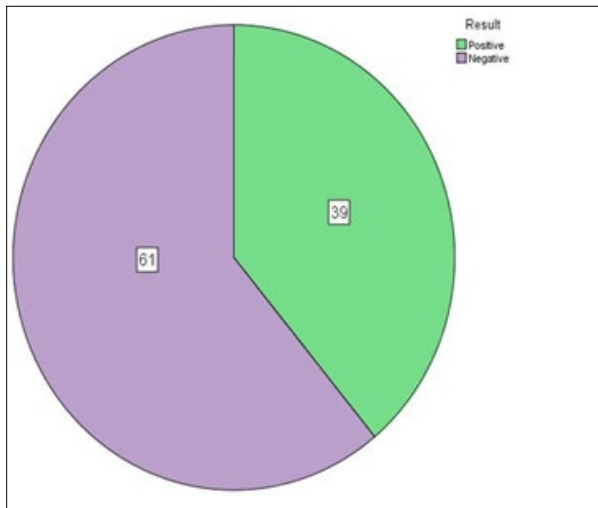
## DATA ANALYSIS

The data that collected from questionnaire and laboratory results were analysed 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  $6.0 \pm 6.74$  (**Table 2**). The results showed that out of one hundred (n=100) participants screened, only 39 (39%) were positive for Mantoux test (show  $\geq 10$  mm induration diameter), while 61 (61%) were negative (show  $\leq 10$  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  $13.96 \pm 3.29$  than female  $13.81 \pm 2.22$  (**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**).

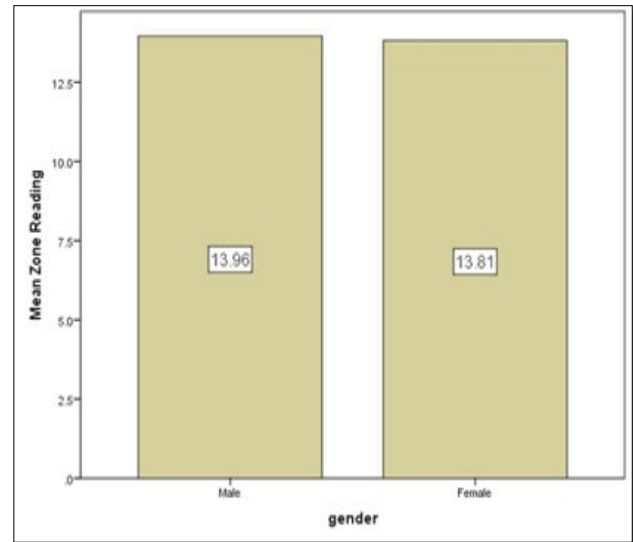


**Figure 1.** Result of Montoux test, describe that more than half of participant (61% the violet branch) were negative for mantoux test while (39% green) were positive showing induration in their PPD injecting site.

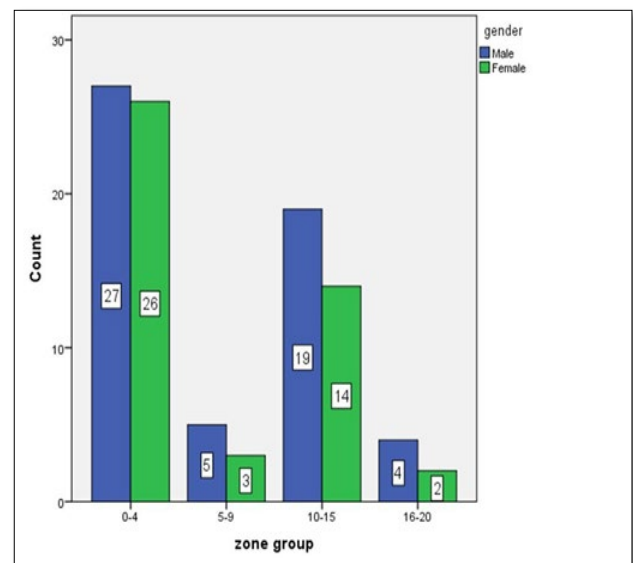
**Table 1.** Distribution of participants according to the gender.

| % Percent | Frequency | Gender |
|-----------|-----------|--------|
| 55        | 55        | Male   |
| 45        | 45        | Female |
| 100       | 100       | Total  |

It describe that the participants were 55 (55%) male and 45 (45%) female all of them were over 20 years



**Figure 2.** Describe the variance between male and female, who show induration post PPD injection, see that male induration zone reading mean (13.96) higher than female mean (13.81).



**Figure 3.** Describe that among total 100 participants in this study 53 (27 male, 26 female) show induration zone 0-4 and 8 (5 male, 3 female) show induration zone 5-9. While 33 (19 male, 14 female) show induration zone 10-15 and only 6 (4 male, 2 female) show zone reading 16-20.

**Table 2.** Describe mean median mode maximum and minimum of induration zone reading.

| Standard dev. | Minimum | Maximum | Mode | Median | Mean |
|---------------|---------|---------|------|--------|------|
| 6.742         | 0.00    | 20.0    | 0.0  | 3.00   | 6.00 |

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

|              | Results   |              |           |              | Total |
|--------------|-----------|--------------|-----------|--------------|-------|
|              | Positive  |              | Negative  |              |       |
|              | Frequency | Percentage % | Frequency | Percentage % |       |
| Male         | 23        | 58.97        | 32        | 52.46        | 55    |
| Female       | 16        | 41.03        | 29        | 47.54        | 45    |
| Gender Total | 39        | 100%         | 61        | 100%         | 100   |

It describes the result of Manteaux test according to the gender, male were more responder to vaccine than female with percentage of 58.97 and 52.46, respectively

## DISCUSSION

Currently, Bacillus Calmette-Guerin (BCG) is the only vaccine approved by FDA for use to prevent TB. Immunologically, following the BCG intradermal inoculation, resident epidermal macrophages interact with BCG via several pattern-recognition receptors (PRRs) resulting in Stimulation of T lymphocytes and protective immunity [26,27]. The tuberculin skin test (TST) is used as a diagnostic tool to assess the latent infection with Mycobacterium tuberculosis. But, it was also widely used as BCG vaccination indicator. 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 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 results showed that out one hundred (n=100) participants screened, only 39 (39%) were positive for Manteaux test (show  $\geq 10$  mm induration diameter), while 61 (61%) were negative ( $\leq 10$  mm induration diameter). These results reflect relatively intermediate BCG efficacy

rate but we must be taken in consideration the fact that the absence of induration zone among vaccinated adults after manteaux test is not clear-cut for loss of cell-mediated immunity against TB. Besides, the manteaux test is only screening approaches and gives the only Idea about the immunity status of the vaccinated person. So, we need to use standard IFN $\gamma$  measurement to clarify the result because CD4 $^{+}$  T cells, as well as the cytokines IL-12, IFN- $\gamma$  and TNF, are critical in the control of Mycobacterium tuberculosis. Furthermore, interferon-gamma release assays (IGRAs) have become common in clinical use in the 2010s and in some contexts they are used instead of TSTs, whereas in other contexts TSTs and IGRAs both continue to be useful.

The result shows that more than half of participants failed to develop induration post tuberculin test and this may be explained by either they are improperly vaccinated or they take the invalid vaccine (improper vaccine storage) at time of vaccination. 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 interpretation supported by finding of Moliva et al. [28] who reported that the mycobacterial “immunity” has been attributed to immunological memory. However, the immunity wanes over time. Also, our study group explanation is powered by Shen et al. [29] report who find the BCG efficacy against TB in humans is predicted to be 60% and wanes with increasing age, respectively. In addition, Differences in the mechanisms of Ag recognition,

Ag uptake, Ag processing and Ag presentation may contribute to the reasons behind why BCG is not fully protective and some they did not mount a successful immune response [30,31].

The result obtained by this study agreed with that obtained by Rosenthal et al. [32] and Smith [33] 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. [34] 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 most participants have a scare in their vaccine injecting site and this is a very strong evidence that the person has been mount immunity against TB but antithesis to that our result demonstrated more than half of the participants were negative to mantoux test it is not surprising when we take the fact that the immunity to TB has waned with increasing age in consideration. In 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 $\gamma$  measurement) should be done to clarify the results.

## ACKNOWLEDGEMENT

Special thanks to all who participated in this study, with our best wishes to be kept from tuberculosis infection. Also, we would like to acknowledge ALSHAB HOSPITAL vaccination department staff for their support in mantoux test performance.

## REFERENCES

1. WHO (2015) Global Tuberculosis Report 2015. 20<sup>th</sup> edn.
2. WHO (2015) Tuberculosis Factsheet.
3. Mandell G, Bennett J, Dolin R (2010) Mandell, Douglas and Bennett's principles and practice of infectious

- diseases. 7<sup>th</sup> Edn. Philadelphia, PA: Churchill Livingstone, Elsevier, p: 250.
4. CDC (2012) Basic TB facts. Retrieved February 06, 2016.
5. Center for Disease Control (2018) How TB spreads? Retrieved March 14, 2018.
6. Kumar V, Abbas AK, Fausto N, Mitchell RN (2007). Robbins Basic Pathology. 8<sup>th</sup> edn. Saunders Elsevier, pp: 516-522.
7. Skolnik R (2011). Global health 101. 2<sup>nd</sup> edn. Burlington, MA: Jones & Bartlett Learning, p: 253.
8. Mainous AG III, Pomeroy C (2009) Management of antimicrobials in infectious diseases: Impact of antibiotic resistance. (2<sup>nd</sup> rev. ed.) Totowa, N.J.: Humana Press, p: 74.
9. Khan (2011) Essence of Paediatrics. Elsevier, India, p: 401.
10. Agarwal R, Malhotra P, Awasthi A, Kakkar N, Gupta D (2005) Tuberculosis dilated cardiomyopathy: An under-recognized entity? BMC Infect Dis 5: 29.
11. Bozzano F, Marras F, De Maria A (2014) Immunology of tuberculosis. Mediterr J Hematol Infect Dis 6: e2014027.
12. Konstantinos A (2010) Testing for tuberculosis. Australian Prescriber 33: 12-18.
13. World Health Organisation (2018) BCG vaccines: WHO position paper, February 2018 - Recommendations. Vaccine 36: 3408-3410.
14. Ryan KJ, Ray CG (2014) Sherris medical microbiology. 4th edn. MC Graw Hill.
15. Meena R, Meena LS (2011) Unique characteristics feature of MTB in relation to the immune system. Am J Immunol 7: 1-8.
16. WHO (2017) Global tuberculosis report 2017. Accessed from: [www.who.int/tb/publications/global\\_report/](http://www.who.int/tb/publications/global_report/)
17. <http://www.emro.who.int/sdn/programmes/stop-TB-sudan.html>
18. Vienna (2007) Accelerating advocacy on TB/HIV.
19. Nguipodop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P (2016) Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: A retrospective population-based cohort study. Lancet Infect Dis 16: 219-226.
20. Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, et al. (2004) Long-term efficacy of

- BCG vaccine in American Indians and Alaska natives: A 60 year follow-up study. JAMA 291: 2086-2091.
21. Peter A, Timothy M (2005) The success and failure of BCG - Implications for a novel TB vaccine. Microbiology 3: 656-662.
22. Ahmad NA, Abd Hamid HA, Sahril N, Mohd Yusoff MF, Naidu BM, et al. (2013) Bacille Calmette-Guerin (BCG) revaccination: Is it beneficial for tuberculosis control? 2: 656.
23. Chadha VK (2001) Tuberculin test. Indian J Pediatr 68: 53-58.
24. Kiwanuka JP (2005) Interpretation of tuberculin skin-test results in the diagnosis of tuberculosis in children. Afr Health Sci 5: 152-156.
25. Surajit N, Basanti A (2012) Mantoux test and its interpretation. Indian Dermatol 3: 2-6.
26. Juan IM, Joanne T, Jordi BT (2017) Immune responses to Bacillus Calmette-Guérin vaccination: Why do they fail to protect against Mycobacterium tuberculosis? Front Immunol 8: 407.
27. Sendide K, Reiner NE, Lee JS, Bourgoin S, Talal A, et al. (2005) Cross-talk between CD14 and complement receptor 3 promotes phagocytosis of mycobacteria: Regulation by phosphatidylinositol 3-kinase and cytohesin-1. J Immunol 174: 4210-4219.
28. Moliva JI, Turner J, Torrelles JB (2015) Prospects in Mycobacterium bovis Bacillus Calmette-Guerin (BCG) vaccine diversity and delivery: Why does BCG fail to protect against tuberculosis? Vaccine 33: 5035-5041.
29. Shen Y, Zhou D, Qiu L, Lai X, Simon M, et al. (2002) Adaptive immune response of Vgamma2Vdelta2+ T cells during mycobacterial infections. Science 295: 2255-2258.
30. Afonso-Barroso A, Clark SO, Williams A, Rosa GT, Nobrega C, et al. (2013) Lipoarabinomannan mannose caps do not affect mycobacterial virulence or the induction of protective immunity in experimental animal models of infection and have minimal impact on *in vitro* inflammatory responses. Cell Microbiol 15: 660-674.
31. Sidobre S, Nigou J, Puzo G, Riviere M (2000) Lipoglycans are putative ligands for the human pulmonary surfactant protein A attachment to mycobacteria. J Biol Chem 275: 2415-2422.
32. Rosenthal SR, Loewinsohn E, Graham ML, Liveright D, Thorne G, et al. (1961) BCG vaccination against tuberculosis in Chicago: A twenty year study statistically analyzed. Pediatrics 28: 622-641.
33. Smith PG (1987) Case-control studies of the efficacy of BCG against tuberculosis. In: International Union against Tuberculosis, ed. Proceedings of the XXVIth IUAT World Conference on Tuberculosis and Respiratory Diseases. Singapore: Professional Postgraduate. Services International, pp: 73-79.
34. Clemens JD, Chuong JJ, Feinstein AR (1983) The BCG controversy: A methodological and statistical reappraisal. JAMA 249: 2362-2369.