

Research Paper: Comparing Tuberculin Anergy Skin Test Reactions and Lymphoblastic Transformation in Medical Students



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ABSTRACT

Background: Tuberculin Skin Test (TST) reaction is an accepted screening test for diagnosing acute and latent Tuberculosis (TB) infection among at-risk populations. It is performed with a standard Protein Purified Derivative (PPD) solution. The skin reaction of PPD is unreliable to distinguish natural infection from the Bacillus Calmette Guerin (BCG) effect. The present survey aimed to determine the prevalence of Tuberculin Anergy (TA) reactions among medical students. Accordingly, we evaluated its sensitivity, specificity, and efficiency by applying the Recall Panel Antigen Test (RPAT) and the Lymphoblastic Transformation Test (LTT).

Methods: The participants were sequentially enrolled in this study according to the designed protocol. The current study was conducted on healthy medical students before registering for the hospital training course. All research subjects were healthy, having a cicatrix of Bacillus Calmette Guerin (BCG) vaccination on the arm, and at a young age (20-24 years).

Results: In total, 180 medical students met the inclusion criteria of the study. The study subjects' Mean±SD age was 22±3.07 years (male=89, female=91). Moreover, 75 (42%) subjects presented a TA reaction in the first step of TST screening. Furthermore, 13 (64%) individuals presented a negative result of the RPAT. The sensitivity and specificity of the TST reactions, compared with the LTT were 100% and 94.4 %, respectively.

Conclusion: Significant TA reactions were found among medical students with positive cicatrix post-vaccinated. The obtained data suggested that the BCG protection for individuals may be decreased as time expired. Consequently, the medical students will especially be at high risk for TB infection during the clinical training course. The collected data highlighted the patient-safety from the viewpoint of forensic medicine.

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1. Introduction

The incidence of Tuberculous (TB) infection was 13.7% per 100.00 among the Iranian population in 2013 [1]. The burden of TB in Iran was 6% in the eastern part of the Mediterranean region (2010) [2]. The trend of new cases of TB was increasing from 6.1 to 6.3 million from 2015 to 2016. Multidrug-Resistant strains were also raised in recent years worldwide [3]. Extensive evidence indicated that TB is a threat to individuals.

Subsequent involvement to latent TB and the emergence of the resistance of strains may be a diagnostic problem in healthcare staff and medical students [4, 5]. Infected individuals may present mild or nonspecific symptoms and signs and lead to either failure or delayed diagnosis of TB [6]. The prevalence of undiagnosed TB was estimated to be 67% infected cases after death [7]. Furthermore, the prevention of infections among healthcare workers and the setting is an important point of patient safety [8] concerning mortality, morbidity, hospital stay, and cost [9]. Forensic medicine has a role in the prevention and control of communicable diseases, medical liability, and health-related and protected problems.

The effective prevention strategy of TB transmission is based on the setting of an infection control program. It included detecting suspected and confirmed infected individuals, risk assessment, TB screening, environmental assessment, and risk classification on the number of beds [10].

Bacillus Calmette-Guérin (BCG) vaccine is the only universally-accepted vaccine against human Mycobacterium (MB) tuberculosis and is used since 1921. BCG plays a protective role concerning the mortality and morbidity among TB-infected populations.

The Iranian national policy documented a BCG vaccination protocol in infants. BCG produces a superficial ulcer after the intradermal injection. The healing process occurs several months post-vaccination and their cicatrix skin ulcer remains for life.

BCG skin cicatrix is on the upper arm representative as a post-vaccination sign. The protective effect of the BCG vaccine is variable and controversial in adolescences and adults [11]. The interval period of the protective effect of BCG takes almost 10 years [12] and can induce effector memory T cells [13]. The power of the current property of BCG is more related to previous TB infection, before vaccination, and sensitization to environmental MB [14].

The Tuberculin Skin Test (TST) reaction is a delayed-type hypersensitivity response. Purified Protein Derivative (PPD) is used as a material for performing the standard TST. It is a reliable screening test for diagnosing Latent Tuberculosis Infection (LTBI) and suspected TB with culture-negative MB among the at-risk population [15]. The prevalence of LTBI was reported to be 16% in Iranian healthcare workers [16]. Besides, the frequency of Iranian PPD positive was published among the general population and medical students as 26.2% and 38.9%, respectively [15].

The anergy skin reaction is antigen-specific immunosuppression and can be generalized and cause false-negative TST reactions [17]. BCG vaccination may interfere with TST reaction interpretation. The current report suggested that the TST reaction provides a significant rate of negative values in previously young-vaccinated populations; it is associated with a high prevalence of TB infection in their countries [18]. Besides, PPD negative post-vaccinated healthcare workers had remained lower resistant to TB infection, compared to PPD positive subjects [19].

Tuberculin Anergy (TA) reaction is considered a health problem for high-risk groups, like medical students. Moreover, its reaction is unreliable for distinguishing natural infection from than BCG effect [20]. However, TA reaction depends on several factors, such as the strain and dose of the BCG vaccine, age, nutrition status, the number of years passed since vaccination, and the repeated TST [21].

The present survey aimed to determine the prevalence of TA reactions among medical students. We also evaluated its sensitivity, specificity, and efficiency by applying the Recall Panel Antigen Test (RPAT) and Lymphoblastic Transformation Test (LTT).

2. Materials and Methods

The present cross-sectional and descriptive was conducted in Loghman Hospital affiliated to Shahid Beheshti University of Medical Sciences (SBUMS).

The study sample was sequentially recruited among the first- and second-year students in the universities of medical sciences of Tehran and Shahid Beheshti. A total of 300 undergraduate medical students participated in the current study. Moreover, 180 medical students met the inclusion criteria of the study. All study participants provided written informed consent forms.

The inclusion criteria included being undergraduate medical students with a history of BCG vaccination at birth; the presence of BCG cicatrix scar formation on the arm; being healthy; no history of past or current TB infection, and contact with identified TB-infected patients. Besides, the age, gender, as well as presence or absence of a BCG scar on the arm were considered as the inclusion criterion.

The exclusion criteria of the study were as follows: Active TB infection; Human Immunodeficiency Virus (HIV) infection; immunosuppressive diseases; a history of malnutrition; a history of viral infection one month before the study; repeated TST; malignancy, and undergoing corticosteroids. The skin cicatrix of BCG vaccination was classified as a real scar (>2 mm), tiny (≤ 2 mm), and not visible [22].

Initially, all selected participants conducted PPD standard test screening. The TST was performed with PPD, containing 0.1 mL of the solution of 5 Tuberculin Unit (TU). PPD was applied intradermally into the dorsal aspect of the left forearm using a plastic tuberculin syringe and a disposable 25 G needle.

The test results were recorded 48-72 hours after the first injection. The obtained information was interpreted based on the presence of a skin induration. PPD skin reaction transversely measured the long axis of the forearm using the ballpoint method and was recorded in millimeters. The interpretation of PPD was instructed as per the Centers for Disease Control and Prevention (CDC) guideline. The skin indurations of PPD <6 mm were defined specifically as negative; undetectable skin induration was determined as TA reaction.

TA subjects were selected in the second stage. The CDC recommended the additional use of ≥ 2 control antigens with delayed-type hypersensitivity, including mumps antigen and tetanus toxoid. Anergy is defined as zero millimeters of skin induration for all delayed-type hypersensitivity antigens, i.e., administered in a given panel antigen. Skin tests with three recall antigens were administered intradermal. Skin induration >5 millimeters was considered a positive response after 24 or 48 hours from the onset of injection. The healthy subjects consisted of skin reactions with ≥ 2 antigens, and that an anergic subject has no response to any antigens.

LTT is the reflection of cell-mediated immunity and T cell function; as a rule, the LTT is reported to be well-correlated with a skin reaction. Next, the LTT was applied to the donors' peripheral lymphocytes. They have considered anergic if both PPD and panel skin test reac-

tion results were negative. The lymphocytes were cultured *in vitro* with PPD and Phytohemagglutinin (PHA).

Furthermore, 9.5 ccs of a venous blood sample were obtained and mixed with 0.5 cc heparin. It was incubated at 4°C and immediately transferred to the immunology laboratory. Defibrinated whole blood was shaken with glass beads for 10 min. Lymphoid cells are separated by centrifugation on a ficoll-isopaque gradient for 30 minutes at 2500 RPM. Centrifuged products and lymphocytes were collected at the interface by disinfected Pasteur pipette. The cells were washed three times at 1640 medium RPM. It was suspended at a concentration of 5×10^6 lymphocytes/mL and was set up on flat-bottomed microplates.

The cells were stimulated with PPD and PHA solution antigens. Then, they were washed with PBS. It contains 2% Autologous Plasma (AP). Lymphocytes were cultured in plastic tubes (polyethylene tube) in a volume of 1 mL of 10% human AB serum at 1640 medium RPMI. Antigens and mutagens (PPD, PHA) were added to the tubes in 0.1 mL volume. Its cultures were incubated for 2-8 days at 37°C within a 5% CO₂ atmosphere. It remained for 20 hours in the presence of 1 Ci of 3 H-labelled thymidines. The uptake of radioactivity was measured with a gamma counter after cell harvest on a semiautomatic harvester. The stimulation index was defined as follows: transformation (as counts per minute) in the presence of antigens/transformation in the absence of stimulants. Mean radioactivity is expressed as counts per minute (CT/min). It is determined using triplicate cultures by calculating the geometric mean [23].

Data analysis was performed in SPSS. The normality of the data was detected with the Kolmogorov-Smirnov test ($P > 0.5$). The frequency was reported by percentage. Mean scores were performed using the Pearson correlation test, Chi-squared test, and Independent-Samples t-test. $P < 0.05$ was set as the significance level.

3. Results

One hundred and eighty undergraduate medical students were recruited in the current study. The study participants' Mean \pm SD age was 23.5 ± 6.4 years. In total, 92 females and 89 males participated in this study. BCG vaccination was performed in the birthing period, and the entire subjects had real skin BCG cicatrix. Of them, 78% had >2 mm diameter, and 22% presented a tiny scar.

A total of 105 (58%) subjects were positive skin tests for PPD. Of them, 58% were males and 42% females. Furthermore, 75 (42%) of the studied students had TA

Table 1. The results of stimulation indexes of PPD antigen and PHA mutagen

Stimulation Index	Mean±SD		P
	Case	Control	
PPD antigen	1.4±0.54	10.4±5.9	<0.001
PHA mutagen	16.3±10.6	15.2±4.8	>0.05

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skin reacted, (males=37 & females=38). No significant difference was found between gender and PPD responses ($\chi=1.36$). Moreover, 47 subjects were enrolled from the TA skin reaction subset for the next step.

The RPAT was applied to the forearms of the TA skin reaction in the research subjects. Besides, 17 (36%) participants indicated positive responses to RPAT. In total, 30 (64%) students had negative RPAT responses (General Anergy; GA) (males=16, females=14).

A total of 17 subjects with negative RPAT entered in the next step for LTT as the gold standard detection test for true TA (males=9 & females=8). The results of the Stimulation Index (SI) are signified between the cases and controls for PPD antigen and PHA mutagen in [Table 1](#). One female subject revealed a positive LTT.

The sensitivity and specificity of the TST reactions concerning LTT were 100% and 94.4%, respectively. However, efficiency, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of the recent concept were reported to be 97%, 94%, and 100%, respectively. The TST and RPAT could detect true TA skin reactions among students with positive BCG skin scars.

4. Discussion

TB infection is the main causative human infectious source globally. The rate of infection is 100-300 per 100000 in Asia. One-Third of the world's population is clinically-asymptomatic infected and known as latent TB. Eight million new cases of TB infection are found annually; 60% of those will die without treatment [24].

The World Health Organization (WHO) is the first organization that attempted the control and prevent the outbreak of TB worldwide. Mass BCG vaccination and case management are the accepted effective strategy suggested since August 1947 [25]. BCG vaccine is a living and weakened Mycobacterium Bovis strain, and was first used in 1912. BCG is the most popular vaccine and the current vaccine for protection against TB world-

wide. Besides, its coverage efficacy is equal to 80%. The frequencies of protective effect are estimated to be 50% overall and in serious infection are calculated as 78%. It provides the most protective effect on TB in children (meningitis); it presents a variable efficacy in protection against pulmonary tuberculosis [26].

BCG vaccine may induce false-positive responses to the outcome of TST reactions. An essential causal factor that interferes with the BCG effect consists of the differences between BCG vaccines; the presence of the environmental mycobacteria; human genetics, and the differences of TB strains. The duration of BCG protection remains undiscovered; post-vaccination BCG reactivity has various responses concerning the TST reactions. It often persists for <10 years in the absence of exposed-M, TB, and infection. Evidence revealed a protective effect of BCG against pulmonary TB lasting until 15 years [27]. Post-vaccinated protection efficacy BCG reactivity is up to 80% in children 11. Its protective efficacy declines after 5 years and reaches 40% of the desired effect [28]. Our study findings disclosed a positive TST of 58% among medical students, i.e., higher than the current report.

The prevalence of TA reactions is low in the healthy population (0%-5%) [29]. The present study data indicated that the TA reactions were performed among hospitalized patients with a rate of 33.7% [30]. The study outcomes presented a 42% of TA reactions between focus populations.

5. Conclusion

Significant tuberculin anergy reactions were found among medical students with positive cicatrix post-vaccination. The obtained study suggested that the BCG protection for individuals may be decreased as time expired. Consequently, the medical students will especially be at high risks for TB infection during the clinical training course. The resulting data is an important point in the patient-safety from the viewpoint of forensic medicine.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article. The participants were informed about the purpose of the research and its implementation stages; they were also assured about the confidentiality of their information; Moreover, They were allowed to leave the study whenever they wish, and if desired, the research results would be available to them. All authors read and accept the publication of the article.

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Author's contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflicts of interest.

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