

Evaluation of Relative Potency of Human Tuberculin Skin Test in the Guinea Pigs Sensitized with Mycobacterium Tuberculosis, M. Bovis BCG and M. Avium

Davoud Sadeghi-Gariz,¹ Nader Mosavari,^{*2} Behnam Rafiee,³ Shojaat Dashtipour,² Ahmad Sakhravi,² Emran Heshmati,¹ Abbas Zare²

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Razi Vaccine and Serum Research Institute, Karaj, Iran
3. Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran

| Article information | Abstract |
|--|--|
| <p>Article history: Received: 17 Apr 2012 Accepted: 5 July 2012 Available online: 15 Apr 2013 ZJRMS 2013; 15(12): 1-4</p> <p>Keywords: Human tuberculin skin test Mycobacterium tuberculosis Mycobacterium avium Mycobacterium bovis BCG</p> <p>*Corresponding author at: Razi Vaccine and Serum Research Institute, Karaj, Iran E-mail: dawoodsadeghi64@yahoo.com</p> | <p>Background: The tuberculin skin test is the most commonly used test for diagnosing tuberculosis (TB) infection. The basis of tuberculin testing is the induction of a delayed hypersensitivity reaction to the intradermal injection of tuberculin. Unfortunately, this test is incapable of distinguishing <i>Mycobacterium tuberculosis</i> infection from Bacille Calmette-Guerin (BCG) vaccination or infection with non-tuberculous mycobacteria. The aim of this study is to evaluate the relative potency of human tuberculin skin test (produced by Razi Vaccine and Serum Research Institute) in the guinea pigs sensitized with <i>M. tuberculosis</i>, <i>M. bovis</i> BCG and <i>M. avium</i>.</p> <p>Materials and Methods: For skin test, different groups of guinea pigs were sensitized with <i>M. tuberculosis</i>, <i>M. avium</i> and <i>M. bovis</i> BCG. Guinea pigs were injected intradermally with 0.1 ml of 0.4, 2 and 10 µg/ml of tuberculin. Skin reactions (diameters of erythema, in millimeters) were independently measured 24 h after injection and results were calculated.</p> <p>Results: The results showed that the specificity index of human tuberculin test for guinea pigs sensitized with <i>M. bovis</i> BCG in compare of guinea pigs sensitized with <i>M. tuberculosis</i> was equal and for guinea pigs sensitized with <i>M. avium</i> was not equal.</p> <p>Conclusion: This study demonstrated that human tuberculin test produced by Razi Institute for diagnosis of latent infection to <i>M. tuberculosis</i> has lower specificity for <i>M. bovis</i> in comparison with <i>M. avium</i>.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p> |

Introduction

Tuberculosis is a disease with global spread. This infectious disease is contagious and easily transports from one person to another. It has been estimated that one third of the global population is poll infected by this disease [1, 2]. Although most of these infections are hidden but one tenth of these infections are converted to the evident tuberculosis and results in more than 2 million deaths in global level [3, 4]. Timely determination of this disease is important in the tuberculosis treatment. The disease determination is based on injection techniques of tuberculin test, incubation and the techniques based on genome. Due to the lack of existence of laboratory facilities, the experts in formed with the technique, cost, determine this disease is not conducted except in especial laboratories with incubation techniques, and the determinations based on genome. Tuberculin skin test is general test that is used to determine the hidden infection or tuberculosis active infection and is based on establishment of delayed hypersensitivity reactions after injecting the protein PPD (Purified Protein Derivative).

Skin test is a cheap and simple test which can be done without experimental expertise. PPD is extracted proteins from culture filtrate of *M. tuberculosis* which include mycobacterium antigenic proteins with variable molecular

weight which most of them are a part of mycobacterium secreted proteins [5, 6].

PPD is injected to under the skin upper layer or in other hand intraderma and the reaction is determined as a red sign around the injection point. Tuberculin skin test is not able to distinguish between the patients and the persons who received BCG vaccine or the persons who are infected to non-tuberculous mycobacteria (NTM) [7, 8].

This test is used in Iran and the world as a standard test to determine tuberculosis in spite of tuberculin skin test detects and so far the more suitable test has not been replaced to determine the tuberculosis. The goal of conducting this study was comparison of the results of human tuberculin skin test produced in Razi Vaccine and Serum Research Institute on the guinea-pigs sensitive with *M. tuberculosis*, *M. avium* and *M. bovis* BCG.

Materials and Methods

The kind of study in this research was intervention. Thirty male albino guinea-pigs weighing 500-700 g, of uniform type prepared from Razi Vaccine and Serum Research Institute were used throughout the study. The guinea-pigs were classified into three ten number groups. Ten of the guinea-pigs were sensitized with *M. avium* D₄ standard strain, and ten of the guinea-pigs were sensitized

with *M. bovis* BCG, and the remaining ten guinea-pigs were sensitized with mixture of equal amount of *M. tuberculosis* (C, DT and PN standard strain). The guinea-pigs were sensitized by intramuscular injection with 0.5 ml of a suspension in liquid paraffin (containing 4 mg/ml of heat-killed freeze-dried tubercle bacilli of avian, bovin or human type).

Forty-five days after sensitization, animals were shaved on the back and were prepared for intradermally injection. In this study, human tuberculin (1 mg/ml concentration) produced in the Razi Vaccine and Serum Research Institute was used. Three viscosities 0.4, 2 and 10 mg/ml were prepared from human tuberculin solution (to dilute, tampton phosphate having tuin was used). Intradermal injection was done from the prepared viscosities (according to the random table) near to the guinea-pigs waist bead. The injections were conducted by insulin syringe and as intraderma and the level of every injection was 0.1 ml. The skin reaction of antigens injection as the red sign around the injection point was measured 24 hours after injection. Two diameters resulting from the injection perpendicular to the injection point were measured and the result was reported in terms of millimeter.

Finally, ordering the results in the table and obtaining the total and average in the table, placing the results in the related formula, the human tuberculin sensivity amount relative to the guinea-pigs sensitive to *M. tuberculosis* was compared to the guinea-pigs sensitive to *M. avium* and *M. bovis* BCG. Also specific index was obtained for *M. avium* and *M. bovis* BCG comparison with *M. tuberculosis* (specific index amount of human tuberculin test was obtained from the skin reaction size division of guinea-pigs sensitive to *M. tuberculosis* in the skin

reaction size of guinea-pigs sensitive to *M. avium* and *M. bovis* BCG) [9].

Results

The red sign around the injection point (Fig. 1) was measured and recorded 24 h after in oculation of human tuberculin test to sensitized guinea-pigs (Table1). The most diameter zone (19.7 mm) was observed in sensitized guinea-pigs to *M. tuberculosis* after inoculation of 10 µg/ml of human tuberculin test and the least diameter zone (4.75 mm) was observed in sensitized guinea-pigs to *M. avium* after inoculation of 0.4 µg/ml of human tuberculin test. Also, the sum of diameter zone for three dose so fin oculation in sensitized guinea-pigs to *M. tuberculosis*, *M. bovis* BCG and *M. avium* were 450 mm, 444.5 mm and 259.5 mm, respectively. In this study, comparing there sult softest son sensitized guinea-pigs to *M. tuberculosis* are reference test with there sult softest son sensitized guinea-pigs to *M. bovis* BCG and *M. avium* showed that relative potency for the guinea-pigs sensitive to *M. bovis* BCG in comparison to the guinea-pigs sensitive to *M. tuberculosis* was equal to 107% (in the range of 80-125) and was equal to 767% for the guinea-pigs sensitive to *M. avium* (there was not in the range). The specificity index of skin test was calculated by division of skin reaction rate of sensitized guinea-pigs to *M. tuberculosis* on its for on sensitized guinea-pigs to *M. bovis* BCG and *M. avium* and it showed that this skin test had amore specificity than *M. bovis* BCG (as a vaccine) for differentiation of *M. tuberculosis* from *M. avium* as nonpathogenic mycobacteria (Fig. 2).

Table 1. Signs diameter 24 hours after injecting human tuberculin to the guinea-pigs sensitive to *M. tuberculosis* and *M. bovis* BCG and *M. avium*

| Concentration (µg/ml) | <i>M. tuberculosis</i> | | | <i>M. bovis</i> BCG | | | <i>M. avium</i> | | |
|-----------------------|------------------------|-----------|-----------|---------------------|-----------|-----------|-----------------|----------|-----------|
| | 10 | 2 | 0.4 | 10 | 2 | 0.4 | 10 | 2 | 0.4 |
| Average (mm) | 19.7±2.06 | 15.3±2.90 | 10.0±2.50 | 19.1±2.11 | 15.1±2.61 | 10.3±1.92 | 12.2±2.77 | 9.0±2.83 | 4.75±2.12 |
| Total (mm) | 197 | 153 | 100 | 191 | 150.5 | 103 | 122 | 90 | 47.5 |
| Sum (mm) | 450 | | | 444.5 | | | 259.5 | | |



Figure 1. The red sign around the injection point 24 hours after intradermal injection

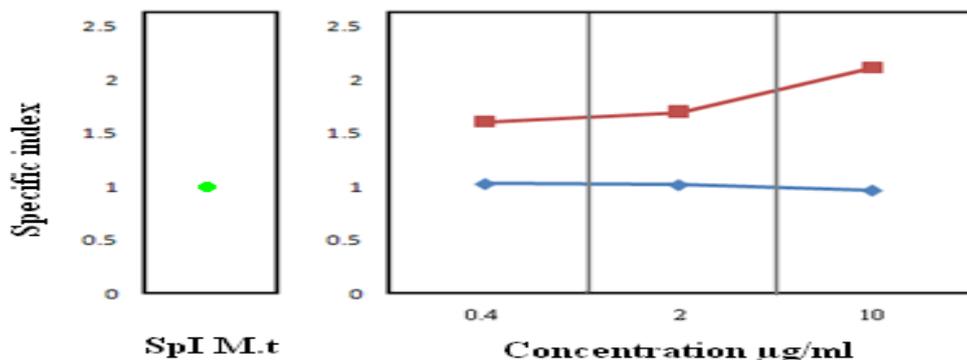


Figure 2. The specific index (SpI) comparison of human tuberculin test to distinguish between the infection to *M. tuberculosis*, *M. avium*, *M. bovis* BCG (SpI rates for *M. tuberculosis* was considered equal to 1, SpI rate for *M. tuberculosis* in comparison with *M. bovis* BCG, SpI rate for *M. tuberculosis* in comparison with *M. avium*)

Discussion

This study demonstrated that human tuberculin test produced by Razi Institute for diagnosis of latent infection to *M. tuberculosis* has upper specificity for *M. avium* in comparison with *M. bovis* BCG. About one-third of people on the world have infected with *M. tuberculosis* [1, 2]. However the most of the are latent, but about 0.1 of those will be converted to activated tuberculosis and more than 2 millions of people die from this disease annually [3, 4]. Tuberculin skin test is used for diagnosis of latent and active tuberculosis throughout the world. It is produced in Razi Vaccine and Serum Research Institute in Iran and it is used for diagnosis of tuberculosis in health centers. In a study conducted by Huebner et al., Tuberculin skin test is based on the delayed hypersensitivity reactions that produced by a mix of *M. tuberculosis* antigens [10]. In studies conducted by Huebner et al. and Harboe et al., the specificity of tuberculin skin test is so weak because the antigens of PPD are common with other mycobacterium species including BCG and non-tuberculosis mycobacteriums [6, 10].

In this study, in attempt to characterizing the human tuberculin skin test produced in Razi Vaccine and Serum Research Institute, we compared the results of test on sensitized guinea-pigs to *M. tuberculosis*, *M. bovis* (BCG) and *M. avium*. As mentioned our results showed that the human tuberculosis test produced in Razi Vaccine and Serum Research Institute is unable to distinguish between *M. tuberculosis* and BCG and its specificity is low to identify *M. tuberculosis* infections from BCG vaccine. But, this test on the other hand can distinguish *M. tuberculosis* from *M. avium* (as non-tuberculosis mycobacteriums) and also has higher specificity in distinguish between latent infections to *M. tuberculosis* in comparison with non-tuberculosis mycobacteriums.

References

1. Baumann S, Nasser-Eddine A, Kaufmann SH. Progress in tuberculosis vaccine development. *Curr Opin Microbiol* 2006; 18(4):438-48.
2. de Jonge M, Pehau-Arnaudet GR, Fretz MM, et al. ESAT-6 from *Mycobacterium tuberculosis* dissociates from

In a study conducted by Lyashchenko et al., the specificity level of human tuberculin test was considered for *M. bovis* BCG in comparison to *M. avium* and their specificity index level was equal to 1.11 relative to each other but the specificity index level of produced human tuberculin in Razi institution was obtained in the present study for *M. bovis* BCG in comparison to *M. avium* was equal to 1.71 relative to each other [9].

Concludingly it is predictable that human tuberculin test for diagnosis of latent infection to *M. tuberculosis* has lower specificity for *M. bovis* BCG in comparison with *M. avium* because *M. tuberculosis* is genetically more similar to *M. bovis* rather than *M. avium* [11].

At present, many studies exist to produce one new determination test against tuberculosis by antigens present in RD₁ genetic region which is placed in *M. tuberculosis* and *M. bovis* genome but it doesn't exist in other species including *M. bovis* BCG and non *M. tuberculosis* such as *M. avium* and intra cellular. But tuberculin test is used as standard test to determine tuberculosis in Iran and the world [12-14].

Acknowledgements

This study is related to the Davud Sadeghi thesis which is part of research design with 2-18-18-89048 related to Razi serum vaccine making.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Razi Vaccine and Serum Research Institute, Karaj.

itsputative chaperone CFP-10 under acidic conditions and exhibits membrane-lysing activity. *J Bacteriol* 2007; 189(16): 6028-6034.

3. Shiloh MU, DiGiuseppe-Champion PA. To catch a killer: What can mycobacterial models teach us about

- Mycobacterium tuberculosis pathogenesis? Curr Opin Microbiol 2010; 13(1): 86-92.
4. Zeinali M, Jammalan M, Ardestani S and Mosaveri N. Immunological and cytotoxicological characterization of tuberculin purified protein derivative (PPD) conjugated to single-walled carbon nanotubes. Immunol Lett 2009; 126(1-2): 48-53.
 5. de la Rúa-Domenech R, Goodchild AT, Vordermeier HM, et al. Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Res Vet Sci 2006; 81(2): 190-210.
 6. Harboe M. Antigens of PPD, old tuberculin, and autoclaved Mycobacterium bovis BCG studied by crossed immunoelectrophoresis. Am Rev Respir 1981; 124(1): 80-7.
 7. Ravn P, Munk ME, Andersen AB, et al. Prospective evaluation of a whole-blood test using mycobacterium tuberculosis-specific antigens ESAT-6 and CFP-10 for diagnosis of active tuberculosis. Clin Diagn Lab Immunol 2005; 12(4): 491-6.
 8. Mortal M. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am Thoracic Soc 2000; 49(6): 1-51.
 9. Lyashchenko K, Manca C, Colangeli R, et al. Use of Mycobacterium tuberculosis complex specific antigen cocktails for a skin test specific for tuberculosis. Infect Immun 1998; 66(8): 3606-3610.
 10. Huebner RE, Schein MF, Bass JB. The tuberculin skin test. Clin Infect Dis 1993; 17: 968-975.
 11. Brosch R, Gordon M, Marmiesse P, et al. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc Natl Acad Sci USA 2002; 99(6): 3684-9.
 12. Weldingh K, Andersen P. ESAT-6/CFP10 skin test predicts disease in M. tuberculosis infected Guinea pigs. PLoS One 2008; 3(4): e1978.
 13. Ganguly N, Siddiqui I, Sharma P. Role of M. tuberculosis RD-1 region encoded secretory proteins in protective response and virulence. Tuberculosis 2008; 88(6): 510-517.
 14. Farshadzadeh Z, Sankian M, Yousefi F, et al. Cloning, expression and purification of early secretory antigenic target 6kDa protein (ESAT-6) of Mycobacterium tuberculosis. Jundishapur J Microbiol 2010; 3(2): 53-60.

Please cite this article as: Sadeghi-Gariz D, Mosavari N, Rafiee B, Dashtipour S, Sakhravi A, Heshmati E, Zare A. Evaluation of relative potency of human tuberculin skin test in the guinea pigs sensitized with Mycobacterium tuberculosis, M. bovis BCG and M. avium. Zahedan J Res Med Sci (ZJRMS) 2013; 15(2): 1-4.