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 the techniques based on genome. Due to the lack of injection techniques of tuberculin test, incubation and determination of this disease is important in the existence of laboratory facilities, the experts in formed the techniques based on genome. Due to the lack of injection techniques of tuberculin test, incubation and determination of this disease is important in the existence of laboratory facilities, the experts in formed the techniques based on genome.

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 vaccinate 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 by 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, tampont 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 sensitivity 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 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*

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>M. tuberculosis</th>
<th>M. bovis BCG</th>
<th>M. avium</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>19.7±2.06</td>
<td>15.3±2.90</td>
<td>10.0±2.50</td>
</tr>
<tr>
<td>2</td>
<td>15.1±2.11</td>
<td>15.1±2.61</td>
<td>10.3±1.92</td>
</tr>
<tr>
<td>0.4</td>
<td>19.1±2.11</td>
<td>12.2±2.77</td>
<td>9.0±2.83</td>
</tr>
<tr>
<td></td>
<td>4.75±2.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (mm)</td>
<td>197</td>
<td>153</td>
<td>100</td>
</tr>
<tr>
<td>Total (mm)</td>
<td>450</td>
<td>444.5</td>
<td>259.5</td>
</tr>
<tr>
<td>Sum (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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].

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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.

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