Mycobacterium Tuberculosis Heat Shock Protein 16 as a Potential Marker for Latent TB: A Preliminary Findings

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Abstract

Identifying latent Tuberculosis (TB) is a key component of global efforts to eliminate TB. Heat shock proteins (Hsps) of mycobacteria are up regulated in response to various environmental stresses during infection. The present study was undertaken to evaluate the diagnostic efficacy of Mycobacterium tuberculosis (MTB) Hsp16, MTB Hsp65 and MTB Hsp71 in latent and active TB in a high TB prevalence area by ELISA method. The mean optical density for Hsp16 as compared to Hsp65 and Hsp71 in latent TB subjects was significantly higher than the active subjects with significant P value of 0.0004. A preliminary finding indicates that the Hsp16 is more specific to latency and may be useful as a diagnostic marker for latent TB infection.

Keywords: Latent TB; Heat shock proteins; Chaperones; ELISA

Background

Tuberculosis (TB) remains one of the world’s top ten leading causes of death. More than 2.0 million deaths were attributed to TB in 2000 [1]. One third of the world’s population infected with Mycobacterium tuberculosis (MTB) contains the infection in a latent form without any clinical symptoms [2,3]. It is assumed that protective immunity maintains MTB infection in a latent state [4]. Following primary infection, the lifetime cumulative risk for active TB is estimated to be 10-20%. Certain factors (HIV infection, malnutrition, low age, immunosuppressive drugs etc) increases the risk of reactivation when first infected [5,6].

Identifying latent TB is a key component of global efforts to eliminate TB. Due to unavailability of gold standard methods to identify the latent TB cases are increasing exponentially [7]. For the past many years, tuberculin skin test (TST) have been used worldwide, however this test was not able to differentiate MTB infected individuals from those vaccinated with Bacillus Calmette-Guerin (BCG) [8]. Recently, QuantiFERON®-TB Gold test is developed alternative to TST for diagnosis of latent TB [8,9]. However, in developing and underdeveloped countries this is under trial and not currently used in many laboratories due to its high cost and time with reference to availability of results. In absence of simple and reliable diagnosis, this latent infection becomes relatively resistant to current treatment regimen and is converted into active disease at favorable condition. This situation renders the global eradication of TB even more difficult to achieve than is the already ambitious goal embraced by the World Health Organization (WHO) of treating all active cases of TB [10]. This stress the urgency of identification of biomarkers which can be used in differential diagnosis of latent TB from active infection.

Heat shock proteins (Hsp) of MTB have received a great deal of attention recently because Hsp appears to be one of the major immunologically active mycobacterial antigens following infection and is expressed at high levels by bacterial pathogens during adaptation for intracellular survival [11,12]. Earlier from our laboratory and various other workers have reported the diagnostic applications of Hsps in pulmonary and extra-pulmonary TB [13-16], however the role of Hsp is not known in patients who are in contact with TB patients. To the best of our knowledge, not much work is done on MTB specific Hsp in latent or exposed TB population despite of lot of experimental in vitro work is done to study latent TB. It will be interesting to see whether Hsps can be useful for differential diagnosis of active from latent TB.

In the present study, we have evaluated the diagnostic efficacy of Hsp16, Hsp65 and Hsp71 in cases of latent and active TB population in high TB prevalence area of Nagpur district, Maharashtra, India.

Materials and Methods

Study subjects

We prospectively selected serum samples from 22 active TB (04 male, 18 female; age 6 to 50 years) and 24 latent TB subjects (11 male, 13 female; age3 to 75 years) from Macca Masjid, Teka Naka, Nagpur, India having high prevalence of TB and also from Central India Institute of Medical Sciences (CIIMS), Nagpur, India. All the latent TB patients, having normal chest radiographs and no signs of clinical impairment. Serum samples were obtained from active TB patients before initiation of Anti Koch Treatment (AKT) and were stored at -20°C until they were tested. In India, BCG vaccination is given within one week of the birth of child; all subjects included in the study had been vaccinated with BCG. Samples were collected from all study groups for which patient’s consent was obtained. The CIIMS Ethical Committee, Nagpur, India approved the study and all the analyses were performed double blinded. All patients were grouped as follows:

Active TB (n=22): To diagnose active TB, sputum microscopy was done on two serial sputum samples by staining with Ziehl Neelson Stain as per the guidelines of Revised National TB Control Programme (RNTCP, India).

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**Results**

The sample populations included 22 TB patients and 24 latent TB patients. There were 04 males and 18 females, with a mean age of 27.86 years (range 6 to 50 years) in TB group. In the latent TB group, there were 11 males and 13 females, with a mean age of 32.02 years (range 6 to 50 years). None of the patients were positive for anti-HIV antibodies, and none were receiving immunosuppressive drugs. Among 22 TB patients, only 2 patients had initial positive results for AFB in sputum samples. Final cultures for MTB on Lowenstein-Jensen medium obtained after 6 weeks were positive.

The results of heat shock proteins detection in serum were analyzed for all cases, expressed as OD are shown in Table 1. The mean OD for Hsp16 was 1.101 [0.495-1.904] for latent TB significantly higher than the active group 0.892 [95%CI: 0.437-1.203] (P = 0.0004) along with control group having mean OD of 0.755 [0.623-0.943]. The area under the ROC curve for latent TB by Hsp16 detection was 0.755 [95%CI: 0.623-0.943] (P = 0.0004) along with control group having mean OD of 0.755 [0.623-0.943]. The area under the ROC curve for latent TB by Hsp16 detection was 0.755 [0.623-0.943] (P = 0.0004) along with control group having mean OD of 0.755 [0.623-0.943]. The area under the ROC curve for latent TB by Hsp16 detection was 0.755 [0.623-0.943] (P = 0.0004) along with control group having mean OD of 0.755 [0.623-0.943]. The area under the ROC curve for latent TB by Hsp16 detection was 0.755 [0.623-0.943] (P = 0.0004) along with control group having mean OD of 0.755 [0.623-0.943]. The area under the ROC curve for latent TB by Hsp16 detection was 0.755 [0.623-0.943] (P = 0.0004) along with control group having mean OD of 0.755 [0.623-0.943].

The statistical analysis was performed in SPSS V10.0 and MedCalc® Software using T test for continuous variables and Mann-Whitney for the non-parametric data analysis. Results are expressed as means of OD and 95%CI. The yield of test, the selection of cut-off points and the comparison of three Hsps (Hsp16, 65 and 71) were performed with a Receiver Operating Characteristic Curve (ROC) analysis.

**Table 1:** Distribution of Hsps across each study group.

<table>
<thead>
<tr>
<th>Hsp test in serum</th>
<th>Latent TB</th>
<th>Active TB</th>
<th>Control Group</th>
<th>P-value**</th>
<th>P-value **</th>
<th>P-value **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp16, mean OD (95% CI)*</td>
<td>1.101 [0.495-1.904]</td>
<td>0.892 [0.437-1.203]</td>
<td>0.705 [0.623-0.943]</td>
<td>P = 0.0001</td>
<td>P = 0.0004</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>Hsp65, mean OD (95% CI)*</td>
<td>0.856 [0.528-1.401]</td>
<td>0.871 [0.585-1.105]</td>
<td>0.777 [0.527-1.371]</td>
<td>P = 0.03</td>
<td>P = 0.5582</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>Hsp71, mean OD (95% CI)*</td>
<td>0.637 [0.431-1.103]</td>
<td>0.694 [0.435-1.218]</td>
<td>0.682 [0.426-1.208]</td>
<td>P &gt; 0.05</td>
<td>P = 0.5886</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

*95% CI: 95% confidence intervals; **Mann-Whitney test; OD = Optical Density
Table 1 shows the occurrence of Hsp65 antigen in sera from the TB and latent TB groups as determined by the ELISA method along with mean absorbance. The mean OD was almost equal (0.871 [0.585-1.105]) for active TB as compared to latent cases 0.856 [0.528-1.401], P = 0.5582. The mean OD for control group was 0.777 [0.527-1.371]. Scatter plots of the Hsp65 activity in sera from TB and latent TB patients with mean value are shown in Figure 2. The area under the ROC curve for latent TB by Hsp65 detection was 0.55 [95% CI 0.397-0.697] with 59.09% of sensitivity and 62.5% of specificity for a cut-off value of > 0.825 (Table 2).

Scatter plots of the Hsp65 activity in sera from TB and latent TB patients with mean value are shown in Figure 2. The area under the ROC curve for latent TB by Hsp65 detection was 0.55 [95% CI 0.397-0.697] with 59.09% of sensitivity and 62.5% of specificity for a cut-off value of > 0.825 (Table 2). Scatter plots of the Hsp71 activity in sera from TB and latent TB patients with mean value are shown in Figure 3.

**Discussion**

In absence of specific test for latent TB, majority of patients remain undiagnosed and serve as a pool from which active cases of TB are drawn annually. Secondly 5–10% of recently exposed individuals develop clinically active TB in the first two years after exposure, together with the often casual nature of exposure; makes diagnosis of latent TB extremely difficult among recently exposed and potentially infected individuals [14]. In our laboratory we have identified few cases of latent TB infections which developed into active TB within one and half year of tenure (personal observations). This stresses not only the development of diagnostic tool for latent TB and but also to perform differential diagnosis with active infections.

To the best of our knowledge, this is the first published study, where we are evaluating Hsp16, Hsp65 and Hsp71 as a biomarker for latent TB in adult population.

Hsp are believed to play a role in the pathogenesis of TB. Earlier various workers have reported the diagnostic value of Hsp in serum samples of pulmonary TB [11,18,19]. Similarly, elevated levels of Hsp have also been noted in patients with cerebral TB [15]. It is not surprising that due to their wide distribution and their homology among different species, Hsp represent target antigens of the immune response [20,21]. In the present study, we have assessed the role of Hsp in Latent TB infection patients.

We have shown among all the Hsps which were used in the present study, expression of Hsp16 is elevated latent TB cases as compared to active TB and control population, demonstrating its potential as a
biomarker for latent TB in adult population. In earlier studies, using in vivo models various scientists have demonstrated that it is the most abundant protein found in latent state of MTB Infection [16,22]. Similarly Yanmin et al. [23], have proposed that Hsp16 plays an active role in slowing the growth of MTB in vivo immediately following infection. Demissie et al. [24], have shown that bacteria which are Hsp16 deficient showed reduced ability to grow inside the macrophages while reactivation revert its expression back to the low level. There are also many studies which suggest that maximum expression of Hsp16 is found in stationary phase of MTB growth [22]. These all evidences make this protein a strong candidate in the list of markers of latent infection.

Similarly the Hsp65 and 71 were also evaluated in the same set of samples active and latent infection. The expression of Hsp65 was elevated in both latent and active cases in comparison to control population. On the other hand Hsp71 level was not raised in either latent or TB cases. Overall, this finding of our study suggests that Hsp71 is not helpful in diagnosis of latent TB while Hsp65 can be used as potential markers for latent TB diagnosis but useful in differential diagnosis of latent from active TB. However, Hsp16 is expressed significantly high in latent TB groups as compared to active TB and control population. With this results it seems that Hsp16 can be used for differential diagnosis of latent TB with active TB However, we are not able to make solid conclusion unless we will evaluate this in high number of samples.

Earlier several others markers have been identified and evaluated for the latent TB diagnosis. Whitaker et al, have shown elevated level of IP-10 following MTB antigen specific stimulation of whole blood in both active and latent TB cases [25]. Similarly Ravn et al., have suggested monocyte derived chemokines inducible protein (MCP-10) as biomarkers for latent TB [26]. All the above mentioned biomarkers have diagnostic potential however like IFN-gamma and Hsp65, these markers does not distinguish between active and latent TB.

Conclusion

In conclusion, we have evaluated Hsp16, Hsp65 and Hsp71 for latent TB. Among these, Hsp16 appears the most promising marker for latent TB as its expression in very high in latent TB subjects as compared to active TB and control population. Additional studies with high number of Latent TB subjects recommended determining the sensitivity and specificity of this potential protein as latent TB specific marker.

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References