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Evaluation of heat shock proteins for discriminating between latent tuberculosis infection and active tuberculosis: A preliminary report

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Summary The diagnosis of a latent tuberculosis infection (LTBI) is of the utmost concern. The available tests, the tuberculin skin test (TST) and the Quantiferon-TB Gold test (QFT-G) cannot discriminate between active TB and LTBI. Therefore, the aim of the study is to identify new biomarkers that can discriminate between active TB and LTBI and can also assess the risk of the individual developing active TB. In total, 55 blood samples were collected, of which 10 samples were from the active TB infection group, 10 were from the high-risk exposure group, 23 were from the low-risk exposure group, and 12 were from healthy controls living in a non-TB endemic area. A panel of heat shock proteins (Hsps), including host Hsp25, Hsp60, Hsp70, and Hsp90 and *Mycobacterium tuberculosis* (MTB) Hsp16, were evaluated in all of the collected samples using ELISA. The levels of the host Hsp(s) (Hsp25, Hsp60, Hsp70 and Hsp90) and MTB Hsp16 were significantly ($p < 0.05$) elevated in the active TB group compared to the high-risk exposure group, the low-risk exposure group and the control group. Notably, the levels of the same panel of Hsp(s) were elevated in the high-risk exposure group compared to the low-risk exposure group. On follow-up, out of the 10 high-risk exposure participants, 3 converted into active

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TB, indicating that this group has the highest risk of developing TB. Thus, the evaluated panel of Hsp(s) can discriminate between LTBI and active TB. They can also identify individuals who are at the highest risk of developing active TB. Because they can be rapidly detected, Hsp(s) have an edge over the existing diagnostic tools for LTBI. The evaluation of these proteins will be useful in designing better diagnostic methods for LTBI.

Introduction

Tuberculosis (TB) is the largest health problem in the world. Approximately one-third of the world's population is latently infected with *Mycobacterium tuberculosis* (MTB), and 10% of latently infected individuals will develop active TB during their lifetime. Identifying latently infected individuals is part of the global plan for TB control [1]. Many studies have reported markers that can differentiate between active and latent TB-infected (LTBI) individuals [2,3]. Traditionally, the diagnosis of LTBI has relied on the tuberculin skin test (TST) [4]; however, the TST has several limitations, including a high rate of false-positive results among individuals vaccinated with Bacillus Calmette-Guérin (BCG) or exposed to non-TB mycobacteria [5,6]. Regardless of their reported sensitivities, T-cell-based interferon gamma (IFN-γ) release assays (IGRAs) [7,8] have been approved for the diagnosis of LTBI [9]. However, the two commercially available IGRAs, the QuantiFERON TB-Gold test (Cellestis, Victoria, Australia) and the T-SPOT TB test (Oxford Immunotec, Abington, UK), fail to distinguish active disease from LTBI [10,11]. Therefore, there is a need to explore new markers that can discriminate between active TB and LTBI and can serve as alternative or additional immunological biomarkers of the disease status.

Heat shock proteins (Hsps) have recently received a great deal of attention as promising biomarkers for TB infection [12—14] and have been shown to share diverse functions, such as the control of protein degradation, thermotolerance [15—17], immunomodulation and regulation of development and evolution [18]. Hsp(s) provide a natural link between the innate and adaptive immune responses [19]. They function as chaperones and co-chaperones, binding intracellular polypeptide chains and misfolded proteins, preventing aggregation and supporting folding and transport [20]. Our earlier findings suggest that Hsp65 and Hsp71 can be used for the diagnosis of TB [14,21]. We have also reported that Hsp16 is more specific to latency and can be used as a diagnostic marker for LTBI [22]. Therefore, with regard to the available literature and our previous findings, we aim to evaluate a panel of host Hsp25, Hsp60, Hsp70, and Hsp90 and MTB Hsp16 in the serum samples of participants that have household contact with active TB cases to screen for LTBI. The selected population has high crowding index in their homes, poor socioeconomic status and unhygienic living conditions. These risk factors may increase the transmission of TB in the population. We hypothesize that the selected panel of host and MTB Hsp(s) will be differentially expressed among the selected groups.

Materials and methods

The present study was conducted in a high TB endemic area in the Nagpur district of Maharashtra, India. As per the local health care center report, the annual risk of TB infection in this community region is very high. The community has a high household crowding index, with an average of 6—8 members living in small, closed rooms, which increases the risk of disease transmission among family members. A total of 43 blood samples were collected from eight families living in this TB endemic area. Twelve participants were recruited from an area where the annual risk of TB infection is very low, as reported by a local health care center. The blood samples collected from these participants were considered to be healthy controls for the analysis.

The participants were given an oral explanation of the study, and written consent was obtained from all of them. The study was approved by Ethical Committee of Central India Institute of Medical Sciences, (CIIMS), (01/CIIMS/Hsps/09/13) Nagpur.

Study group definition

Active TB group (*n* = 10)
This group includes family members (TB index cases) aged 17—55 years who have confirmed