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Comparison of hspX gene sequence in the Beijing and non-Beijing *Mycobacterium tuberculosis*

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ABSTRACT

Purpose: The pathogenicity of various lineages of *Mycobacterium tuberculosis* (MTB) is different. This could be due to the difference in survival ability within the host macrophage. The alpha crystalline secretion protein, a product of the *hspx* gene, is one of the bacterial protection factors in these stressful situations. The Beijing family, part of the East Asian lineage, was reported to be more virulent. Regarding the importance of this protein in pathogenicity, this study was conducted to investigate the polymorphism of the *hspx* gene in Beijing family compared to non- Beijing strains.
Method: DNA of 50 MTB isolates were extracted by boiling method. The existence of *hspx* gene was determined using PCR-specific primer and finally PCR product was sequenced to examine the polymorphism in both direct and reverse directions. Sequencing results were aligned by chromas software.
Results: The *hspx* gene was detected in all of the Beijing and non-Beijing isolates. The polymorphism in the sequences of this gene were not observed in all of the MTB isolates.
Discussion: This study indicated that *hspx* gene is protected. Also it has showed that lineage type was not related to the sequence of *hspX* gene, but the expression of this protein may be different, which requires further studies.

1. Introduction

Unlike many known pathogens, Mycobacterium tuberculosis (MTB) does not have classical virulence factors and its pathogenicity is related to the unique compounds of the cell wall and structural and secretion proteins [1,2]. The progression of **tuberculosis** (TB) can have different consequences which is determined largely by the response of the host immune system [1,2]. The bacterial survival within the macrophage is one of the main cause of the bacterial pathogenicity. In this environment, the oxygen pressure is very low and the immune system attempts to prevent bacterial growth. One of the factors contributing to this stress is the secretion of an Alpha-crystalline (Acr) protein, which is part of the hspX thermal shock proteins group. This protein helps bacteria to tolerate oxidative stress [1–3].

So far, several lineages of MTB have been identified in the world, which vary in terms of drug resistance, pathogenicity and distribution. One of the most pathogenic sub-lineages is the Beijing which was first found to be prevalent in Asia [4–6]. In the recent years this lineage caused a serious problem worldwide and detected in the reports from some African, European and American counties too [7–9]. Due to the drug resistance, treatment failure, the high rate of transmission and the ability to high replication in human macrophages considered as the predominant characteristics of this sub-lineage [10]. The reasons for the difference in the pathogenicity of MTB sub-lineages are not clear, but it seems that greater survival rate in the macrophages can be a leading feature. Indeed different genotypes of MTB have different potency in pathogenesis. It's not clear why this difference are acquired. In this study among the various known genes which is related to MTB virulence and intracellular stability on macrophage, hspX gene was selected and compare its presence and sequences in Beijing (as important pathogen genotype) and the isolates belong to other genotypes. In fact we focused on the association between polymorphism in the *hspx* gene of MTB strains and Beijing and non-Beijing families isolated from north of Iran, near Caspian Sea, which has high rate of tuberculosis.

2. Methods

Among 227 MTB strains isolated from patients of Golestan province,

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Table 1
Sequences of Primers designed for the *hspx* gene.

Primer (Name)	Sequence (5′ to 3′)	Product Size (bp)
***hspx* forward**	TCAAAGGCATCCGTTTCCATCG	**466**
***hspx* reverse**	GGTGGACCGGATCTGAATGTGC	

north of Iran near Caspian Sea, the identification of the Beijing sub-lineage was determined by using the real time PCR method described by Hilleman study [11]. This study was carried out on 25 MTB strains belonged to the Beijing sub lineage in addition to 25 strains belonged to non-Beijing sub lineage that were matched the age, gender, place of residence and ethnicity of patients harbor Beijing family.

DNA of MTB isolates were extracted by boiling methods [6]. The specific primers for the *hspx* gene was designed using primer 3 plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) which sequences is shown in the Table 1.

The PCR cycle for the *hspx* gene was as follows; 95° C for 3 min and then 29 cycles of 95° C for 30 s, 61° C for 30 s, 72° C for 30 s and finally 72° for 5 min.

The PCR product was sent to the Macrogen Corporation (Seoul, *South Korea*) for sequencing in both forward and reverse directions to investigate the polymorphism of *hspx* gene. Sequencing results were analyzed with Chromas software. Then, all the sequences were compared with the *hspx* gene of H37Rv strain present in the NCBI gene bank, GeneID: 887579, by the compatible MUSCLE software.

3. Results

The *hspX* gene was present in all 50 (100%) isolates of Beijing and non-Beijing sub lineages with a 466 bp band length. The sequences didn't show polymorphism by chromas software analysis. The sequences of *hspX* gene in Beijing and non-Beijing strains did not differ. The sequences of the *hspX* gene were compared to the standard sequence of H37Rv MTB and had 99% identities with this strain.

4. Discussion

There are several lineages of MTB in the world and one of the most important sub-lineage is Beijing. As reported by several studies different genotypes of MTB have different pathogenesis. The reason of this difference is not obvious. Among various known genes which is related to MTB virulence and intracellular stability on macrophage, hspX genes is one of the factors that is contributing to bacterial pathogenesis and its survival in the macrophage.

The alpha crystalline protein encoded by *hspX* gene of MTB is expressed during hypoxia, and low pH condition and stationary phase, similar to conditions present within areas of some granulomas [2,3]. The aim of this study was the comparison of the presence and sequences of *hspX* gene in Beijing and the non-Beijing MTB that was isolated from north of Iran.

This study showed that the sequences of *hspx* gene was conserved in all isolates of MTB, so that in Beijing and non-Beijing isolates didn't observed any difference. Therefore, this gene can be used as an appropriate target gene for the detection of MTB by methods like PCR. Although our results displayed that there was no difference in the sequence of the *hspx* gene in the different sub-lineage of MTB, the difference in expression of this gene was not determined. Consequently we suggest further studies to determine the association between expression of this gene and differences of the sub-lineages in terms of growth rate, pathogenesis, and survival in the stress conditions.

Also in the recent years, a lot of efforts have been made to use alpha crystalline protein as a stimulant for the immune system and the produce a protein vaccine alone or in combination with BCG for the control of tuberculosis [3,12]. It is also possible to use it to detect tuberculosis infections, especially latent tuberculosis, by detecting the anti-HspX IgM antibody or itself as an antigen [13].This suggests that the *hspx* gene encoding this protein should have a significant stability in the isolates of MTB.

For future studies, the determination of antigenicity of the Acr protein for the production of a vaccine against tuberculosis, study of polymorphism and mutation in the promoter regions of this gene and the level of gene expression are suggested.

5. Ethics approval statement

The studies approved by Ethics Committee of Golestan University of Medical Sciences (Ethics Code: IR.Goums.REC.1395.255). The patients/participants provided their written informed consent to participate in this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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