Prevalence of proline racemase/hydroxyproline epimerase gene in human brucella isolates in Iran

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Abstract
Background: Human brucellosis is a zoonotic disease caused by Brucella melitensis, Brucella abortus, and Brucella suis. Brucella causes a chronic disease, which subverts the immune defense system of their hosts. In this study, the prevalence of an important Brucella virulence determinant, PrpA, which can modulate immune response, was determined in human isolates.

Methods: Polymerase chain reaction (PCR) assay was standardized and applied to 37 isolates obtained from patient’s specimens. Primers for prpA gene were designed and evaluated using bioinformatic tools. DNA sequencing was performed for further verification.

Results: In the 37 Brucella isolates (31 Brucella melitensis and 6 Brucella abortus), 32 (86.4%) carried prpA gene.

Conclusion: Presence of prpA gene in most isolates indicates the high prevalence of this gene among Iranian isolates and emphasizes its role in pathogenicity of this organism.

Keywords: Brucella species, Brucellosis, Proline Racemase, PrpA

Introduction

Brucella, a Gram negative cocccobacillus, is responsible for brucellosis disease in domestic and wild animals and is transmittable to human hosts. Although humans are not the main sources of infection, the disease causes socio-economic problems in many countries (1). Broad spectrum of mammalians is at risk of becoming infected with Brucella. Humans are mainly infected by Brucella melitensis, Brucella abortus, and Brucella suis (2-3). Human brucellosis is transmitted through several routes such as infection of contaminated dairy products, inhalation, and direct contact with infected animal tissues; however, person to person transmission is extremely rare (4-5). The disease is febrile and debilitating for human hosts, with particular problems and constitutional symptoms (6). Additionally, brucellosis a chronic and persistent infection with a capacity of becoming granulomatous (7).

Pathogenicity in Brucella and other intracellular organisms such as Leishmania spp., Trypanosomes and Salmonella typhi depends on survival and replication of the organism inside the host cell. These pathogens develop multiple approaches to subvert the host immune responses. The ability to hide and survive in host cells leads to establishment of a chronic infection (8-9). Accordingly, Brucella utilizes numerous factors such as type IV secretion system (VirB), cyclic β1, 2-glucans, and LPS to manipulate the host’s immune system. Identification of new putative factors has opened many doors for better understanding of pathogenicity (10).

Brucella PrpA (Proline Racemase Protein A), homologous to a proline racemase with mitogenic activity in the human protozoan parasite Trypanosoma cruzi, is a T-independent B lymphocyte mitogen and a potent IL-10 inducer required for the establishment of chronicity and the early immune suppression observed in mice after infection (10). PrpA uses NMM-IIA (nonmuscular myosin IIA) for attachment to macrophages to activate lymphoproliferation (11). Although PrpA has been described as immunomodulatory molecule in several pathogens (12-14), there had been no information on the prevalence of PrpA in Brucella species in Iran. In the present study, a

↑ What is “already known” in this topic:
PrpA as an immunomodulatory molecule activates IL-10 and stimulates B cell replication. Organism such as Brucella requires PrpA to establish chronic infections.

→ What this article adds:
High prevalence of prpA gene in human Brucella isolates may increase the virulence capacity among Iranian isolates of B. melitensis and B. abortus.
Brucella isolates, PrpA, Human and animal samples

PCR assay was developed to detect prpA gene that was applied to 37 B. melitensis and B. abortus isolates obtained from patients in cities of Tehran, Arak and Hamadan in Iran.

Methods

Bacterial strains

From a total of 37 isolates, 31 B. melitensis and 6 B. abortus were identified based on bacteriological tests such as colony morphology, Gram staining, oxidase, catalase, CO2 growth, H2S production, and dye tolerance such as basic fushin and thionin.

DNA preparation

A loopful of colonies of each isolate on Brucella agar plates was picked and suspended in 200 μL of distilled water. After vortexing, the suspension was boiled for 5 minutes, and 50 μL of the supernatant was collected after spinning at 14 000 rpm for 10 minutes. The DNA concentration of the boiled extracts was determined with a spectrophotometer.

PCR assay

PCR amplifications were performed in a final volume of 25 μL in PCR tubes. The reaction mixtures consisted of 2 μL of the DNA template, 1 μL of each primer, 8 μL of mastermix (Taq DNA polymerase Mastermix Mix Red, MgCl2, Amplicon), and the total volume was adjusted to 25 μL using distilled deionized water. PCR program for amplification of prpA consisted of initial denaturation at 94°C for 4 minutes, 30 cycles of application with denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. Electrophoresis of PCR products was performed on 1.5 % agarose gels using 100-bp DNA ladder as molecular size marker. Gels were visualized under UV and documented using Uvitec System DOC-008.XD (EEC).

Primer designation and DNA sequencing

The nucleotide sequence of Brucella prpA gene was obtained from B. melitensis 16M chromosome II complete sequence (AE008918.1) in NCBI GenBank database. Specific primer set was designed based on pairwise and multiple sequence alignment for the corresponding gene using CLC Sequence Viewer Version 6.8.2. The primer sequences and their amplicon size are demonstrated in Table 1.

| Target gene | Primer designation | Oligonucleotide sequence (5’-3’) | PCR product (bp) |
|-------------|--------------------|----------------------------------|-----------------|
| PrpA        | PrpA-F             | AACTCAATGATGATGCACC              | 672             |
|             | PrpA-R             | ACGGTGATAGCCTGTGTC               |                 |

Table 1. PCR primer sequence used for amplification of the Brucella prpA gene.

| NUMBER | GENDER | SPECIMEN TYPE | BRUCELLA SPECIES | STRAIN | PROVINCE/CITY |
|--------|--------|---------------|------------------|--------|---------------|
| 1      | Female | CSF           | B. melitensis    | 1-A    | Arak          |
| 2      | Female | CSF           | B. melitensis    | 2-A    | Arak          |
| 3      | Male   | Blood         | B. melitensis    | 3-A    | Arak          |
| 4      | Male   | Blood         | B. melitensis    | 4-A    | Arak          |
| 5      | Female | Blood         | B. abortus       | 5-A    | Arak          |
| 6      | Male   | CSF           | B. melitensis    | 6-A    | Arak          |
| 7      | Male   | Blood         | B. abortus       | 7-A    | Arak          |
| 8      | Male   | Blood         | B. melitensis    | 8-A    | Arak          |
| 9      | Male   | Blood         | B. melitensis    | 9-A    | Arak          |
| 10     | Female | Blood         | B. melitensis    | 10-A   | Hamadan       |
| 11     | Male   | Blood         | B. melitensis    | 11-A   | Hamadan       |
| 12     | Female | Blood         | B. melitensis    | 12-A   | Hamadan       |
| 13     | Male   | Blood         | B. melitensis    | 13-A   | Hamadan       |
| 14     | Male   | Blood         | B. melitensis    | 14-A   | Hamadan       |
| 15     | Female | Blood         | B. melitensis    | 15-A   | Hamadan       |
| 16     | Male   | Blood         | B. melitensis    | 16-A   | Hamadan       |
| 17     | Male   | Blood         | B. abortus       | 17-A   | Hamadan       |
| 18     | Male   | Blood         | B. melitensis    | 18-A   | Hamadan       |
| 19     | Male   | Blood         | B. melitensis    | 19-A   | Hamadan       |
| 20     | Male   | Blood         | B. melitensis    | 20-A   | Hamadan       |
| 21     | Female | Blood         | B. melitensis    | 21-A   | Tehran        |
| 22     | Female | Blood         | B. abortus       | 22-A   | Tehran        |
| 23     | Female | Blood         | B. melitensis    | 23-A   | Tehran        |
| 24     | Male   | CSF           | B. abortus       | 24-A   | Tehran        |
| 25     | Female | Blood         | B. abortus       | 25-A   | Tehran        |
| 26     | Male   | Blood         | B. melitensis    | 26-A   | Tehran        |
| 27     | Male   | Blood         | B. melitensis    | 27-A   | Tehran        |
| 28     | Female | Blood         | B. melitensis    | 28-A   | Tehran        |
| 29     | Male   | Blood         | B. melitensis    | 29-A   | Tehran        |
| 30     | Male   | Blood         | B. melitensis    | 30-A   | Tehran        |
| 31     | Male   | Blood         | B. melitensis    | 31-A   | Tehran        |
| 32     | Male   | Blood         | B. melitensis    | 32-A   | Tehran        |
| 33     | Female | Blood         | B. melitensis    | 33-A   | Tehran        |
| 34     | Female | CSF           | B. melitensis    | 34-A   | Tehran        |
| 35     | Female | CSF           | B. melitensis    | 35-A   | Tehran        |
| 36     | Male   | CSF           | B. melitensis    | 36-A   | Tehran        |
| 37     | Male   | Blood         | B. melitensis    | 37-A   | Tehran        |

Table 2. Demographic characteristics of human Brucella isolates.

http://mjiri.iums.ac.ir
Med J Islam Repub Iran. 2017 (7 Sep); 31:57.
The amplified fragment of prpA gene was purified using Silica Bead DNA Gel Extraction Kit (Thermo Scientific, USA). Sequencing was performed on both strands using an automated sequencer system (ABI 3730xl DNA Analyzer). DNA sequences were edited by Chromas Lite Version 2.5.1 (Technelysium Pty Ltd, Australia) and BioEdit Version 7.2.5 (Hall, 1999).

**Results**

From a total of 37 isolates, 31 (83.7%) B. melitensis and 6 (16.3%) B. abortus were identified from 29 blood and 8 CSF human samples. Of the patients, 23 (62%) were male and 14 (38%) were female. Detailed information on specimen type, Brucella species type, and areas of sample collection are presented in Table 2. As expected, prpA gene assay produced an amplicon of 672 bp (Fig. 1).

In the current study, all B. melitensis isolates had prpA gene; however, just one of the six B. abortus isolates was positive for this gene (Table 3). Analysis of the sequenced gene with Chromas software and blast in the NCBI (National Center for Biotechnology Information) site showed the same DNA sequences; therefore, all the PCR assay results were confirmed.

**Discussion**

*Brucella* has an amazing ability to adapt to hosts’ cellular atmosphere and evade immune responses. Other organisms which cause chronic diseases such as *Mycobacterium tuberculosis, Salmonella* spp, and *Trypanosoma cruzi* use the same mechanism to reach their replication niche (15). Although little is known about the molecular mechanisms of *Brucella* virulence factors, numerous putative factors have been identified (16).

The present study aimed at identifying *B. melitensis* and *B. abortus* strains, which were carriers of prpA gene. Our results confirmed the presence of prpA gene in 32 (86.4%) *B. melitensis* and *B. abortus* isolates collected from human patient specimens. Interestingly, 5 out of 6 *B. abortus* isolates lacked this gene, which is not completely consistent with the result of Spera et al. (10). Our findings revealed the presence of prpA gene in human isolates of *Brucella* (100% *B. melitensis*, and 16.6% *B. abortus*) and not in animal isolates, which may be responsible for variations in results. It can be concluded that the present gene is more frequent in *B. melitensis* strains compared with *B. abortus* strains in Iran; however, there is no warrant for expression of the gene.

Proline racemase protein, a homodimeric enzyme, was initially identified in *Clostridium sticklandii* protobacterium; however, a eukaryotic proline racemase was first isolated from *Trypanosoma cruzi* (TcPRAC), the causative agent of Chagas disease (17). According to Goytia et al., Proline racemase function is similar to hydroxy proline epimerase activity as a lymphocyte mitogen (18). This enzyme is capable of converting L and D-proline enantiomers reversibly (19); D-amino acids are often found in eukaryotes and bacterial cell walls (20). Proline racemase antibodies and inhibitor, and pyrrole-2-carboxylic acid (PYC) exert influence on the infection of *T. cruzi* in vitro. PYC can also interfere in intracellular *T. cruzi* differentiation (19). PrpA in *Brucella* is a molecular virulence factor, which can put the host’s immune system in an anergic state (10).

**Conclusion**

The present study demonstrated a very high level of prpA gene presence in human *Brucella* isolates. The design of a PCR test to assess the presence of aforementioned gene was a first attempt to understand the mechanism of virulence, especially in Iranian isolates of *Brucella melitensis* and *Brucella abortus*. For further understanding of prpA genetic epidemiology, similar studies on animal isolates may be required.

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**Table 3. Distribution prpA genes among 37 human Brucella isolates**

| Virulence gene | Human isolates | B. melitensis (n=31) | B. abortus (n=6) |
|---------------|----------------|---------------------|-----------------|
| prpA          |                | 31 (100)            | 1 (16.7)        |

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Fig. 1: Representative PCR products of Brucella prpA gene from human strains of B. melitensis and B. abortus. Lanes M, DNA Ladder Mix; Lanes 2,3,7,8,9,10,12,13 are Brucella melitensis strains positive for prpA. Lane 14 Brucella abortus strain positive for prpA, Lanes 4,5,6,11,15 are Brucella abortus strains negative for prpA.
Conflict of Interests

The authors declare that they have no competing interests.

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