Full Length Research Paper

Detection of mycobacterial skin infections by polymerase chain reaction (PCR) amplification of deoxyribonucleic acid (DNA) isolated from paraffin-embedded tissue

Kasra Behrouznasab1*, Mohammad Reza Razavi2, Hassan Seirafi3, Taheer Nejadsattari4, Kumarass Amini5 and Kumarss Amini5

1Department of microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2Department of Parasitology, Pasteur Institute, Tehran, Iran.
3Department of Dermatology, Tehran Medical Sciences University, Tehran, Iran.
4Department of Biology, Faculty of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
5Department of Microbiology, Saveh Branch, Islamic Azad University, Saveh, Iran.

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Atypic mycobacterial granulomatous skin infections are often caused by Mycobacterium marinum, Mycobacterium ulcerans, Mycobacterium fortuitum, Mycobacterium chelonae, and rarely Mycobacterium avium. The lesions appear as papules, nodules in hands, plaque blisters, wart ulcers and markers transmission (sorotrichosis) in the path of lymph glands and lesions; and display a granulomatous accumulation of giant cells. Infection is limited to the skin, and in other cases it could lead to immunosuppression. To determine if mycobacteria were present in granulomatose skin lesion, a total of 58 paraffine tissue blocks were obtained and deoxyribonucleic acid (DNA) isolated the polymerase chain reaction (PCR) that was used to amplify the 16S rRNA gene. PCR amplification demonstrated the presence of Mycobacterium spp. in 18 blocks (31%). Among these 18 blocks, 8 (44%) positive for M. marinum, 33 (17%) for M. ulcerans, 5 isolates (27%) M. fortuitum and M. chelonae, 2 (12%) M. avium. We conclude that mycobacteria ought to be considered in the treatment of skin granulomas in Iran.

Key words: Mycobacterial, granuloma skin infection, polymerase chain reaction (PCR).

INTRODUCTION

Atypic mycobacterial infection typically includes pulmonary infections, lymphadenitis, and granulomatous skin and transmitted infections (in people with immunodeficiency) (Wagner and Young, 2004) skin infections are often caused by Mycobacterium marinum, Mycobacterium ulcerans, Mycobacterium fortuitum, Mycobacterium chelonae are typically In from of swimming pool granuloma (Palenque, 2000). Skin infections are probably caused by a common environmental source and due to exposure to aquatic animal, exposure to fish farming ponds, swimming pools, injections, and by surface wounds or skin scratching, truma (Mahaisavariya et al., 2003). Granulomatous cutaneous lesions, are often appeared on the elbows, fingers, hands as papules, nodules on the hands, blisters, plaques, wart ulcers, sorotrichoid transmission lesions and sometimes ulcerate. Most of lesion will remain within 1 to 3 years by leaving Scar (Bartralot et al., 2005). The infection is limited to the skin and in case of immunosuppression would be changed to lymphatic transmitted spread infection. Transmission from person to person is rare (Tan and Chan, 1999). Polymerase chain reaction (PCR) is a specific, sensitive and accurate
method for diagnosis of bacterial infections. This method can recognize a few atypic Mycobacterial genomes in samples (paraffin-embedded tissues) and its differentiation from granulomatous cutaneous lesions similar to parasitic and fungal. (Cook et al., 1994; Ghossein et al., 1992). The PCR with atypic mycobacterial 16S rRNA and specific primers have been applied for detection of a variety of atypic mycobacterial species (Boddinghaus et al., 1990; Abed et al., 1995; Vanechoutte et al., 1993). With this diagnostic method cutaneous lesion caused by atypic Mycobacterial can be diagnosed from other granulomatous infections such as leishmaniasis and sporotrichosis and treatment of such cutaneous disease with use of appropriate antibiotics and if necessary using surgery (Sanchez et al., 2000 Wolinsky et al., 1972; Frevel et al., 1999). The aims of this study were to detect atypic Mycobacterial (M. marinum) in paraffin-embedded tissues and to differentiate it from similar cutaneous lesion (Leishmaniasis and Sporotrichosis) by PCR method.

MATERIALS AND METHODS

Fifty five (58) formalin- fixed, paraffin- embedded tissue samples of granulomatous lesion from the period 2006 to 2010 were obtained from the Pathology archives of Razi Hospital, Iran. All paraffin-embedded skin tissues had clinical signs of granuloma. Mycobacterium smegmatis (PTCC 1307) was used as a positive control. Were cultured in Lewenstein-Jensen (LJ) agar medium. At 14 days they were checked for Mycobacterium colony appearing on the agar media.

PCR method

DNA was extracted from paraffine-embedded tissue following the method of Goldmann et al., (1998). In this study two primers (forward and reverse) which had been designed and amplified at a 136 bp region of 16S rRNA gene of member of the genus mycobacterium and two primers to amplified at a 250 bp region of 16S rRNA gene of M. marinum were used (Table 1).

Deoxyribonucleic acid (DNA) amplifications were carried out in a total volume of 35.25 μL containing 17.5 μL DNA, 0.1 μL of each primers, 0.5 μL dNTP mix (10 mM) (Cinnagen Inc., Tehran, Iran), 4 μL MgCl₂ (25 mM), 2.5 μL PCR buffer (10x) (Cinnagen Inc., Tehran, Iran), and 0.25 μL Taq DNA polymerase (5 unit/μL) (Cinnagen Inc., Tehran, Iran). Reaction mixture were thermocycled 30 times beginning with an initial denaturation step of min at 94°C. The temperature and time profile of each cycle was as following: 94°C for 1 min (Annealing) and 72°C for 1 min (extension), reactions were finished with a final extension step at 72°C for 5 min (Folgeira et al., 1993). PCR products (1 μL) were mixed with 2 μL Loading buffer (6x) and the PCR products and 100 bp DNA Ladder were then separated by electrophoresis (100 volts for 1 h) in 1% agarose gel and stained with 0.5 μL/ml ethidium bromide.

RESULTS

From a total of 58 samples that were paraffin-embedded tissues, 18 (31%) samples had granulomatous skin infections caused by atypic Mycobacterial and 40 (69%) samples of granulomatous skin infection had recourse of non-Mycobacterium (Parasitic and fungal).

PCR identification of atypic mycobacterial species in samples was successful in 18 strains and showed specific amplicon at 136 bp (Figure 1). This proved that 31% suspected granuloma tissue were infected with Atypical Mycobacterium in PCR test. The results of PCR samples are present in Tables 2 and 3. On the PCR test, 8(44%) isolates examined were positive for M. marinum, 3(17%) isolates for M. ulcerans, 5(27%) isolates for M. fortuitum-chelonae and 2(12%) isolates for M. avium showed specific amplicon at 250 bp. (Figure 2). This method established that 8 granuloma tissues out of 58 were infected with M. marinum. PCR results of total samples, in 40 samples were negative, in 40 PCR negative samples (69%) of skin infections had other than parasitic, and fungous from paraffine- embedded tissue samples.

DISCUSSION

The results reported in this paper confirmed the detection of M. marinum as major agent of swimming pool granuloma for the first time in Iran.

These results reported occurrence of M. marinum in Iran such as other countries with intensive reaving of granuloma tissues (Sciacce, 2006). Microorganism with characteristics of atypic mycobacterial was isolated from granuloma skin tissues. The identification of the isolates was performed by the PCR test. Routine identification of atypic mycobacterial species is usually based on classic methods including acid-fast staining, culture, biochemical tests, BACTEC and immunofluorescence examination.
Specificity of the PCR detection assay using the specific primers. M: Marker 100 bp; PC: positive control [M. smegmatis (PTCC 1307)]; NC: Negative control, 1-9 suspected samples. The formation of 136 bp bands in 9 Atypical Mycobacterium positive samples.

Table 2. Differentiation of granulomatous skin lesion samples for atypical mycobacterium PCR methods results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PCR</th>
<th>Granulomatous skin infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>+</td>
<td>Atypical Mycobacterium</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>Non Mycobacterium (parasitic-fungal)</td>
</tr>
<tr>
<td>58</td>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

Table 3. Identification of Atypical Mycobacterium species of samples, Atypic Mycobacterial-PCR and M. marinum-PCR results.

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>Atypical mycobacterium- PCR</th>
<th>Mycobacterium species- PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>18</td>
<td>+</td>
</tr>
<tr>
<td>58</td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>44</td>
<td>M. marinum (8)</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>M. ulcerans (3)</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>M. fortuitum chelonae (5)</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>M. avium (2)</td>
<td>+</td>
</tr>
<tr>
<td>58</td>
<td>Total</td>
<td></td>
</tr>
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</table>

(Tsukamura, 1981). PCR is faster than the routine tests and it can be used as trusty and supersede test in the detection of M. marinum from skin tissues. In this present study this technique showed a distinct advantage over the culture method of identification. In this study, examination of 58 granuloma skin tissue samples provides 18 atypical mycobacterium species. All of Mycobacterium positive samples that analyzed for M. marinum infection by PCR method was positive (44%) on the other hand 58 skin tissue samples were collected that detected 18 atypical mycobacterium species (31%) and 40 samples were caused by non Mycobacterium (69%). This study suggests that, among different collecting sites, granuloma skin tissue samples are suitable for detection of
M. marinum and the number of atypical mycobacterial in granuloma tissue may vary considerably. This established that main agent of fish tank granuloma was M. marinum and other strains may infect granuloma.

In our examination PCR-RFLP technique and sequencing genes 16S r RNA, hsp65, ITS were used for separating different Mycobacterium species (Marie-Claude et al., 2000) and in other study used the PCR technique of 16S rRNA and 23S rRNA genes and identified species of Mycobacterium (Abed et al., 1995). Reaserchers proved that the infection limited to the skin infected with M. marinum which in most cases was associated with fish and the majority of these skin lesions were on the hands with clinical sporotrichosis appearance (Sanchez et al., 2000). During an epidemiological study patients with cutaneous infection M. marinum were doing fishery, were exposure with fish due to their job, it can be concluded that the most dangerous places to get infected with this bacterium are swimming pools and fish farming ponds (Johnson and Tzumi, 1987).

Granulomatous skin infections caused by atypical Mycobacterium the infection generally occurs in soft tissues, skin and sometimes deep wounds, necrotic ulcers and subcutaneous on the skin and lesions has granulomatous transmission sporotrichosis in the path of lymph glands similar cutaneous lesion (Leishmaniasis and Sporotrichosis), (Sciaccce-Kirby, 2006).

In conclusion, this report demonstrates that PCR methods can accurately identify atypical mycobacterial contamination, but the culture method can not only be costly and time consuming but may also show some false negative results. The PCR method is rapid, reliable, and simple method for the detection of mycobacterium contamination in granuloma tissues and PCR technique can be successful for the detection of M. marinum and were differentiated from similar parasitic and fungal in granulamous skin lesion samples.

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