Identification of Leishmania Spp. in the Infected Foot Wounds of Patient with Mycobacterium Fortuitum

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Abstract

A common intramacrophage niche for leishmanial and mycobacterial pathogens, and a similar spectrum of immune response and disease phenotypes, led to the prediction that the same genes/candidate gene regions might be responsible for genetic susceptibility to mycobacterial infections. There are several reports of atypical cutaneous presentation of leishmaniasis such as paronychial, whitlow, lid, scar, palmoplantar and chancriform in immunocompetent patients. Numerous Leishmania organisms were observed within the histiocytes and extracellular in patient suffering from swelling, pain and itching. An unknown strain of mycobacterium was also isolated from biopsy specimen of the patient. Phenotypic and molecular tests carried out for identification of the strain based on standard methods. The susceptibility of the strain to anti-mycobacterial agents was performed by CDC standard Method. Numerous Leishmania organisms were observed within the histiocytes and extracellular space. PCR amplifications tests for detection of leishmania have been positive. Acid-fast staining and culture tests have been positive. PCR amplifications tests for detection of M. tuberculosis and M. avium have been negative. Phenotypic tests mainly including, positive result for growth at 25°C and 37°C and PNB test,
growth on MacConkey agar without crystal violet, arylsulphatase, urease, iron uptake and 68°C and 22°C catalase and negative for tween 80 hydrolysis, tolerance to 5% NaCl, niacin production and nitrate reduction test provided evidences that the strain belong to M. fortuitum species.

Our findings show that M. fortuitum however rare its incidence may be is capable of causing infection in immune-compromised patients and could be co-infection in the human population.

**Keywords:** Leishmania; Co-infection; foot wounds; Mycobacterium fortuitum

1 Introduction

Leishmaniases are a group of diseases caused by several species of the genus Leishmania. Human leishmaniasis is usually classified as cutaneous and visceral or old world type and new world type. The species involved in old world type are L. major, L. tropica, L. aethiopica and L. donovani infantum [1]. The species responsible for new world type of cutaneous leishmaniasis are L. Mexicana and L. brasilienensis. More than 90% of all cases of cutaneous leishmaniasis occur in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Peru, Saudi Arabia and the Syrian Arab Republic [1, 2]. Visceral and cutaneous leishmaniasis has been reported in Iran [2]. More recent reported incidence of cutaneous and visceral leishmaniasis in Iran estimates about 42.2 in 100000 and 0.3 in 100000, respectively [1, 2, 3]. In Iran, where tuberculosis is prevalent and considers as one of the most challenges of public health, reports of disease caused by Non tuberculosis mycobacteria (NTM) is increasing [4, 5]. Co-infection of leishmaniasis and different species of Mycobacterium in the same lesions have been reported from different parts of world [6, 7]. We report here the first well documented of a unique coetaneous ulcer resulting from coinfection of Leishmania spp. parasite and M. fortuitum from a patient. Different approaches including smears microscopy of biopsies, phenotypic tests and molecular assays have been used for diagnosis.

2 Case report

In Jun 2013, a 28 years old male civil engineer from south of Khozestan (south east, Iran) admitted to Masoud laboratory (Tehran, Iran), presented with non healing wound over right side of his foot for last 19 months. Patient had recently returned from Mashhad city as endemic region of leishmaniasis at North east of Iran. Initially the lesions started as two painless acne like lesions. On examination, the lesion was raised, rim, dome-shaped and dull red in color. The disease typically follows a slow chronic course over many years with painless swelling and intermittent discharge of pus. He had a deep itching sensation. After 12 months of appearing of lesions, massive swelling of the area occurs, with indurations, skin rupture, and sinus tract formation. As the infection spreads, old sinuses close and new ones open (Figure 1). The exudates are typically granular. As the infection spreads, old sinuses close and new ones open. For isolation and identification of causative agent, five independent biopsies were obtained from patient. The samples were investigated for fungal infection, tuberculosis, mycetoma, leishmaniasis, NTM and anaerobic pathogens which sometimes involve this area.
2.1. Microbiology Testing

Five independent biopsies were obtained from patient. The samples were subjected to batteries of phenotypic test including direct microscopy examination of acid fast staining and Giemsa staining, Gram staining, partial acid fast staining and culturing on Blood agar, Saboraud Dextrose Agar, Lowenstein-Jensen (LJ) medium and Thioglycollate broth. A part of each biopsy also examined using standard primers for detection of DNA of M. tuberculosis (750bp segment of the IS6110) and M. avium (411bp segment of rpoB gene) by commercial kits (DNA technology kit, Russian company). Reference strain M. tuberculosis H37Rv was used as control. The samples also were examined by constant region of the kinetoplast minicircles, [8]. For each, PCR amplification were carried out in 50 µl tube containing 2 µl KCl, 2 µl Tris (pH 8.0 ),1.5 µl MgCl2, 0.5 µl dNTP, 1U Taq polymerase, 27 µl water ( DDW molecular grade), 20 pmol of each primer and 6-10 µl of DNA template. The following thermocycling parameters were applied: initial denaturation at 95°C for 5 min; 36 cycles of denaturation at 94°C for 1 min; primer annealing at 56°C for 1 min; extension at 72°C for 1 min and a final extension at 72°C for 10 min. PCR amplified products were run onto a 1% agarose gel and stained with ethidium bromide and visualized under ultraviolet (UV) light.

3 Results

Direct microscopy examination of samples containing the granules was negative for fungal infection using 10% KOH. Partial acid-fast staining also yielded negative results but acid-fast staining shown few acid-fast bacilli on the smear (Figure 2). Leishmania spp. identified in biopsies of patient using Giemsa staining method (Figure 3).

Culture of clinical specimens on Blood agar, Saboraud Dextrose Agar and Thioglycollate broth were negative but rapidly growing mycobacteria (RGM) was grew in pure culture on LJ medium from five samples.

Grown colonies of RGM isolates were identified to the species level using phenotypic tests including aroylsulfatase activity, catalase production, growth rate, niacin accumulation, nitrate reduction tests, pigment production, tehurita reduction, TCH (2-thiophen carboxylic acid) and urease activity according to CDC standards methods, which confirmed the identity of the isolates as M. fortuitum like species.

Further identification of isolates by using hsp65 PCR restriction length polymorphism analysis (hsp65-PRA) method using two specific primers Tb11 (5’-ACCAACGATGGGTGTCCAT-3’) and Tb12 (5’-CTTGTCGAACCGCATACCT-3’ and digestion with two restriction enzyme (BstEII and HaeIII), confirmed the identity of the isolates as M. fortuitum species [9].

Direct PCR amplification of clinical samples for M. tuberculosis and M. avium were negative but PCR was positive for Leishmania spp (Figure 4).
Figure 2 (A&B): leishman body form that were seen in gimsa staining of lesion of his foot

Figure 3: M. fortuitum were seen in asid fast staining

Figure 4: Electrophresis results of leishmania from biopsy and smears. Lane1, Negative control, lane 2, 6 leishmania 120 pb from smear.Lane 3, 4, 5 represent 120bp leishmania fragment from biopsy. M: 50bp size marker (Fermentas).

The susceptibility of the strain to common antimycobacterial agents was performed by the proportional method and revealed that the isolates were resistant to isoniazid and rifampin. After detection of Leishmania spp and M. fortuitum from obtained clinical sample, the patient was put on Lomidine for leishmania therapy and Amikacin and Ofloxacin for M. fortuitum treatment. Complete recovery was observed after three months of treatment.

4 Discussion

A number of clinical cases of concomitant VL-TB have been reported in Sudan [10] and in other parts of the world [10]. However, little is known about the degree of interaction between both infections at community level.
Co-infection of tuberculosis and leishmaniasis has been reported previously in patients with altered immune response due to primary or secondary immunodeficiency. [10, 7] These intracellular pathogens have a unique interaction in the host immune response and their development depends on impaired cell-mediated immunity. A case of chronic non-healing cutaneous ulcer with chronic fistulating soft-tissue mass is presented.

We can suppose three strategies for immune compromised in this patient. Leishmania spp were used a mechanism for inhibition of phagolysosome biogenesis that prevent elimination of pathogen using reactive free radicals [11] and presentation of Leishmania antigens using MHC molecules [12]. Second, this pathogen used antigenic shedding and antigenic variation that lead to polyclonal activation of lymphocyte in immune system [13]. Finally, polyclonal activation promotes immune response exhausted [14]. Recruitment of regulatory T lymphocytes (Treg) to the site of infection is other way for diminishing of immune response [15].

Regulatory T cells secrete inhibitory cytokine such as IL-10 that lead to decrease antigen presentation and expression of costimulatory molecules such as CD80 and CD86 [16]. These events provide a situation for beginning of opportunist infection such as M. fortuitum. Taken together presence of Leishmania spp in five independent biopsies of patient and positive PCR test for Leishmania spp parasite and grown of pure culture of M. fortuitum from patient samples in addition to presence of acid fast bacilli confirm a coinfection and well documented case of disease caused by Leishmania spp parasite and M. fortuitum.

As leishmaniasis was suspected, biopsies were taken and examined by microscopy of smears (Giemsa stain), culture on Novy-McNeal-Nicolle (NNN)-medium, and by mini-exon repeat PCR.

References


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