Original Articles

Cutaneous Leishmaniasis in Multan: Species identification

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Abstract

Objective: To identify the Leishmania species being responsible for cutaneous leishmaniasis in Multan.

Method: Parasites were isolated from clinically and parasitologically confirmed lesions of cutaneous leishmaniasis from 30 patients by fine needle aspiration (FNA). The bioptical materials were then cultured in Evans Tobie's medium and parasites isolated were identified by isoenzyme electrophoresis technique.

Results: Successful Leishmania isolates were obtained from 16 patients. All strains were identified by biochemical techniques as belonging to Leishmania tropica zimodeme MON7 variant PGD.

Conclusion: The causative species was identified as Leishmania tropica. (JPMA 53:445; 2003)

Introduction

Cutaneous leishmaniasis (CL) more popularly known as Oriental Sore or Delhi Boil is a parasitic infection caused by different species of protozoa Leishmania and transmitted by sandfly of genus Phlebotomus in the Old World. The disease is widespread in tropical and subtropical areas.

In addition to cutaneous leishmaniasis, the other clinical variants of leishmaniasis in human beings are attributed to different leishmania species and depending upon the extent and severity of involvement are, diffuse cutaneous leishmaniasis, mucocutaneous leishmaniaisis and visceral leishmaniasis.

In Pakistan both visceral and cutaneous leishmaniasis prevails over several endemic foci with different climatic and geographical conditions[1] (Figure). The visceral form is restricted to the northern areas like Gilgit, Baltistan and Azad Jammu and Kashmir, but alarmingly cases have been reported from areas of NWFP as well as Punjab which neighbour Azad Jammu and Kashmir.[2,3] On the other hand the cutaneous leishmaniasis is endemic and highly prevalent in the provinces of Baluchistan and North West Frontier Province (NWFP).[4] In Sindh province, cases have been reported from Dadu, Larkana, Jacobabad and Karachi, while in Punjab region, the disease is mainly present in Multan and Dera Ghazi Khan.[5] In Multan, the oldest city of southern Punjab with a population of 1.5 million,the disease is mainly concentrated in endemic form in interior old part of the city.[6,7]

Most studies in Pakistan on Leishmania species identification have been carried out in Baluchistan, NWFP and Azad Kashmir where two species, Leishmania tropica and Leishmania infantum[8,9], were identified as being responsible for leishmaniasis. However in Punjab region very limited work has been done. Therefore this study was carried out to identify the causative Leishmania species in Multan and to compare it with the clinical appearance.

Patient and Methods

a) Leishmania Isolation in culture media from CL patients

Thirty patients from Multan area with active lesions of cutaneous leishmaniasis, presenting in the Dermatology Outpatient Department of Nishtar Hospital from December 1999 to March 2000, were included in the present study. Patients showing negative smear for amastigotes (LD) to the microscopical examination, having scar or history of previous treatment for leishmaniasis were excluded.

Bioptical material was isolated from the edge of the lesion with the help of syringe (FNA), after cleaning it with normal buffer saline and was directly inoculated into the biphasic culture Evans Tobie's medium.[10] Initially the isolates were maintained at 22°C at Nishtar Hospital Multan and then sent to the Department of Parasitology of Istituto Superiore di Sanità of Rome, Italy, in four different sendings from December 1999 until April 2000, for culture examination, Leishmania stocks cryopreservation and

and Leishmania strains biochemical identification.

b) Isoenzyme characterization

At the Leishmania Reference Centre of Rome, all Leishmania isolates were maintained at the cryobank, mass cultured in Brain Heart Infusion culture medium[11] then characterized by starch-gel electrophoretic analysis of 13 isoenzymes (15 enzymic loci).

The principle of enzyme electrophoresis is that soluble enzymes are extracted from the parasites and then placed in a gel matrix containing a buffer, rendering the isoenzyme a negative charge. When current is passed through the gel, the isoenzymes migrate according to their net charge. So the isoenzyme with the greatest negative charge moves the farthest towards the positive pole while those with least negative charge travel the shortest. Then the isoenzymes bands are visualized by appropriate staining techniques.

The enzymes studied are: PGM, phosphogucomutase (E.C.2.7.5.1); GPI, glucose-phosphate isomerase (E.C.5.3.1.9); GOT, glutamate-oxaloacetate transmainase (E.C.2.6.1.1); ME, malic enzyme (E.C.1.1.1.40); 6PGD, glucose 6 phosphate dehydrogenase (E.C.1.1.1.49); G6PD, glucose 6 phosphate dehydrogenase (E.C.1.1.1.37); NP, nucleoside purine phosphorylase (E.C.3.2.2.1); MDH, malate dehydrogenase (E.C.1.1.1.37), MPI, mannose phosphate isomerase (E.C.5.3.1.8); ICD, isocitrate dehydrogenase (E.C.1.1.1.42); DIA, diaphorase nicotinamide adenine dinucleotide (reduced form) (E.C.1.6.2.2); GLUD, glutamate dehydrogenase (E.C.1.4.1.3); FH, fumarate hydratase (E.C.4.2.1.2). The techniques employed and the zymodemes nomenclature adopted were those of Montpellier centre.[12] World Health Organization (WHO) reference strains of L.tropica, L. donovani, L. major and L. infantum were used as references; furthermore L. tropica strains isolated in Pakistan and in neighboring countries were used as reference.

Results

All thirty patients were from the interior old city. Fourteen (47%) of these 30 were from two different madrasas, located in the same vicinity. Eighty six percent of the patients were children and young adults in 11-20 years of age group (range 6-35 years). The disease was more common in males (77%) as compared to females. Clinically all the lesions were of dry type and occurred on the exposed parts of limbs and face.

The in vitro isolation was attempted from all 30 patients. Two cultures were negative, while 7 cultures were immediately eliminated for fungal contamination and another 5 were initially positive but eliminated for subsequent fungal contamination. Finally a total of sixteen Leishmania stocks were obtained.

All strains were isoenzymaticaly identified as Leishmania tropica belonging to the same zymodeme MON7 in the enzyme PGD.

Discussion

The leishmania species identification is important not only from epidemiological point of view but has also relevance to clinical illness and management.[13] There are several methods available for species identification including isoenzyme technique, which was used in this study. Initially material was inoculated from 30 patients of cutaneous leishmaniasis but only 16 samples were successfully cultured and all the isolates were identified, by isoenzyme electrophoresis, as L tropica zymodeme MON7 PGD varient. This species has also previously being identified as cause of cutaneous leishmaniasis in Quetta, Baluchistan region.[14] The difference is the clinical appearance because in this study, all the lesions were of dry type while in Baluchistan mostly wet type of the disease is seen.[15] So it seems that the morphological pattern of the disease depends upon immune response of the host rather than on the specific strain of leishmania.

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