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Localized *Leishmania Lymphadenitis* Etiologic Agent Identified as *Leishmania tropica* Using Gene Sequencing

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Dear Editor-in-Chief

Leishmaniasis include several clinical manifestations, mostly cutaneous, visceral and mucosal (1, 2). Various species can lead to diverse clinical presentations. Thus species identification contribute to proper management (3). Localized *Leishmania* Lymphadenitis (LLL) is a distinct entity in clinicopathologic field presenting with isolated lymphadenitis and possible cocomitant cutaneous lesion in the absence of systemic visceral involvement (4). Species identification has been acceptable by conventional microscopic evaluation, serologic methods such as isoenzyme and monoclonal antibody detection and particularly PCR. However, definite identification and confirmation of parasite without gene sequencing have always been doubted (5).

In this study, we tried to identify underlying agent of LLL via gene sequencing. To the best of our knowledge, no previous study has fulfilled this method for parasite species identification in this rare clinicopathologic entity.

Two prototypical cases of LLL with evidently countable leishman bodies in histologic slides were selected from a previously gathered collection (6). Then, paraffin blocks of excised lymph nodes were cut to 10 micrometer thick sections. DNA extraction was carried out using QIAamp FFPE kit in accordance with manufacturer's instruction. Subsequently, A pair of primers were designed for rRNA Internal transcribed Region (GenBank Accession number: FJ948464.1). The forward (L.ITS.R: 5'-TTTAATAATCCTGGTCACAGCC-3') and reverse (L.ITS.F: 5'-CAAATACACGCATGCACTCTC-3') primers were analyzed using AlleleID 6.0 software. The primers NCBI BLAST revealed a 201 bp product for *L. tropica* (1). In next step, a 25 µL of reaction mixture composed of 12.5 µL mastermix, 1 µL of forward primer, 1 µL of reverse primer, 5.5 µL H2O and 5 µL of extracted DNA was prepared and PCR amplification was performed with susequent thermal cycling: 95 °C for 2 min, 95 °C for 20 sec (denaturation) and 60 °C for 30 sec (annealing and extension).

Finally, real time PCR end product was purified with Bioneer AccuPrep PCR Purification kit and the

Purified DNA was delivered to Bioneer Corporation on proper cold chain, then its sequencing was carried out according to AccuPower and Top DNA sequencing kit.

Two cases belonged to 7 and 12 yr old girls. Both of them had submental lymphadenitis. Histologic patterns of lymph nodes revealed anergic histiocytes, necrotizing and epithelioid granulomas with countable leishman bodies. Gene sequencing of real time PCR end product according to Bioneer corpo-

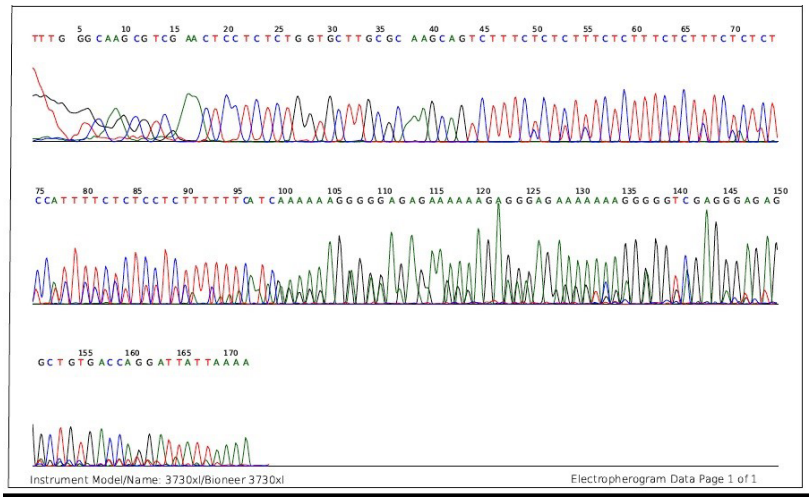

Fig. 1
rRNA-ITS sequence of isolates from LLL after one tail trimming showing 172 nucleotides

ration protocol resulted in a 172 bp sequence, which was approximately equivalent to the length of PCR product after one tail trimming (Fig.1). The resultant PCR product sequence BLAST in NCBI database led to the same accession number used for primer design that was related to *L. tropica* isolate.

Demographic data revealed this clinicopathologic entity as a rather childhood and adolescence disease, which usually involves unprotected areas. Similar findings have been reported in previous studies concerning cutaneous leishmaniasis, which tends to involve unprotected areas especially face with more prevalence in childhood and adolescence possibly due to lack of previous exposure and immunity (5).

The detected sequence BLAST in NCBI databank was most compatible with *L. tropica* GenBank Accession number: FJ948464.1. The sequence was 98% identical and only 3 bases and two 1mer gaps were different in the 172 nucleotide sequence. This difference might be due to single nucleotide polymorphism. The alignment of this sequence was very similar to sequence of *L. tropica* isolated from cutaneous lesions of the same geographic region (southeastern part of Iran) with 98% identical queue of nucleotides (7). Single nucleotide polymorphism is a possible reason for this slight variation in sequence; it might introduce a new subspecies of *L. tropica* with a tendency to involve lymph node.

Anyhow more accurate judgment mandate a larger scale study with more recent and fresh specimen for more detailed sequencing.

Acknowledgments

The authors declare that there is no conflict of interests.

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