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Lupoid leishmaniasis among the known cases of cutaneous leishmaniasis in Herat Province, western Afghanistan

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Summary Lupoid cutaneous leishmaniasis (LCL) is an uncommon form of chronic cutaneous leishmaniasis, which is mostly caused by *Leishmania tropica* in the Old World and has a high incidence throughout early life. Between 2012 and 2013, patients with active lesions suspected to be cutaneous leishmaniasis (CL) were examined. Diagnosis was performed through a combination of methods, i.e., clinical examination, direct smears and kDNA polymerase chain reaction (PCR). Overall, 162(4.2%) subjects, through clinical examination and PCR confirmation alone, were diagnosed as having LCL, with the duration of the lesions varying from 2 to 5 years. Most (85.8%) of the subjects with LCL were <20 years of age. No amastigote was found in direct smears. Moreover, direct PCR on the negative smears for identifying *Leishmania* provided a specificity of 100%, and the species was identified as *Leishmania tropica* using specific kDNA PCR. Performing PCR on skin smears appears

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to offer a valuable method for the diagnosis of LCL because it is highly specific and sensitive, especially for clinical correlative studies.

Introduction

Cutaneous leishmaniasis (CL) is endemic in approximately 70 countries, and approximately 90% of the cases occur in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria [1]. Leishmaniasis still remains as a major public health challenge in 14 of 22 countries of the Eastern Mediterranean Region, including Afghanistan [2]. CL in Afghanistan (known locally as Saldane) occurs either as zoonotic cutaneous leishmaniasis (ZCL) due to *L. major* or anthroponotic cutaneous leishmaniasis (ACL) due to *L. tropica* in seven provinces of the country [3,4]. The ACL form of leishmaniasis has long been reported in various areas of Afghanistan, including Kabul and Herat Provinces [5].

Lupoid cutaneous leishmaniasis (LCL), also known as recidivans or relapsing leishmaniasis, is an uncommon chronic form of ACL that is mainly caused by *Leishmania tropica* in Africa, Europe, and Asia. Lupoid leishmaniasis occurs through approximately 4—10% of *L. tropica* infections in the Old World [6]. The progressive lesions, which are commonly on the face, are identified by a scar with marginal papules, which are multiple and recurrent [7,8]. *Leishmania* amastigotes are usually absent on microscopy, and the culture is often negative; PCR assay is significantly recommended for diagnosis [9]. The low density of amastigotes in direct smears and tissue samples could lead to delayed diagnosis or clinical and histological misdiagnosis often with *Lupus vulgaris* [9].

Among the endemic regions of CL in Afghanistan, Herat Province in the west of the country has been considered to be an important focus of ACL in past decades [3,4]. Over the last decade, the incidence of CL has increased in many districts of Herat Province [10]. Little information is available on the true burden of LCL and the main agents of the disease in the area. The small amount of epidemiological data about CL, especially LCL in Afghanistan, is most likely caused by difficulties related to war and conflicts with terrorist groups in the country. This study, for the first time, was aimed at determining the demographic features and prevalence of lupoid leishmaniasis and the characterization of its main causative agent in Herat Province, western Afghanistan.

Patients and methods

Sample collection

This study was conducted in Herat Province, western Afghanistan, from January 2012 to December 2013. Overall, the patients with active lesions who were clinically suspected of CL and registered at the leishmaniasis referral laboratory of the WHO sub-office in Herat were enrolled in the present study, and 4189 suspicious cases of CL were recorded. The demographic and clinical information for each patient were registered in our leishmaniasis database. All of the cases of new lesions in the center or margin of a scar of a healed leishmaniasis dermal lesion with a duration of more than 1 year were classified as having the lupoid form of leishmaniasis.

Cutaneous leishmaniasis was diagnosed clinically and was confirmed by preparing a Giemsa stained (for 30 min) skin scraping smear, by using material that was scraped with a sterile blade, air-dried, and fixed with absolute methanol from the lesion to determine the presence of amastigotes under a light microscope. The data analysis was performed by Chi-square, using SPSS 16. The differences were considered to be statistically significant when $P \leq 0.05$.

PCR assay

DNA extracted from skin smears that belonged to all of the clinically suspected lupoid forms was used for PCR. For DNA extraction using a modified salting-out procedure, the samples were scraped from each slide with a clean blade and transferred