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Research paper

Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015

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ABSTRACT

Cutaneous leishmaniases (CL) are vector-borne parasitic diseases endemic in many countries of the Middle East including Palestine. Between 1994 and 2015, 2160 clinically suspected human cases of CL from the Jericho District were examined. Stained skin tissue smears and aspirates were checked by microscopy and cultured for promastigotes, respectively. For leishmanial species identification, amplification products from a PCR-ITS1 followed by RFLP analysis using *Hae* III. Data were analyzed using Epi Info free-software. The overall infection rate was 41.4% (895/2160), 56.3% (504/895) of the cases were male, 43.7% (391/895) female, 60.5% (514/849) children under age 14, 41.3% (259/627) of the cases were caused by *Leishmania major* and 57.3% (359/627) by *Leishmania tropica*. The case numbers peaked in 1995, 2001, 2004, and 2012. Statistically-significant clusters of cases caused by *L. major* were restricted to the Jericho District; those caused by *L. tropica* were from the districts of Jericho, Bethlehem, Nablus and Tubas. CL is seasonal and trails the sand fly season. Distribution of cases was parabolic with fewest in July. The monthly total number of cases of CL and just those caused by *L. major* correlated significantly with temperature, rainfall, relative humidity, evaporation, wind speed and sunshine ($P < 0.05$, $r^2 = 0.7–0.9$ and $P < 0.05$, $r^2 = 0.5–0.8$, respectively). Cases caused by *L. tropica*, significantly, had a single lesion compared to cases caused by *L. major* ($P = 0.0001$), which, significantly, had multiple lesions ($P = 0.0001$). This and previous studies showed that CL is present in all Palestinian districts. The surveillance of CL has increased public awareness and molecular biological methodology for leishmanial species identification is an essential addition to classical diagnosis. The overall results are discussed, correlated to climatic and environmental changes and large-scale human activities.

1. Introduction

Within the last century starting from 1910, cutaneous leishmaniasis (CL) in Palestine has received attention by scientists including Palestinians, French and Germans with appearing and disappearing foci over time. Then Palestinian and Israeli scientists worked extensively on wider and deeper scales in the Jordan Valley. Within few years of discovering that the diseases now known as the leishmaniases were caused by protozoan parasites, amastigotes were seen by light microscopy in stained smears of skin tissue from dermal lesions of inhabitants living in the area specified (Canaan, 1916, 1929, 1945; Huntemüller, 1914). The means for characterizing and differentiating leishmanial parasites were not available then and all cases of human CL were described as being caused by a single species, i.e., *Leishmania tropica*. Separation of this clinically defined 'species' into the two definite species *L. tropica* and *Leishmania major* came much later and was based firstly on biological and epidemiological criteria, then also on serological, indicating antigenic, differences, and finally corroborated by biochemical and genetic differences (Bray et al., 1973). Classically, the species *L. tropica* is considered to be anthroponotic, being transmitted from person to person by female sand flies of the species *Phlebotomus sergenti* (Al-Jawabreh et al., 2004; Schnur et al., 2004), rather than zoonotic and being transmitted from infected animals to people. However, recent studies have indicated that in some cases, at least, the species *L. tropica* is also zoonotic with the rock hyraxes, *Procavia capensis*, serving as the animal reservoir

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(Jacobson et al., 2003; Svobodova et al., 2006; Talmi-Frank et al., 2010). In a focus north of the Sea of Galilee (Lake of Tiberias), female sand flies of the species *Phlebotomus arabicus* were found harbouring parasites of the species for *L. tropica* and are considered to be their specific local vectors in addition to the more ubiquitous sand flies of the species *Ph. sergenti* (Jacobson et al., 2003; Svobodova et al., 2006). Human infections caused by either species occur seasonally in parallel with sandfly vector abundance, which increases from mid-spring through to mid-autumn (Muller et al., 2011; Orshan et al., 2010; Sawalha et al., 2003; Schlein et al., 1982).

The geographical area encompassing the southern part of the Jordan Valley down to and including the northern shores of the Dead Sea with the city of Jericho at its center is a typical focus of zoonotic cutaneous leishmaniasis (CL) caused by the species *Leishmania major* with female sand flies of the species *Phlebotomus papatasi* and desert rodents, i.e., sand rats, of the species *Psamommys obesus* serving as the vectors and animal reservoir, respectively (Gunders et al., 1968a, 1968b; Schlein et al., 1982, 1984). Despite the Jordan Valley and Dead Sea area being known as a focus of zoonotic CL caused by the species *L. major*, the species *L. tropica* has also been implicated in causing human cases of CL in this area and those bordering it. Human cases of CL caused by the parasites of both species, *L. tropica* and *L. major*, have been reported in all the other West Bank Palestinian districts (Al-Jawabreh et al., 2003, 2004; Azmi et al., 2012; Jaffe et al., 2004). Those caused there by *L. major* are probably importations, following visits to the Jordan Valley and Dead Sea area. The situation concerning leishmaniases in general in the Gaza Strip is unknown with no autochthonous infection reported.

Implementation of the Oslo accords signed in 1993 led to significant changes in all the Palestinian districts relinquished to the Palestinian authorities among them the refurbishing of government institutions like the Palestinian Ministry of Health and new development in urbanization and agriculture (Arabic-Islamic States, 2016). It also enabled the return of Palestinians domiciled in other countries, in some of which various leishmaniases are endemic, leading to the possible importation of non-indigenous types of leishmanial parasite. In addition to urbanization and agriculture; conflict is another factor highly correlated to the CL outbreak as in the case of civil war in Syria (Al-Salem et al., 2016). This article is a study of human CL that occurred in the Jericho District during the 21-year period between 1994 and 2015. It aimed to correlate fluctuations in the numbers of human cases with changes in climatic and environmental conditions, and human activities like immigration, urbanization and agriculture while also comparing the number cases caused by *L. major* with those caused by *L. tropica* where possible.

2. Materials and methods

2.1. Study design

A long-term cross-sectional study on the prevalence of human CL was conducted, covering the 21-year period between 1994 and 2015, which included a descriptive analysis of the clinical manifestations seen and demographic parameters of the cases encountered.

2.2. Study area

The study was conducted in the Jericho District, which includes the Palestinian part of the Jordan Valley, which is part of one of the classical foci of human CL. The city of Jericho (A'riha) (latitude of 31° 52′ N and longitude 35° 28′) is the only city in the Palestinian part of the Jordan Valley with a large population of 22,609 inhabitants, reaching 52,154 inhabitants when the outlying villages and refugee camps are included (Palestinian Central Bureau of Statistics, 2015a). In addition to part of the Jordan Valley, the Jericho District incorporates the eastern part of Jerusalem (Al-Quds) and the cities of Bethlehem, Ramallah, Nablus, and Tubas and the countryside surrounding them. The total area of the Jericho District is about 593 km^2 and it has the lowest population density of all the Palestinian districts at 71 persons per km^2 (Palestinian Central Bureau of Statistics, 2009, 2015a). The Jordan Valley in the vicinity of Jericho has a unique topography with an elevation of 300 m below sea level, which drops to 400 m below sea level at the southern end of the Dead Sea (Applied Research Institute Jerusalem, 2011c). The Jericho District is a hot, dry, semi-arid area with a rainy season, approximately, from October to March, an annual cumulative rainfall averaging 166 mm, a mean average temperature of 20.4 °C and absolute minimum and maximum temperatures of −0.4 °C and 46.4 °C, respectively (Palestinian Central Bureau of Statistics, 2015b; Palestinian Metrological Authority, 2015).

2.3. Human cases

Between February 1994 and June 2015, 2260 patients with skin lesions suggestive of CL attended or were referred to the Leishmaniases Research Unit (LRU) in Jericho for laboratory diagnosis and confirmation as cases of CL. The patients were Palestinians living in the Jericho District and its vicinity with the exception of a few patients who were temporary sojourners working on international projects in the aforementioned areas. A patient's data sheet was filled in for each patient prior to taking tissue samples. The data sheet included: demographic data such as name, address, age and sex; a clinical description; and epidemiological information such as travel history and number, positions and duration of lesions.

2.4. Diagnosis

Diagnosis included conventional and molecular diagnostic methods.

2.4.1. Microscopy

Five touch smears were prepared from lesion(s), stained with Giemsa stain, and examined microscopically for amastigotes (Al-Jawabreh et al., 2006).

2.4.2. In-vitro culture

Beginning in 1998, dermal tissue aspirates were cultured as described previously (Al-Jawabreh et al., 2003).

2.4.3. DNA extraction

Beginning in June 1997, dermal tissue scrapings from lesions were blotted onto autoclaved Whatman no. 4 filter papers (Whatman International Ltd., England) for leishmanial DNA extraction as described previously (Al-Jawabreh et al., 2004). However, samples before that date were extracted from Giemsa-stained smears. In this case, the stained dermal tissue was removed by spreading 50 μl of lysis buffer onto the surface of the slide and scratched off with a sterile surgical blade into a sterile 1.5 ml micro-centrifuge tube. The procedure was repeated several times until a total volume of 250 μl of lysis buffer accumulated in the tube. Then, the DNA was extracted as described above for filter papers.

2.4.4. PCR amplification

The ribosomal internal transcribed spacer 1 (ITS1) region separating the genes coding for ssu rRNA and L5.8S rRNA was amplified by a PCR, using the primers LITSR and L5.8S as described elsewhere (Al-Jawabreh et al., 2004, 2006; El Tai et al., 2000; Schonian et al., 2003). At a later stage, DNA was accurately quantified, using the Thermo scientific NanoDrop 2000 spectrophotometer. Also, commercial master mix kit PCR-Ready from Syntezza (Syntezza Bioscience Ltd., Jerusalem) was used for amplification of DNA.

2.4.5. Restriction fragment length polymorphism (RFLP)

PCR products derived from the ITS1 region were digested with the restriction enzyme *Hae* III according to the conditions recommended by the supplier (Promega, Promega Corporation, USA) and as described

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