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Serologic survey of leishmaniasis in Ibn Sina patients, Sirte, Libya.

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A B S T R A C T

The clinical and parasitological features of leishmaniasis were investigated, retrospectively, in 162 patients who attended the out-patient clinic of Ibn Sina hospital during January 2017 and June 2018. The aim was to evaluate the epidemiological characteristics of leishmaniasis. Sera from all the suspected patients were examined for slit and smear examination.

Seventy one represent (43.82%) of 162 were positive. Of them, 52 (73%) were males and 19 (26.76%) females. The most affected age group was found to be between 21 and 30; the least affected was age group below 10 years..

1.0 Inroduction

Leishmaniasis is a parasitic disease caused by a haemoflagellate Leishmania. There are more than 21 species causing human infection. The infection is transmitted to humans through the bites of female sandflies (infected phlebotomine sandfly) belonging to 30 species. Depending on the causative species, it can manifest as Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), Diffused Cutaneous Leishmaniasis (DCL) or Visceral Leishmaniasis (VL). Leishmaniasis is prevalent in at least 88 countries. More than 90 percent of the cutaneous cases occur in Afghanistan, Saudi Arabia, Algeria, Brazil, Iran, Iraq, Syria and Sudan; while more than 90 percent of visceral cases occur in India and Sudan. Mucocutaneous form is mostly found in Latin America (El-Hassan and Zijlstra 2001, Hoogstraal and Heyneman 1969). Approximately 350 million people live in the area of active parasite transmission.

In humans, Leishmania parasites are the causative agents of a wide spectrum of diseases of variable severity, ranging from a localised cutaneous lesion, often benign and self-healing, to a visceral dissemination that may be fatal (Ashford and Bettini 1987). Over the last 20 years, visceral leishmaniasis has become increasingly common in the Mediterranean basin, largely as the result of the HIV pandemic spreading to areas where visceral leishmaniasis already occurred (Ribera *et al*., 1996, Desjeux P 1999).

The clinical and epidemiological findings in various forms of leishmaniasis are non-pathognomonic and these can mimick several other conditions. Hence a laboratory diagnosis is required to confirm the clinical suspicion (Grimaldi Jr 1993). The diagnostic tools used for each leishmanial syndrome viz. visceral, cutaneous, and mucocutaneous form vary but the gold standard in each case remains to be the demonstration and isolation of the parasite from appropriate tissues.

The diagnosis of leishmaniasis is reliably made by the demonstration of the parasite in smears and by isolation, either in culture or by animal inoculation of a biopsy sample or tissue aspirate from the spleen, or the bone marrow. The sensitivity is highest for splenic aspiration (as high as 98%) but so is the risk of complications such as haemorrhage (Herwaldt BL 1999). Occasionally the amastigotes have also been demonstrated in liver biopsy (50-85% sensitive), lymph node aspirates and buffy coat smears, particularly in HIV-Leishmania co-infection,cases.

The parasitological diagnosis of CL is made by demonstration of amastigotes in skin lesions on skin biopsy and on culture of these specimens (Rajasekariah *et al*., 2001). Culture based diagnosis of mucocutaneous leishmaniasis has very low sensitivity as the organisms are often scant. The individual sensitivities for the methods for

patients and Montenegro-positive healthy controls were: histopathology: 14% and 16%; impressions smear: 19% and 21%; dermal scraping: 19% and 26%; aspirate-culture: 58% and 64%; aspirate-hamster: 38% and 41%; biopsy-culture: 50% and 55%; and biopsy-hamster 52% and 57%, respectively (Jensen *et al*., 1996). The sensitivity is slightly better for younger lesions than for lesions older than 6 months.

2.0 Materials and Methods

The subjects of the present, retrospective study were the suspected patients who attended the outpatient clinic of Ibn sina hospital between January 2007 and June 2008. The presence of anti-Leishmania spp. antibodies was determined using both an IFA and an enzyme linked immunosorbent assay (ELISA). Smears of buffy coats produced from peripheral blood and of biopsies (of bone-marrow and sometimes other tissues) had been Giemsa-stained and checked for amastigotes, under the microscope.

The IFA, a test commonly employed in field studies, was performed as described by using Leishmania infantum promastigotes cultured in Evans' modified Tobie medium (Evans DA, 1978). The lag-phase promastigotes were washed three times in 0.15 M NaCl, air dried on 12-well multi-test slides (Flow Laboratories, Milan, Italy) and fixed with cold acetone for 10 min. Ten micro liters of diluted sera from the suspected patients were placed on duplicate slides containing fixed promastigotes. Slides were incubated in a moist chamber at 37 ^{o}C for 30 min, and washed in phosphate buffered saline solution (PBSS, pH 7.4). Ten microliters of a fluorescein-labeled rabbit anti-human Immunoglobulin G (IgG) (BioMakor, Rehovot, UK) were added at a dilution of 1:40 to each well. The slides were re incubated at 37 C for 30 min in a moist chamber, washed in PBSS, and mounted using 50% buffered glycerin solution. Sera from Leishmania-infected patients and sero negative persons were used as positive and negative controls, respectively.

The ELISA was performed using L. infantum-soluble antigen. Lag-phase promastigotes were washed as previously described and incubated at 4 C for 15 min in 0.08% Triton X 100 (Sigma Chimica, Milan, Italy) diluted in 0.1 M Tris buffer (Farmita!ia Carlo Erba S.P.A., Milan, Italy), pH 8, containing NaCl 0.15 M ethylene diamine tetra-acetic acid (EDTA) (Sigma Chimica, Milan, Italy), and protease inhibitors (Sigma Chimica, Milan, Italy). The supernatant was clarified by centrifugation at 15,000 x g for 30 min, and stored at -20 C until needed. The ELISA was performed using 0.1 µg per well of antigen diluted in carbonate buffer 0.05 M (Farmitalia Carlo Erba, Milan, Italy), pH 9.6. After washing with PBSS containing 0.05% Tween 20 (Riedel de Haen AG, Seelze, Hannoven, Germany) (PBSS Tween 20), plates were post-coated with PBSS containing 1 % bovine serum albumin (Fluka Chemie AG, Buchs, Switzerland) (PBSS BSA) for 12 hr. After washing, sera diluted 1/200 in PBSS BSA 0.1% was added and incubated for 3 hr. Plates were washed and a peroxidase conjugate rabbit anti-human IgG antiserum (Sigma Chimica, Milan, Italy) was added at each well at a dilution of 1/4,000. All steps were performed at 20 C. The enzyme reaction was carried out with o-phenylenediammne dihydrochloride amine (Sigma Chimica, Milan, Italy) in citrate phosphate buffer with a pH of 5.5 (Farmitalia Carlo Erba S.P.A., Milan, Italy). The reaction was stopped after 50 min with 4N H2SO4.

3.0 Results

Serum samples collected from out patients who had attended for treatment of certain aliments and suspected with leishmaniasis infection were diagnosed using IFA and ELISA tests. Out of 71 positive cases 52 (73%) were males and 19 (26.76%) females. (Table 1).

Table-1: Positive cases of leishmaniasis by age and sex in Sirte, Libya

Age group/years	Frequency	No. of positive	Male	Female
Less than 10	35	10	7	3
11-20	30	12	10	2
21-30	52	30	21	9
31-40	20	09	5	4
41-50	12	05	4	1
51-60	08	03	3	-
Above than 60	05	02	2	-
Total	162	71	52	19

The least affected group that tested positive for the leishmaniasis infection was in below10 years age group, 10 out of 35 (28.57%). 12 out of 30 patients of the age group between 11 – 20 was affected (40%). A highest of 30 out of 52 cases were tested positive for the age group 21-30 (57.67%) (Figure 1). 9 cases out of 20 were tested positive for the age group 31-40 (45%); 5 cases out of 12 between the age 41 – 50 (41.6%); 3 cases out of 8 (37.5%) were positive between age group 51-60 and 2 cases were tested positive out of 5 (40%) in the age group above 60.

Figure 1: Leishmaniasis infection among tested population in Sirte, Libya

Seventy one (43.82%) of 162 suspected patients' sera had Leishmania antibodies with titers ranging from 1:40 to 1:320. Twenty six of the seventy one had an IFA titer of 1/80 with optical density in ELISA ranging from 0.854 to 0.960, thirty four sera showed an IFA titer of 1/40, with optical density from 0.632 to 0.746; remaining eleven positive samples had an IFA titer of 1/320 corresponding to optical density 1 . 087. The low antibody titer response may be due to recent infection. The highest IFA antibody titers corresponded with overt disease signs such as weight loss, dermatitis and skin ulcers.

4.0 Discussion

Leishmaniasis can be transmitted in many tropical and sub-tropical countries, and is found in parts of about 88 countries (Pampiglione *et al*., 1975). Approximately 350 million people live in these areas. The settings in which leishmaniasis is found, range from rainforests in Central and South America to deserts in West Asia. More than 90 percent of the world's cases of visceral leishmaniasis are in India, Bangladesh, Nepal, Sudan, and Brazil. Leishmaniasis is present in Iraq and was contracted by a number of the troops involved in the 2003 invasion of that country and the subsequent occupation. The soldiers nicknamed the disease as Baghdad boil. It has been reported by Agency France-Press that more than 650 U.S. soldiers may have experienced the disease between the start of the invasion in March 2003 and late 2004 (WHO., 1990).

Within Afghanistan, in particular Kabul is a town where leishmaniasis occurs commonly partly to do with bad sanitation and waste left uncollected in streets, allowing parasite-spreading sand flies an environment they find favorable. During our observation in Sirte, we found most of the places very tidy and the garbage dumps from the streets were collected regularly and disposed of outside the city in a proper way. But there are some places especially, outskirts of the city where population is less but, due to dumping of garbage in that area collected from the city and even some places where garbage was not cleared frequently, there is more probability of leshmaniasis infection due to the conditions that favor sand flies which can spread the parasite.

The Enzyme Linked Immunosorbent Assay (ELISA) is a valuable tool in the serodiagnosis of leishmaniasis. The test is useful for laboratory analysis as well as for field applications (Hommel *et al*., 1978, Martin *et al*., 1998, Jensen *et al*., 1999). However, the sensitivity and specificity of ELISA is greatly influenced by the antigen used. More recently, several recombinant antigens like rGBP from L. donovani, rORFF from L. infantum, rgp63, rK9, rK26 and rK39 from L.chagasi have been developed and tested. The rK39 strip test has been found highly sensitive and a reliable indicator of kala-azar in India (Singh *et al*., 1995).

In our study most of the positive cases (41 of 71; 58%) were found to be from the localities which are outside the city zone and areas where improper sanitary conditions are prevailing due to garbage accumulation near to the residential area. Only 31 of 71 (42%) are from various places with in the city and most of the negative cases (66 out of 91; 73%) are from this area. Only 25 (27%) negative cases were from city out skirts. So our observations suggest that if those areas where mainly garbage dumps are not removed frequently and are not disposed properly, if the municipal authorities are asked to take action and even if public are educated regarding infections that spread through improper sanitary conditions, infections like leishmaniasis can be controlled effectively.

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