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Epidemiology of Leishmaniasis in South Kordofan Region, Western Sudan

A.A. Osman
Department of Family and Community Medicine, College of Medicine,
King Khalid University, Abha, P.O. 641 Zip 61421, Saudi Arabia

Abstract: This study aimed to identify cases of leishmaniasis in the Nuba mountain area which is situated in a unique geographical site as it is located in the centre of Sudanese leishmania belt. The study was conducted in the Green Valley village (Rashad province, South Kordofan state) with a population of 332. This study applied in field situation collection of epidemiological and demographic data, clinical examination, Leishmanin Skin Test (LST) and PCR (Polymerase Chain Reactions). Most of the villagers have presented with sub-clinical form of leishmaniasis, presented with minor symptoms and signs that can occur in clinical form of visceral leishmaniasis such as fever, diarrhoea, epistaxis, enlarged lymph nodes, spleen and liver. As many conventional diagnostic methods such as direct microscopy, culture and serology have some drawbacks and failures in diagnosing subclinical cases of leishmaniasis, here in this study the Leishmanin Skin Test (LST) and PCR (Polymerase Chain Reactions) tested on blood spotted finger pricks spotted on filter papers collected from the villagers under field condition. Almost all of the 32 positives by PCR have presented with sub-clinical pattern of the disease indicating the predominance of sub-clinical form in this study area. Positive LST; 43.6% in August, 45.7% in February and 51.2% in October reflected the exposure rate to the disease among the villagers who showed variable magnitude of reactions. All age groups were involved indicating indoors transmission. Most of villagers were subclinical and no gender predilection.

Key words: Visceral, cutaneous, leishmaniasis, LST (skin test), sandflies, Nuba mountain

INTRODUCTION

Leishmaniasis is an important parasitic disease with a great public health problem to many countries. The clinical presentation of the disease depends mainly on parasite genotype versus host immunogenetic profile. In old world transmission to human via a bite of female sandfly mainly due to *Phlebatomous* sp. (*papatasi, orientalis*). *L. donovani* complex (*donovani*, *infantum* and *chagasi*) is causing visceral leishmaniasis while *L. tropica* (arthroponotic) and *L. major* (zoonotic) are responsible for cutaneous leishmaniasis.

Leishmania donovani is the main causative species for visceral leishmaniasis while *Leishmania major* needs an animal reservoir and it the main causative parasite for cutaneous leishmaniasis in Sudan (Neave, 1904; Kirk and Sati, 1940). In Sudan the leishmania belt extends from Gadarif region from east where visceral leismania is a predominant form to Darfor region to the west where the cutaneous form has been reported (El-Hassan and Zijlstra, 2001).

The Nuba mountain is situated in Kordofan region which lies in the mid way in the leishmania belt this area has rarely been studied (Abdalla *et al*., 2001, 2003) while several studies in the field of leishmaniasis have been conducted mainly in the eastern and southern Sudan (Zijlstra *et al*., 1994; Siddig *et al*., 1990). Many mobile, nomadic tribes are settled in this area from various ethnicity such as Massaleet, Arabs and Nuba they work as shepherds and farmers. Variable types of domestic and wild animals are found such as camels, cows, sheep, dogs and rodents. The ecology is suitable for the vector (sandflies) existence and breeding, this is shown by the presence of sub-savna climate and the plants forests belonging to Acasia Balanitis (Kirk and Lewis, 1955). The cracked dry muddy sand offer sandflies optimum breeding sites.

Wild range of investigations are available for detection of leishmania cases but still the most reliable and easy test used in screening and epidemiological tool to be used in field studies is the skin test LST (Montonegro test) (Ho *et al*., 1983). So in this study LST was used in three consecutive field visits. Clinical, demographic and epidemiological data were collected by special questionnaire.

This study aimed to identify the leishmania cases among the inhabitants of The Green Valley villages (three adjacent villages each one consist of around 24 cottages, each cottage represent a family) and to find out the epidemiological factors. These villages were deserted for 20 years due to a killing disease that resemble

leishmaniasis clinically as described by the villagers. Resettling in these villages have lead to re-appearance of similar illness.

MATERIALS AND METHODS

Study area: The Green Valley village, a small village lies in Nuba mountain, west of Sudan (12N-9S and 32E-29W). The total population was around 332. All inhabitants of this village were included.

Demographic and epidemiological data were collected using special questionnaire. Clinical examination of all villagers was conducted. Screening looking for the symptoms and signs related to leishmaniasis which include; fever, epistaxis, abdominal pain, anaemia, enlarged liver, spleen and lymph nodes for visceral and skin ulcers, scars and mucocutaneous lesions for cutaneous.

Three consecutive field intervention were performed in months August, October and February as they reflect the summer, winter and autumn, respectively or pre-raining, raining and post-raining seasons as these periods affect greatly the breeding of the disease vector (sandflies) so the variation of the vector population will lead to variation in transmission rate. Leishmanin Skin Test (LST) (donated kindly by Prof. El-Hassan *et al*., 1990, LRG (Sudanese leishmania research roup), Khartoum) was done to all villagers in three consecutive occasions August (summer), February (winter), October (autumn). About 0.1 mL leishmanin was injected subcutaneously in the upper extensor part of the left arm. The ball pin technique was applied after 48-72 h indurations, redness and swelling >5 mm was considered positive. Actually the LST grades was applied to blot the test results. A finger prick blood spotted filter papers were collected from all the villagers.

All the samples were tested for leishmania parasite detection using specific donovani primers: A J S 3 (5'CCAGTTTTTCCCGCCCCT3') and DB8 (5' GGGTTGGTGTAAAATAGGGC3') (Barker, Cambridge). The DNA was extracted from blood spotted filter papers collected from all the villagers. SDS detergent and proteinase K were used, after spinning the supernants were treated with phenol/chloroform/isoamyl alcohol. DNA were precipitated in absolute ethanol and preserved in Tris-EDTA (TE). The chelex extraction method was proved not to be more sensitive when compared with phenol/chloroform/isoamyl alcohol. Parasite DNAdetect ion: all samples were amplified by PCR using kDNA primers (AJS3 and DB8) and Taq polymerase enzyme was added after the first PCR cycle hot start (95°C for 5 min). After the PCR cycles ended the amplified DNA was examined using horizontal agarose electrophoresis technique visualized by Ethidium bromide. DNA profile was compared with known L.d isolates (positive control). PCR reaction conditions using Leishmania primers (AJS3 and DB8) composed of: Hot start temp 94°C for 5 min, Denaturation temp 94°C for 1 min, Annealing temp 64°C for 1 min, Extension temp 72°C for 2 min, Incubation temp 72°C for 10 min, Store temp 12°C for overnight. Contents of 24 mL total PCR reaction mixture: 4 mL d NTPS Nucleotides, 2.5 mL 10×Reaction Buffer, 1.5 mL Primer AJS3 dilution, 1.5 mL Primer DB8 dilution, 9.5 mL H_2O, 5 mL Taqpolymerase dilution. All samples were run in 1.4% agarose gel stained with Ethidium bromide at 80 V.

RESULTS AND DISCUSSION

The demographic, clinical and epidemiological data showed that the total population under study was 332; 155 children age <15 years and adults represents the rest (48% of the population) females were predominant (Table 1). Clinically both groups (children and adults) did not complain of weight loss, epistaxis and cough. Only 3% complained of diarrhea. Fever was a complain of 34% of children and 16% adults while abdominal pain experienced by 6.7% of children and 13.5% of adults. Clinical assessment of liver and spleen enlargement among the study group 14 individuals sowed enlarged liver while 50 ones showed enlarged spleen (Table 2). Only 63 individuals (51 children, 12 adults) showed enlarged lymph nodes, localized to inguinal region. Enlargement of spleen and liver are shown in (Fig. 1). No skin ulcers, scars and mucocutaneous lesions for suspected cutaneous cases.

The positive LST estimated during the three consecutive field intervention was 43.6% in August, 45.7% February and 51.2% in October among the same studied population (n = 332). The distribution of the final LST results (after October field intervention) among children 53 positives (34.2%), 102 negatives (65.8%) and among adults 117 positives (66.2%), 60 negatives (33.8%) among the total population; 170 positives (51.2%), 162

Table 1: Distribution of the study population by gender and age group

Age group	Male No. (%)	Females No. (%)	Total No. (%)
0-5 years	26 (46.4)	30 (53.6)	56 (16.9)
6-10 years	22 (37.9)	36 (62.1)	58 (17.5)
11-20 years	27 (35.1)	50 (64.9)	77 (23.1)
>20 years	71 (50.4)	70 (49.6)	141 (42.5)
Total	146 (43.9)	186 (56.1)	332 (100.0)

Table 2: Clinical assessment of liver and spleen among the study group

Organ\size	0 cm	1 cm	2 cm	3 cm	4 cm	6 cm	8 cm	12 cm
Spleen	282	4	11	1	21	8	2	3
Liver	318	0	7	0	6	1	0	0

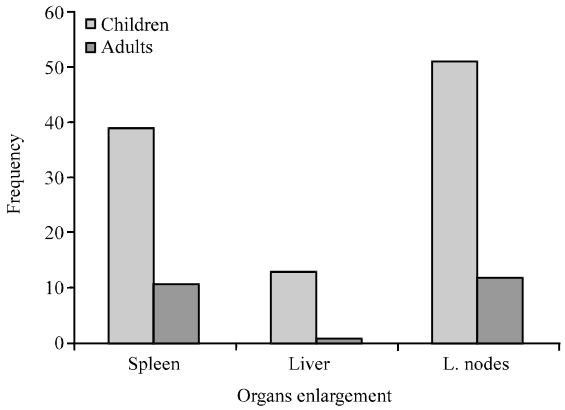

Fig. 1: Organs enlargement among children and adults

Fig. 2: AJS3 and DB8 primers used on field samples to detect leishmania DNA (positive samples from down upward on the left side of the gel No. 113, 135, 55, 23, M (DNA marker) and from the right side No. 6, 8, 4, C-, C+ controls

Table 3: Distribution of LST results by clinical grades in relation to gender

LST-clinical grades	LST size in cm	Females	Males	Total number
0 and I	<5 cm	98	66	164
II	5-8 cm	13	6	19
III	>8 cm	73	70	143
IV	>8 cm with skin bolus	2	4	6

(48.8%). Distribution of LST results by clinical grades in relation to gender is shown in (Table 3). AJS3 and DB8 primers used on field samples to detect leishmania DNA (Fig. 2) showed 32 positive leishmania parasite.

Leishmaniasis is a parasitic disease transmitted to human by an infected female sandfly bite of the genus Phelbotomus (Killick-Kendrick, 1990). *Leishmania donovani* is the main parasite species causing visceral leishmaniasis in Sudan (El-Hassan *et al*., 1990) while Leishmania major is responsible for most infection leading to cutaneous leishmaniasis known as Zoonotic Cutaneous Leishmaniasis (ZCL) that confer a zoonotic reservoir (El-Safi *et al*., 1991). Leishmaniasis is a worldwide distributed and endemic in around 82 countries. The annual incidence is estimated at some 600.000 new clinical cases, officially reported of 12 million cases and a population at risk about 350 million (WHO, 1990).

The leishmania belt in Sudan crossing the country from east to west at the sub-savana region in this area several nomadic tribes are living. They composed of variable ethinic population, they are farmers and shepherds. Annually they migrate north and south within this region following the rains and grass for better condition of living. These villages were deserted for 20 years due to a killing disease that resemble leishmaniasis clinically as described by the villagers (fever and abdominal swelling then death). Resettling in these villages have lead to re-appearance of similar illness.

In this study the positive leishmanin test reflected the transmission rate among the population under study. Clinical examination (performed by the researchers as he is a medical professional) showed subclinical or minor clinical presentation. Epidemiological studies from endemic regions worldwide showed the predominance of cases among children in indoors infections (Jahn *et al*., 1986; Badaro *et al*., 1986). This is explained by the low immunity in children in comparison to adults. Kala-azar is a potential fatal disease characterized by long term fever, spleen enlargement, immunosuppersion and weight loss. The parasites inhabit the macrophages of the spleen, liver and bone marrow in the aflagellated amastigotes. During the course of the disease, there is a marked depression of cellular immunity to leishmania antigens (Carvalho *et al*., 1981) and a poly-clonal B cell activation with high titer of both specific and non-specific antibodies. After successful treatment both T cell proliferation to leishmania antigens *in vitro* and delayed type hypersensitivity to killed leishmania promastigotes *in vivo* developed (Sacks *et al*., 1987).

Leishmaniasis is mainly T cell-mediated disease (Liew, 1989), so selection of an efficient clinic-epidemiological tool has lead to chose the leishmanin skin test (Montonegro test; LST) which proved to be a good screening and epidemiological tool for identification of leishmania transmission and attack rate; clinical and sub-clinical ones.

In comparison to other tools such as skin smear or biopsy for microscopic identification of parasites although, the later is considered as gold standard test for leishmaniasis but the majority of specimens showed negative results when the number of parasites used to be scarce. Molecular tools such as PCR used to be expensive and impractical in field conditions.

CONCLUSION

LST (Leishmanin Skin Test) results have shown the continuous conversion of this test among negatives after consecutive testing, this denotes the continuous transmission of the disease in the area. Estimation of positive rates reflect the attack rate within the study group, the progressive rising means that some of negative LST cases have changed to positive and this seroconversion was noticed among all age groups. This study have screened these villages and showed that the capability of leishmaniasis to exist in deserted areas can be explained by the disease capability to maintain internal circulation within the vectors and animal reservoirs and this can last as long as 20 years.

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