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Assessment of demographic and nutritional factors on Leishmaniasis in Sudan

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Abstract

The objective of this study was to evaluate the magnitude of le ishmaniasis in the study area and to assess the role of demographic and nutritional status in the disease transmission. It was a prospective cohort study on leishmaniasis. It was conducted in 3 villages in West of Sudan. Leishmanin skin test (LST) was assessed as an epidemiological tool in a sub-clinical focus of leishmania in Sudan. The total number of the population in all villages is 332. Methods used in this study, Demographic data were taken and for assessment of nutritional status of children in regard to their type of infection, height and weight were measured using health meter, leishmanin skin test was done to all villagers in first visit. The study was conducted in three field visits, during raining season in 2008 to 2009. In the last two visits, the test was performed to those who had negative LST results in the first visit, so as to detect LST conversion.

The results of the positive cases in the three visits were as follows: 42.7%, 46.3% and 51.2%. Almost half of the population had the disease and these subsequently increasing rates indicate continuous transmission. Evaluation the nutritional status which based on weight-for-age and height-for-age The frequency of seriously low weight for age is significantly higher among the children with clinical visceral leishmaniasis than children with subclinical infection.

Key words: *Leishmania*, leishmanin skin test "LST", Sudan.

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Introduction

Sudan is a largest country in Africa with variable climates and environments. The Savna and Sub-Savna occupy a large belt crossing from east to west of Sudan, providing suitable inhabitant for many tropical diseases including leishmaniasis [1,2,3,4,5]..

Balanitis and Accacia forests provide ecological areas for vector {sand fly}[6] and reservoir {zoonosis} [7,8] hence the perfect medium is available for the disease to be endemic.

Worldwide Leishmania classified as a multifactorial disease and a lot of Micro and Macro environmental factors may be responsible for development of the disease. In the endemic areas like in India, Tunisia and Brazil many studies done to elicit those factors. (i.e., age, sex cytotoxic immune responses, living in same geographical region, way of living , Water lodging, Plantation, Fumigation product and way of making food) [9,10,11]. Most of these studies related malnutrition with outcomes of Leishmania infection . Since the disease predominantly affects the people of low income group in whom the nutritional status is very poor and malnutrition has been considered as a major risk factor for the development of VL. Children are at great risk of developing VL when they are malnourished. The relationship between malnutrition and VL is poorly understood especially among children. The various nutritional status and laboratory related tests have been carried out in different categories of malnourished VL patients. i.e., cholesterol level, vitamin A level . all test showed sever malnutrition with leishmaniasis and revel low level of previous elements with leishmania infection .by comparing children with VL to relatives. [11,12]

LST test is considered to represent an evaluation *in vivo* of cell mediated immunity (CMI) in leishmaniasis [13,14, 15, 16, 17]. Together with the determination of circulating

antibodies, it is a useful tool in epidemiological studies [18]. During active infection, the test is usually negative, but it has been reported to be positive in some cases of sub-clinical or very early visceral leishmanisis (VL) and after successful treatment [19]. Since VL in its active stage is associated with both specific and non-specific suppression of CMI, a previously positive tuberculin reaction may become negative during active VL. After successful treatment, the tuberculin reaction may revert to positive earlier than the leishmanin reaction [20].

LST specificity in healthy controls is nearly 100% for all antigens. Sensitivity increased minimally with increasing dose. Side effects such as vesiculation and ulceration at the site of LST application increased with antigen dose. Storage under hard conditions decreased LST potency but not sensitivity, while storage at 2-8 °C affected neither potency nor sensitivity. 85% of parasitologically diagnosed, LST-positive cases of leishmaniasis remained LST-positive when retested six months to three years later [14].

The LST, which measures the delayed-type cutaneous hypersensitivity (0TH) response to *Leishrnania-derived* antigens, is useful tool for both clinical diagnosis and epidemiological studies [21]. However, it fails to distinguish current from prior leishmanial infection, a characteristic most problematic in areas where the prevalence of LST reactivity is high [22].

The relative pathogenicity (probability of illness, given infection) in specific endemic foci have been estimated by the fraction of LST reactive individuals who manifest signs or a history of prior leishmanial lesions [23].

Aim of the study

The objective of this study was to evaluate the magnitude of leishmaniasis in the study area.

Specific objectives:

To assess the role of demographic characteristic in accordance to leishmaniasis

To assess the role of nutritional status in the disease transmission

Rational of the study

This area was not subjected to previous studies for leishmaniasis [24, 25] although it is situated within the leishmania belt which extends across Sudan between two hyperendemic areas on the east for visceral and on the west for cutaneous type of leishmaniasis. The study area is characterized by Unique natural, geographic and ethinic forms. Leishmaniasis was thought to be hyperendemic in this area during the seventies with high mortality among the villagers that led the inhabitants to desert their village for more than 15 years, only few years ago they return back to their village.

Material and Methods

It was prospective cohort epidemiological study, held in three visits during pre-raining, raining and post raining seasons of year 2008 to 2009 (July, September and February) which reflect the transmission load according to breading of sand flies(leishmania vector).

Study Area

The study was conducted in three Villages in Rashad Province, West of Sudan.

Sample size

All inhabitants of the three villages were included. They were 332 person.

Clear explanation of this study has been provided to all villagers, so the human right have been preserved according to the research ethical requirement of the Gezira university Sudan.

Demographic data were collected using a special questionnaire using personal interviewed to all villagers visiting them in their cottages. For assessment of nutritional status of children in regard to their type of infection, height and weight were measured using health meter

Analysis of nutritional status was evaluated on the basis o f weight-for-age and height-for-age. The standards from the National Centre for Health Statistics, for expected weight for age and height for age were used in the nutritional status calculations. Nutritional values were assessed using the age, height and weight using health-meter apparatus and depending on Gomes classification system. All children were classified into one of three categories; normal, mild and moderately to severe malnutrition. Normal children those were above 90% for mean weigh for age **(WAM)** and above 95% for median height to age **(HAM)**. Mild malnutrition describes those who fall between 75% and 90% of median of weight for age or between 90% and 95% of median of height for age. Moderate to severe malnutrition describes individuals of < 75% of the median weight for age or < 90% of the median height for age.

Leihmanin Skin Test - LST (Montenegro Test)

Skin test (delayed-type hypersensitivity [DTH]) antigens used in this study for all villagers . The antigens were prepared from washed suspension of *L infanturn* promastigotes (Roma), grown in blood agar medium. The promastigotes were resuspended in 0.5% phenol-saline to reach a concentration of 10^8-10^{10} cells/ml. A total of 0.1 ml of the antigen was injected intradermally into the alcohol-cleansed volar surface of the patient's right forearm. The diameter of the induration was measured 48 hrs later by outlining the indurated border with a ball-point pen. The induration with a diameter of 5 mm or bigger has

been reported as positive [26]. Grade of induration of LST was measured. It was classified to 4 grades < 5cm is negative, from5 to 8 cm was grade **I&II**, grade **III** is more than 8cm. While grade **IV** is more than8cm with skin bolus reaction. Data collection and measuring height and weight were done by the author who is medical professional. Leihmanin Skin Test was done by lab technician

Results

In our study population females were 186 and males were 146 (56.1% and 43.9%, respectively). Population under 20 years of age were 40.6 %. Many mobile, nomadic tribes are settled in this area from various ethnicity such as Massaleet, Arabs and Nuba they mainly work as shepherds and farmers. Results showen in Table 1.

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

Age group	Male No. & %	Females No. & %	Total No. & %
0-5 years	26 (7.8%)	30 (9.03%)	56 (16.9%)
6-20 years	49 (14.75%)	86 (25.9%)	135 (40.66%)
> 20 years	71 (21.38%)	70 (21.08%)	141 (42.46%)
Total	146 (43.9%)	186 (56.1%)	332 (100%)

Table 2. *LST final results among children (<16 yrs.) and adults (>=16 yrs.)*

LST Results	Children <16 yrs.	Adults >=16 yrs.	Total
Positive PLST	53 (34.2%)	117 (66.2%)	170 (51.2%)
Negative LST	102 (65.8%)	60 (33.8%)	162 (48.8%)
Total	155 (51.2%)	177 (48.8%)	332 (100%)

LST

The final LST results showed that 170 (51.2%) were positive and 162(48.8) were negative, detail information about children and adults was shown in Table (2).

LST-conversion

In the second visit, more 12 cases became positive, the total positives cases were 46.3%. Distributed as follows; 2, 1 and 9 cases in sub-village 1, 2 and 3 consecutively. Sixty-seven percent of them were children (<l6years) and 33% adults (>=l6years). Male/female ratio was 1:1.

LST-grades were; 11 out of those 12 cases were grade 3 (>8mm.). The largest induration size was 16 mm. All 12 cases were subclinical.

In the last visit there were 16 positive cases more, their distribution as follows; 5, 4 and 7 cases in sub-village 1, 2 and 3 consecutively. Fifty percent of them were children (<16years) and 50% adults (>=l6years). Thirty-one percent were male and 69% were female.

Table 3. *Distribution of LST final results by clinical grades*

LST-grades were; 14 out of 16 were grade 3 (>8mm.). The largest induration size was 20 mm. Details were shown in Table 3. All 16 cases were subclinical.

LST-pattern in studied homes during the study

Village 1 (24 cottages): All positive homes (all family members) were 7 on first visit and in the second visit increased to 8. While there were no total negative homes which might suggest that the infection was widely spread and involved all homes in this village. Village 2 (36 cottages): Positive homes (all family members) were 4 and remain constant throughout the study duration. 8 families (homes) were all negatives this might be due to a large number of absents, denoting that members of this village were not constantly settled due to internal and/or external migrations. Village 3 (17 cottages): Positive homes (all family members) were 4 in first visit raised to 5 on second one. While total negative homes were 2 reduced to one home in first visit and non on the third visit which suggest the infection transmission.

LSTR Clinical Grades	--ve	1 & II	III	IV
LST cm.	<5cm.	5-8 cm.	>8cm.	>8cm. with skin bolus reaction
Total number	164	19	143	6

Table 4. *Details of nutritional status of converted cases among children (<16 years) during the second visit*

Village	HAM	WAM	Nutritional status(Gomes classification)
V1	92.03	87.52	Mild
V3	88.89	68.90	Moderate to sever
V3	92.47	97.30	Normal
V3	93.49	75.07	Mild
V3	98.10	75.85	Mild
V3	95.19	76.38	Mild
V3	99.35	93.50	Normal
V3	95.32	75.77	Mild

HAM = Median of Height; WAM = Median of weight for age

Table 5. *Details of nutritional status of converted cases among children (<16 years) during the third visit*

Village	HAM	WAM	Nutritional status(Gomes classification
V1	95.43	85.68	Mild
V3	111.80	99.20	Normal
V3	101.40	107.50	Normal
V3	82.85	74.30	Moderate to sever
V3	94.82	102.50	Normal
V3	84.12	85.08	Mild
V3	100.30	112.70	Normal
V3	92.31	85.31	Mild

N.B. reduction of WAM usually occur in acute conditions while reduction of HAM usually occur in chronic conditions

Analysis of the nutritional status

The frequency of seriously low weight for age is significantly higher among the children with clinical visceral leishmaniasis than children with subclinical infection. The effect of nutritional status on LST conversion among children (<l6yrs) in this study showed in Tables (4,5). Almost two third of children (8 children) which their LST test converted from negative to positive had malnutrition according to Gomes classification, in the first visit and 50% of them in the third visit.

Discussion

The frequency of exposure to leishmaniasis among the inhabitant under study was 51.2%. All age group were involved and there were no gender variation. Since the disease predominantly affects the people of low income group in whom the nutritional status is compromised and malnutrition has been considered as a major risk factor for the development of VL. (12) This was clearly showed in children under study whom their LST converted from negative to positive in second visit, 62.5% of them were mild malnourished. In the third visit the percentage of children converted to positive was 8 children 50% of them were malnourished both mild and moderate according to Gomez scale. Final results according to the standard LST grades (1-4 grades); 20% of children group lies in the 3rd grade, while 51% of the adult group had grade III and grade 4 (bolus reaction) which was observed only among adults. This can be explained by the immature immune system among children. The continuous conversion of the negatives to positive LST, is clear in both groups children and adults, this may indicate the continuous transmission after rain season as well it indicates indoors transmission.

There are few reports on the use of leishmanin skin test in the Sudan. Most information comes from Kenya, where Manson- Bahr reported the test to be characteristically negative during active kala-a.zar and to become positive in 80% of cured cases after two years [19]. The first report from the Sudan is from the NAMRU-3 team, who found that 11 patients out of 14 treated kala-azar patients positive three or more months after treatment. Also an

important epidemiological observations were made by them in Upper Nile Province, areas of active transmission of kala-azar (Palai-Tir), that 59% of individuals tested were positive while in areas where the disease was unknown Bantiu (Southern West of Sudan), only 10% showed positive reactions. While in Shilluk village (South of Sudan), an average of 44% leishmanin positive reactions was obtained; no clear increase with age was seen [27].

In a hospital based study *95%* of patients with active disease were leishmanin negative [28]. Six Americans who contracted leishmaniasis only four with skin lesions were leishmanin positive at diagnosis, the other two had kala-azar became leishmanin positive after treatment [29].

A leishmanin test survey in Sudan was done in the southern Blue Nile area, 42% of individuals were found positive, compared with 77% in a known endemic area for cutaneous leishmaniasis (Darfour West Sudan) and 4% in Khartoum (central Sudan) where all forms of leishmaniasis are uncommon [30]. It is important to evaluate whether repeated skin testing is sensitizing or not. The sensitizing capacity of LST is of interest in setting where they will be administered repeatedly, e.g. vaccination trials rely on LST conversion being due to vaccination and not to repeated skin testing [31,32]. Because potency and sensitivity may vary by the patient group and clinical setting, LST antigens are best compared when administered simultaneously to the same individuals. Comparison of potency in contrast to the size of the induration, which accompanied the DTH response. The sensitivity of a given antigen is defined as the percent of persons with leishmaniasis in whom the antigen induced a positive leishmanin reactions. The specificity of a given antigen is defined as the percent of healthy volunteers in whom the antigen failed to induce a positive leimshmanin reaction. The duration of LST reaction is widely considered to be for whole life [16].

Conclusion

Malnutrition and associated alterations in resistance to infectious disease contribute to the poor health of populations in much of the world. In fact that malnutrition is a risk factor for developing severe disease after infection by an opportunistic parasite.

The gender has no significant role in disease distribution in this study and as all ages were affected this indicates indoors transmission.

Future recommendation

Further prolong studies are highly recommended to provide useful clinical and epidemiological information of the disease in this area. These clinical and epidemiological data will aid in disease control strategies.

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