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Original Article

Role of Leishmanin Skin Test (LST) as Epidemiological Indicator for *Cutaneous Leishmaniasis* in Al-tragma Village, River Nile State, Sudan

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Abstract

Introduction: Cutaneous leishmaniasis (CL) is a common health problem in the world and its zoonotic form has been reported to occur in Sudan. Leishmaniasis has been considered the second most important parasitic disease after malaria. Therefore, the role of leishmanin skin test (LST) as an epidemiological indicator for CL infection was determined in Al-tragma village, one of the endemic areas for the disease in Sudan.

Materials and Methods: In this cross-sectional study, 410 individuals inhabiting Al-tragma village were enrolled. Two methods of data collection were used in this study, a structured questionnaire and LST. A structured questionnaire was designed to collect and test socio-demographic characteristics. The LST was performed by intradermal injection of 0.1 mL of LST antigen on the volar surface of the left forearm. The result of LST was read after 48-72 hours using the ballpoint pen technique. SPSS version 20.0 was used for data analysis.

Results: LST results showed that 70.7% of respondents were positive and 29.3% were negative. The most infected age groups were 31–40 years (80%) and more than 40 years (82%). The results showed there was a significant difference in LTS results based on age groups, occupations, domestic animals and pets found in and around the houses, and previous experience of the disease ($P < 0.05$).

Conclusion: The overall positive rate for LST was 70.7%. The exposure duration had no effect on LST results. It seems that the patients acquired long-lasting immunity. LST is still a promising tool for evaluating the epidemiological status of Leishmaniasis in endemic areas.

Keywords: Leishmanin skin test, Leishmaniasis, Cutaneous leishmaniasis, *Leishmania major*, Sudan

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Introduction

Leishmaniasis is one of the neglected tropical diseases and vector-borne diseases, present in different clinical forms (1,2). Leishmaniasis comprises a group of diseases caused by intracellular kinetoplastid protozoan parasites of the genus *Leishmania* or *Viannia* that live in macrophages of some vertebrates as amastigote form (3).

The *Leishmania* parasites consist of different species and strains with epidemiological diversity that are pathogenic to humans, leading to different pathological and clinical diseases such as visceral leishmaniasis, mucocutaneous leishmaniasis (MCL), diffuse-cutaneous leishmaniasis (DCL), and cutaneous leishmaniasis (CL), in which the parasite is restricted to cutaneous and subcutaneous tissue (4).

CL is a common disease in the world, which is divided into zoonotic cutaneous leishmaniasis (ZCL) and anthroponotic cutaneous leishmaniasis (ACL) (5). ZCL was reported in Sudan and has been considered to be the second most important parasitic disease after malaria. According to the reports of the World Health Organization (WHO), many cases have been reported in

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Khartoum State (6), and there was an increased incidence of the disease from October to last March 2014, and about 560 patients have been reported to be infected, most of whom were from East Nile (Shrug Al-Nile), Salha and Al-Klaklah (personal communication). Another survey conducted in Sudan showed that of the 1236 individuals enrolled in this survey, 688 were diagnosed with CL, with an infection rate of 55.7% (6). Leishmaniasis is prevalent in the 4 continents and it is considered endemic in 88 countries, 72 of which are developing countries. Worldwide, the annual prevalence of new cases is estimated to be 0.2–0.4 million for visceral leishmaniasis and 0.7–1.2 million for CL, while 12 million people are currently affected by the disease (7). The diagnosis of CL depends on clinical examination coupled with parasitological and molecular techniques. The parasitological investigations, which are considered as a gold standard technique, face many problems, including low parasite count in the lesion and the presence of the parasite restricted to skin only. Traditional methods, such as direct culture, are time-consuming and can be performed in specialized laboratories by expert microscopists, while molecular methods have many advantages, including high sensitivity and specificity (8).

The current study aimed to assess the role of leishmanin skin test (LST) as an epidemiological indicator for CL in the study area.

Materials and Methods

Study Area

This study was conducted in El Tragma Elgabha Village. It is located at the eastern bank of the River Nile (16.4 ° North and 33.25 ° East), 13 Km north of Shandi city, River Nile State, and 175 km north of Khartoum, the capital of Sudan (Figure 1). The population is around 5000 individuals. The area is endemic for CL where an outbreak of CL was reported in 1978. It was agricultural in nature that provided a good environment for survival and multiplication of insect vectors (9).

Experimental Design

This cross-sectional study was conducted to describe the epidemiological status of CL using the LST and a questionnaire.

Questionnaire Design

A structured questionnaire was designed and used to collect socio-demographic characteristics. Data concerning exposure, background of inhabitants, their past complaints, and accompanied skin lesions were also included.

Sample Size

The sample size was determined in a systematic way according to the following simple formula of Daniel ($n = Z^2P(1-P)/d^2$), where n is the sample size, Z is the Z statistic for the level of confidence, P is expected prevalence or proportion (in proportion of one; if 20%, $P = 0.2$), and d is the precision (in proportion of one; if 5%, $d = 0.05$). Z statistic (Z): For the level of confidence of 95% which is conventional, Z value is 1.96 (10).

Leishmanin Skin Test

Leishmanin is a reagent recommended for skin tests in humans for Immuno-epidemiological studies (11).

The Leishmanin antigen obtained from Pasteur Institute of Iran and *L. major* (reference strain MRHO/IR/75/ER) was used for the preparation of the leishmanin. The preparation contained a final concentration of *L. major* promastigotes of 6×10^6 in 1 mL of phosphate-buffered saline.

The LST was performed by intradermal injection of 0.1 mL of skin test antigen on the volar surface of the left forearm using a sterile disposable insulin syringe. The results of LST were read after 48-72 hours using the ballpoint pen technique; induration size of ≥5 mm

Figure 1. Location of Al-Tragma Elgabha Village on the Map (13 Km North of Shandi City).

was taken as positive according to a study conducted by Ibrahim (9).

Test Establishment and Validation

At the start of the study, the investigator trained the medical personnel in filling out the questionnaire and performing the LST, with the help of a dermatologist from Shandi Teaching Hospital. The medical team consisted of a medical officer, nurses, lab technicians, and the investigator (9 persons). In the field, the team was divided into two groups, one group of four persons under the supervision of the medical officer and the second team consisting of five persons under the supervision of the investigator.

Results

Most respondents were female (69.1%) and 30.9% were male. The mean age of the respondents was 38.5±2. Most of the respondents were housewives (39.2%), followed by students (22.6%), farmers (13.6%), building construction workers (4.9%), unemployed (15.3%), office workers (3.2%), and pensioned persons (1.2%). The domestic animals and pets found in and around the houses were domestic ruminants (47.2%), pets (40.1%), and dogs (1.0%). Moreover, the rodent burrows were abundant, in and around houses, which were considered as potential reservoir hosts in the area. Most of the respondents slept outdoors (87.8%) and few of them slept indoors (12.2%). Additionally, 86.6% did not use bed nets, 4.4% used bed nets and 9.0% were irregular users of bed nets. Moreover, 75.4% used protective clothes when sleeping and the rest (24.6%) did not. 91.0% were not affected by CL before, and only 9.0% had the disease before.

It was found that 59.5% of the respondents had CL for more than six years, 35.1% for 1-3 years, and 5.4% for 4-6 years. Concerning the sites of scars, 73% of the scars were found in the lower extremities, 16% in the upper extremities, 8.1% in all the exposed areas, and 2.9% in the face. Regarding the number of scars, 89.2% of them had 1-3 scars, 5.4% had 4-6 scars, and 5.4% had more than six scars. In 62.2% of the respondents, the ulcer healed in 1-3 months, 10.8% in 4-6 months, and 27% in more than 6 months.

Results of Leishmanin Skin Test (Montenegro Skin Test)

LST results showed that 70.7% of respondents were positive while only 29.3% of the respondents were negative. The diameter of indurations ranged from 5 mm to 27 mm, 48.8% of the group had an induration size of 5-15 mm, 40.5% had an induration size of 20-25 mm, and only 10.7% had an induration size of more than 25 mm, (Figure 2; Table 1).

Factors Affecting LST Results

Among males, 72.2% (91/126) were positive and 27.8% (35/126) were negative. Among females, 70% (199/284) were positive and 30% (85/284) were negative. There was no significant difference between them ($P = 0.658$) (Table 2).

Table 3 shows the relationship between age groups and leishmanin test results. In the age group 10-20 years, 49% were positive, in the age group 21-30 years, 69% were positive, in the age group 31-40 years, 80% were positive, and in the age group over 40 years, 82% were positive. The results showed that there was a significant difference between them ($P = 0.005$).

Concerning the effect of occupation in the result of LST, of the 56 farmers, 46 (82%) were positive, of the 20 building constructors, 14 (70%) were positive, of the 13 office workers, 9 (69%) were positive, of the 93 students, 44 (47%) were positive, of the 205 housewives, 158 (77%) were positive, of the 5 pensioned respondents, all 5 (100%) were positive, and of the 18 unemployed respondents, 14 (77%) were positive. The results showed that there was a significant difference between them ($P = 0.000$) (Table 4).

As shown in Table 5, concerning the domestic animals and pets found in and around the houses, dogs were found in four houses, and all the people found in these houses were positive, cats and dogs were found in three houses, indicating two positive and one negative results. The results showed that there was a significant difference between them ($P = 0.013$).

Concerning the sleeping habits of the respondents, of the 361 respondents sleeping outdoors, 71.7% (259/361) were positive and 28.3% (102/361) were negative. Of the 49 respondents sleeping indoors, 63.3% (31/49) were positive and 36.7% (18/49) were negative. The results showed no significant difference between them ($P = 0.220$) (Table 6).

The effect of using bed nets on LST results showed that of the 37 irregular users of bed nets, 67.5% (25/37) were positive and 32.5% (12/37) were negative. Of the 18 users of bed nets, 78% (14/18) were positive and 22% (4/18)

Figure 2. Diameter of Indurations (>25 mm).

Table 1. The Size of Induration in LST Positive Cases

Induration size	No.	Percent
(5-15 mm)	142	48.8 %
(16-26 mm)	117	40.5 %
(27 ≥ mm)	31	10.7 %
Total	290	100.0 %

$\chi^2 = 6.134$, $P = 0.040$

Table 2. Relationship between LST and Gender

	Leishmanin Results		
	Positive	Negative	Total
Males	91 (72.2%)	35 (27.8%)	126 (100%)
Females	199 (70%)	85 (30%)	284 (100%)
Total	290	120	310

$\chi^2 = 0.195$, $P = 0.658$

were negative. Of the 355 non-users of bed nets, 71% (251/355) were positive and 29% (104/355) were negative. The results showed no significant difference between them ($P = 0.737$) (Table 7).

The LST results were positive in all the respondents (100%) who have experienced the disease before (Table 8).

Discussion

The results of this study using LST revealed that the positivity rate of *Leishmania major* infection was higher in males than in females although the number of females in the study population was higher than males, which could be related to the randomized cluster sampling technique used in this study.

The rearing of domestic animals and the presence of rodents affected the infection rate among the population in the study area. Cats had a major role in the transmission of the disease ($P = 0.013$). Other animals such as ruminants had also a contributory role in transmission. It was observed that rodents, which are considered potential reservoir hosts, were abundant in the study area and burrowed around the houses of residents, not necessarily at the level of the house but at the level of the village at large. This may help in maintaining the transmission cycle. In a study conducted in Palestine, where the causative agent was confirmed to be *Leishmania infantum*, infected dogs were the primary reservoir hosts in endemic regions, and they were the most significant risk factor predisposing humans to infection (12).

Most of the participants (88%) used to sleep outdoors, of whom 71.7% were positive, while only 63.3% of those sleeping indoors were positive, which may support the claims that outdoor sleeping habits facilitate the transmission of the disease. This finding agreed with the various previous studies done in endemic areas in Sudan (13-16).

LST results revealed that the use of bed nets has no effect on the acquisition of the disease, as most of the participants who did not use bed nets were negative and some who used bed nets were positive. This may be due to the fact that sandflies are active from dusk to dawn and the mosquito bed nets with large mesh sizes are preferred by the community in endemic areas in a hot climate. Nets

Table 3. Comparison of LST Results in Different Age Groups

Age groups	Total	Leishmanin Results		LST% (+ve)
		Positive	Negative	
10-20	91	45	46	49%
21-30	121	84	37	69%
31-40	124	100	24	80%
More	74	61	13	82%
Total	410	290	120	70.7%

$\chi^2 = 30.788$, $P = 0.005$

Table 4. Comparison of LST Results in Different Occupations

Occupations	Total	Leishmanin		LST% (+ve)
		Positive	Negative	
Farmer	56	46	10	82%
Building constructors	20	14	6	70%
Office workers	13	9	4	69%
Students	93	44	49	47%
Housewives	205	158	47	77%
Pensioned	5	5	0	100%
Unemployed	18	14	4	77%
Total	410	290	120	70.7%

$\chi^2 = 34.663$, $P < 0.000$

Table 5. Comparison of LST Results in People Having Different Domestic Animals and Pets

		Animals					N	Total
		C	Do	G, S, Ca	C, G, S, Ca	D, C		
Leishmanin	+ve	113	4	20	147	2	4	290
	-ve	52	0	20	47	1	0	120
Positive %		165	4	40	194	3	4	410
		68%	100%	50%	89.7%	66.6%	0%	70%

C: Cat, G: Goats, S: Sheep, Ca: Cattle, D: Dog, N: Not found, T: Total
$\chi^2 = 14.423$, $P = 0.013$

Table 6. The Comparison LST Results in People with Different Sleeping Habits

		Sleeping Habits		Total
		Indoor	Outdoor	
LST	+ve	31 (63.3%)	259 (71.7%)	290
	-ve	18 (36.7%)	102 (28.3%)	120
	Total	49	361	410

χ^2= 1.499, *P* = 0.22

Table 7. Effect of Using Bed Nets on LST Results

		Use of Bed Nets			Total
		Rare	Always	Not use	
LST	+ve	25 (67.5%)	14 (78%)	251 (71%)	290
	-ve	12 (32.5%)	4.0(22%)	104 (29%)	120
Total		37 (100%)	18 (100%)	355 (100%)	410

χ^2 = 0.611, *P* = 0.737

Table 8. Relationship Between LST Results and Having a Previous History of the Disease

		Having dieses		Total
		Yes	No	
LST	**+ve**	37 (100%)	253 (67.8%)	290
	-ve	0 (0%)	120 (32.2%)	120
Total		37	373	410

χ^2 = 16.829, *P* = 0.000

with a large mesh size (50 holes/inch^2) can prevent the passage of anopheline mosquitoes, but they would allow the passage of the smaller sandflies (3-5 mm).

The LST results showed that those who have suffered from CL before, as indicated by the presence of scars, were all infected. Scars were reported to be present in different parts of the patients' body, mostly in the lower extremities and to a lesser extent in the upper extremities, and this was because of sandflies lobbing at the lower level. The results also revealed that only one infected child, less than 7 years of age, developed a scar on his face. This finding was contrary to the results reported in Iran by Pourmohammadi et al (17), who indicated that upper extremities are frequently affected sites for the development of scars.

The overall positive rate for LST was 70.7%, which indicates active transmission of the disease in the study area. Similar results were obtained by Ibrahim (9) in Tuti Island and Al-Tragma Elgabha. The author postulated that the disease is endemic in Al-Tragma Elgabha, and the situation appears to be different from Tuti Island with more clinical cases and features of an outbreak in its peak, either in the ascending or descending phase. It may not be easy to differentiate between these phases without longitudinal follow-up data, which may possibly lead to the initiation of an outbreak in the area (9).

Conclusion

The overall positive rate for LST was 70.7% with a higher positivity rate among males compared to females. Furthermore, the exposure duration had no effect on LST results. It seems that the patients acquired long-lasting immunity. LST is still a promising tool for evaluating the epidemiological status in endemic areas, which could help in planning, prevention, and vaccination programs. Effective control can be achieved by the use of bed nets with a small mesh size or insecticide-treated bed nets.

Availability of Data and Materials

Data are presented within the manuscript and can be provided by the corresponding author upon reasonable request.

Conflict of Interests

The authors declare that they have no competing interests.

Ethics Issues

The study protocol was approved by the Research and Ethics Committee of the
Faculty of Medical Laboratory Sciences, University of Gezira and Ministry of Health, River Nile State, Sudan. Written informed consent was obtained from all participants or their guardians/ legal representatives before data and sample collection.

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Authors' Contributions

AMO, ADA, NMA, and KH conceived and designed the study. ADA and SBM supervised the study. AMO, NMA, KH, and RSH wrote the initial draft. AMO, ADA, NMA, and KAM analyzed the data and wrote the final draft of the manuscript. AMO, NMA, KH, and RSH contributed to data collection and laboratory investigations. All authors critically read, revised, and approved the final draft of the manuscript submitted to the journal.

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