

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

**Abdelgani Mohamed Osman<sup>1</sup>, Adam Dawoud Abakar<sup>2</sup>, Nazar Mohamed Abdalla<sup>3</sup>, Kamal Hussain<sup>1</sup>, Rasha SlahEl-deen Hassan<sup>4</sup>, Khalid Abdelsamea Mohamedahmed<sup>5,6\*</sup> and Saseed Bakiker Mahmoud<sup>7</sup>**

## Abstract

**Background:** Cutaneous Leishmaniasis (CL) is a common health problem in the world and the zoonotic form is reported to occur in Sudan. Leishmaniasis has been considered the second parasitic health problem after malaria. Therefore we determine the role of Leishmanin Skin Test (LST) as an epidemiological indicator for CL infection in Al-tragma village; one of the endemic areas for the disease in Sudan.

**Methods:** The study is a cross-sectional study, 410 individuals inhabiting Al-tragma village were enrolled in the study. Two methods were used in this study; structure questionnaire and LST. A structured questionnaire was designed to collect and tested socio-demographic characteristics. The LST was performed by intradermal injection of 0.1 ml 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 (v 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 result showed there was a significant difference between LST and age groups, occupation, domestic and pets animals found in and around the houses, and those having the disease before (P-value<0.05).

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

**Keywords:** Lishmanin skin test; Leishmaniasis; Cutaneous leishmaniasis; *Leishmania major*

**Abbreviations:** ACL: Anthroponotic Cutaneous Leishmaniasis; CL: Cutaneous Leishmaniasis; DCL: Diffuse-Cutaneous Leishmaniasis; LST: Leishmanin Skin Test; MCL: Mucocutaneous Leishmaniasis; NTDs: Neglected Tropical Diseases; PBS: Phosphate-Buffered Saline; SPSS: Statistical Package of Social Sciences; VL: Visceral Leishmaniasis; WHO: World Health Organization; ZCL: Zoonotic Cutaneous Leishmaniasis.

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## Introduction

Leishmaniasis is one of the neglected tropical diseases (NTDs) and vector-borne diseases, present in different clinical forms [1,2]. Leishmaniasis comprises a group of diseases caused by intracellular parasite kinetoplastid protozoan 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 diverse that are pathogenic to human, leading to different pathological and clinical diseases such

as Visceral leishmaniasis (VL), Mucocutaneous leishmaniasis (MCL) Diffuse-cutaneous Leishmaniasis (DCL), and Cutaneous Leishmaniasis (CL), in which the parasite is restricted to cutaneous and subcutaneous tissue [4].

Cutaneous Leishmaniasis (CL) is a common health problem in the world; it 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 parasitic health problem after malaria. According to the record of the WHO, many cases have been reported in Khartoum State

<sup>1</sup>Department of Parasitology, University of Technology Merowe, Merowe, Sudan

<sup>2</sup>Department of Medical Parasitology, Gezira University, Wad Medani, Sudan

<sup>3</sup>Department of Internal Medicine, Abaha University, Abaha, KSA

<sup>4</sup>Department of Clinical Pharmacy, University of Khartoum, Khartoum, Sudan

<sup>5</sup>Department of Hematology, Gezira University, Wad Medani, Sudan

<sup>6</sup>Department of Immunology, Gezira University, Wad Medani, Sudan

<sup>7</sup>Department of Dermatology and Venereal Diseases, Gezira University, Wad Medani, Sudan

**Corresponding author:** Khalid Abdelsamea Mohamedahmed, Department of Hematology, Faculty of Medical Laboratory Sciences, Gezira University, Wad Medani, Sudan

✉ khalid.gu89@gmail.com

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[6], and there was an increased incidence of the disease from October to last March 2014, and about 560 patients have been reported, Most of them from East Nile (Shrug Al-Nile), Salha and Al-Klakah (Personal communication). In another survey in Sudan showed that 688 were diagnosed as cases of CL out of the 1,236 individuals enrolled in this survey, giving an infection rate of 55.7% [6]. Leishmaniasis is prevalent in the four continents and considered endemic in 88 countries, 72 of which are developing countries. Worldwide, the annual prevalence of new cases is estimated as 0.2-0.4 million of VL and 0.7–1.2 million of 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; low parasite count in the lesion and the parasite restricted to skin only. Traditional methods such as direct culture were time-consuming and can be performed in specialized laboratories with expert microscopists so that molecular methods have many advantages; 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 site

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 was around 5000 individuals. The area is endemic for CL where an outbreak of CL was reported in 1978. It was agricultural in nature that facilitated a good environment for survival and multiplication of insect vectors [9].



**Figure 1:** Al-Tragma Elgabha village (13 Km north of Shandi city).

### Experimental design

A cross-sectional study conducted to describe the epidemiological status of CL using the Leishmanin Skin Test (LST) and questionnaire.

### Questionnaire design

A structured questionnaire attends to collect socio-demographic characteristics was designed and tested. Data concerning

demography for exposure, historical background of inhabitants, and their past complaint and accompanied skin lesions.

### Sample size

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

### Leishmanin Skin Test (LST)

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

The Leishmanin antigen obtained from Pasteur Institute of Iran *L. major* (reference strain MRHO/IR/75/ER) was used for the preparation of the leishmanin. The preparation contained a final concentration for *L. major* promastigotes of  $6 \times 10^6$  in 1 ml of Phosphate-Buffered Saline (PBS).

The LST was performed by intradermal injection of 0.1 ml skin test antigen on the volar surface of the left forearm using a sterile disposable insulin syringe. The result of LST was read after 48-72 hours using the ballpoint pen technique; induration size  $\geq 5$  mm was taken as positive reading according to Ibrahim study [9].

### Test establishment and validation

At the start of the study, the investigator trained the medical personnel in filling 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 consisted of five persons under the supervision of the investigator.

## Results

Most respondents were females, 69.1% and 30.9% males; the mean age was  $38.5 \pm 2$ . The occupation of most of the respondents were housewives 39.2%, students 22.6%, farmers 13.6%, building construction workers 4.9%, unemployed 15.3%, office workers 3.2% and pensioned 1.2%. The domestic and pet animals found, in and around the houses, were domestic ruminants 47.2% and pets were 40.1% and dogs 1.0%. Moreover, the rodent's burrows were abundant, in and around houses, which consider as potential reservoir hosts in the area. Most of the respondents used to sleep outdoors 87.8% and fewer indoors 12.2%. 86.6% did not use bed nets, 4.4% using bed nets and irregular users of bed nets were 9.0%. 75.4% used protective cloths when sleeping and the rest 24.6% didn't. 91.0% were not affected by CL before, and only 9.0% had the disease before.

The onset of CL among the respondents was 59.5% for more than six years, 35.1% for 1-3 years, and 5.4% for 4-6 years. Concerning the sites of scars, 73% 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% 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)

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, the group had diameter 5-15 mm were 48.8%, 20-25 mm were 40.5% but only 10.7% LST had more than 25 mm (**Figure 2** and **Tables 1** and **2**).



**Figure 2:** LST diameter of indurations (>25mm).

**Table 1:** The indurations size of LST positive among study group.

Indurations size	Frequency	Percent
(5-15 mm)	142	48.80%
(16-26 mm)	117	40.50%
(27 ≥ mm)	31	10.70%
Total	290	100.00%
<b>Note:</b> $\chi^2=6.134$ ; P-value=0.040.		

**Table 2:** Relationship between LST and sex.

	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
<b>Note:</b> $\chi^2=0.195$ ; P-value=0.658			

### Factors affecting LST results

Out of the 126 males in the study population, 72.2% (91/126) of them were positive and 27.8% (35/126) negative. Out of the 284 females, 70% (199/284) were positive and 30% (85/284) were negative. There was no significant difference between them (P-value=0.658).

**Table 3** shows the relation of age groups with the leishmanin test results, in the age group 10-20 years out of 91, 45 (49%) of them were positive, age group 21-30 years out of 121, 84 (69%) of them were positive, the age group 31-40 years out of 124, 100 (80%) of them were positive and more than 40 years out of 74, 61 (82%) of them were positive. The result showed there was a significant difference between them (P-value=0.005).

**Table 3:** Comparison of LST with age among study group.

Leishmanin results				
Age groups	Total	Positive	Negative	LST% (+ve)
Oct-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	70.70%
<b>Note:</b> $\chi^2=30.788$ ; P-value=0.005.				

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

**Table 4:** Comparison of LST with occupation among study group.

Leishmanin				
Occupations	Total	Positive	Negative	LST% (+ve)
Farmer	56	46	10	82%
B. 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	70.70%
<b>Note:</b> $\chi^2=34.663$ ; P-value<0.000.				

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

Comparing sleeping habits of the respondents with LST, out of 361 sleeping outdoors, 71.7% (259/361) of them were positive and 28.3% (102/361) of them were negative. Out of the 49 sleeping indoors, 63.3% (31/49) of them were positive and 36.7% (18/49) of them were negatives. The result showed no significant difference between them (P-value=0.220) (**Table 6**).

**Table 5:** Comparison of domestic and pets animals with LST results.

		Animals						
		C	Do	G, S, Ca	C, G, S, Ca	DC	N	Total
Leishmanin	+ve	113	4	20	147	2	4	290
	-ve	52	0	20	47	1	0	120
		165	4	40	194	3	4	410
Positive %		68%	100%	50%	89.70%	66.60%	0%	70%
Note: X²=14.423; P-value=0.013; Abbreviations: C: Cat; G: Goats; S: Sheep; Ca: Cattle; D: Dog; N: Not found; T: Total.								

**Table 6:** The comparison of sleeping habits with LST results.

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

**Note:**  $X^2=1.499$ ; P-value=0.22.

The effect of using bed nets on LST results showed that out of the 37 rare using bed nets, 67.5% (25/37) were positive and 32.5% (12/37) were negative. Out of the 18 always using bed nets, 78% (14/18) were positive and 22% (4/18) were negative. Out of the 355 not using bed nets, 71% (251/355) were positive and 29% (104/355) were negative, the result showed no significant difference between them (P-value=0.737) (**Table 7**).

**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

**Note:**  $X^2=0.611$ ; P-value=0.737.

The results of LST among the respondents having the disease before, all 37 (100%) were positive (**Table 8**).

**Table 8:** Relationship between LST results and those having the disease before.

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

**Note:**  $X^2=0.611$ ; P-value=0.737.

## Discussion

The results of this study using LST revealed that infection by *Leishmania major* the positivity rate was higher in males than females although the number of females in the study population was more than males, this could be related to the randomized clustered design sampling technique used in this study, the female's participants were more than males.

The rearing of domestic animals and the presence of rodents did affect the infection rate among the population in the study area. Cats had a major role in the transmission of the disease (P-value=0.013). Other animals like ruminants had also a contributory role in transmission. It was observed that rodents, which are considered potential reservoir hosts, were prevailing in the study area and usually burrows around houses of residents, not necessary 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 in Palestine, where the causative agent was confirmed to be *Leishmania infantum*, infected dogs were the primary reservoir host in endemic regions, and were the most significant risk factor predisposing humans to infection [12].

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

LST result revealed that the use of bed nets has no effect on acquiring the disease, as, most of the participant not using bed nets were negative and some using bed nets were positive that because the activity of the sand flies mostly from dusk to dawn and the mosquitoes bed nets been used, which were preferred by the community in endemic areas in a hot climate, were wider mesh size. Wider mesh size (50 holes/inch<sup>2</sup>), can prevent the passage of anopheline mosquitoes, and would allow the passage of the smaller sand flies (3-5 mm).

The LST result showed that exposures who suffered from CL before, as indicated by the presence of scars, were all infected. The presence of scars among the infected individuals was found distributed in different parts of the patients' body, mostly in the lower extremities and to a lesser extent in the upper extremities, this was because sand flies lobbing at the lower level. The results also revealed that only one infected child, less than 7 years,



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 and possibly lead to the initiation of an outbreak in the area [9].

## Conclusion

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

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## Author's 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 data and wrote the final draft of the manuscript. AMO, NMA, KH, 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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## Availability of Data and Materials

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

## Declarations

### Ethics and consent to participate

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.

## Competing Interests

The authors declare that they have no competing interests.

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