

Evaluation of Subclinical Cutaneous Leishmaniasis Using Leishmanin Skin Test in Keana North-Central Nigeria

L Manu, C Uneke, M Njoku, S Agwale, P Inyama

Citation

L Manu, C Uneke, M Njoku, S Agwale, P Inyama. *Evaluation of Subclinical Cutaneous Leishmaniasis Using Leishmanin Skin Test in Keana North-Central Nigeria*. The Internet Journal of Parasitic Diseases. 2005 Volume 1 Number 1.

Abstract

Subclinical Cutaneous Leishmaniasis (SCL) was evaluated using the Leishmanin Skin Test (LST) in Keana, North-Central Nigeria. Fifty-three individuals were studied. Fifteen, 28.30% had lesions indicative of previous and active leishmaniasis, 9 (16.98%) had scars while 29(54.72%) had neither scars nor lesions. Lesions and scars were multiple and mostly found on the hind limbs. Mice inoculated with tissue from lesions sampled did not develop lesions. Results showed that 18(34.0%) of the subjects were LST positive with the prevalence higher in the females than the males (42.9% vs 24.0%). The LST positivity occurred with the highest frequency amongst individuals who had scars (44.5%). There was significant difference in the association between sex and the LST positivity ($\chi^2 = 2.11$, df. = 1, $P < 0.05$). Individuals of 41 – 50 years old had the highest LST positivity (42.9%). Results provide evidence of presence of Cutaneous Leishmaniasis in Keana and confirmed the effectiveness of LST in identifying of cutaneous leishmaniasis associated symptoms.

INTRODUCTION

The Leishmania is a group of globally widespread protozoa parasitic organisms, which are causative agents of leishmaniasis. Leishmania species, an obligate intracellular parasite of mammalian macrophages (1), manifests in multifaceted clinical forms as: Cutaneous leishmaniasis (CL), Mucutaneous leishmaniasis (MCL), Diffuse cutaneous leishmaniasis (DCL) and Visceral leishmaniasis (VL) (2). Leishmania species are transmitted exclusively by the bite of the female Phlebotomine sandfly. The sandfly becomes infected when taking blood from reservoir hosts, which include man and domestic animals. Worldwide prevalence of leishmaniasis exceeds 12 million cases with estimated annual incidence of 400,000 cases in 80 countries among a population at risk of about 400 million (3).

Different strains cause similar and yet peculiar diseases and symptoms. For instance, cutaneous leishmaniasis of the Old World is called oriental sore and is caused by Leishmania belonging to the L. tropica complex, L. major and L. aethiopica. The disease is distributed throughout the Mediterranean and non-Mediterranean areas including West Africa (4). Clinical features of CL include nodular form and oriental sore, infiltrating ulcerating, erythematous types and multiple papular lesions (5). Cases of cutaneous leishmaniasis in Nigeria since the mid 19th century have

been documented (6, 7). The lack of data on parasitological tests is partly due to difficulty in growing the parasite in vitro (4). Parasitological diagnostic techniques are tedious, inconveniencing, does not detect current and previous infections, hence not good enough for mass diagnosis of CL (8).

Cutaneous leishmaniasis is on the increase in Nigeria (9, 10). There is need therefore to adopt an easy, rapid, less invasive, specific and sensitive method for mass screening and detection of current and previous infections. This is achieved through immunological techniques, which encompass molecular and cellular biology of antigen recognition and of specific immune reactions and non-specific (1).

The Leishmanin Skin Test (LST) has been used by several researchers (2, 12), and has been reported to measure delayed type hypersensitivity and is being used to assess prevalence of subclinical infections and measures CL transmission rate over time. In this study we used LST to evaluate subclinical cutaneous leishmaniasis in Keana, a known CL endemic area.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Keana, the administrative headquarters of Keana L.G.A of Nassarawa State, North

Central Nigeria. Its vegetation is generally Guinea Savannah consisting of tall grasses and patchy trees. The annual rainfall of this zone is about 1500mm and lasts between 6 to 8 months (late March to late October). The weather is relatively hot throughout the year. Sandfly resting sites such as caves, tree holes, cracks in rocks, cavities between boulders, fissures in the ground, buildings, termite hills and animal burrows abound in the area.

STUDY POPULATION

The study population comprised of 53 individuals (25 males, 28 males) aged 3 to 60 years. After a successful community mobilization through religious and community leaders in conjunction with the Primary Health Care (PHC) Centre in the locality, the 53 individuals willingly indicated interest to participate in the study. Clinical assessment of subjects was done with respect to the presence of lesions and/or scars and suspected cases were identified by the collaborating PHC Centre. Identification of lesions/scars was done according to the criteria outlined previously (5). However, it was obvious that suspected cases may be having the subclinical leishmaniasis, and that is if other tropical infections with similar manifestations such as tuberculosis and leprosy, are ruled out. Informed consent was duly obtained from each participant, with the assurance that the information obtained would be treated with utmost confidentiality and for the purpose of the research only. Demographic information (age, sex, marital status, occupation, travel history and duration of stay in locality) was obtained from each participant by interview.

PARASITOLOGICAL DIAGNOSIS

The most recent lesions were selected from 3 subjects cleaned with 70% alcohol and small incision was made (through the intact skin to about 2mm deep) at the nodular edge of the sores. Two smears were prepared from slit-skin scrapings of the same lesions, air-dried, fixed with methanol, stained with Giemsa's stain, kept for 20 minutes and washed. Each stain was then screened microscopically for amastigotes. Tissues recovered from skin scraping of the 3 subjects were inoculated into BALB/c mice through the upper footpad. Mice were then observed for the development of lesions as described previously (13, 14).

LEISHMANIN SKIN TEST (LST)

The leishmanin antigens used for the skin test were donated by the Laboratory of Leishmaniasis/AIDS Research, University of Jos, Nigeria. The antigens consisted of pooled

heat-killed promastigotes derived from *Leishmania major* and *Leishmania amazonensis* suspended in PBS with 0.5% phenol. Before the study commenced the leishmanin antigens were standardized using known positive and negative controls. Each individual was injected intracutaneously with a dose of 0.1ml of the antigens on the deltoid area of the forearm. The test (reactions measured as skin induration), were assessed after 48 hours using the ballpoint pen method (15). The freshly inked outline of the induration areas were transferred on white clean papers and measured in the laboratory. Average indurations diameter of equal or more than 5mm more considered as positive Leishmanin test (12,16).

RESULTS

Of the 53 individuals studied, 15 (28.30%) had lesions. Scars were found on 9 (16.98%) of the population; while 29 (54.72%) had no noticeable lesions or scars and hence were considered apparently healthy. Lesions and scars were mostly multiple and present on the legs. Most lesions observed were already resolving and parastiological confirmatory test carried out on a few samples by Giemsa's stain preparation gave no positive result. Mice inoculated with tissue from lesions sampled did not develop lesions. Results from the LST indicated that 18 (34.0%) of the subjects were LST positive. The prevalence was higher among the female than the males (42.9%: 24.0%). The LST positivity occurred with the highest frequency (44.5%) amongst individuals who had scars, compare to the apparently healthy (37.9%) and those with lesions (20.0%) (Table 1).

Figure 1

Table 1: Sex-related prevalence of positive Leishmanin skin test (LST) among subjects studied in Keana, North-central Nigeria

Sex	Clinical Picture						No. Overall	
	Lesions No. Tested	No. (%) Positive	Scars No. tested	No. (%) Positive	Apparently healthy No. Tested	No. (%) Positive	No. Tested	No. (%) Positive
Male	8	0(0.0)	1	0(0.0)	16	6(37.5)	25	6(24.0)
Female	7	3(42.9)	8	4(50.0)	13	5(38.5)	28	12(42.9)
Total	15	3(20.0)	9	4(44.5)	29	11(37.9)	53	18(34.0)

Statistically there was no significant difference observed in the trend ($\chi^2 = 2.11$, $df = 1$, $p \leq 0.05$). Age related prevalence of positive LST amongst the subject showed that individuals belonging to age group 41 – 50 years had the highest prevalence of 42.9% followed by those in their second decade of life (38.5%) and those below ten years old (33.3%) (Table 2). There was no statically significant difference in the association between age and positive LST ($\chi^2 = 1.933$, $df. 5$, $p \leq 0.05$).

Figure 2

Table 2: Age-related prevalence of positive leishmanin skin test (LST) among subjects studied in Keana.

Age (Yrs)	Clinical Picture						No. Overall	
	Lesions No. Tested	No. (%) Positive	Scars No. tested	No. (%) Positive	Apparently healthy No. Tested	No. (%) Positive	No. Tested	No. (%) Positive
≤ 10	6	0(0.0)	0	0(0.0)	3	3(100.0)	9	3(33.3)
11-20	9	3(33.3)	8	4(50.0)	9	3(33.3)	26	10(38.5)
21-30	0	0(0.0)	0	0(0.0)	1	0(0.0)	1	0(0.0)
31-40	0	0(0.0)	0	0(0.0)	9	2(22.2)	9	2(22.2)
41-50	0	0(0.0)	0	0(0.0)	7	3(42.9)	7	3(42.9)
≥ 51	0	0(0.0)	1	0(0.0)	0	0(0.0)	1	0(0.0)
Total	15	3(20.0)	9	4(44.5)	29	11(37.9)	53	18(34.0)

DISCUSSION

A number of previous documented investigations of CL in Keana indicated that the area was endemic (_{10, 13}). In this study the cases of lesions (28.30%) and scars (16.98%) which occurred mostly among individuals of age group 11-20 years confirmed that the infection is still endemic in the area (₁₇). The occurrence of active lesions on legs agrees with the findings of Dedet et al. (₁₈) that this might be because of exposure of this part of the body during period of high temperature.

The lesions/scars ratio was calculated as an important factor in determining how long disease has been present in the community. However, the ratio does not represent their absolute magnitudes. Factors such as difficulty in finding small leishmanial, scars, women not likely to admit they have scars; and not all inhabitants screened, were possible factors for not calculating the absolute magnitudes (₁₉).

The Leishmanin Skin Test (LST) is still frequently used for clinical diagnosis of the disease and epidemiological surveys on the prevalence of leishmanial infection (₁). The prevalence of positive LST of 34.0% observed among the study population in Keana where there is a high risk of infection by *Leishmania major* may be regarded as being of a fairly high magnitude. Cumulative data from some earlier studies suggested that the percentage of positive LST result increase with age (₂₀). However, in our study no observation of this kind was made, although individuals of 41 – 50 year age group had the highest prevalence, most of the infection occurred among the younger people and this conformed to the observations made in Brazil (₁₆).

A few samples made by Giemsa's stain preparation did not confirm the disease parasitologically. Mice inoculated with tissues from sampled lesions did not develop lesions. It is reported that in CL, LST would become positive during active phase when lesions are present and may remain

positive for life (_{9, 21}). However, many of the individuals with lesions were negative to LST. This could mean that lesions observed may not have been due to leishmaniasis.

This study looked at the possibility of using pooled leishmanin derived from New world and Old world *Leishmania* species to identify individuals with CL due to Old world *Leishmania*, using possible results from clinically-positive and high risk individuals from known endemic communities with *Leishmania* infection due to Old World parasites found in Nigeria. This study is the first attempt to use pooled antigens derived from both New and Old World for LST in sub-Saharan Africa. In a similar investigation, earlier conducted in the study area, leishmanin derived from *L. major* produced LST positivity rate of 12.5%, that from *L. amazonensis* produced LST positivity of 15.8%, while the pooled antigen derived from *L. mexicana*, *L. amazonensis*, and *L. guyanensis* produced LST positivity rate of 25.5% [9]. The results showed that New World *Leishmania*-derived leishmanin was effective for detecting Old World CL in humans, which was in agreement with the findings of Akuffo et al. (₂₂), where leishmanin prepared from *L. major* was capable to detect CL caused by *L. aethiopica* or *L. braziliensis*. In addition, in terms of sensitivity, the pooled heat-killed *L. mexicana*, *L. amazonensis*, and *L. guyanensis* promastigotes was apparently superior to both the other products (*L. major* or *L. amazonensis*-derived leishmanin) (₉). The LST is reported to be specific for leishmaniasis but is not species specific (₂₀). Our results showed that the leishmanin caused indurations greater than 5.0mm in all patients with CL due probably to *L. major* infection. Thus, the leishmanin showed that patients with CL responded with classic DTH responses to New World *Leishmania*-derived skin test antigen. Correlations between in vivo immune responses and in vitro cellular proliferation of cells have been described (_{22, 23}). It is however still not clear which type of antigens induced the indurations observed in the LST in this study.

Reports about cross-reactivity between *Leishmania* species and other infections have been documented (₁₈). These cross-reactions could warrant the use of standardized leishmanin, but some factors associated with the preparation of the selected strain of New World *Leishmania* used in the leishmanin preparations may result in antigens that are adequate for detecting immunity in some types of leishmaniasis but not others. To understand what these factors are, it may be important to identify specifically what

antigens induced the indurations. This is largely due to our limitations in the results from our mice study and insufficient requirements for further in vitro studies. Though more results are still needed, we have been able to demonstrate the current status of leishmaniasis in Keana communities and hence north central Nigeria. It calls for a public health attention for the control of the diseases and its causative agents. This becomes more important in the current migrations in north central Nigeria due to civil unrest. It may be the same in current global village, where great distances are bridged by transportation and communication resulting in accelerated movement and population mixing.

This study is not without a few major limitations. Conclusions with respect to age-related susceptibility/exposure could not be properly drawn, largely due to our limited sample size. Strategies should be devised to increase sample size in future studies especially in this part of the globe, where community-based epidemiological studies involving sampling of human population often suffer setbacks because of the unwillingness of many members of the target population to participate. Secondly, our parasitological analysis using the Giemsa staining and the BALB/c mice could not confirm the infection. This may be because the few individuals suspected to have CL from whose lesions, materials were obtained for the parasitological analysis, may not be infected with leishmania. Although the antigens used were standardized using positive and negative controls, the possibility of cross-reactivity by other tropical infections could interfere with results may be excluded. Future study using isoenzyme electrophoresis technique and PCR analysis is advocated in order to clarify this.

ACKNOWLEDGEMENT

Authors wish to thank the traditional ruler of Keana for logistic support in the community mobilization of participants in this study.

CORRESPONDENCE TO

C.J. Uneke Department of Medical Microbiology/Parasitology, Faculty of Clinical Medicine, Ebonyi State University, P.M.B 053 Abakaliki, Nigeria
Telephone: 234-08038928597 Fax number: 234-04300222
E-mail: unekecj@yahoo.com

References

1. Grimaldi G Jr., Tesh RB. Leishmaniasis of the New

- World: Current concepts and Implications for future Research. Clin Microbiol Rev 1993; 6: 230 - 50.
2. Schaefer KU, Kurtzhals JA, Sherwood JA, Githure JI, Kager PA, Muller AS. Epidemiology and Clinical Manifestations of Visceral and Cutaneous leishmaniasis in Baringo District, Rift valley, Kenya. A literature Review. Trop Geogr Med 1994; 46: 129 - 33.
3. Noyes HA, Arana BA, Chance ML, Maingo R. The Leishmania hertigi (Kinetoplastid: Trypanosomatidae) complex and the Lizard Leishmania. Their classification and evidence of a Neotropical origin of the Leishmania endotrypanum clads. J Euk Microbiol 1997; 44: 511 - 7.
4. Ashford RW, Bettini S. Ecology and Epidemiology of Old World Leishmaniasis. Volume I. London: Academic Press Inc, 1987: 365-424.
5. El-safi SH, Peters W, El-Toam B, El-Kadarow A, Evans DA. Studies on Leishmaniasis in the Sudan 2. Clinical and Parasitological studies on Cutaneous Leishmaniasis. Trans R Soc Trop Med Hyg 1991; 85: 457.
6. Elmes GT, Hall RN. Cutaneous leishmaniasis in Nigeria. Trans R Soc Trop Med Hyg 1994; 37: 437 - 9.
7. Jellief RS. Cutaneous Leishmaniasis in Nigeria and the Western Sudan. West African Medical Journal, 1955; 4: 92-4.
8. Hepburn NC. Cutaneous Leishmaniasis: an overview. J Postgrad Med 2003; 49:50-4.
9. Agwale SM, Duhlińska DD, Grimaldi G. Jr. Response to Heterologous Leishmanins in Cutaneous Leishmaniasis in Cutaneous Leishmaniasis in Nigeria-Discovery of New Focus. Mem Inst Oswaldo Cruz. 1998; 93:23-7.
10. Ikeh EI, Ajayi JA, Bello CSS. Outbreak of Human Leishmaniasis in Keana District Nigeria. A preliminary communication. Nig Med J 1993; 24: 101-2.
11. Jaffe CL, Benett E, Grimaldi G Jr., McMahon-Pratt D. Production and Characterization of species-specific monoclonal antibodies against Leishmania donovani for immunodiagnosis. J Immunol 1984; 133: 440-7.
12. Shiddo SA, Akuffo HO, Mohamed AA, Hult G, Nilsson LA, Ouchterlony O, Thorstensson R. Visceral Leishmaniasis in Somalia: Prevalence of Leishmanin Positive and Seropositive inhabitants in an Endemic Area. Trans R Soc Trop Med Hyg 1995; 89: 21-4.
13. Agwale SM, Donji B, Okolo CJ, Duhlińska DD. Clinical and Parasitological prevalence of Leishmaniasis in school Children in Keana, Awe LGC of Plateau State, Nigeria. Mem Inst Oswaldo Cruz 1993; 88: 347.
14. Maingon R, Feliciangeli D, Guzman B, Rodriguez N, Convit J, Adamson R, Chance M, Petralanda I, Dougherty M, Ward R. Cutaneous Leishmaniasis in Tachira State, Venezuela. Ann Trop Med Parasitol 1994; 88: 29 - 36.
15. Marty P, Le Fichoux Y, Giordana D, Brugentti A. Leishmanin reaction in the human population of a Highly Endemic Focus of Canine Leishmaniasis in Alpes-Maritimes, France. Trans R Soc Trop Med Hyg 1992; 86: 249-50.
16. Souza WJ, Sabroza PC, Santos CS, de Sousa E, Henrique MF, Coutinho SG. Montenegro Skin Test for American Cutaneous leishmaniasis carried out on school children in Rio de Janeiro, Brazil: An Indicator of Transmission Risk. Acta Trop 1992; 52: 111 - 9.
17. Lysenko AJ, Beljaev AE. Quantitative Approaches to Epidemiology. In: Peters W, Killick-Kendrick R, eds. The Leishmaniasis in Biology and Medicine Vol. 1. London: Academic Press, 1987; 263 - 90.

18. Dedet JP, Pradinaud R, Gay F. Epidemiological aspects of Human Cutaneous Leishmaniasis in French Guiana. *Trans R Soc Trop Med Hyg* 1989; 83: 616 - 20.
19. Dye C, Killick-Kendrick R, Vitutia MM, et al. Epidemiology of Canine Leishmaniasis: Prevalence, incidence and Basic reproduction number calculated from a cross-sectional serological survey on the Island of Gozo, Malta. *Parasitology* 1992; 105: 35 - 41.
20. Pampiglione S, Manson-Bahr PE, La Placa M, Borgatti MA, Musumeci S. Studies in Mediterranean Leishmaniasis 3. The Leishmanin skin test in Kala-azar. *Trans R Soc Trop Med Hyg* 1975; 69: 60 - 8.
21. Reed SG, Badaro R, Masur H, et al. Selection of a skin test antigen for American visceral leishmaniasis. *Am J Trop Med Hyg* 1986; 35: 79 - 85.
22. Akuffo D, Darce M, Maasho K, Berhan T Y. In vivo evaluation of Immune Responses in Leishmaniasis: The use Cross-species Leishmanin preparations for skin testing. *Am J Trop Med* 1995; 53:16-22.
23. Alvarado R, Enk C, Schnur L, Frankenburg S. Delayed-type Hypersensitivity and Lymphocyte proliferation in Response to Leishmania Major infection in a group of children in Jericho. *Trans R Soc Trop Med Hyg* 1989; 83:189-92.

Author Information

Lois Manu, M.Sc.

Applied Parasitology and Entomology Unit, Department of Zoology, University of Jos

Chigozie J. Uneke, M.Sc.

Applied Parasitology and Entomology Unit, Department of Zoology, University of Jos

Moses O. Njoku, Ph.D.

Applied Parasitology and Entomology Unit, Department of Zoology, University of Jos

Simon M. Agwale, Ph.D.

Applied Parasitology and Entomology Unit, Department of Zoology, University of Jos

Petrus U. Inyama, M.Sc.

Applied Parasitology and Entomology Unit, Department of Zoology, University of Jos