Original article

A survey on leishmaniasis and the leishmanin skin test profile in Lower Awash Valley, northeast Ethiopia

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Abstract

Background: The prevalence of both visceral and cutaneous leishmaniasis in various parts of Ethiopia is evident. Earlier discrete works also give some clue of the circulation of some sort of leishmanial parasites in northeast Ethiopia. This necessitated further work in the area designated as the Lower Awash in northeast Ethiopia.

Objectives: The study was undertaken to explore exposure to leishmanial parasites in representative sites in the Lower Awash Valley, northeast Ethiopia.

Methods: After an assessment for clinical leishmaniases, a cross-sectional leishmanin skin test was carried out between March 1996 and August 1997 on 789 individuals of whom 767, mostly apparently healthy study participants returned for the reading of the reaction.

Results: The rate of positive leishmanin response in the study sites ranged from 19 to 85%, the overall prevalence being about 40%. The difference in leishmanin reaction among the major study localities was found to be statistically significant, the highest (50.8%) was observed in Mile locality with the lowest in Asayta (24.7%). Over 40% of males and a third of the females showed positive response, the gender difference in leishmanin response being significant. The rate of positive leishmanin reaction appeared to increase with age, the increase being more apparent in males.

Conclusion: The ascending positive leishmanin reaction rate with age, with a higher prevalence in the indigenous Afars with relative preponderance in males, parallels findings in other endemic areas and reflects the relevance of outdoor exposure to infection. [*Ethiop.J.Health Dev.* 2004;18(3):159-163]

Introduction

The leishmanin skin test is an immunological procedure resembling the mantoux test. The test measures delayed hypersensitivity reaction to an antigen prepared from a culture of different species of *Leishmania.*

The leishmanin reaction varies in the different clinico-epidemiological forms and spectra of leishmaniases. It turns positive in late oriental sore, in late East African and Mediterranean kala-azar and South American leishmaniasis, but is reckoned to be negative or barely positive in Indian kala-azar (1, 2). In infections with *L. tropica* and *L. mexicana*, the leishmanin reaction gets positive at the onset of infection. However, this does not confer immunity to re-infection unless the leishmanial leision has healed completely (3). In *L. braziliensis*, the test becomes positive during the active phase of infection. In *L. donovani*, nevertheless, it may not become positive until six to eight weeks following recovery from the disease (3). Post kala-azar dermal leishmaniasis (PKDL) cases give weak positive or negative reactions, suggesting an incomplete immune mechanism. Active (untreated) diffuse cutaneous leishmaniasis (DCL) cases are expected to express negative responses, with a possibility of reversing to positive following protracted course of treatment with pentavalent antimonials (4). Unlike earlier notions, a relatively recent work demonstrated a positive leishmanin not to be necessarily a life long phenomenon (5). A positive leishmanin test is perceived to reflect cryptic (sub-clinical) infection with leishmaniases (3, 6-9). The relevance of a leishmanin skin test, the epidemiological significance of a positive reaction and the application of the technique have been examined elsewhere (10-13).

Leishmanin skin test surveys have been conducted in the highland (14) and arid lowlands of Ethiopia (15-17) to map out the distribution of both cutaneous leishmaniasis due to *L. aethiopica* and visceral leishmaniasis caused by *L. donovani*, respectively. Recent studies in areas commonly designated as Upper Awash and Middle Awash as well demonstrated a considerable rate of leishmanin positivity in representative sites (11, 12). Concurrent entomological studies over a large stretch of land have as well generated supportive evidence (18, Institute of Pathobiology unpublished data). In this study, a cross-sectional leishmanin skin test was conducted in 1996 and 1997 to assess the burden of exposure to leishmanial infection in the area demarcated as the Lower Awash.

Methods

After a reconnaissance assessment of the study area, representative study sites were sampled from major localities of Lower Awash, which included Mile, Dubti and Asayta between March 1996 and August 1997. The criteria for inclusion of the sites comprised ecology,

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pertinence to the transmission of leishmanial parasites, urban settlement, sites of agricultural development and or temporary inhabitation. As per the inclusion parameters, 14 study sites were selected from the three principal study localities. Since the area is remote with access problems, purposive sampling was employed. To get a better picture regarding exposure to leishmanial parasites, settlements with regular pesticide pressure were excluded and thus villages in the outskirts of the towns of Mile, Dubti and Asayta were considered for the study.

All study sites are located below 700 meters of altitude above sea level. With the exception of those situated along the course of Mile and Awash rivers, the rest of the sites are warm and dry for most of the year. To ensure autochthonous exposure, individuals of both sexes who either stayed in the respective study sites for five years and above or natives above five years of age were included in the study. Although the precise size of the denominator population was difficult to estimate at the time of the investigation, the size engaged (767) is reckoned to be about 10-15% of the target population. Though the Afar were the dominating ethnic group, migrant laborers were also included when available. Informed verbal consent was secured prior to involvement into the study. Study individuals were identified by relevant socio-demographic and epidemiological characteristics which included age, sex, ethnic group, duration of stay and the like. The administration of the leishmanin test was preceded with general physical examination and screening of the study subjects for further confirmation of suspects. Confirmation was done by standard serological and parasitological tests, with provision for treatment with Sodium stibogluconate (Pentostam) for confirmed cases.

Ethical clearance for conducting this study was secured from the Institute of Pathobiology (IPB) and standard procedures such as aseptic techniques were strictly followed. Examination of patients was carried out under the local health facilities by qualified members of the research team with assistance from the local health personnel.

The *Leishmania major* antigen used for the survey was obtained from the Institute of Pasteur, Tehran, Iran kindly provided for the project by the Special Program for Research and Training in Tropical Diseases (TDR). The antigen was validated at IPB for potency and safety using volunteers and formely crued cutaneous leishmaniasis cases (staff of IPB) prior to its administration in the field. This particular antigen had also been previously validated and utilized (13). Following cleaning of the skin over the volar surface of the forearm with 70% alcohol, 0.1 ml of the antigen containing standard amounts of promastigotes was administered intradermally employing a one ml syringe and 26 G 3/8 0.45x10 microlance needles. Needles were changed for all individuals without drawing back fluid. Skin test indurations were mostly read at 48 hours, with very few tracked for a 72 hours reading. The skin reactions were read according to the techniques recommended by the World Health organization (19). By applying a perfectly moderate pressure, a line was slowly drawn with a ballpoint pen about 1-2 cm from the margin of the skin indurations towards its center. On sensation of resistance to further movement, the pen was lifted from the skin. The procedure was repeated on the opposite side of the skin reaction. This procedure was further repeated at right angles, to get a two-dimensional measurement of the indurations. Then, the diameter of the indurations was established by taking the mean of the lengths between the two pairs of opposing lines. An induration of five milimeters or above was defined as a positive reaction.

Results

A total of 789 participants of both sexes and different age groups were included in the study from 14 sites of the three major localities of Mile, Dubty and Asayta. No clinical case suggestive of cutaneous or visceral leishmaniasis was encountered. Several individuals, however, had various medical complaints and pertinent findings and were managed accordingly. Seven hundred and sixty seven of the skin tested (97%) returned for the reading of the response (Table 1). Variation in skin test reaction was evident among the different sites. Relatively higher positive reaction was observed in sites around Mile and Dubty, mainly from sites selected along the courses of Mile and Awash rivers.

About half of the participants in the locality of Mile had positive leishmanin reaction, while close to 40% and only about 25% of those from Dubty and Asayta localities, respectively, had positive leishmanin responses (Table 1). This variation among the major study localities was found to be statistically significant ($X^2 = 35.6$; d.f = 2; P< 0.001). The prevalence of leishmanin positivity ranged from about 19% in Bakaitu around Asayta to 85% in the former Mile banana plantation site. Sites in the vicinity of Asayta had lower leishmanin rates, the one with the least prevalence, Bakaitu and others also being from the same locality.

Positive leishmanin reaction was minimal in the age group of 5-9 years. An increase in positive response with age appears to be a rule in both sexes, with higher rates in the age group 30-59 years in both sexes. Just over 45% of males and 33% of the females assessed demonstrated a positive reaction (Table 2). The difference was statistically significant ($X^2 = 176.4$; *d.f.* = 15; P < 0.001). The overall prevalence of leishmanin positivity was 38.3%.

Table 1. **Leishmanin skin test reaction in Lower Awash, northeast Ethiopia, 1996/97**

Locality	Sites	Tested	Pos. (%)
Mile	Bekeri Deear	66	20 (30.3)
	Harsis	49	26 (53.1)
	Former Mile farm	74	63 (85.1)
	Gessyo	39	13 (33.3)
	Upper Mile	38	13 (34.2)
	Sub-total tested	266	135 (50.8)
Dubty	Logia	41	14 (34.1)
	Irolaf	56	32 (57 1)
	Urfura	44	18 (40.9)
	Ayderus	85	24 (28.2)
	Haile Baire	44	14 (31.8)
	Sub-total tested	270	102 (37.8)
Asayta	Hellomeli	47	10 (21.3)
	Hamintole	54	14 (25.9)
	Handag	78	23 (29.5)
	Bakaitu	52	10 (19.2)
	Sub-total tested	231	57 (24.7)
	Grand Total	767	294 (38.3%)

Table 2: **Leishmanin positivity by age and sex in Lower Awash, northeast Ethiopia, 1996/97**

Age group	Male			Female			Total		
(years)	n	Positive	(%)	N	Positive	(%)	n	Positive	(%)
5-9	64	8	12.5	77	5	6.5	141	13	9.2
10-19	132	49	37.1	86	13	15.1	218	62	28.4
20-29	49	28	57.1	75	34	45.3	124	62	50.0
30-39	46	28	60.9	77	32	41.6	123	60	48.8
40-49	38	26	68.4	33	12	36.4	71	38	53.5
50 & above	54	39	72.2	36	20	55.5	90	59	65.6
Total	383	178	46.5	384	116	32.8	767	294	38.3

The indigenous Afar people made up the majority of the study population and a higher prevalence of positive skin test induration (42%) was observed in the same ethnic group compared to the rest (Table 3). The lowest prevalence was observed in members of the Amhara ethnic group, while a combination of a few other ethnic groups residing in the area also manifested relatively lower rates of leishmanin reaction. The difference in leishmanin reaction observed among the different ethnic groups residing in the area was also significant (X^2 = 26.6; d.f. = 2; P<0.001).

Table 3: **Leishmanin skin test reaction by ethnic group in Lower Awash, northeast Ethiopia 1996/97**

Ethnic group	N	Positive	Percent positive
Afar	646	273	42
Amhara	88	15	17
Others	33	6	18
Total	767	294	38

Discussion

The highland and low-lying regions of Ethiopia are reckoned to be endemic for the spectra of cutaneous leishmaniasis and the visceral form of the disease respectively. The Rift Valley region of Ethiopia, which bisects the country into two western and eastern highlands is among the areas that are principally for harabouring visceral leishmaniasis (15, 17, 20). Most recent studies along this line have also thrown some light on the epidemiological importance of the disease down the extensive course of the Valley (10-13, 21).

The current leishmanin survey has been undertaken to reveal additional information pertaining to exposure to leishmaniasis and overt disease in northeast Ethiopia, the northern most tip of the Rift Valley in Ethiopia. The rate of leishmanin positivity in some of the study sites is not should not be undermined. However, confirmed cases of either cutaneous or visceral leishmaniasis were not encountered in the survey. Neither old scars suggestive of

cutaneous leishmaniasis nor clinical suspects of visceral leish-maniasis were apparent in contrast to what has recently been observed in Upper Awash (11). On top of the cross-sectional nature of the study, various other explanations could be made as speculated earlier (15) for the virtual absence of overt cases or suspects. One among others is the fact that clinical leishmaniasis is not occurring in any great numbers in the area since overt cases of the disease entities may form only the tip of the iceberg of the infection, with sub-clinical cases expected to make the bulk of the infections (7-9, 15, 22-24). The other explanation could be the attribution of the high leishmanin positivity to non-pathogenic *Leishmania* species or to closely related organisms cross-reacting with the administered antigen (15).

Variation in leishmanin response was noted among the different study localities and the respective sites. The micro-ecological variation favouring the breeding of potential vectors to sustain the propagation of leishmaniasis is reckoned to be the most plausible explanation for the wide variation in leishmanin positivity. The plethora of potential vectors of different forms of leishmaniasis collected from the area designated as the Upper Awash located to the south of the current study area of the Lower Awash (18, IPB Unpublished data) is very much in agreement with this assertion. The fact that the Asayta locality is relatively drier and devoid of vegetation shelter, with lower density of *Phlebotomus orientalis* (18, IPB Unpublished data) could explain the low exposure to leishmanial parasites. *Phlebotomus orientalis,* the presumed vector of visceral leishmaniasis in NW and SW Ethiopia was the most common specimen in our collection (18, I PB Unpublished data) from Mile locality. Further, the leishmanin profile in some sites notably in the former Mile farm area and the rest is typical of an endemic site, with a positive reaction building up progressively to adulthood as shown in other endemic localities (12, 15-17, 25, 26). The low occurrence of skin test positivity in the youngest members of the study community (under ten years of age) possibly indicates an out door exposure to the circulating leishmanial parasites, associated with activities mainly concerning older children and adults. The relative disparity in leishmanin positivity in gender, might as well entail a particular exposure in the predilection of males.

Variation in leishmanin positivity by ethnic group was noted. Investigators are of the conviction that this variation could be a function of the duration of stay in the locality, resulting in intense exposure to leishmanial parasites rather than inherent differences in susceptibility by ethnic background. As observed earlier in other parts of the Awash Basin (11, 12, 15), higher rates of leishmanin positivity were evident in the native Afaris.

The relevance of a leishmanin test to gauge exposure to leishmanial parasites is irrefutable. However, the interpretation of a positive reaction most often remains a challenge. The manifestation of an alarmingly high positive leishmanin rate in some of the study sites, nevertheless, remains cause for great concern. Due to the frequent occurrence of leishmaniasis in immuno-compromised individuals, including in HIV/AIDS cases, it has been some time since certain authorities suggested its consideration in the list of opportunistic microbial infections (27). Experts warrant that AIDS could increase the risk of visceral leishmaniasis by many-folds in endemic areas (28). If a person with IHV is bitten by infected sand flies he would develop severe leishmaniasis. The development of full-blown leishmaniasis in HIV infected individuals is also indicated to expedite HIV multiplication and worsen the patient's status by further suppressing the immune status. The very recent report of HIV infection in the area, including in the native Afar (29), makes the impending threat of the HIV/leishmania association and interaction evident.

The area commonly designated as the Lower Awash, besides being in close proximity to the major outlet to the Red Sea, is known to be home to various cash crops cultivated by commercial farms. Some of the farms are reviving once more with immense potential for further expansion. The enormous geothermal reserve in the vicinity is also an additional indicator of the economic significance of the area. Such development efforts inevitably augment the influx of non-immune and non-indigenous migrant labourers. Given the predictable expansion of HIV/AIDS in rural areas and the conducive conditions for indigenous exposure to leishmanial parasites, we are now of the firm conviction regarding the need for continuous scrutiny of visceral leishmaniasis and HIV/AIDS in the study localities.

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