Original article

Leishmaniases survey in the Awash Valley: The magnitude of positive leishmanin reaction and its pattern in the Middle Awash

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Abstract

Background: Both visceral and cutaneous leishmaniasis are reckoned to be endemic in Ethiopia in magnitudes of undetermined prevalence and distribution. There is considerable information pertaining to the public health importance of leishmaniases in the lower course of the Rift Valley of Ethiopia. Nevertheless, there is evident gap of knowledge on these disease entities in the middle course of the Valley, where the current investigation has been carried out.

Objectives: The study was conducted to get pertinent information regarding exposure to leishmanial parasites and to screen study subjects in the delineated course of the Valley for possible occurrence of clinical cases.

Methods: Following screening for overt cases of leishmaniasis, a cross-sectional leishmanin skin test was undertaken in 926 individuals. The overall prevalence of leishmanin positivity in 889 (96%) of those who returned for reading of the test was about 40%. Nearly 60% of the males and about 25% of the females tested positive for the antigen. Positivity appeared to be increasing with age in both sexes. Variation in leishmanin response among the study localities was evident and this appeared to be influenced very much by the duration of stay in the area. This difference was found to be statistically significnt (X^2=13.7; P<0.005).

Conclusion: the leishmanin response profile of the study area is typical of an endemic locality, with positivity building up with age as experienced in other endemic sites. This suggests an outdoor exposure to infection. The implication of this finding will be further discussed.
[*Ethiop.J.Health Dev.* 2002;16(2):157-163]

Introduction

The leishmanin skin test has been conducted in various endemic areas of leishmaniasis for a considerable period of time. The test is a delayed hypersensitivity reaction to an antigen prepared from a culture of various species of *Leishmania*. It is accounted to be a good tool for epidemiological surveys of leishmaniasis (1-9). The significance of a positive test and the application of the procedure have been reviewed (10).

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The Middle course of the Awash Valley where this study was carried out has been suspected to be endemic for visceral leishmaniasis. However, the only survey regarding the importance of the disease in the area was that of Fuller *et al.* (7) which was carried out over two decades ago and concentrated on few, probably self-presenting subjects originating from selected urban centers. In the survey, a higher rate of leishmanin positivity was reported in the indigenous Afars compared to the migrant labourers. The sketchy reports from the neighbouring countries also throw some light pertaining to the importance of leishmaniasis in the area. We presume that Courtois' (11) report of two cases of childhood visceral leishmaniasis (VL) in Djibouti indicate the continuity of this potential focus to

the neighbouring country and probably across the Red Sea to the Arabian Peninsula. Among the five cases of VL reported later from Djibouti (12), two were suspected to be originating from a village very close to the Awash Valley in Ethiopia. The latter group also described six cases of cutaneous leishmaniasis (CL), of which three were thought to be imported from either Ethiopia or Yemen. Our own report of two confirmed cases of VL implicated to be originating from the then Wello-Assab area (part of the current Afar National Regional State), just north of the current study site (13) is also a further indication of the significance of leishmaniasis in the area. There is also local entomological evidence for the importance of leishmaniasis (14) in the study area. Our simltaneous entomological study (manuscript under preparation) as well has revealed the richness of the flora to host a number of Phlebotomid sandflies incriminated as vectors of visceral and cutaneous leishmaniasis nationally and across the Red Sea, in the near and Middle East.

The aim of this study was thus to get sound information pertaining to exposure to leishmanial parasites and to screen study subjects in the designated course of the valley for possible occurrence of clinical cases of the different entities of the disease.

Methods

Area and population: A preliminary survey was conducted to select representative study sites in different ecological set ups of the Middle Awash, as designated by the agricultural development schemes of the former Awash Agricultural Estates. The inclusion criteria for study sites were the same as described earlier (10) and included: ecology suggestive for the transmission of leishmanial parasites (eg. low altitude, high temperature, favourable micro-habitats for breeding of potential vectors, etc.), settlement, agricultural development, etc.. Based on the inclusion criteria, pertinent investigation sites were selected from Melka Sadi, Melka Werer, Amibara and Gewane localities. Generally, the area lies in the Awash Valley occupying the eastern part of central Ethiopia, situated between latitude 8 to 10^0 N and longitude 38 to 42^0 E. All target sites are situated at lower and descending altitudes from 850 m.a.s.l. in the Melka Sadi/Melka Werer area to 700-750 m.a.s.l in the Gewane locality. The sites are found along the Addis -Djibouti asphalted highway, with the nearest Melka Sadi and remotest Gewane located at 270 and 380 km. from Addis Ababa respectively.

The climate of the area is varied and usually hot and dry. The land, especially along the course of the Awash River, is fertile with extensive grazing. Most of the vast area is an open plain with some acacia forest and savannah. With decreasing altitude, towards the east of the study area, the land gets dry with marked reduction in the natural vegetation. The introduction of large scale irrigated agricultural development since the 1960's has considerably modified the ecology and the Middle Awash is now among the areas noted for its extensive cash crops comprising of cotton, banana and oil seeds.

With the exception of the farm sites, the area is generally sparsely populated with the indigenous population, the Afars. The Afars are predominantly nomads, roaming the area seeking grazing and water for their herds. The introduction of the enormous agricultural development in the area has attracted an influx of migrant labourers form the surrounding highlands. The incursion of the migrant labourers has boosted the population size.

The study population was sampled form Melka Sadi, Melka Werer, Amibara and Gewane. Owing to the paucity of exposure-related information to leishmaniasis, it was difficult to estimate the required sample size. However, an effort was made to include about 10 to 15% of the population of the selected villages for the study. To get a better picture of exposure, the indigenous population was well represented and the focus of the study was mainly on the peripheral villages with less opportunity for residual insecticide spraying which could as well have a deleterious effect on the potential vectors of leishmaniasis. All

native subjects above five years of age and those with history of residence in the selected study villages of at least about five years were eligible for the study.

Screening of study subjects and leishmanin skin testing: Study subjects were identified by pertinent demographic and epidemiological attributes and screened for leishmaniasis by physical examination. Clinical suspects of the disease were subjected for further serological screening for anti-leishmanial antibodies using an IgG based ELISA. Subsequent spleen/bone marrow aspirations were also conducted for parasitological confirmation of the disease on few strong clinical suspects. Following identification and screening, the study subjects were skin tested to assess exposure to leishmanial parasites.

L. major antigen from the Institute Pasteur, Tehran, acquired for this project by the Special programme for Research and Training in Tropical Diseases, TDR (WHO) was used for the leishmanin skin test. The antigen was administered following validation for its potency. After thorough cleaning of the forearm with antiseptics, 0.1 ml of the antigen with quantified number of promastigotes was delivered intradermally utilizing one ml syringes and 26 G 3/8 0.45 x 10 microlance needles. The needle was changed for every subject without drawback of fluid. Most skin test indurations were read at 48 hours, the remaining few being cases for a reading at least at 72 hours. Skin test reactions were read as per techniques recommended by the World Health Organization (15). Exerting a mild pressure, a line was drawn with a ballpoint pen approximately 1-2 cm from the edge of the skin induration towards its centre. On encounter of resistance to further progress, the pen was lifted from the skin. The procedure was repeated on the opposite side of the skin reaction. The same procedure was further reproduced at right angles to get a two dimensional measurement of the skin induration. The diameter of the reaction was then drawn by taking the mean of the measurements between the two pairs of the opposing lines. A positive leishmanin response was defined as an induration of five mm or more in diameter.

Results

Although there were six clinical suspects (four from Melka Werer and two from Gewanie) who underwent further serological and parasitological assessments, no confirmed cases of visceral leishmaniasis were encountered during the course of the leishmanin assessment in the study area. Of the 10 sites identified for the study in part of the Awash Valley designated as the Middle Awash, 926 apparently healthy individuals were skin tested. Eight hundred and eighty nine of the tested (96%) returned for the reading of the induration (Table 1). Variation in skin test positivity among the various study sites was apparent. Higher positive response was generally noted in sampling sites close to Melka Werer, Halaysumale having the highest prevalence of nearly 70%. Sites close to Gewane had generally lower prevalence rates, with the least prevalence documented in some of the sites selected from the same locality.

Table 1: **Leishmanin skin test positivity in the Middle Awash by study site 1994-1996.**

Locality	Sites	Tested	Pos. (%)
Melka Sedi	4th Camp	105	32(31)
	Halaysumale	77	53(69)
Melka Were	Mahdol	66	37(56)
	Woidolele	25	16(64)
Amibara	Sheleko	84	46(55)
	Hassoba	103	64(62)
	Idolokore	118	47(40)
Gewane	Old Gewane	69	16(23)
	Medema	128	18(14)
	Meteka	114	19(17)
	Total	889	348(39)

The overall prevalence of leishmanin positivity was about 40%. Close to 60% of males and about a quarter of the females tested revealed a positive induration (Table 2). Leishmanin positivity seems to start at an early age in both sexes. Positive response as well appears to be increasing with age in both sexes, with higher rates in the age group 30-39 years in both gender. Variation in the skin test reaction among the four major studied localities was marked as depicted in Table 3. As evidenced in the same Table, Melka Werer has the

highest prevalence (63%), followed by Amibara (59%), with intermediate prevalence in Melka Sedi (30%) and least prevalence in the Gewane (23%). The increase in prevalence of a positive leishmanain reaction in age in all localities appears to be consistent. The investigation demonstrated that over 80% of those in the age group 20-39 years in Melka Werer and 50 years and above in Amibara were positive for the test.

Table 2: **Age and sex specific profile of leishmanin reaction in the Middle Awash, 1994-1996.**

Age group (years)	Male			Female			Total		
	N	Positive	(%)	N	Positive	(%)	N	Positive	(%)
5 - 9	47	11	23	43	4	9	90	15	17
10 -19	104	43	41	107	21	20	211	64	30
20 - 29	75	48	64	102	30	29	177	78	44
30 - 39	57	48	84	121	36	30	178	84	47
40 - 49	45	32	71	61	13	21	106	45	43
50 & above	56	37	66	71	25	35	127	62	49
Total	384	219	57	505	129	25	889	348	39

Table 3: **The magnitude of positive leishmanin reaction (%) by age and study locality in the Middle Awash, 1994 to 1996.**

Age group (years)	Study locality			
	Melka Sedi (n=105)	Melka Werer (n=168)	Amibara (n=187)	Gewane (n=429)
5 - 9	-	33	10	9
10 - 19	20	54	57	9
20 - 29	27	81	72	26
30 - 39	44	87	60	33
40 - 49	43	70	64	26
50 & above	-	68	80	34
Total	30	63	59	23

Duration of stay in the study locality seems to influence positive skin test reaction (Table 4). Those born and brought up in the area had a higher prevalence compared to those staying in the area for a relatively less period of time and this difference was found to be statistically significant (X^2=13.7;P<0.05). The indigenous Afars made the majority of the study population and a higher prevalence of positive skin test induration (44%) was observed in the same ethnic group compared to the rest (Table 5). The lowest prevalence was observed in the members of the Amhara Ethnic Group.

Table 4: **Leishmanin skin test positivity by duration of stay in the study sites in the Middle Awash, 1994-1996.**

Duration of stay (years)	Leishmanin reaction		
	n	Positive	(%) positive
Born there	719	301	42
About 5 years	30	7	23
5 to 10	60	17	28
11 to 15	47	16	34
16 to 20	11	3	27
>20 years	22	4	18
Total	889	348	39

X^2=13.7; P<0.05

Table 5: **Leishmanin skin test reaction by ethnic group in the Middle Awash, 1994-96.**

Ethnic group	n	Positive	Percent positive
* Afar	671	298	44
Amhara	119	17	14
Wolayta	54	14	26
Kembata	11	4	36
Others	34	15	44
Total	889	348	39

* Indigenous population

Note: Decimal points in percentages of all tables are rounded to the nearest whole numbers.

Discussions

The visceral form and the different spectrum of cutaneous leishmaniasis are known to prevail in an undetermined magnitude in various localities of Ethiopia. The visceral form of the disease principally caused by *Leishmania donovani* is known to be endemic in areas lying at lower altitudes, while cutanous leishmaniasis predominantly caused by *Leishmania aethiopica* is wide spread in highland Ethiopia. There is paucity of information pertaining to the relative importance of other species of leishmania in the causation of cutaneous leishmaniasis

mainly in lowland areas of the country. There is as well a dire need of substantial information on the distribution and magnitude of both forms of the disease in the country. In parts of the country where the epidemiology of especially visceral leishmaniasis has been soundly established, the disease is considered to be alarming, contributing to about a third of the crude mortality rate in the absence of provisions for early diagnosis and prompt treatment (16). Leishmanin skin test surveys have been conducted to circumscribe potential endemic localities of both cutaneous and visceral leishmaniasis. The prior aim of this assessment was to add information to bridge the gap of knowledge concerning the distribution of leishmaniasis in the study area.

In spite of the high leishmanin positivity in some of the study sites, it might look a paradox to note the virtual absence of confirmed cases of either cutaneous or visceral leishmaniasis. This is, nevertheless, to be expected since overt cases of the different forms of the disease may form only the tip of the iceberg of the overall infection, with possibilities of a wide spectrum of reaction to infection with cryptic cases recognized to make the bulk of the infections (17-21). The wide variation in leishmanin response in the respective study localities/sites could be explained by the marked ecological variation influencing presence of conducive conditions for potential vectors to perpetuate the transmission of whatever form of leishmaniasis. As will be reported elsewhere, the large collection of potential vectors of different forms of leishmaniasis in Melka Werer locality compared to the rest is very much in conformity with this assumption. The cross-sectional nature of the study could as well partly explain the absence of confirmed cases. The leishmanin response profile was, however, typical of an endemic environment, with a positive reaction building up steadily to adulthood as shown in other endemic localities (5-8,22). The relatively low rate of skin test positivity in the under 10 year old indicates of mainly an out door exposure to the circulating leishmanial parasites, associated with activities principally involving older children and adults. The evident variation in leishmanin positivity in sex, notably in older age groups of the society also implies a particular exposure in favour of males.

There is a marked variation in the altitude, vegetation and weather condition in the four study localities. The diversity in the micro-habitats of the respective localities, favouring or deterring propagation of potential vectors of both visceral and cutaneous leishmaniasis, is the only plausible explanation for the variation in exposure and diverse rate in the prevalence of leishmanin positivity among the study localities. The very fact that the Gewane area is apparently drier, relatively devoid of vegetation cover and markedly degraded environmentally is very much in agreement with the preceding assumptions.

Duration of stay in the locality, amplifying intensity of exposure, intuitively influences leishmanin positivity. The variation in leishmanin positivity by ethnic group as well appears to be a function of duration of stay in the locality rather than variation in ethnic background per se. Higher rates of leishmanin positivity in the indigenous Afars residing in the Middle and the Lower course of the Awash Valley were also noted earlier (7), with similar recent observations in members of the same ethnic group residing in parts of the upper Awash (10). The variation in leishmanin reaction in the different ethnic groups representing the non-indigenous population could have been confounded by the differences in the duration of stay in the respective study localities, rather than by the susceptibilities attributable to ethnic variation.

The leishmanin test has been shown to be a valuable tool to measure exposure to leishmanial parasites. Although the role of non-pathogenic leishmaniae or cross reaction of the antigen with related organisms (4, 7) could not be ruled out, the demonstration of a high leishmanin response in some of the study sites is a cause for major concern. Owing to the exaggerated observation of leishmaniasis in immuno-compromised subjects, including in HIV/AIDS cases, it has been some time since

certain authorities suggested the inclusion of leishmaniasis in the ever-growing list of opportunistic microbial infections (23). Some of the very recent observations as well indicate that AIDS increases the risk of visceral leishmaniasis by 100-1000 times in endemic areas (24). Further, a person with HIV infection whose immune system is suppressed and who gets bitten by infected sandfly is indicated to develop severe leishmaniasis. The development of full blown leishmaniasis in HIV infected individuals is also noted to accelerate HIV multiplication and worsen the patient's status by further immuno-suppression.

The study area is very productive with enormous potential for further expansion of an agricultural development. The development endeavour is very likely to enhance the further influx and sharp turn over of the non-immune and non-indigenous labour force. With the inevitable ruralisation of HIV/AIDS and favourable conditions for local exposure to leishmanial parasites, with further indication of the existence of potential vectors of both cutaneous and visceral forms of the disease, we suggest the need for surveillance of especially visceral leishmaniasis in potential endemic areas of the country in general and the study locality in particular.

Acknowledgements

This study was supported by a Re-entry Grant from the UNDP/World Band/ WHO Special Programme for Research and Training in Tropical Diseases and the Addis Ababa University. We would like to thank the late Ato Mulatu Zafe and Ato Tesfaye Getachew and the rest of the staff of the Institute of Pathobiology for their technical assistance. The overall support and encouragement by the Institute to undertake this work is as well very much appreciated.

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