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The current status of cutaneous leishmaniasis and the pattern of lesions in Ochollo primary school students, Ochollo, Southwestern Ethiopia

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Abstract: Background: Leishmaniasis is a vector borne disease caused by the obligate intracellular protozoan leishmania. Objective:To assess the prevalence of cutaneous leishmaniasis and pattern of lesions in Dega Ochollo primary school students, Ochollo, Southwestern Ethiopia. Methods: A cross-sectional study was carried out among 523 school children aging between 6 to 25 years. The students were physically examined for the presence of scar and active lesions. Skin slit and blood were collected from students with suspected active lesions of cutaneous leishmaniasis. Scraps were cultured in Nicolle-Novy-MacNeal(NNN) medium and serological tests were performed using direct agglutination test (DAT). Results: The overall prevalence of tegumentary leishmaniasis including both scar and active lesions among the 523 students which underwent physical examination was 65.8 %. Besides, the study revealed that 64.8% of the participants had current and/or past lesion of cutaneous leishmaniasis. The prevalence of Mucocutaneous leishmaniasis and recidivan was 0.2% and 0.8%, respectively. Three hundred and thirteen (59.8%) students were with scar and 21(4.01%) were with active lesions whereas 8(1.5%) of the cases had both scar and active lesions. Majority (49.71%) of the participants belonging to the age group 11-15 years old were the most affected group (p-value<0.05). The average number of scars and lesions per patient was calculated to be 1.5 and 1.7, respectively. Majority (64.17%) of the cases had single scars while 22.74%, 7.48%, and 5.61% of them had double, triple, and four and above, respectively. The scars were more localized above the neck (82.16%) where the highest (54.56%) proportion of the scars was distributed on check. Of the 29 participants who had active lesions, 4(13.8%) of them were found to be culture positive and one patient was also positive for DAT out of these culture positive patients. One smear positive sample was also found among the samples which were positive for NNN medium. Conclusions: cutaneous leishmaniasis is prevalent in the area causing disfigurement and resulting social stigmatization. This calls for the implementation of prevention and control measures including treatment of infected individuals.

Keywords: Cutaneous Leishmaniaisis, Scar, Active Lesion, Ochollo

1. Introduction

Leishmaniasis is a vector-borne disease caused by obligate intracellular protozoan of the genus *Leishmania* [1, 2]. The disease is considered to be endemic in 88 countries (21 in the New World and 66 in the Old World), 16 are developed countries, 72 are developing countries, and 13 of them are among the least developed [1, 3]. The overall prevalence of leishmaniasis in the world is 12 million with an estimated 1.5 to 2 million new cases each year with 1-1.5 million for CL [1, 4-6]. More than 90% of the CL cases occur in Afghanistan, Iran, Saudi Arabia, and Syria Arab Republic in the Old World and Brazil and Peru in the New World [3, 6-8]. According to 2007 WHO report, one person becomes infected by CL every 20 seconds in the world [9].

Cutaneous leishmaniasis (CL) is an important disease with a wide spectrum of clinical manifestations posing a public health problem in many parts of the world. It is manifested in the following clinical forms: local cutaneous leishmaniasis (LCL),

diffuse cutaneous leishmaniasis (DCL), and mucocutaneous leishmaniasis (MCL) [3]. LCL occurs in both the New and the Old World [5]. The species responsible for Old World cutaneous leishmaniasis (OWCL) are mainly *Leishmania major*, *L.aethiopica* and *L. tropica,*and sporadically *L. infantum* and *L. donovani* can also cause localized CL in the Old World [10, 11]. CL in the Old World is mostly due to *L.major* which produces self-healing lesions or skin sores on the face, nose, forehead, and lower limbs. However, when the lesions are multiple and disabling with disfiguring scars, it creates a lifelong aesthetic stigma. CL due to *L.tropica* is usually more chronic and its most severe form recidivans leishmaniasis (characterized by micro satellite and confluent lesions) is very difficult to treat, often becoming long-lasting, destructive, and disfiguring [3]. DCL affects only the skin but with generalized skin lesions. It is uncommon and is caused by *L.aethiopica* in Africa (Ethiopia and Kenyan Highlands) in the old world and by *L.mexicana amazonensis* in Latin America) [12]. In MCL, patients suffer from progressively destructive ulcerations of the mucosa, extending from the nose and mouth to the pharynx, larynx and even the cartilage producing hideous disfiguring lesions, mutilation of the face, pulmonary aspiration and great suffering for life [5, 13]. It most cases, *L. braziliensis* is responsible for most cases of muco-cutaneous leishmaniasis [13].

In Ethiopia, CL caused by *L.aetiopica,* is a major public health affecting thousands of people in the highlands [14]. The species is naturally harbored by rock hyraxes (*Procavia spp*., and *Hetrohyrax brucei*) and transmitted by two types of sand flies: *Phlebotomous longipes* and *P.pedifer*. The disease as well as the sand fly vectors are widespread over much of the highlands of Ethiopia, ranging between 1,400m and 2,700m altitude [10, 15, 16]. A study of the epidemiology of CL in three areas of Ethiopia was begun in 1969 by Ashford et al [15]. Those areas were; Kutaber (North West of Dessie) in 1969, Aleku (in the Wollega province) in 1969/70 and Ochollo (south west Ethiopia) in 1971. Previous studies in Ochollo in 1971 and 1981 reported a prevalence of active CL of 10.7% and 3.8%, and of healed lesions of 30.4% and 32.8%, respectively [17]. Another study conducted in the same study area between 1987 and 1990 also showed that the prevalence of cutaneous leishmaniasis was found to be 3.5% in 1987 and 3.9% in 1989 [17]. There are also reported cases of cutaneous leishmaniasis from southeast highlands of Sidamo [18] and in the Meta Abo district of shoa province [19]. A survey conducted in Silti woreda following an outbreak of CL showed total prevalence of active CL lesion in the study population was 4.8% [16].

About 20 years have been elapsed since the last epidemiological survey was conducted by Mengistu et al [17] in Ochollo. Though Ochollo was known for its endemicity for cutaneous leishmaniasis (locally known as 'bolbo') before, the current status of the disease in the area is not clearly known. This study, therefore, will be important in high lighting the distribution of the disease and addresses the gaps in information about the status of cutaneous (integumentary) leishmaniasis in the area. Hence, study on the prevalence of CL is a prerequisite not only for formulation of appropriate control strategies but also to predict risk for communities under consideration. Hence, the study assessed the current status of cutaneous leishmaniasis and the pattern of lesions in ochollo primary school students.

2. Methods and Materials

2.1. Study Design

A cross-sectional study design was employed.

2.2. Study Area and Period

The study was conducted between December 2011 and April 2012 in Dega Ochollo complete primary school. The school is located in a village called Ochollo which is found in the south west Ethiopia, in the Gamogofa administrative region in Arbaminch Zuria Wereda, lying half way between Chencha and Arbaminch. Ochollo farmers' association, with a population of around 5000 clustered densely on the top of a hill, is located on the Rift Valley above the west shore of Lake Abaya at 60° 11`N and 37° 41'E. The area covers approximately 25 square kilometers and is divided in to four sub-kebeles namely: Domma, Gucha, Keya and Zuza. The altitude of the study area ranges between 2000m and 2200m above sea level. The walls and roofs of most housing units in Ochollo are built of wood and have mud walls and thatched roofs often cramped, with dirt floors, leaking roofs and no windows leaving occupants vulnerable to adverse weather conditions, insects and rodents.

Source population and study population: The source population for the study were all students of Dega Ochollo complete primary school.

Inclusion criteria:Students who were volunteer were eligible to participate.

Exclusion Criteria: Students with mental problem and non-volunteers were excluded from the group.

2.3. Sampling Technique and Sample Size

A convenient sampling technique was employed. All the students (which were voluntary to participate in the study) of the school were examined for the presence of CL. For ethical reasons, all students were considered for the study. Hence, all the 600 students which were attending school in that academic year were sampled for the study.

2.4. Questionnaire Administration

Pre-prepared questionnaire was used to collect sociodemographic data and assess other factors. After getting of oral/written consent from the study participants, they went under physical examination for the presence of active and/or scars of CL by an experienced medical officer.

2.5. Clinical Sample Collection

Blood: 5 ml of blood was collected from 29 students who had suspected active lesion of CL using the vacuntainer

needle and syringe by experienced personnel. After blood was collected from the voluntary students, the specimens were transported to Arbaminch Hospital, DNDi Leishmaniasis center. The specimens were centrifuged and the serum was collected in serum vials (cryovials). Then the samples were transported using ice box to Addis Ababa University, Medical faculty.

Skin slit scrapings: Skin scrapings were taken from the inflamed (peripheral) part of the lesion with sterile disposable blade after cleaning the area with 70% ethanol using sterile gauze. A small skin slit scrap specimens were taken from the affected areas of the suspected patients. The skin scrapings were inoculated immediately onto Novy-MacNeal-Nicolle (NNN) medium in duplicates. Skin slit smear were also done in duplicates on microscopic slides. The NNN medium (culture) was placed in an Ice box, the slides were placed in slide box and both were transported to Addis Ababa University, Medical faculty.

2.6. Laboratory Diagnosis

Direct agglutination test (DAT): After arrival at Addis Ababa University, Medical faculty, the serum vials containing serum were placed in a refrigerator until processed. All 29 frozen sera were tested using *L. donovani* (*L.d*) antigens. Using V-shaped microtitre plates, sera were serially diluted (1:100 up to 1:102,400 titers) in 0.85% physiological saline and 0.78% (vol/vol) 2-mercaptoethanolm. The antigen prepared from *Leishmania donovani* (obtained from KIT Royal Tropical Institute, Belgium) were reconstituted in 5ml of 0.85% physiological saline. In the series of serum dilutions, and control, 50µL of the test antigens (prepared from *Leishmania donovani*) were added to each well of the plates. Plates were incubated overnight at room temperature (20–23°C). Endpoint titers were read as the last well (highest dilution) in the series of dilutions in which agglutination could be read.

Culture (NNN medium): In order to culture promastigotes, the skin scrapings of the specimen were inoculated in duplicate into NNN medium with overlay Locke's solution immediately at the study site. The cultures were then incubated at 20-24 ^{0}C. Cultures were inspected under light microscope in seven days interval afterwards to detect the growth of *Leishmania* promastigotes. Contaminated samples were discarded immediately. Negative and positive samples were examined every week for consecutive three weeks. Those cultures that remained negative after two weeks (in the third week) were recorded as negative and discarded as mentioned and positive samples were recorded.

Smear: The already prepared smears in the above procedure were then fixed in methanol, air dried and then stained by Giemsa solution. The slides were examined under binocular microscope using the 100x (oil immersion) objective for the detection of leishmania amastigotes before considering the samples as negative.

2.7. Variables

Dependent variable: Skin Lesion, Test status, Lesion size, Scar size, time of onset of scar/lesion

Independent variables: Socio-demographic characteristics such as Age, Sex, Location of residence.

2.8. Ethical Clearance

Ethical clearance was obtained from Addis Ababa University, Institutional Review Board (IRB) of the Aklilu Lemma Institute of Pathobiology. Further permission was also obtained from the Ochollo Farmers' Association and Ochollo complete primary school. Written or oral consent was obtained from the students/guardians before commencing the study. Sodium stibogluconate (SSG) were given to treat the severely affected individuals with active CL.

2.9. Data Analysis

After data collection, it the data was checked for its completeness and internal consistency. Data was entered using Epi Info version 3.0 and exported to SPSS version16.0 software package for statistical analysis.

3. Results

3.1. Sociodemographic Data

Sociodemographic data showed that out of the 600 students sampled for the study, 523 students had participated in the study of which 272 were males and 251 were females. The age distribution of the students showed that 239(45.7 %) students were 10 years old or less, 245 ranged from 11 to 15 years, and 39 students were 16 and above years of age. The mean age for the students was recorded to be 11.36 $\pm$ 2.96 years ranging from 6 to 25 years. Of the study participants, 356(68.1%) of them were Orthodox and 167(31.9%) were protestant.

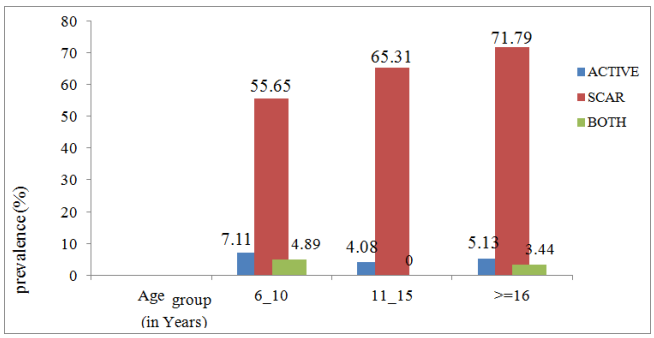

Figure 1. *The prevalence of active lesions and scars of cutaneous leishmaniasis among Dega Ochollo primary school students, 2012.*

Physical examination showed that the prevalence of LCL in the study was found to be 64.8% whereas the prevalence of MCL and Recidiva was 0.2% and 0.8% respectively whereas 0.8% of the cases were found to be infected with both LCL and MCL. The overall prevalence of cutaneous leishmaniasis including both scar and active lesion was 65.8% of which 313 (59.8%) were with scar and 21(4.01%)

were with active lesions whereas 8(1.5%) of the cases had both scar as well as active lesions (Figure 1). The disease was more prevalent in females (51.75%) than males (48.25%) and the difference was statistically significant (P-value=0.018, F=0.018). However, there was no statistically significant difference in the number of lesions between males and females ($p > 0.05$).

Meanwhile, the study also showed that the disease (including scars and active lesions) was more predominant on the age group 11-15 years (49.71%) followed by the age group of 6-10 years (41.81%) and the difference was found statistically significant(p-value<0.05)(Table 1). Though it was not statistically significant, active lesions were more prevalent in students aged between 6 and 10 years (7.11%) with slightly higher in females than males followed by 4.08% and 5.13% for the age groups 11-15 years and 16 and above years of age, respectively. Similarly, healed lesions (scars) were more prevalent in the age group between 11 to 15 years old but it was not statistically significant (P-value>0.05). Single, double and multiple scars as well as active lesions were observed in different body parts of the study subjects. Scars, the characteristics of healed LCL lesions, were observed in about more than half of the study population.

Table 1. *Distribution of leishmaniasis by age among Dega Ochollo primary school students, 2012.*

Age Group(in years)	Positive cases for leishmaniasis	P-value	F-test
6-10	143(41.81%)		
11-15	170(49.71%)	0.041	0.013
>=16	29(8.48%)		
Total	342(100.00%)		

The distribution of the scars was more localized above the neck (82.16%) where the highest proportion of the scars (54.56%) was found to be distributed on cheeck. The rest of the scars were observed in the upper and lower extremities with distribution rate of 15.97% and 1.87%, respectively. On the other hand the distributions of the lesions showed that majority of the lesions (84.86%) were localized above the neck and predominately on cheeck (Table 2).

Table 2. *Localization and distribution of scars and lesions of cutaneous leishmaniasis on different body parts of the Dega Ochollo primary school students, 2012.*

Localization of the scar/lesion	No of scars (%)(n=482)	No of Lesion (%)(n=53)
Cheeck	263(54.56)	19 (35.85)
Forehead	58(12.03)	3 (5.66)
Hand	54(11.20)	2(3.77)
Nose	39(8.09)	10 (18.87)
Chin	16(3.33)	1(1.87)
Lower arm (including Elbow)	13(2.70)	3(5.66)
Upper arm	10(2.07)	2(3.77)
Thigh/leg/feet	9(1.87)	1(1.87)
Earlobe	8(1.66)	10(18.87)
Lip	7(1.45)	1(1.87)
Eyelash/eyebrow	5(1.04)	1(1.87)
Total	482(100.00)	53(100.00)

The average number of scars per person was calculated to be 1.5 while the average number of lesions was found to be 1.7 per patient being 8 the maximum number of lesions/or scars per patient. As it is shown in table 3, 64.17% of the cases had single scars while 22.74%, 7.48%, and5.61% of the cases had double, triple and multiple scars(scars above three), respectively. On the other hand, single lesions were observed in 41.38% of the cases who had active lesions, two lesions in 24.14% and triple lesions were observed in 10.34 of the cases whereas more than 24% of the cases had multiple lesions higher than three.

Of those who had active lesions, 12 (25%) of them had single lesion while 8.33% and 6.25% had double and triple lesions on their cheeck, respectively. It was also shown that 13.2% and 1.87% of the lesions were localized on the upper and lower extremities, respectively.

The pattern of lesions also showed that the most common lesions were single and of ulcerative type, appearing as reddish plaques with irregular borders. Butter fly shape extensive lesions and scars, multiple satellite small scars and lesions were also present in some of the cases.

Moreover, the clinical onset of the disease was determined by directly interviewing the patients and majority, 90.6 % (309) of the cases who had scars and/or lesions had reported that as the lesion/scar was emerged when they were less than 5 years of age during their childhood time (Table 4). Laboratory findings also showed that of the patients who had active lesions, 4(13.8%) were positive for NNN medium (culture). Besides, one patient was also positive for DAT out of these culture positive patients. Cultures from 2 (6.9%) active cases were identified as positive by the first week, cultures from two active cases (6.9%) of them were identified as positive at 14 days, and at 21 days no new positive results were observed. One smear positive sample was also observed from the above culture positive patients.

Table 3. *The number of active lesions and scars of cutaneous leishmaniasis among Dega ochollo primary school students, 2012.*

No of scar/lesion	Frequency (%)	
	Scar	lesion
1.00	206(64.17%)	12(41.38%)
2.00	73(22.74%)	7(24.14%)
3.00	24(7.48%)	3(10.34%)
4.00	11(3.43%)	3(10.34%)
5.00	4(1.25%)	2(6.90%)
7.00	2(0.62%)	1(3.45)
8.00	1(0.31%)	1(3.45%)
Total	321(100%)	29 (100%)

Table 4. *The clinical on set of leishmaniasis among Dega Ochollo primary school students, 2012.*

Clinical on set (in Years)	Frequency	Percent
<5	309(341)	90.62
5-7	7(341)	2.05
8-10	14(341)	4.11
>10	11(341)	3.23
Total	341	100.0

4. Discussion

Cutaneous leishmaniasis is a serious public health problem and has been reported in this area since 1969 [15]. The overall prevalence of cutaneous leishmaniais including both scar and active lesion was 65.8 % of which 313 (59.8%) were with scar and 21(4.01%) were with active lesions whereas 8(1.5%) of the cases had both scar as well as active lesions. This rate is higher than the previous findings reported from the same study area [15, 17] and elsewhere [20]. Hence, this study indicated that the disease is more prevalent currently than ever in the area and is progressively increasing in the area in the past 20 years. This increase in prevalence might be correlated with climatic, environmental and sociodemographic changes in the area. Reinfection of cases was rare of which 1.5% of the cases had both scar and active lesions. This finding goes in accordance with the previous study conducted in Ochollo [17] in which reinfection was seldom.

Of the total number of individuals who had active skin lesions, 84.86 % of the lesions were above the neck being majority of the lesions (35.85%) on cheeck. This is also true for scars where 82.16% of the scars were above the neck and of which 54.56% were on cheeck. This might be due to several factors such as; the face is the exposed body part which is easily accessibility to sandfly bites (as it is more exposed), and sleeping outdoors or indoors without a bed net which increases the exposure to sand fly bite during its active hours. This finding is similar to a study conducted in Iran [21]. Besides, the softness of the skin on the face makes it preferable site of feeding for the sandfly as it shown by a study conducted elsewhere [22].

In the present study, presentations of most lesions were single, healing and of ulcerative type appearing as reddish plaques and irregular borders. Moreover, butterfly shape extensive lesions and scars, multiple small satellite lesions, and scars were observed. A considerable number of students had multiple scars and lesions in their different body parts. Similar results have been reported by some other researchers in some endemic areas [23]. It was also shown that CL cases were more frequent in females(51.75%) as compared to males (48.25%) with significant difference ($P<0.05$) and females seemed to be at a higher risk of acquiring CL than males. This finding goes in agreement with studies conducted earlier in elsewhere [20, 24]. The reason why females showed a higher infection rate than males is unclear, but could be due to a variety of environmental and behavioural factors [25] or due to other genetic or immunological characteristics [23]. However, this finding contradicts with a study reported earlier in Iran in that males were more compared to females [21].

The study also showed that the ulcers were observed among all age groups; however, the highest rate of both scar and active lesion was recorded in the age group of 11–15 years followed by the age group 6-10 (41.81%) putting the former age group at higher risk of contracting the disease and the difference was found statistically significant ($P<0.05$). This is to be expected because school children in this age group play outdoors more than other age groups. This finding was in agreement with those reported by other authors [15, 16, 26]. Furthermore, the study also revealed that early age of first infection in which scars of old infection dated back from childhood. Accordingly, the clinical onset of the disease was before 5 years of age in 90.62% of the cases. This indicates that children are bitten at home/surroundings and this could possibly reflect the domestic behavior and proximity of major resting sites of the sand fly as well as major hyrax colonies to human dwellings.

In the study it was also observed that culture and serological tests were found to produce low positive results. These very low positive results might be associated with many factors. Smears and cultures obtained from the patients showed very low or more or less negative results due to fungal contamination of the samples, bacterial superinfection of ulcers, application of local herbs on the lesion or burning of the lesion might resulted in low parasitaemia or undetectable level of the parasites. In this study, undetactable levels of antibody titers were observed in the patients and this could be probably linked to one of the following factors. The first could be due to the reason that the type of antigen used was heterologous antigen (derived from *L.donovani*) and the other is in LCL patients, antibody levels are generally considered to be very low unlike in DCL and VL patients where antibodies may reach exceedingly high as it is reported elsewhere [27].

5. Conclusion and Recommendations

Cutaneous leishmaniasis in Ochollo is becoming more prevalent currently than ever. This increase in prevalence might be due to environmental changes such as deforestation, new settlements and displacement of populations that happened in the past 20 or 30 years. Although the morbidity associated with CL is not significant, and the disease is not lethal, the disfigurement and resulting social stigmatization may cause or precipitate psychological disorders. The clinical on set of cutaneous leishmaniasis as depicted from the study is on the early age of child hood showing that children are bitten at home. This probably indicates that the domestic behaviors of the sand fly as well as the proximity of the hyrax colonies to the houses. This calls for the entomological measures and control of the sand fly and provision of protective sandfly nets to the dwellers will have a significant role in preventing the disease.

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