CORRELATION OF THE CLINICAL DIAGNOSIS OF CUTANEOUS LESHMANIASIS WITH SKIN SLT SMEAR,HISTOLOGY AND CULTURE AT DERMATOLOGY CLINIC,ALERT CENTER FROM MAY 2018 TO MAY 2020 G.C ADDIS ABABA, ETHIOPIA

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

BY: NETSANET AZENE (MD)

ADVISORS:Dagnachew Shibeshi(MD,Dermatoenerologist,Allergologist,PhD)

Fewzia Mohammed MD, Dermatoenerologist,Assistant professor)

Eliyas Mulatu (MD, Dermatoenerologist,Assistant professor)

A THESIS TO BE SUBMITTED TO THE DEPARTMENT OF DERMATOVENEREOLOGY, SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE SPECIALITY CERTIFICATE IN DERMATOVENEREOLOGY

SEPTEMBER, 2021

ADDIS ABABA, ETHIOPIA

AKNOWLEDGEMENT

First, I would like to thank AAU, dermatovenereology department for giving me this opportunity to exercise Research Methodology that would enable me to do more in my future,I would like to thank my advisers.Last but not least,I am also grateful to all AAU dermatovenerology seniors and everyone who helped me in the completion of this Research .

Contents

List of tables

Li st of figures

Acronyms

A.A=Addis Ababa

AAU= Addis Ababa University

ALERT= All Africa Leprosy Rehabilitation and Training

CL=Cutaneous leshmaniasis

DCL=Diffuse Cutaneous leshmaniasis

ETB=Ethiopian Birr

MCL=Mucocutaneous leshmaniasis

NTDs= Neglected Tropical Diseases

PI= Principal Investigator

PCR=polymerase chain reaction

WHO= World Health Organization

VL=visceral leshmaniasis

Abstract

Background: Leishmaniasis is a complex of diseases caused by the protozoa Leishmania and transmitted by the bite of infected phlebotomine sandfies.

clinical diagnosis of CL may be possible in endemic areas.since the treatment is potentially toxic laboratory confirmation is mandatory.

Objective: To asses correlation of clinical diagnosis of cutaneous leishmaniasis with positive laboratory tests used for the identification of leishmania parasite (skin slit smear ,culture and histopathology)among clinically diagnosed cases at dermatology clinic ,ALERT center from May 2018 to May 2020 G.C Addis Ababa, Ethiopia.

Methodology: retrospective review of medical records of patients diagnosed with cutaneous leishmanias in ALERT Hospital, AA, Ethiopia from May 2018 to May 2020 was performed . Patient medical records retrieved and analyzed *using SPSS Version 24.*

The results will be submitted to ALERT hospital, AAU College of Health Sciences, Department of Dermatovenerology, School of Public Health and FMOH.

Results: *Total of* 215 were clinically diagnosed as cutaneous leishmaniasis by the dermatologist and dermatology residents.

Out of 215, 158 (73.5%) patients were slit-skin-smear (SSS) positive,culture was done for 57 patents ,25 /215(11.6%) and histopathology done for 32/215(14.9%), from these LD body seen in 4/32(12.5%) and in 25/32(78.13%) suggestive histology result and in 3/32(9.34%) diagnosis of other diseases was made.

Conclusion: *In our study ,* slit-skin-smear (SSS) positivity was 73%(158/215), culture positivity was 43.84%(25/57) and histopathology positivity was 90.6% (29/32).

From total of 215 clinically suspected case 208 cases was diagnosed with one of the investigation modality SSS,culture or histopathology.Therefore clinical suspected cases by the clinician became 96.7% (208/215) positive.

Recommendation: In limited resources setting and clinical accuracy of 96.7 %, clinical diagnosis by a dermatologist showed significantly comparable positive result with the laboratory investigation

1.Introduction

1.1. Background

Worldwide, Cutaneous leishmaniasis is the most common form of leishmaniasis and approximately 90% occurring in the Middle East and southern America countries [4].

The routine diagnosis of CL patients depends on examination of skin lesions using smears and cultures of dermal scrapings or examination of sections obtained from a skin biopsy (2). The investigations available have a wide range of reported sensitivities. The sensitivity of direct microscopy is not high, and tissue culture is not uniformly available and successful[7].

The diagnostic methods available at present are mostly based on clinical and epidemiologic evidence and parasite detection. Parasitologic tests of a skin biopsy specimen are not always conclusive in patients with a clinical diagnosis of cutaneous leishmaniasis . Several PCR assays have been developed for the detection of the Leishmania parasite. PCR based methods often have high sensitivities [5].

It can usually be clinically diagnosed in patients coming from an endemic area and having discrete, relatively painless skin lesions (nodules, plaques, ulcers, or noduloulcerative lesions), mostly on exposed parts of the body[26-29].slit-skin smears are used for the confirmation. It has an advantage that it requires only Leishman staining and a good quality microscope equipped with oil immersion lens. The yield of the test, however, depends upon the quality of prepared smear, microscope and expertise of the pathologist. There is a lot of false positivity due to wrong technique of smear preparation, poor fixing and staining, less sensitive microscope, and misinterpretation of Leishman-Donovan (LD) bodies in the smear[6].

Few health facilities in the country that have the capacity to diagnose and treat CL with the majority located in cities far from endemic areas complicates CL health promotion and control measures in these communities[5].

Immunological studies have classified Leishmania parasites into Old World species including Leishmania major, Leishmania infantum and Leishmania tropica that are commonly found in the Middle East, Mediterranean basin and the Horn of Africa and the New World species commonly found in the southern America countries [31].

In Ethiopia, CL is principally caused by Leishmania aethiopica and rarely by Leishmania tropica and Leishmania major [32-34]. The spectrum of the disease and its response to treatment vary according to the species. Therefore, a species-specific treatment was recommended [36].

Leishmania aethiopica is the main cause of CL in Ethiopia causing the most severe forms of CL such as diffuse CL with multiple skin lesions characterised by non-ulcerating papular, nodular and plaque involving most parts of the body[24].

Management ranges from observation to systemic therapy, primarily with antimonials, and vaccines in development[23].

Local topical treatment is the choice for patients with small and single lesions. If systemic treatment is indicated fluconazole or ketoconazole are recommended for L. major and pentavalent antimonials are effective for L. tropica [37]. However, the studies available on the treatment response of L. aethiopica showed that it is less responsive to currently available chemotherapy [38,39]. Although in vitro studies indicate L. aethiopica is highly susceptible to miltefosine[40]clinical evidence is not yet available. Paromomycin is recommended as one of the options in the Ethiopian national guidelines, but the only evidence available is from a small case series on DCL patients [39]in which two patients were successfully treated with paromomycin. However, both relapsed and were subsequently successfully treated with an extended course of paromomycin with systemic SSG. Small studies were done using metronidazole , but was not promising [41].

1.2. Statement of the problem

The clinical manifestation CL are not always pathognomic. It can look like other common condition. Treatment and superinfection can also change its clinical presentation. Atypical variants can be a presentation occasionally. In such cases confirmatory diagnostic test are important.

1.3. Significance of the study

The aim of the study is to correlate clinical diagnosis with skin lit smear, histopathology and culture diagnosis of cutaneous leishmaniasis. The study will provide information on the accuracy of clinical diagnosis in the diagnosis of CL.

2.Chapter Two: Literature review

Cutaneous leishmaniasis is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability or stigma. In 2018 over 85% of new CL cases occurred in 10 countries: Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iran (Islamic Republic of), Iraq, Pakistan, the Syrian Arab Republic and Tunisia[2].In endemic countries, diagnosis is often made clinically and, if possible, by microscopic examination of lesion biopsy smears to visually confirm leishmania parasites as the cause. The use of more sophisticated diagnostic techniques that allow for species identification is usually restricted to research or clinical settings in non-endemic countries [42].

One study done on epidemiological and laboratory characteristics of patients with American cutaneous leishmaniasis ,Nineteen patients presenting active lesions who had been diagnosed through clinical evaluation and laboratory tests were selected. The tests included direct investigation, in vitro culturing, Montenegro skin test, indirect immunofluorescence and polymerase chain reaction. The Montenegro Skin Test showed positive results in 89% of the patients; indirect immunofluorescence, in 79%; direct investigation, in 58%; and polymerase chain reaction in 75%. Seven Leishmania (Viannia) braziliensis samples were isolated from these patients and were characterized by means of specific monoclonal antibodies.American cutaneous leishmaniasis diagnosis is achieved through an association of clinical, epidemiological and laboratory characteristics. In the present study, the diagnosis was confirmed by means of direct investigation, parasite isolation in culture medium, Montenegro skin test, indirect immunofluorescence and PCR. The patients showed positive results in at least one of the diagnostic tests[30].

Study done In India showed ,out of 60 registered cases, 60% were smear-positive and 50% demonstrated Leishman Donovan (LD) bodies in histological sections. Twenty-six of the remaining (parasite-negative) cases showed one of the recognizable histological patterns seen in CL[14].

From Punjab, north-west frontier province, and northern areas of Pakistan,study showed ,Out of the 60 patients registered as clinical cases, 2 (3.33%) were only smear-positive (histology negative), 4 (6.66%) were only positive on histology (smear negative), and 30 (50%) demonstrated Leishman Donovan (LD) bodies in skin smears as well as in histological sections. Of the remaining 24(40%)parasitologically negative cases, 20 showed one of the recognizable histological patterns seen in CL, three patients had non suggestive histology but responded to a therapeutic trial of pentavalent antimony compound, and one turned out to have a deep mycosis(chromoblastomycosis) on histology. The clinical diagnosis was thus confirmed parasitologically in 36 (60%) cases, histologically supported in 20 (33.3%), and was suggested in further 3 (5%) cases by a satisfactory therapeutic response [14].

One Study done In Sri Lanka ,Out of 114 patients who were included in the study,103 were clinically diagnosed as cutaneous leishmaniasis by the dermatologist. Out of 103, 87.4% (n=90) were typical clinical cases while 12.6% (n=13) were clinically suggestive cases. There were 14.6% (n=15) patients who were slit-skin-smear (SSS) positive but histopathology was negative for LD bodies; 26.2% (n=27) were histopathology positive but SSS negative; 19.4% (n=20) were both SSS and histopathology positive. Therefore total parasite positivity in the study group was 60.2% (n=62). Overall, slit-skin-smear (SSS) positivity was 33.9% (35/103), while histopathology showing parasite positivity was 45.6% (47/103) [25].

From 123 suspected CL cases in Selti wereda,Ethiopia, culture, histopathology and PCR were carried out on 71 samples. Of the 71 samples 46.5% (33/71) were culture positive, 31.0% (22/71) were contaminated and 22.5% (16/71) were culture negative. These 71 samples were tested with PCR and 83.1% (59/71) were positive for the presence of Leishmania amastigote DNA in the sample. Eleven of the culture-contaminated and five of the culture-negative samples were PCR positive. Histopathological analyses of these 71 samples confifirmed that 58 were positive for the presence of amastigotes in tissue sections and macrophages. For two samples, only culture and histopathology were done and both were found to be positive. Culture and smear were done on 50 samples, of which 15 (30.0%) were culture positive, 23 (46%) contaminated and 12 (24%) culture negative. With direct smear, 13 of the 23 culture-contaminated and three of the 12 culture-negative samples were found to be smear positive [17].

One study done in 45 patients whose tentative clinical diagnosis included CL referred from the All Africa Leprosy Rehabilitation and Training Centre (ALERT) dermatology clinic to Armauer Hansen Research Institute (AHRI) ,sensitivity and specificity of parasite demonstration methods (smear, culture and histology) and serological assays (enzyme-linked immunosorbent assay [ELISA], direct agglutination test and immunoblot) were compared in the diagnosis of leishmaniasis in Ethiopia.The duration of disease ranged from 3 months to 10 years and , 37.5%, 40% and 55.5% were positive by smear, histology and culture respectively. Culture showed the highest sensitivity when cases histologically suggestive of CL (17/45) were excluded. However, due to the longer procedural time (l-6 weeks), the possibility of contamination and relatively high cost, the use of culture as a routine diagnostic procedure may be limited except in selected diagnostic problem cases when smears do not give a positive result. Unlike smear and culture, histology gives a diagnosis by exclusion criteria for most of the amastigote-negative, clinically suspected CL caseA45 patients whose [20].

3.Objectives

3.1. General objective

To correlate the clinical diagnosis of CL with positive laboratory tests (Skin slit sear ,culture and histopathology) among clinically diagnosed case at ALERT Hospital, Dermatology clinic.

3.2 SPECIFIC OBJECTIVES

- To determine sociodemographic characteristics of patients with CL
- To see the clinical sub type
- To assess the duration of lesion
- To describe body sites affected
- To assess how the diagnosis was established
- To assess histopathologic pattern

4.Chapter Four: Methods and Materials

4.1. Study area and period

The study was conducted at ALERT Center Dermatology clinics from May 2020- September 2020. ALERT hospital is located in south west Addis Ababa, Ethiopia.

ALERT's activities focus on rehabilitation of leprosy patients, training programs on leprosy for personnel from around the world and leprosy control. The hospital currently provides a wide range of services in various departments including Dermatology, emergency services, gynecology and obstetrics, pediatrics, HIV treatment, orthopedics and plastic surgery.

4.2. **Study design**

Hospital based, retrospective study design was used to conduct this study

4.3 **SourcePopulation**

All medical records of patients seen for skin problem at ALERT center

4.4 Study population

All medical records of patients suspected of CL at ALERT center, Dermatology clinic between May 2018 and May 2020

4.5. **Eligibility**

4.5.1 Inclusion criteria:

All medical records of the patients suspected of CL at ALERT center dermatology clinics during the study period.

4.5.2 Exclusion criteria

Incomplete cards

4.6 Sampling method and sample size

convenience sampling method used and a total of 215 CL patient cards will be included

$$\text{Sample size} = \frac{Z(a/2)^2 \; p(1-p)^2}{d^2}$$

$$= \frac{1.96^2 \; 0.142(1-0.142)}{0.05^2} = 215$$

4.7. Data collection

Data was collected by retrieving and reviewing patients' medical records from ALERT center Dermatology clinic using structured questionnaire and analyzed according to demography, site of the lesions,duration of lesion, clinical types , confirmatory laboratory tests,histopathologic pattern.

4.8. Data analysis

The results was coded and analyzed by SPSS version 24 statistical package.

4.9. Data quality assurance

The questionnaire checked for completeness .

4.10 Variables

4.10.1. Independent variables

Age, sex, residence/region, location and duration of the lesion, laboratory tests used to confirm the diagnosis

4.10.2. Dependent variables

Type of CL

4.11 Limitations

Lack of documentation about travel history

4.12. Operational Definitions

Clinical Diagnosis

Localized Cutaneous Leishmaniasis (LCL):

Lesions start as erythematous painless papules that enlarge over a few weeks to form nodules/plaques and often ulcerate and become crusted.

Mucocutaneous Leishmaniasis (MCL):

MCL typically causes infiltrative inflammation of the mucocutaneous borders of the nose

and/or lips, chronic and progressive spread of lesions to the nasal, pharyngeal, and buccal mucosa

Diffuse Cutaneous Leishmaniasis(DCL):

Diffuse CL begins as a single nonulcerative nodule that grows and becomes surrounded by other similar lesions. The nodules are shiny and often slightly red; their edges are usually distinct, with normal intervening skin.

4.14 Ethical considerations

Ethical clearance was obtained from the institutional review board of the medical college of Addis Ababa University and was taken to ALERT center administrators. Accordingly, permission letter was secured . Anonymity and confidentiality of the patient information was kept private. The name of the patients was not be included .

4.15 Dissemination plan

The study result will be submitted to the department of Dermatovenereology and after approval will be submitted to Ethiopian Federal Ministry of Health, FMHACA and concerned NGOs in the form of power point presentation. The manuscript of the study will be submitted to relevant national and international journals for publication. Copy of the article will be placed in the library of College of Health Sciences, AAU for the future reference.

5.Results

5.1 Participants Socio-demographic Characteristics

Data was collected from 215 cutaneous and cutaneous leshmaniasis suspected patients cards.The age ranged from 1 to 78 years. The mean (±SD) age of presentation is 27.4(±16.98) .Majority were in the age group ranging from 10-19 year,66(30.7%) followed by 20-29 ,60(27.9%) and 20(9.3%) were below 10 year .

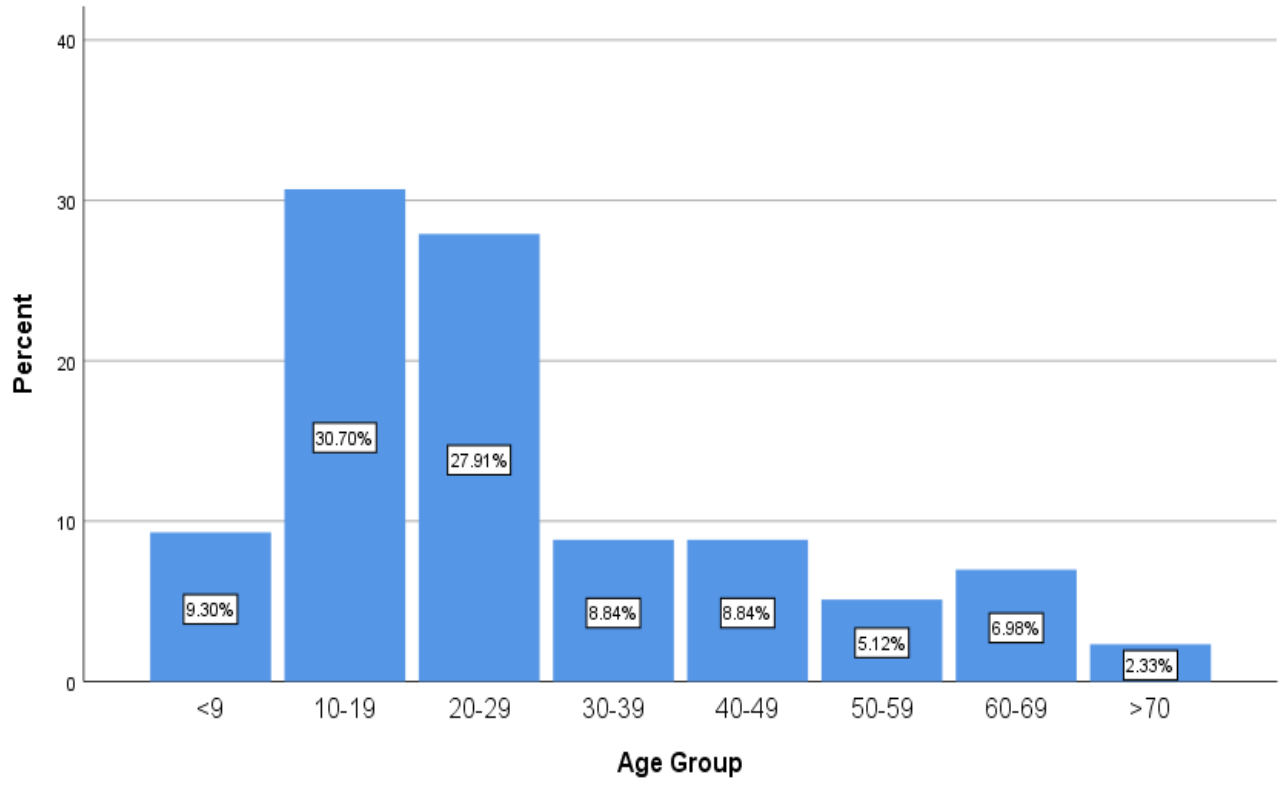

Figure 1 Age frequency of participants

	Frequency	Percent(%)
Sex		
Male	113	52.6
Female	102	47.4
Total	215	100.0
Residency		
A.A	91	42.3
SNNP	23	10.7
Oromia	83	38.6
Amhara	14	6.5
Tigray	3	1.4
Other	1	.5
Total	215	100.0
Age Group(year)		
<9	20	9.3
10-19	66	30.7
20-29	60	27.9
30-39	19	8.8

40-49	19	8.8
50-59	11	5.1
60-69	15	7.0
>70	5	2.3
Total	215	100.0

Table 1 Socio-demographic data

From the total of 215 patents,majority were men 113(52.6%) and 102(47.4%) were female with male to female ratio of 1.1:1.

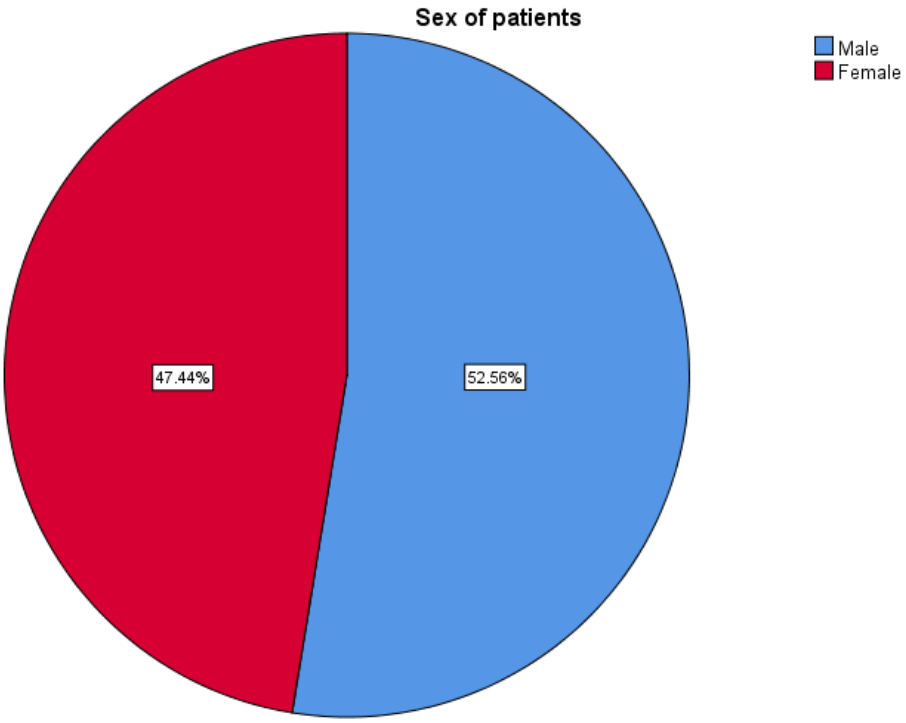

Figure 2 Sex of patents

Majority ,91(42.3%) were from Addis Ababa followed by Oromia ,83(38.6%).The rest are from SNNP,Amhara ,Tigray and Afar region.

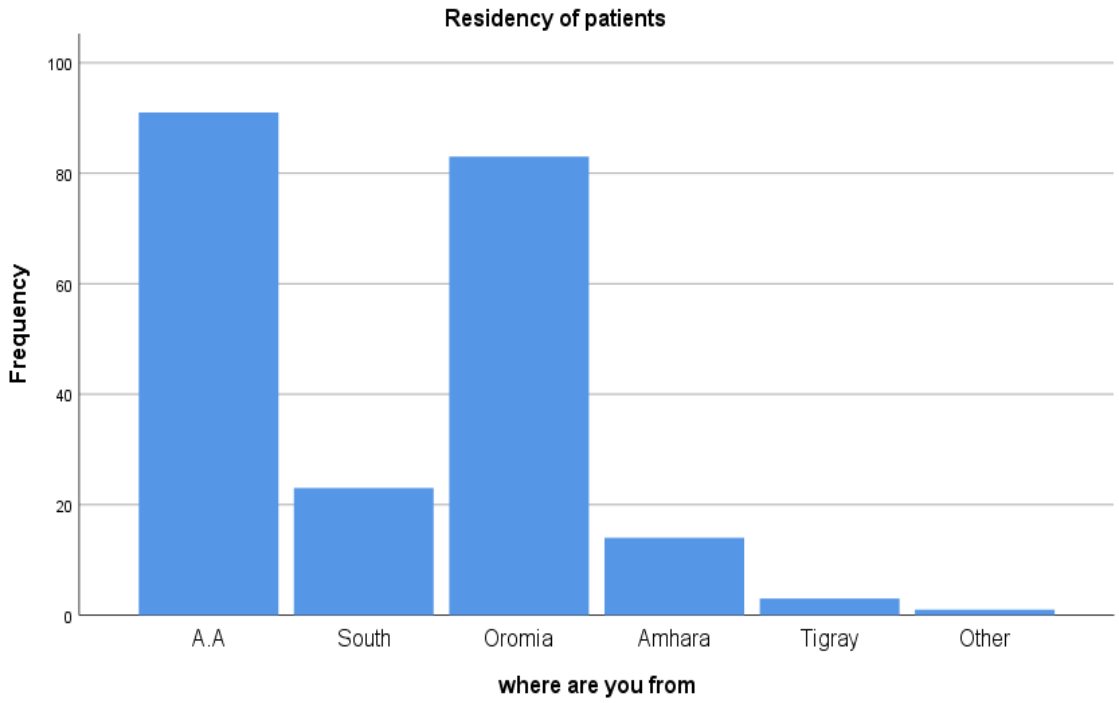

Figure 3 Residency of patients

5.2 Clinical classisification of cutaneous Leshmaniasis

Most frequent clinical presentation was LCL 168(78.1%), followed by MCL 45(20.9%) and DCL 2 (0.9 %).

		Frequency	Percent(%0
Type of CL	LCL	168	78.1
	MCL	45	20.9
	DCL	2	0.9
	Total	215	100.0

Table 2 Clinical subtype of CL

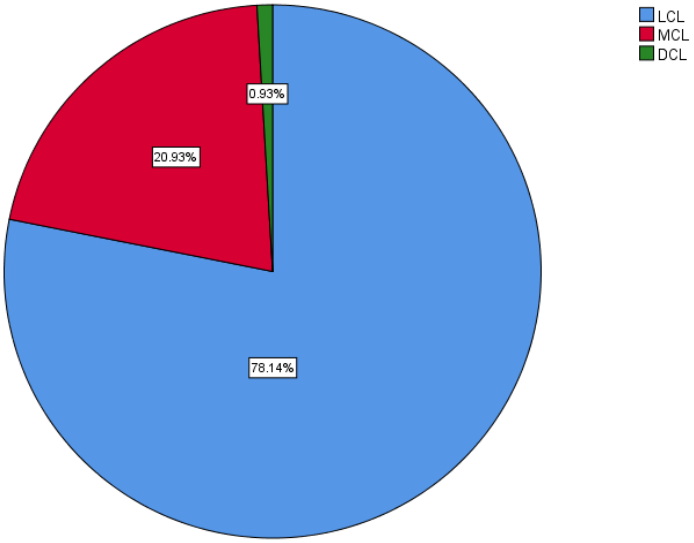

Figure 4 ***Clinical subtype of CL***

Affected body part by Leshmania

Almost all of the affected sites are exposed body parts,but non-exposed areas are also affected . 211(98%) involve exposed areas, in 2 case (1%) non-exposed part of the body and the rest 2(1%) involve both exposed and non exposed sites.

Majority 61(28.4%) has lesion on cheek and 59(27.4%) had lesion on nose,for head lesion was seen in 22(10.2%), lip involvement 11(5.1%) and for arm lesion in 10(24.2%). Other site than the mentioned one and multiple site of involvement seen in 52(24.2%) of patient .

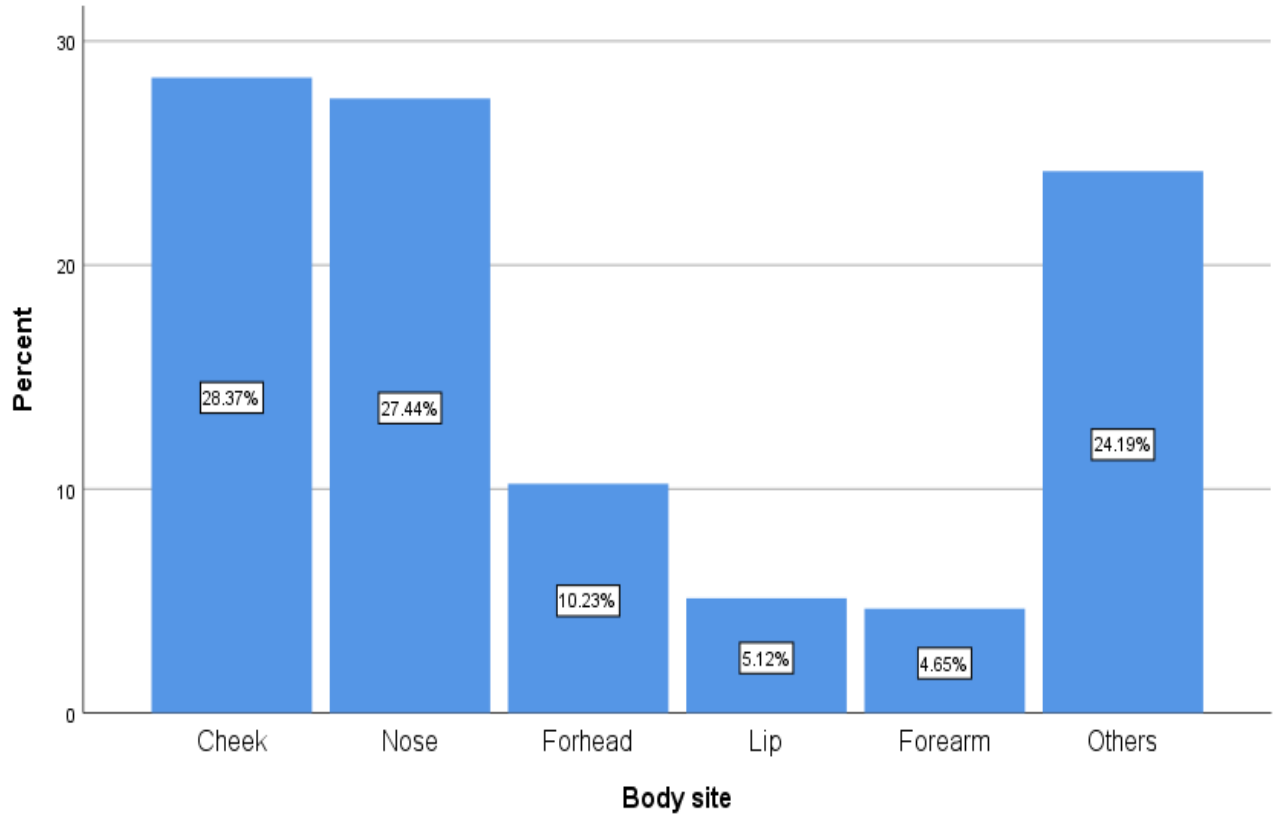

Figure 5 Affected Body Part by Leshmania

The duration of the lesions varied from 4 weeks to 15 years with mean duration of lesion 13.95 months.

Majority ,124(57.7%) present with in 1-11 month of development of lesion, 50 (23.3%) within 12-23 months and 41(19%) of patents presented after 2 year and above the development of lesions.

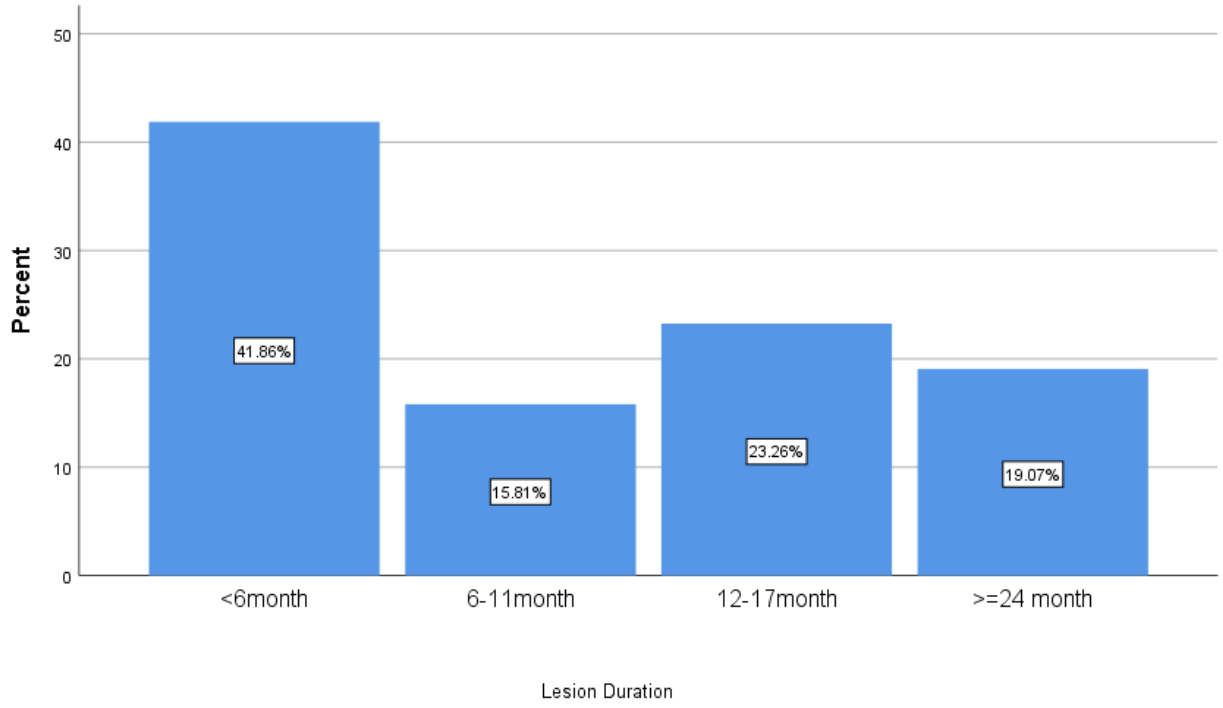

Figure 4a Duration of lesion

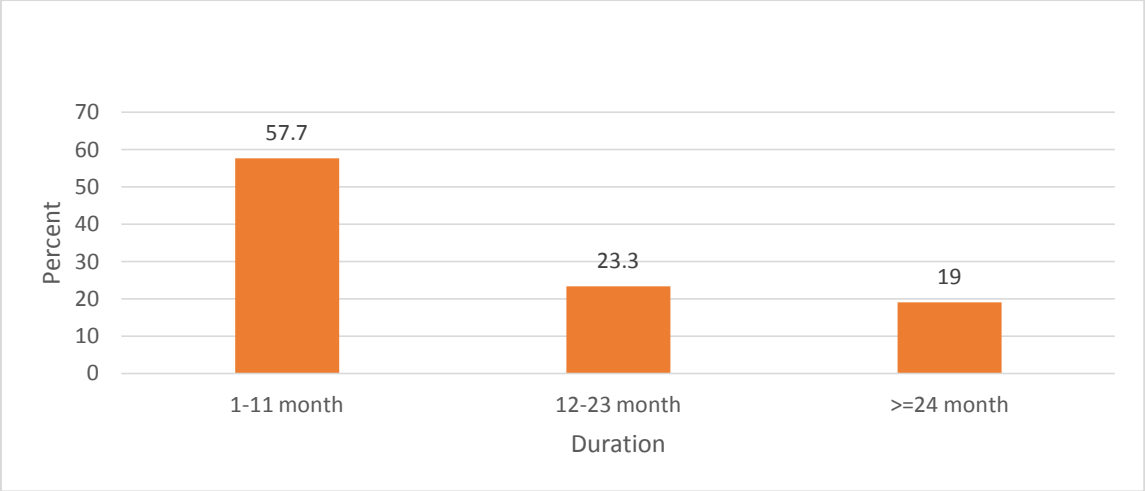

Figure 4b Duration of lesion

Duration Of Lesion	Frequency	Percent
1-11 month	124	57.7
12-23 month	50	23.3
>=24 month	41	19
Total	215	100

Table 3 Duration Of Lesion

5.3 Co-relation of clinical diagnosis with SSS,Culture and Histopathology

In this study, 215 cases were clinically diagnosed as cutaneous leishmaniasis by the dermatologist and dermatology residents.

Out of 215, 158 (73.5%) patients were slit-skin-smear (SSS) positive,those 57/215(26.5%) patents having negative SSS result was investigated by culture and histopathology done for both SSS and culture negative patents, 32/215(14.9%).From these, LD body seen in 4/32(12.5%) and in 25/32(78.1%) suggestive histology result and in 3/32(9.4%) diagnosis of other diseases was made.

In general, slit-skin-smear (SSS) positivity was 73%(158/215), culture 43.84%(25/57) and histopathology positivity was 90.6% (29/32).

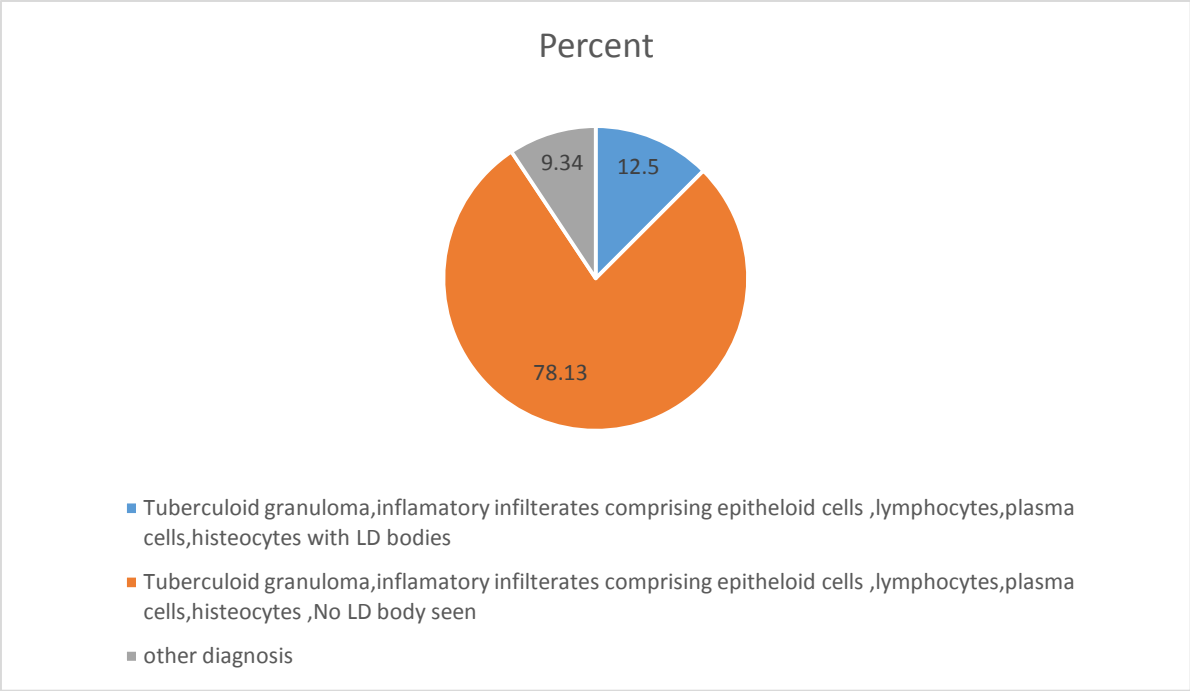

Figure 6 Histopathologic Result

	Frequency	Percent
Tuberculoid granuloma,inflamatory infilterates comprising epitheloid cells ,lymphocytes,plasma cells,histeocytes with LD bodies	4	12.5
Tuberculoid granuloma,inflamatory infilterates comprising epitheloid cells ,lymphocytes,plasma cells,histeocytes ,No LD body seen	25	78.13

other diagnosis	3	9.34
Total	32	100

Table 4 Histopathologic Result

6. DISCUSSION

In this study ,Majority were in the age group ranging from 10-19 year,66(30.7%) followed by 20-29 year age range patients ,60(27.9%) and 20(9.3%) were below 10 year .The mean (±SD) age of presentation is 27.4 (±16.98) being one year as minimum age of presentation and seventy eight year as maximum age of presentation which is close to the presentation in study done in Silti woreda,Ethiopia ,46.7% (43/92), were in the age group 11—20 years followed by 33.7% (31/92) in the age group 0—10 years.(17 E. Negera et al.May 2008).Another study done in North-Western Yemen showed highest age specific active lesion prevalence was noted in the age group 10–19 (12.8%; 64/499) followed by age group of 0–9 (9.8%; 51/520) (12).

Study done at the ALERT Hospital Dermatology Clinic the enrolled patients ranged from 6 to 73 years with a median of 19 years. (43) this show similar maximum age at presentation with this research but low median age at presentatioion.

The Saesie Tsaeda-emba district, eastern Tigray, northern Ethiopia research reported the age at presentation ranged between 10 months and 80 years (median age, 16 years).(22).The Sri Lanka research put the age rang from 4 - 80 years (median 35.4 years) with higher being 4 year as the youngest patient age at presetation and the median age of presentation(35.4) as compared to our result (25).

And the Ochollo,Ethiopia research showed age rang from 1 to 28 years of age with a median of 6 years with 28 year old as a maximum age of presentation, unlike our result (78 year). (43)

The majority ,91(42.3%) were from Addis Ababa followed by Oromia ,83(38.6%) .Similarly one reserch done at ALERT center also showed 81 /160(50.6%) clinically diagnosed CL patients from Addis Ababa (43).

The study showed no significant gender difference in the frequency of leshmania cases,52.56 %(113/215) were male and 47.44% (102/215) female with M: F (1.1:1). Research done in Siliti wereda showed Similar result with this study 47.8% (44/92) male vs. 52.2% (48/92) female.(17).But another research done at ALERT Hospital Dermatology Clinic showed higher numbers of cases in males (64%, 102/160) compared to females (36%, 58/160). (43).

The Saesie Tsaeda-emba district, Eastern Tigray cases also showed male prevalence of the disease(176 males [66.4%] and 89 females [33.6%]) (22)

Simillar male predominance seen in the Sri Lanka research with male to female ratio of Majority were men 2.5 to 1.(25)

In this study,single lesion occur in the majority ,194/215(90.23%) and two or more lesions seen in 21/215(9.77 %) of case.Similiarly,at Ochollo, 97% (33/34) had a single lesion and at ALERT Hospital Dermatology Clinic, 77% (121/157) had single lesions, 16% (25/ 157) had two and 7% (11/157) had more than two lesions. (43)

But lower result seen in Silti woreda , 46.7% (43/92), had single lesions and only 17.4% (16/92) developed three or more. (17).

Our research found that the duration of the lesions varied from 4 weeks to 15 years,mean=13.95 months.

Majority ,124(57.7%) present with in 1-11 month`s after development of lesion,50(23.3%) within 12-23 monthes and 41(19%) lesiones lates for 2 year and above.Less than 6

mon,90(41.9%),6-11 month 34(15.8%),12-17 month,50(23%),24 month and above 41,(19.1%) . Similiarly,the average duration of the lesion at diagnosis was 15 months for those at the ALERT Hospital Dermatology Clinic. (43) The duration of the lesions varied from 4 weeks to 5 years (median 9.2 months) in Sri Lanka.(25).The average duration of the lesion at diagnosis was lower than our result as compaired to the Ochollo researchwhich is 9 months (43)

But in Silti woreda (69/92) 75% of the patients had a lesion that had developed 6 or more months before starting treatment. (17).And also lower mean duration of diagnosis seen in Yemen,the duration of the disease ranged between 3 weeks and 18 months, except in one case with a duration of 8 years (median duration, 5 months).(12).

Our study showed majority 61(28.4%) has lesion on cheek and 59(27.4%)on nose,for-head lesion in 22(10.2%) ,lip involvement 11(5.1%) and forarm lesion in 10(24.2%),involving the nose, cheek, forehead and lip (71.1%). patients at ALERT Hospital Dermatology Clinic and cases at Ochollo,both researches had similar finding with this research having the most common sites of occurrence in order of frequency were on the cheeks, nose, forehead, lips, arms, limbs, eyelid and neck.72% (113/157) involving the nose, cheek, forehead and lip .

More than half of the CL cases, 55.4% (51/92), developed lesions on either or both cheeks. Nose lesions 13.0% (12/92) (17). The majority of active lesions were observed on the face in which the cheeks62 (37.1%) and the nose 45 (26.9%) were most affected.22 (13.2 %), 14 (8.4%)6 (3.6)6 (3.6hand,forhead,lip,ear (12). Similarly Sri Lanka research had ,face lesions (27%), followed by forearm (21%), leg (20%), trunk (17%) and arm (15%). (25)

But one study done in Pakistan showed upper extremities were the most common lesion site (41.6%) (44**)**

From all the CL cases most frequent clinical presentation was LCL 168(78.1%), followed by MCL 45(20.9%) and DCL 2 (0.9 %)., Similar result reported in one research done in Silti wereda Ethiopa,19.2% (14/73) were clinically of the mucocutaneous type. Lower result from the study done at Yemen ,MCL was observed in 22 cases (8.3%) (12)

This study showed out of 215, 158 (73.5%) patients were slit-skin-smear (SSS) positive,those 57/215(26.5%) patents having negative SSS result was investigated by culture and histopathology done for both SSS and culture negative patents, 32/215(14.9%).From these, LD body seen in 4/32(12.5%) and in 25/32(78.1%) suggestive histology result and in 3/32(9.4%) diagnosis of other diseases was made.

Putting slit-skin-smear (SSS) positivity as 73%(158/215) in our study SSS Positivity is higher as compaired Saesie Tsaeda-emba district, eastern Tigray, northern Ethiopia Skin scrapings from a total of 43 participants who had active lesions were smear examined and amastigotes

were visualized from 69.8% of them(22) .Higher result also seen as compared to SSS positivity of Srilanka reserch whch is 33.9% (35/103), culture 43.84%(25/57) similar with the Siliti wereda research, of the 71 samples 46.5% (33/71) were culture positive and higher culture positivity when we compare with result of Saesie Tsaeda-emba district, eastern Tigray, northern Ethiopia culture 32.3% were found positive for CL(22) . Similarly,lower culture positivity in Ochollo village 26% (10/35) (43).Higher result of pure culture result , 44% (71/160) for the samples from ALERT Hospital Dermatology Clinic and .(43)

Histopathology positivity became 90.6% (29/32) but lower result as compaired with the Histopathological analyses of Silti wereda 58/71 (81%) were positive for the presence of amastigotes in tissue sections and macrophages.(17).

In our study histopathology result , LD body seen in 4/32(12.5%) and in 25/32(78.1%) suggestive histology result.

One study done in Srilanka showed higher result, 45.6% parasite positivity and 33% giving supportive histology but lower overall 78.6% (25) comparing with our study which is 78.1%(29/32).

7. Strength and limitation of the study

7.1. Strength

All the data are collected by the researcher ,

7.2. Limitation

Lack of documentation of travel history to endemic areas.

8. Conclusion*:* *In our study* In general, slit-skin-smear (SSS) positivity was 73%(158/215), culture 43.84%(25/57) and histopathology positivity was 90.6% (29/32).

From total of 215 clinically suspected case 208 cases was diagnosed with one of the investigation modality SSS,culture or histopathology.Therefore clinical suspected cases by the clinician became 96.7% (208/215) positive

9.*Recommendation:* Considering the magnitude of the problem, limited resources, and clinical accuracy of 96.7 %, clinical diagnosis by a dermatologist appears to show hiher positive result with misdiagnosing limiled number of cases as cutaneous leshmaniasis.And having higher positivity in SSS result we can recommend microscopy as the test of choice for scarce resource settings but the histopathology can be used in a hospital setting for negative SSSresult case as it showed hihest positivity.

References

1. Cutaneous leishmaniasis in north-central Ethiopia: trend, clinical forms, geographic distribution, and determinants Belayneh Eshetu1 and Hassen MamoTropical Medicine and Health (2020

2. WHO Leishmaniasis March 2020

3. Bailey MS, Lockwood DN. Cutaneous leishmaniasis. Clin Dermatol. 2007;25(2):clindermatol.206.05.

4. Hepburn NC. Cutaneous leishmanisis: current and future management. Expert Rev Anti Infec Ther. 2003;1(4):563–70 PMID: 15482153.

5. Cutaneous leishmaniasis a neglected tropical disease: community knowledge, attitude and practices in an endemic area, Northwest EthiopiaTamiru et al. BMC Infectious Diseases (2019) 19:855

6. Leshmaniasis world wide and global estimate of its incidence,Jorge Alva,Ivan D.Veez,Caryn Bern,Merce Herrero ,PLOS 2012

7. Advances in Immunology of Neglected Tropical Diseases: New Control Tools and Prospects for Disease Elimination Barbara Castro-Pimentel Figueiredo ,Carina Silva Pinheiro ,Fabio Vitarelli Marinho and Nestor Adrian Guerrero,March 2020

8. Guideline for the diagnosis treatment and prevention of CL in Ethiopia,2013

9. PCR detection of Leishmania in skin biopsies Meryem Lemrani1 , Salsabil Hamdi1 , Abderahmane Laamrani2 and Mohammed Hassar,2009Value of diagnostic techniques for cutaneous leishmaniasis William R. Faber, MD, PhD,a Linda Oskam, PhD,g Tom van Gool, PhD,b Nel C. M. Kroon,g Kristine J. Knegt-Junk, MD,e Henk Hofwegen,f Allard C. van der Wal, MD, PhD,c and Piet A. Kager, MD, PhDd Amsterdam and Rotterdam, The Netherlands,2003 by the American Academy of Dermatology

10.Is slit-skin smear an alternate to histopathology in the diagnosis of cutaneous leishmaniasis? Asher Ahmed Mashhood, Salman Ahmad, Muhammad Mumtaz Malik Department of Dermatology, CMH, MultanJournal of Pakistan Association of Dermatologists. 2014;24

11.Comparison of Three Methods for Diagnosis of Cutaneous Leishmaniasis B Pourmohammadi1, 2, * MH Motazedian1 , GR Hatam1, M Kalantari1 , P Habibi1 , B Sarkari1 ,Iranian J ParasitolDec 2010

12.Cutaneous leishmaniasis in North-Western Yemen: A clinicoepidemiologic study and Leishmania species identification by polymerase chain reactionerestriction fragment length polymorphism analysis Khatri, Di Muccio, and Gramiccia J AM ACAD DERMATOL OCTOBER 2009

13.Comparison of parasitological and immunological methods in the diagnosis of leishmaniasis in Ethiopia Gennene Mengistu1’2TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1992) 86, 154-157

14.Correlation of clinical, histopathological, and microbiological findings in 60 cases of cutaneous leishman,Arfan ul Bari,January 2006

15.Cutaneous Leishmaniasis Due to Leishmania aethiopicaS. van Henten et al. / EClinicalMedicine 6 (2018) 69–81

16.Comparison of parasitological and immunological methods in the diagnosis of leishmaniasis in Ethiopia Gennene Mengistu1’2transaction of the royal society of tropical medicine and hygiene (1992) 86, 154-157

17. Outbreak of cutaneous leishmaniasis in Silti woreda,Ethiopia: risk factor assessment and causative agent identifification E. Negera et al.May 2008

18. Value of diagnostic techniques for cutaneous leishmaniasisFaber et alJ AM ACAD DERMATOL JULY 2003

19. Andresen K, Gaafar A, El-Hassan AM, Ismail A, Dafalla M, Theander TG, et al. Evaluation of the polymerase chain reaction in the diagnosis of cutaneous leishmaniasis due to Leishmania major: a comparison with direct microscopy of smears and section from lesions. Trans R Soc Trop Med Hyg 1996;90:133-5.

20. Mengistu G, Akuffo H, Fehinger TE, Negese Y, Nilsen R. The comparison of parasitological and immunological methods in the diagnosis of leishmaniasis in Etiopia. Trans R Soc Trop Med Hyg. 1992;86:154–7.

21. World Health Organization. Country profiles: Ethiopia, 2014. Available: http://www.who.int/leishmaniasis/resources/Leishmaniasis_cp_Ethiopia_2014_updated.pdf?ua=1. Accessed 15 Mar 2018.

22. Epidemiological study of cutaneous leishmaniasis in Saesie Tsaeda-emba district, eastern Tigray, northern EthiopiaBsrat et al. Parasites & Vectors (2015) 8:149

23. Clinical manifestations and classifification of Old World cutaneousleishmaniasis Oleg E. Akilov, MD, PhD, Amor Khachemoune,International Journal of Dermatology 2007

24. Morrone A, Pitidis A, Pajno MC, Dassoni F, Latin O, Padovese V. Epidemiological and geographical aspects of leishmaniasis in Tigray northern Ethiopia: a retrospective analysis of medical records, 2005–2008. Trans R Soc Trop Med Hyg. 2011;105:273–80 Elsevier.

25. Correlation of clinical, parasitological and histopathological diagnosis of cutaneous leishmaniasis in an endemic region in Sri Lanka

26. Herwaldt BL. Leishmaniasis. Lancet 1999;354:1191–9.

27. Grevelink SA, Lerner EA. Leishmaniasis. J Am Acad Dermatol 1996;34:257–72.

28. Mujtaba G, Khalid M. Cutaneous leishmaniasis in Multan, Pakistan. Int J Dermatol 1998;37:843–5.

29. Raja KM, Khan AA, Hameed A, Rahman SB. Unusual clinical variants of cutaneous leishmaniasis in Pakistan. Br J Dermatol 1998;139:111–3.

30. Clinical, epidemiological and laboratory aspects of patients with American cutaneous leishmaniasis in the State of PernambucoRevista da Sociedade Brasileira de Medicina Tropical 41(5):439-443, set-out, 2008

31. Martins AL, Barreto JA, Lauris JR, Martins AC. American tegumentary leishmanisis: correlations and immunological, histopathological and clinical parameters. An Bras Dermatol. 2014;89(1):52–8.

32. Ashford, R.W., Bray, M.A., Hutchinson, M.P., Bray, R.S., 1973. The epidemiology of cutaneous leishmaniasis in Ethiopia. Transactions of the Royal Society of Tropical Medicine and Hygiene 67, 568–601.

33. Abebe, A., Evans, D.A., Gemetchu, T., 1990. The isolation of Leishmania aethiopica from the ground squirrel Xerus rutilus. Transactions of the Royal Society of Tropical Medicine and Hygiene 84, 691.

34. Gebre-Michael, T., Balkew, M., Ali, A., Ludovisi, A., Gramiccia, M., 2004. The isolation of Leishmania tropica and L. aethiopica from Phlebotomus (paraphlebotomus) species (diptera: Psychodidae) in the Awash valley, northeastern Ethiopia. Transactions of the Royal Society of Tropical Medicine and Hygiene 98, 64–70.

35. Hailu, A., Di Muccio, T., Abebe, T., Hunegnaw, M., Kager, P.A., Gramiccia, M., 2006. Isolation of Leishmania tropica from an Ethiopian cutaneous leishmaniasis patient. Transactions of the Royal Society of Tropical Medicine and Hygiene 100, 53–58.

36. Blum, J., Hatz, C., Junghanss, T., 1994. The therapy of cutaneous and mucocutaneous leishmaniasis. Deutsche Medizinische Wochenschrift 119, 1169–1172.

37. Blum, J., Desjeux, P., Schwartz, E., Beck, B., Hartz, Z., 2004. Treatment of cutaneous leishmaniasis among travelers. Journal of Antimicrobial Chemotherapy 53, 158–166

38. Chulay, J.D., Anzeze, E.M., Koech, D.K., Bryceson, A.D., 1983. High-dose sodium stibogluconate treatment of cutaneous leishmaniasis in Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene 77, 717–721.

39. Teklemariam, S., Hiwot, A.G., Frommel, D., Miko, T.L., Ganlov, G., Bryceson, A., 1994. Aminosidine and its combination with sodium stibogluconate in the treatment of diVuse cutaneous leishmaniasis caused by Leishmania aethiopica. Transactions of the Royal Society of Tropical Medicine and Hygiene 88, 334–339.

40.Utaile M.Kassahun A.Abebe T.Hailu A.Susceptibility of clinical isolates of Leishmania aethiopica to miltefosine, paromomycin, amphotericin B and sodium stibogluconate using amastigote-macrophage in vitro model.Exp Parasitol. May 2013; 134: 68-75
41.Belehu A.Naafs B.Touw-Langendijk E.Failure of metronidazole treatment in Ethiopian mucocutaneous leishmaniasis.Br J Dermatol. 1978; 99: 421-422
42.Cutaneous leishmaniasis Richard Reithinger, Jean-Claude Dujardin, Hechmi Louzir, Claude Pirmez, Bruce Alexander, Simon Brooker Vol 7 September 2007

43.Leishmania (Kinetoplastida): Species typing with isoenzyme and PCR–RFLP from cutaneous leishmaniasis patients in Ethiopia E. Gadisa a,¤ , A. Genetu d , T. Kuru a , D. Jirata a , K. Dagne b , A. AseVa a , L. Gedamu c

44.CUTANEOUS LEISHMANIASIS IN KHYBER PAKHTUNKHWA, PAKISTAN Cutaneous Leishmaniasis in Khyber Pakhtunkhwa Province of Pakistan: Clinical Diversity and Species-Level Diagnosis KHAN AND OTHERS 2016 by the American Society of Tropical Medicine and Hygi**ene**