ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 05, pp.31190-31196, May, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

CLINICOEPIDEMIOLOGICAL STUDY ON FOCUS OF CUTANEOUS LEISHMANIASIS IN SAUDI ARABIA

[1,*]Abdalla, N. M., [2]Abdelgani, A. M., [3]Osman, A. A. and [4]Mohamed, M. N.

[1]Department of Microbiology, College of Medicine, King Khalid University, Saudi Arabia
[2]Microbiology Specialist, Faculty of Medical Lab., Gezira University, Wad Medani, P.O.BOX 20, Sudan
[3]Consultant Reproductive Health, Family and Community Medicine, College of Medicine, King Khalid University, Saudi Arabia
[4]Internist Abha General Hospital, 61421 Abha, P.O. 641, Saudi Arabia

ARTICLE INFO

Article History:
Received 23rd February, 2016
Received in revised form
20th March, 2016
Accepted 15th April, 2016
Published online 20th May, 2016

Key words:

Cutaneous leishmaniasis,
Leishmanin skin test (LST),
Leishmania tropica,
Leishmania major.
Clinicoepidemiological.

ABSTRACT

A cross-sectional study conducted in Al-Madinah Almonawarah in KSA held during the period from March 2014-March 2015. This study aiming to identify the epidemiological and clinical pattern of cutaneous leishmaniasis in Al-Madinah Almonawarah area. Epidemiological and clinical data were collected using special questionnaire. Statistical analysis was done using special computer program SPSS. Laboratory specimens were collected using skin scraping and needle aspiration. Staining and direct microscopy were done. LST was conducted. The study includes 164 patients, all of them were men, almost half of them were farmers. 58% indoor transmission. Bed nets were not used by 85.4% of patients, moreover most of the patient 83% were not using protective clothes. Clinically sever form of presentation indicate 4 and more ulcers. Severe form was encountered in (14.6%) among children < 15years and 32.1%. in the Adults. Those with underweight had higher percent in mild and severe form of presentation 61.5% and 38.5% respectively. LST was negative in all cases. The study revealed that Al-Madinah Almonawarah is endemic area for cutaneous leishmaniasis due to the presence of the vector and the reservoir. Age has less effect on clinical presentation (indoors and outdoors transmission are present) compared with social habits of patients and nutritional status.

Citation: Abdalla, N. M., Abdelgani, A. M., Osman, A. A. and Mohamed, M. N., 2016. "Clinicoepidemiological study on focus of cutaneous leishmaniasis in Saudi Arabia", *International Journal of Current Research*, 8, (05), 31190-31196.

INTRODUCTION

Leishmaniasis comprises a group of disease caused by intracellular parasite kinetoplastid protozoan of genus Leishmania or Viannia that live in macrophages of some vertebrate as amastigote form. The Leishmania parasites consist of different species and strains which are epidemiological diverse. Some of these species were pathogenic to man leading to different pathological and clinical spectrum such as Visceral Leishmaniasis, Muco-cutaneous Leishmaniasis (MCL), Diffuse Cutaneous Leishmaniasis (DCL) and Cutaneous Leishmaniasis (CL). The latest form in which the parasite restricted to cutaneous and sub-cutaneous tissue (Lainson and Shaw, 1998). Leishmaniasis are prevalent on four continents and considered endemic in 88 countries, 72 of which are developing countries. The worldwide prevalence of the disease is estimated at 12 million cases, with 400,000 to 600,000 new cases per year for visceral forms and of 1–1.5 million for the cutaneous forms. Over 90% of cases occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru (WHO 2003). The Leishmania species present with a similar clinical appearance, but with different prognosis during the course of the infection. The ulcers caused by parasites of the subgenus Viannia are more aggressive and can recur after treatment. The ulcers caused by parasites of subgenus Leishmania are less severe and more likely to cure spontaneously or after treatment (Pirmez *et al.,* 1999). The diagnosis of cutaneous leishmaniasis is performed using clinical and epidemiological features and through parasitological tests; direct microscopic examination of smears after Giemsa staining, in vitro culture, histopathological techniques, and immunological methods (Sundar, 2002). The different Leishmania species are not equally easy to culture. Contamination is a constant problem, and variations in efficacy among different growth media formulations or even batches may be encountered. Likewise, the success of microscopic

Corresponding author: Abdalla, N. M.,
Department of Microbiology, College of Medicine,
King Khalid University, Saudi Arabia.

identification of amastigotes in stained preparations depends on the number of parasites present and/or the experience of the technician examining the slide. Moreover the different clinical signs of Cutaneous Leishmaniasis depend on the type, duration of clinical lesion and geographical location. One of the most likely factors influencing the clinical manifestations, is genetic variability of parasites (El-Hassan, *et al.,* 2001). Simple Cutaneous Leishmaniasis (oriental sore), is a skin involvement of Leishmania parasite in which the amastigote multiplication is restricted to skin macrophages. The disease is caused by species *Leishmania tropica* complex in the Old World and *Leishmania mexicana* complex in the New World. The disease started as a red papule at the site of sandfly inoculation of promastigotes, this eventually ulcerates, become crusted and then heal leaving a depressed scar. incubation period range from two weeks to several months (Schemidt and Robert, 1989). Spontaneous healing of lesions usually occurs within two months to one year. Lesions of Cutaneous Leishmaniasis are nodular, nodulo-ulcerative or diffuse. Recidivian leishmaniasis is allergic form, in which new isolated or grouped lesions developed around the edge of the scar of the primary healing lesion, they harbor few parasites, which are often undetectable. Heavy lymphocytic infiltrate, giant cells and rare epithelial and histolytic elements characterize these lesions. Leishmaniasis recidiva and Diffuse Cutaneous Leishmaniasis (DCL) are non self-healing chronic forms of disease (El-safi *et al.,* 1989)

The pathogenic complexes to man includes: *L. major*: mainly causes Zoonotic Cutaneous Leishmaniasis (ZCL) and Mucocutaneous Leishmaniasis (MCL) of the Old World. *L. aethiopica*: mainly causes Zoonotic Cutaneous Leishmaniasis (ZCL) and Diffused Cutaneous Leishmaniasis (DCL) of the Eastern Africa. *L. mexicana*: mainly causes Cutaneous Leishmaniasis (CL) and Diffused Cutaneous Leishmaniasis (DCL) of the New World. *L. (V) braziliensis*: mainly causes Cutaneous Leishmaniasis (CL) and Mucocutaneous Leishmaniasis (MCL) of the New World. *L. tropica*: mainly causes Cutaneous Leishmaniasis (ACL) of the Old World. *L. Donovani*: mainly causes Visceral Leishmaniasis (VL), Mucocutaneous Cutaneous Leishmaniasis (MCL) and Post Kala-azar Dermal Leishmaniasis (PKDL) (WHO, 1990). The geographic distribution of cutaneous leishmaniasis is mainly determined by the sandfly vectors (*Phlebotomus sp*. and *Lutzomyia sp*.) (Magill, *et al.,* 1994) . However, *L. tropica* was not the only species identified to be the causative agent of CL. *L. donovani* was isolated from a cutaneous lesion in a tourist from southern France who visited Yemen for two weeks. (Rioux *et al.,* 1989). Similarly documented in Nuba Mountain study (Abdlla, 2010). The predominance of *L. tropica* as a causative agent of CL has been reported from Saudi Arabia (Al-Zahrani *et al.,* 1988). *Leishmania tropica* is commonly stated to be anthroponotic (WHO 1984), although zoonotic transmission has been reported from Greece (Garifallou *et al.,* 1984), Saudi Arabia (Al-Zahrani *et al*., 1989). Vectors in Al-Madinah Almonawarah; Sandflies (Diptera: Psychodidea: Phlebotominae) are of considerable public health importance in the tropics and subtropics. They are obligate vectors of Leishmaniasis. A recent study done by Aymen El-Badry *et al* the entomological survey was carried out in four provinces representing the surrounding of Al-Madinah Almonawarah. Western Saudi Arabia from May 2007 to April 2008. Occurrence and spreading of sand flies have not been systematically monitored and documented in Al-Madinah Almonawarah, a total of 809 sand flies were collected 72.6% females and 27.3% males, they composed of four species of two genera Phlebotomus and Sergentomyia comprising 658 (81.3%) *P. Papatasi*, 27 (3.3%) *P. sergenti* 34 (4.2%) *S. cyldei* and 90 (11.1%) *S. antennata*. *P. papatasi* was most abundant species of the collected sand flies (El-Badry *et al.,* 2008). They are two types of Leishmaniasis with regard to reservoir hosts: Zoonotic Leishmaniasis in which, the reservoir hosts are wild and/or domestic animals. Anthroponotic Leishmaniasis in which, the reservoir host is man. Domestic and sylvatic mammals infected with leishmaniasis may or may not show obvious clinical signs of infection. Often, there are relatively few amastigotes in the skin or viscera with minimal or no host response. Some mammals such as dog, which are considered to be natural reservoir host of leishmaniasis, may be eventually be killed by the infection. Five species of rodents were caught, namely, *Meriories libycus*; *Psammomys obesus*; *Rattus rattus*; *Jaculus jaculus* and *Hystrix indica* with the former was the most dominant (90%). Examination of these rodents revealed detectable leishmanial parasite in only one species, Meriories lybicus. Of the 89 mice of this species examined 18 (20.2%) showed detectable amastigotes in Almadinah Almonawarah and Tabouk regions indicating that this species could be the reservoir host for infection. This study aiming to identify the epidemiological and clinical pattern of cutaneous leishmaniasis in Al-Madinah Almonawarah area.

MATERIALS AND METHODS

The study area

This study was conducted in Almadinah Almonawarah western region of Saudi Arabia, it is elevation above sea level 630 meter, the average annual temperature maximum 30ºC to minimum 20ºC with average humidity 33% and the average annual rainfall was 38.4 millimeter. most cases participated in this study were from the farm in Khulial street in the south of the city, The other two districts of Al-Madinah city, are Al-Yutamah around seventy five kilometer north to Al-Madinah and Shajow located one hundred kilometers south to Al-Madinah Almonawarah.

Study population

The study group were all patients with suspected skin ulcer of cutaneous leishmaniasis attending Al-migat Hospital in Al-Madinah Almonawarah, Utamah and Shajow health centres. During the study period from March 2014 to March 2015.

Data collection

Structured questionnaire was done included demographic, epidemiological & clinical data, (the following information; sex, age, nationality, their socio-demographic characteristics, availability of electricity, domestic animals kept, number and sites of lesions of active lesions). All cases of cutaneous leishmaniasis were clinically and microscopically confirmed.

Clinical diagnosis

All patients were diagnosed clinically by dermatologist and general practitioners. The clinical manifestation of CL can be misdiagnosed and confused with dermal diseases, however the disease in endemic areas can be easily diagnosed by the ulcers in the naked areas and the feature of the ulcers is very characteristic with prominent edged, volcanic appearance, started with papules and takes long term mostly one to six months without pain.

Laboratory Diagnosis of Cutaneous Leishmaniasis

Parasitological diagnosis

(i) Giemsa stain preparation

This was prepared by adding methanol gradually to the Giemsa powder until it completely dissolved. Glycerin was then added and the mixture was stored for 24 hours. The above-prepared Giemsa stain was diluted with phosphate buffer pH 7.2, to final concentration of 10% immediately before use.

(ii) Leishman stain

Leishman stain is a 0.2% (W/V) solution of eosin in methanol

(iii) Preparation of smears

For all patients (n=164) samples from the edges of the lesion were collected using insulin syringe aspiration was taken and put in two clean microscopic slides with drop of normal saline, then the slides left to dry completely. For Giemsa stain the slides were fixed with 100% methanol, left to dry then covered with 10% Giemsa stain and left for 15 minutes then rinsed with distilled water by pouring water on the edge of the slide. The smear were allowed to dry and then examined carefully for Leishmania amastigote microscopically at X100 power using the immersion oil lens. No culture was made for any sample in this study. In some cases using the Leishman stain by covering the slides with stain for 30 mints then left to air dried, finally examined microscopically at X100 power using immersion oil lens. All these technique was performed in the laboratory of Al-miquat Hospital in Al-Madinah Almonawarah.

Ethical considerations

All patients with active lesions who took part in this research project had given informed consent. All the risks had been explained to them by the principal investigator before the beginning of the programme. Participants could choose to withdraw from the study at any time if they wished. This study was conducted after permission of the health authority of the vector born diseases in Al-Madinah region. The procedures followed in this study were in accordance with the ethical standards of the responsible committee on human experimentation (King Khalid University, Saudi Arabia) and in keeping with the Helsinki Declaration of 1964, as revised in 1975, 1983, 1989, 1996, and 2000. Human rights were fully fulfilled.

Data analysis techniques: The collected data were statistically analyzed using the SPSS version 19.

RESULTS

Epidemiological and demographic analysis

All patients included in the study were male (n, 164) due to culture and religious reasons. The distribution of the patient in the study areas. Age distribution, range from, less than one year of age to more than 40years of age. The mean age was 29.2.+ 2.5. More than half of the patients were Saudi. Concerning patient occupation; farmers (48.8%) followed by builders (20.1%). the least group was poultry rising (4, 2.4%). Shown in Table 1. The domestic animals in the areas were goats (67, 40.9%) cats (27, 16.5%) dogs (9, 5.5%) dogs and goats (5, 3%) poultry (4, 2.4%) dogs, goats and cats (11, 6.7%) cat and goat (4, 2.4%) and 37 houses without domestics animals. Moreover the burrows of the rodent is abundant in and around the houses which consider as potential reservoir in the area. The sleeping habits of the participants most of them they sleep indoor (94, 58%) and this mostly depend on the availability of the electricity. Other important factors include usage of the bed nets those who used it always (22, 13.4%), rear users were (2, 1.2%) and non users were (140, 85.4%), moreover most of the patients (136, 83%) they were not using protective cloths.

Table 1. Demographic characteristic

Age in years	No. of pt	Percent%
0-10	12	7.3
11-20	12	7.3
21-30	76	46.3
31-40	46	28.0
41 and more	14	8.5
Occupation	No. of patient	Percent%
Farmer	80	48.8
Builder	33	20.1
Student	12	7.3
Unemployed	15	9.1
Drivers	10	6.1
Poultry rising	10	6.1
Nationality	No. of patient	Percent%
Saudi	84	51.2
Non Saudi	80	48.8
Sleeping habits	No. of patient	Percent%
Indoor	94.5	58
Outdoor	68	42

Clinical presentation of the study group

Concerning the sites of the lesion mostly occur in the upper extreme (67, 40,9%) and the lower extreme (28, 17.1%) and fewer having the lesion in their face five children under the age of 7 years and one Yemeni his age 35 years (6, 3.7%) and the lowest sites of lesion occurrence was on the neck (4, 2.4%). The rest of patients (59, 36%) have multiple lesion sites. Most of the patient having low number of lesion 1-3 (100, 78%) and 4 and more lesions in (64, 22%) of patients. The clinical confirmation and attending of the patient one of the health center or hospital are mostly (144, 86.6%) 1-3 months from the onset of the symptoms and the 4-6 months (19, 11%) but only 0.6% one patient attend the health center more than six month after the lesion appear.

Table 2. Number of lesions According to age groups

No. of Lesion		Age					Total
		0-10	11-20	21-30	31-40	>40	
	1-3	11	8	48	33	10	110
	4-6	1	4	26	12	3	46
	>6	0	0	2	5	1	8
Total		12	12	76	46	14	164

Table 3. The number of lesions with occupation

		Occupation							Total
		Farming	Construction	Office work	Driver	Poultry	unemployed	Student	
No Lesion	1-3	60	17	6	10	2	8	9	112
	4-6	21	13	4	0	2	3	2	45
	>6	3	3	0	0	0	0	0	7
Total		84	31	10	10	4	11	11	164

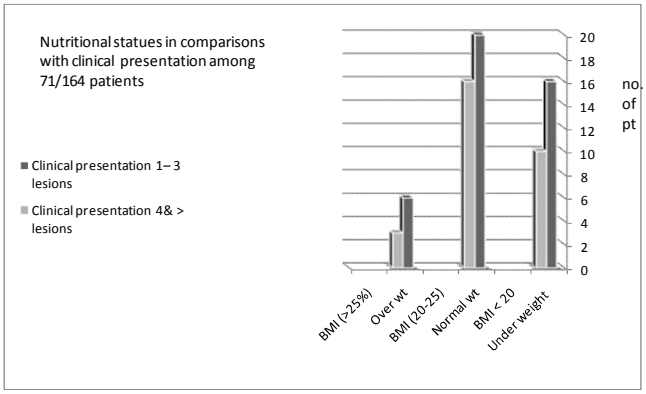

Fig. 1. Nutritional statues in comparisons with clinical presentation among 71/164 patients

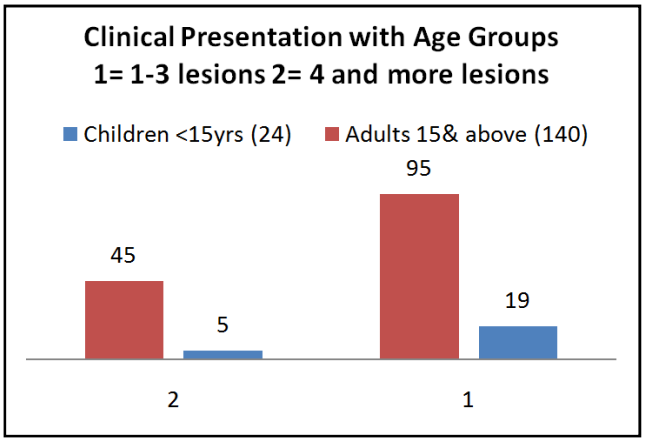

Fig. 2. Number of Lesions according to age (children & adults)

Most of the patients received medicine (150, 91.5%) and mostly they having Pentostam (109, 66.5%) whereas the rest receiving Cauterization therapy (33, 20.1%). The 14 patients (8%) which did not attending any clinic because they are foreigners and have no license for staining and roaming, few of the patient they received both therapy Pentostam and Cauterization (8, 4.9%).

Laboratory results: Concerning the result of the laboratory; results of the microscope examination (n, 88) all were positive and those patients were from Al-Meqate Hospital, secondly clinical suspected (n, 76) all patient receiving treatment in one of health center (Yutamah or Shajow villages) depending on clinical diagnosis as they have no facilities of laboratory investigation, The results of leishmanin skin test (LST) (montonegro test) were negative in all patient, in comparison between the Nutritional statues of patient with the severity of the disease (number of ulcers) among 71/164 patients ; Underweight group showed: those having 1-3 ulcers in(16), 4-6 ulcers (8), >6 ulcers (2). Those with normal weight showed: 1-3 ulcers (20), 4-6 ulcers (14), >6 ulcers (1). Finally patients with overweight showed: 1-3 ulcers in (6), 4-6 ulcers in (3) and >6 ulcers in (1). Fig. (1) In comparison between the age of patient (children below 15 years of age & adults 15 years and more) with the severity of the disease (number of ulcers) 164 patients; first group showed: 1-3 ulcers (110), second group 4-6 ulcers(46), third group >6 ulcers(8). Fig. (2). Number of lesions according to age groups was shown in Table (2). In comparison between the occupation of patient with the severity of the disease (number of ulcers) Among the farmers (highest occupation group 84); first group showed: 1-3 ulcers (60), second group 4-6 ulcers (21), third group >6 ulcers (3). Table (3)

DISCUSSION

This is a descriptive, cross-sectional study conducted in Al-Madinah Almonawarah in KSA in order to study the clinical, epidemiological and nutritional pattern of cutaneous leishmaniasis. Leishmaniasis is parasitic disease presented in different clinical forms, visceral, cutaneous, mucocutaneous and PKDL (Post Kala-azar Dermal Leishmanisis). Cutaneous Leishmaniasis is common health problem in the World, it divided into ZCL and ACL. Cutaneous leishmaniasis is one of the public health in many countries and the incidence are increasing since 1993. Control of animal reservoir host in zoonotic form is one of the best measures against leishmaniasis. All patient included in this study were male due to culture and religious reasons. Total number of 164. The incidence rate of CL infection was 85.4% in patients over 15 years old. This finding is lower than that reported for Colombia (86%), but higher than in Iran (38%) and Turkey (45%) (WHO, 2007). It is postulated that the decrease in incidence with age was due to development of immunity by previous infections. This study indicates that the incidence rate of multiple lesions in CL patients is 58%. This result could be

due to long periods of exposure to Plebotomine sand flies and the high population density of sand flies in this area. Regarding the distribution of CL lesions in this study, we found that the higher proportion of the lesions were located on the upper limbs (57%), face (47%) and lower limbs (15%) and less frequently on the scalp (2%) and ears (1%). These observations are consistent with reports concerning Turkey, Colombia (Ramirez, 2000) and Iran (Yaghoobi, 2002). In comparison, the study by (Alsamarai, 2008) found that CL lesions occurred mainly on upper limbs and lower limbs, less frequently on the face, and much less frequently on the trunk. These observations contrast those of (Nezhad, 2012), who reported that the face was most affected. Ulcerated skin lesions account for the majority of clinical manifestations of cutaneous leishmaniasis. However, the relatively wide range of morphological variations of the skin lesions, which are particularly frequent in New World leishmaniasis, as well as the prevalence of other microbial infections in areas where leishmaniasis is endemic that may mimic the presentation of a Leishmania infection, often complicate the diagnosis of leishmaniasis (WHO 2008). Parasitic diagnosis of CL is therefore necessary before the relatively toxic chemotherapy should be used. However, direct microscopic examination of lesion scraping and fine needle aspirate continue to be the diagnostic method most widely applied because of the ease of performance, low cost, and speed of this technique Phlebotomous attack exposed areas of the body during dusk to dawn. The differences in distribution of lesions noted in the this study mentioned may be explained by the living conditions and habits of the people concerned mainly sleeping indoors and outdoors as well as social activities in evening and night plus the traditional dressing pattern. Half of the occupation in this study were farming (80, 48.8%). This will lead to outdoors transmission, study done in Nuba Mountain, the transmission was indoors and outdoors (Abdalla *et al* 2002).

A study on leishmania reservoir (Abdelgani *et al*., 2003) conducted in The Green Valley Village in Nuba Mountain west of Sudan, using direct microscopy and modified direct agglutination test (DAT-R), ELISA, K39 and PCR. Rabbits, rats, goats, donkeys and human were included in the study. There were no classical clinical sings of leishmaniasis encountered in human or animals. Although the performance of these tests were variable, the DAT-R test was more reliable. PCR results showed the predominance of sub-clinical from and transmission is going through variable reservoirs. In this study the domestic animals in the areas were goats, dogs, cats and poultry which were present in many houses and the surroundings. Moreover the burrows of the rodent is abundant in around the houses which consider as potential reservoir in the area. Most patients in this study were not using the bednets (140, 85.4%) neither using any protective clothes or methods, this lead to increase transmission of the disease. The importance of using bednets and protective cloth have been demonstrated by many studies (Elnaiem, 1998) in Gadareff state eastern Sudan and (Widaa, 2012) White Nile state in Sudan. Typically, a diagnosis of cutaneous leishmaniasis is based on the clinical presentation of patients in geographical regions where the infection normally occurs and on epidemiological criteria, since certain disease manifestations have been associated with particular species or complexes. However, carcinomas or lesions caused by leprosy, syphilis, tuberculosis or Paracoccidioides brasiliensis, all of which are relatively common in Brazil, should be considered in differential diagnosis (Ramos-E-Silva and Jacques, 2002) and atypical infections have occurred such as visceral disease caused by *L. (V.) braziliensis*. Thus, the identification of Leishmania species in clinical samples is necessary to confirm a suspected case of leishmaniasis. Consequently, molecular techniques have several advantages compared to traditional ones, such as the ability to detect parasites present in very low numbers and the ability to be performed with a broad range of clinical specimens (Lima *et al.,* 2013). In this study factors leading to clinical variation from mild (single ulcer) aggressive (multiple ulcers), age and occupation has less effect on clinical presentation (indoors and outdoors transmission were present) compared with social habits of patients and nutritional status. The parasite genotype might affect the clinical presentation of leishmaniaisis (Abdalla *et al.,* 2011). The results of LST for all active case in study were negative because the leishmanin indicator of cell-mediated immunity resulting in a delayed-type hypersensitivity reaction. It is believed that the development of positive LST after successful treatment correlates with long-lasting protection against leishmanial infection. This result agree with study done by (Salib *et al*., 1997). in India; "Evaluation of LST in Indian VL". In different studies; patients had positive Montenegro intradermal tests, having indurations between 5 and 30 mm diameter. Other studies have also shown high sensitivity and its importance for detecting old lesions, where it is difficult to view the parasite. However, it cannot distinguish active, inactive, or past infection. The greatest problem was the low specificity (65%). False positive results were seen in 20 patients (35%) with reactions up to 11 mm in diameter. Similar results have been reported before. All patients (164) received treatment from the health centers (Yutamah and Shajow) or Hospital (Al-Mequat) in form of Pentostam injections or cauterization therapy. Follow up of all patients reviled that all patients were cured expect one. The duration of follow up varied 3-12 months. During the follow up period LST was repeated but remained negative.

Conclusion

In conclusion, this study indicates that CL is endemic in Al--Madinah Almonawarah region. CL is a major health problem occur in variable clinical forms, since secondary bacterial infection was reported in 42% of cases and this infection may influence the natural course of the disease causing further skin damage. Giemsa stain was the most sensitive parasitic diagnostic test. Case detection and treatment as well as control of the vector and reservoir will yield to eliminate this disease

Acknowledgments

We would like to thanks the vector control unit at Al--Madinah Almonawarah Health Affair for their sincere collaboration. Our gratitude to the doctors and health personnel of the health centers (Yutamah and Shajow) and Al-Mequat Hospital.

Conflict of Interest Statement

There is no conflict of interest for this article.

Financial Support

Our Research Project was partially sponsored by {The First University Scientific grant, Scientific Research College, King Khalid University, Abha. KSA. Grant ID number KKU-MED-11-01}.

REFERENCES

Abdalla, N.M, Ibrahim, M.E, El-Hassan, A.M, Osman, O.F, Daifalla, N.S, Barker, D.C, Lambson, B, Miles, M, Mauricio, I, Magzoub, M.M, 2002 Molecular Epidemiology and Clinical Study on Leishmaniasis In Nuba Mountain – Sudan. *Acta. Parasitologica Turcica*,; 26 (1): 23-30. ISSN 1300-6320 .

Abdalla, N.M, Evaluation of Gene Targeted PCR and Molecular Hybridization Used in Diagnosis of Human Leishmania Isolates. 2010. *J. of Biotechnology* 9 (2): 212-217.

Abdalla NM. Comparative study of immune-diagnostic tools with Polymerase Chain Reaction in sub-clinical leishmaniasis isolates. *J of Medicine*, 2011; 12: 34-39.

Abdulghani, M.A., N.M. Abdalla, A.A. Eldosh, Z.A. Helmay and M.M. Magzoub,2003. Assessment of variable diagnostic tests in detection of rabbits leishmania reservoirs in sub-clinical leishmania focus in Sudan. Acta Parasitol. *Turcica*, 27: 111-116.

Alsamarai, A.G,. Faulde, M, Schrader, J, Heyl, G, Amirih, M. 2008 Prevalence of Skin Diseases in Samara, Iraq. MEJIM. In press.13. Differences in transmission seasons as an epidemiological tool for characterization of anthroponotic and zoonotic cutaneous leishmaniasis in northern Afghanistan. *Acta Tropica* 105:131-138.

Al-Zahrani, M.A, Peters, W, Evans, D.A, Chin, C, Smith, V, Lane, R.P, 1988; Phlebotomus sergenti, a vector of Leishmania tropica in Saudi Arabia. *Trans R Soc Trop Med Hyg*. 82:416.

Al-Zahrani, M.A, Peters, W, Evans, D.A, Smith, V, Ching, C.I, 1989. Leishmania infecting man and wild animals in Saudi Arabia. 6. Cutaneous leishmania of man in the south-west. *Trans R Soc Trop Med Hyg*. 83:62l-628.

El-Hassan, A.M, Zijlstra, E.E, 2001 . Leishmaniasis in Sudan. Cutaneous Leishmaniasis. *Trans R Soc Trop Med Hyg*. 95 Suppl 1:S1-17.

El-Safi S.H. Studies on the Leishmaniasis in the Sudan. 1989. Ph.D. Thesis, University of London, England.

El-Badry, A.A, Al-Juhani, A.M, Ibrahim, E.K, *et al*. 2008. Distribution of sand flies in El-Nekheil province, Al-Medina Al-Munawwarah region, western Saudi Arabia. *Parasitol. Res*. 103:151-165.

Elnaiem, D.A, Conners, S, Thmoson, M, Hassan, M.M, Hassan, K.H, Aboud, M.A, Ashford, R.W, 1998. Environmental determinants of the distribution of Phlebotomus orientalis in Sudan. *Ann Trop Med Parasitol* 92: 877-887.

Garifallou, A, Schnur, L.F, Stratigos, J.D, Hadziandoniou, M, Savigos, M, *et al*. 1984. Leishmaniasis in Greece II. Isolation and identification of the parasite causing cutaneous leishmaniasis in man. *Ann Trop Med Parasitol* 78: 369–375.

Hojat, A.N, Mehdi, B, Mojtaba, N, Mohamad, M, 2012. Cutaneous Leishmaniasis in school children in a border area at southwest of Iran. *Sci Parasitol* 13(4):153-158.

Lainson, R., Shaw, J.J, 1987. Evaluation, classification and geographical distribution of Leishmania. The Leishmaniasis in biology and medicine; 1, London: Academic press. 120

Manoel, S.C, Junior, D.C, Rodrigues Z. Maria E.C, Dorval, E.R, Jardim C.P, Elisa T.O, Rodrigo, C, Renato, A, and Maria, F.C,. 2013. Sensitivity of PCR and real-time PCR for the diagnosis of human visceral leishmaniasis using peripheral blood. *Asian Pac J Trop Dis*. 3(1): 10–15. doi: 10.1016/S2222-1808(13)60003-1

Magill, A.J, Grogl M.J, Gasser, R.A, 1994. Visceral infection due to Leishmania tropica in a veteran of Operation Desert Storm who presented 2 years after leaving Saudi Arabia. *Clin. Infect. Dis.* 19: 805–806.

WHO Expert committee. 1984. epidemiological aspects. In: Control of the leishmaniasis. World Health Organization, *Technical Report Series* 701:2-4.

WHO, 2003. Communicable Disease Working Group on Emergencies, HQ Division of Communicable Disease Control, EMRO, WHO OFFICE, Baghdad. WHO Office, Baghdad. Communicable Disease Toolkit, IRAQ CRISIS.WHO 2003:39-44. www.who.int/diseasecontrol_ emergencies/toolkits/Iraq_profile_ok.pdf.

WHO, 2007 WHO—(World Health Organization), 2007. Available from: <http://www.who.int/tdr/diseases/leish/ diseaseinfo.htm>. Accessed April 2007.

WHO, 2008. The global burden of disease: 2004 update. Geneva: World Health Organization.

Yaghoobi-Ershadi, M.R, Hanafi-Bojd, A.A, Javadian, E, Jafari, R, Zahraei-Ramazani, A.R, Mohebali, M. A new focus of cutaneous leishmaniasis caused by Leishmania tropica. *Saudi Med J*. 2002; 23(3): 291-4.

Pirmez, C, Pirmez, V, Silva, T. M, Paes-Oliveira, N. A, Cruz, S.C, Goncalves-da-Costa, M, Catanho, W. D, and Fernandes, O. 1999 Use of PCR in diagnosis of human American tegumentary leishmaniasis in Rio de Janeiro, Brazil, *Journal of Clinical Microbiology* 37, pp. 1819–1823.

Ramirez, J.R, Agudelo, S, Muskus, C, *et al*. 2000 Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. *J Clin Microbiol* 38:3768-3773.

Report of WHO Expert Committee in Control of Leishmaniasis. 1990; WHO Technical Report Series. 793: 11-58.

Rioux, J.-A., Lanotte, G., Serres, E., Pratlang, F., Bastien, P., and Perrieres, J. 1990. Taxonomy of Leishmania, use of isoenzymes, Suggestions for a new classificaiton. Annal de parastitologic Humaine et Comparee, 63 , 111-125.

Ramos, E. Silva and Jacques, M. Ramos-E-Silva and C. De Moura Castro Jacques, 2002, Leishmaniasis and other dermatozoonoses in Brazil, Clinics in Dermatology 20 pp. 122–134.

Sally, O W, Khalid, A.A. Amel, A. E, Mayada, M. A, Mihad, A.I, Mohammed, A. B, Ahmed, H. A, Zakkiah, A.Y, Mo'awia, M. H, 2012. Sandflies (Diptera: Psychodidae) in a focus of visceral leishmaniasis in White Nile, Sudan.

MEM INST OSWALDO CRUZ RIO DE JANERIO, 107 (4): 470-475 DOI: 10.1590/S0074-02762012000400005.

Saliba, E.K, Saleh, N. O, Khoury, S. B, *et al*. 1997. The endemicity of Leishmania tropica (zymodeme MON-137) in the Eira-Yarqa area of Salt District, Jordan. Ann Trop Med Parasitol 91: 453–459.

Shyam, S, and Rai, M, 2002. Laboratory Diagnosis of Visceral Leishmaniasis Clin Diagn Lab Immunol. 9(5): 951–958.

Schemidt, G.D, Robert, L.S, 1989. Foundation of Parasitology. time Mirror/Mosby College Publishing Boston; 4th edition

****