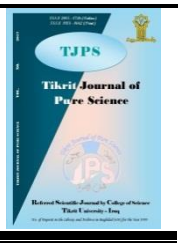
TJPS

Tikrit Journal of Pure Science

Journal Homepage: http://tjps.tu.edu.iq/index.php/j



Detection of Cutaneouse Leishmaniasis species via PCR in Salah Adeen and Baghdad provences

Maysaa Ibrahim Al-Jubori[1] , Abd Alrahman A. Al-Tae[2] , Mohammad A. Al-Faham[3]

[1]*Department of biology, college of science, Tikrit University , Tikrit , Iraq*

[2] *Department of microbiology, college of medicine, Iraqia University , Baghdad , Iraq*

[3] *Department of microbiology , college of medicine, Baghdad University , Baghdad , Iraq*

https://doi.org/10.25130/tjps.v24i1.330

ARTICLE INFO.

Article history:

-Received: 29 / 10 / 2017

-Accepted: 18 / 1 / 2018

-Available online: / / 2019

Keywords:

Cutaneouse, Leishmaniasis , PCR , *Leishmania*, Salah Adeen provence

Corresponding Author:

Name: Maysaa Ibrahim

E-mail:

maysaa.aljubory@gmail.com

Tel:

ABSTRACT

Background: Leishmaniasis is a parasitic diseases that are spread worldwide due to various species of *Leishmania*, which are infect mammales diversity as well as human. *L. tropica*, *L. major*, and *L. aethiopica* which is common causes of cutaneous Leishmaniasis in Salah Adeen and Baghdad provences.

Material and Methods: The present study was conducted to investigate the prevalence of cutaneous Leishmaniasis and to identify *Leishmania* parasites by using polymerase chain reaction (PCR) in some endemic areas of Iraq. A total of 117 samples of patients with suspected cutaneous Leishmaniasis were collected in different age groups. And both sexes 73 male and 44 female patients.

Results: PCR results showed the percentage of infections 62.39% of males while 37.60% of females. The average age was 23.35 years (the range, from 1- 60 years), with the highest percentage of cases in the age group 1-4 years and the lowest rate in the age group (40-60 years). The highest infection was by *L.tropica* of *L.major* and lowest infection caused by *L.aethiopica*, where is considered first revealed in Iraq.

Conclusion: The study found that males were more likely to be infected than females. The study revealed that polymerase chain reaction (PCR) is the most effective and sensitive method for detecting types of Cutaneous Leishmaniasis.

Introduction

Leishmaniasis of the most important vector-borne diseases of humans, which is due to many species of *Leishmania*, mostly zoonotic. Cutaneous Leishmaniasis (CL) is a widespread parasitic disease that can causes serious health problems in the communities throughout the Mediterranean regions and the Middle East, including Iraq [1].

CL has been growing worldwide in both incidence and range, mainly due to the increased human migration. This movement contributes to Leishmanial infections in low or nonendemic areas. The importance of studying the genetic variation of *Leishmania* is mainly attributed to its binding with the epidemiological aspects of the disease, such as geographical location, clinical forms, pathogenecity, virulence, antigenic variation and drug resistance, among others [2].

This parasite is transmitted by females from two genera of sand fly, *Phlebotomus* and *Lutzomyia*, which are of medical importance as the only quality vectors for *Leishmania* species that causing Leishmaniasis to humans [3].

As with many parasites, the classification of the genus *Leishmania* is very complex because the limitation and definition of species are difficult to identify. The exact identification of these species are clinically important and epidemiologically. *Leishamia* parasites have similar morphology and sometimes cause similar clinical manifestations[4].

CL caused by *L. tropica* usually appears as dry, small lesions, located mainly on the face, which after healing, leaving permanent scarring and the inability of a serious, while ulcer because of a *L. major* leads to the production of single or multiple "wet" sore [5].

The symptoms of Leishmaniasis can be range from moderate, self-healing cutaneous lesions to fatal visceral cases. The absence of a human vaccine, increasing resistance to the presently used drugs and their serious side effects, urge the need to research of Leishmaniasis [3].

CL due to *L. tropica* complex is present in many parts of Asia, Africa, Mediterranean Europe and the southern region of the former Soviet Union. Urban Cutaneous Leishmaniasis is thought to be an anthroponosis while the rural cutaneous Leishmaniasis is zoonosis with human infections that occur only sporadically. The reservoir hosts in *L. major* are rodents. *L.aethopica* is endemic in Ethiopia and Kenya. This disease is a zoonosis with rock and tree hyraxes serves as reservoir hosts. The vector for the old world cutaneous Leishmaniasis is the *Phlebotomus* sand fly. The epidemiology of Leishmaniases due to *L. tropica* has not been illustrate fully. The disease is often described as being urban and anthroponotic, and most infections occur in and around quite densely populated cities. However, the rarity of cases in some foci and sudden occurrence of small outbreaks of disease in semi-rural areas suggest that the disease may be zoonotic in some cases [6].

Diagnosis of CL is based on both clinical features (supported by epidemiologic data) and laboratory tests. Several diagnostic methods have been described with significant differences in diagnostic accuracy, including parasitological direct assay (microscopy, anatomy, parasite culture) and / or indirect test with serological and molecular diagnostics [7].

Modern molecular characterization techniques have used polymerase chain reaction (PCR) to amplify parasite DNA from host tissues. PCR-based methods, have been demonstrated as a powerful tool for directly detecting Leishmaniain clinical specimens as well as for characterization of parasites that are highly sensitive and specific compared to conventional methods and provide results in one or two working days. With advances in molecular techniques, a number of molecular markers and protocols have been reported to detect or identify Leishmaniasis at different taxonomic levels (genus, complex, and species) has been reported [1].

PCR is a commonly used technique for typing and pathogen detection, has already been taken to use for *Leishmania* spp. The materials and biopsy Skin obtained by skin scrape have been used for PCR in different studies [8].

There are several methods of laboratory diagnosis of Leishmaniasis including parasitological, molecular, immunological and by using experimental animals [9].

The aim of the study to diagnose the pathogen, epidemiology as a first study, is advanced in our country and for the purpose of providing means to reduce infection with this disease.

Materials and methods

The samples collected from patients attended the dermatology consultant unit of Baghdad, Tikrit, Sammaraa, Aldhuluiya, Balad and Aldujail in Iraq from October 2015 to May 2016. A total number of patients infected with *Leishmania* were 117 samples. These patients were complained from skin lesions in the body. The lesions diagnosed clinically by special dermatologist as the cutaneous Leishmaniasis. The lesion were cleansed with topical antiseptic and take the sample from the nodular part or raised edge of the lesion. Scraping may be performed either from the surface of an ulcer or from skin slits. Ulcer scraping should be taken from both the edges and the center of the lesion. Slit skin scraping should be taken from the nodular part or raised edge of lesion [10].

These samples were kept deep freeze in -20°C.

DNA extraction:

By using the **MagaZorb® DNA Mini-Prep Kit** (promega, USA), DNA was extracted from skin scrapings according to the manufacturer instruction. Where 117 samples were used, these samples were kept deep freeze in -20° until use.

The PCR amplification:

The PCR was performed on 117 swabs samples. The PCR used to amplify the variable area of the minicircle kinetoplastid DNA of *Leishmania* species in the samples. The primers (*L. major*, *L. tropica*, and *L. aethiopica*) are forward MATRAE2 (5'-GGCGATGGTGGAGCAGATGATCT-3') and (*L. tropica*) reverse Tr4.1 (5'- CTCCCCCTTCGGAT-3'), (*L .major*) reverse Ma4.1 (5'-CGGTTCTCGTAGCACACTTGTTG-3'), and (*L. aethiopica*) reverse Ae2.1 (5'-AGTACGTGCACATCAGCACATGGG-3') according to Laurent et al. 2009 [11].

Species-specific *L. major*, *L. tropica*, and *L. aethiopica* PCRs were run in 5 µl of template DNA were amplified in 15 µl Green Master Mix (promega, USA) with 4 mmol/L Mgcl2, 4 µl Nuclease-Free Water and 1 µmol/L of each primer, concentration of DNA 50-150 ng and purification 1.8-2. DNA was amplified using thermal cycler (T-professivnal-Biometra- Germany) Cycling was done as follows: initial denaturation for 5 min at 95 C° followed by 40 cycles consisting of 30 sec at 95C°, 30 sec at 57 C°, and 30 sec at 72 C° and finally, Final Extension of 5 min at 72 C°.

Agarose gel electrophoresis protocol

Dilute 2.5% of Agarose in 1x TBE (Tris- Borate-EDTA) pH 8.3 by using 0.05% red safe (instead of Ethedium bromide to avoid carsinogenic side effect), electrophoresis clone under 100 Volts for 90 mins, visualization of band done by using Gel-Documentation [12].

RESULTS

A total of 117 samples of skin scarping / swabs were collected from the clinical patients in Baghdad, Tikrit, Sammaraa, Aldhuluiya, Balad and Aldujail (Table 1).

Under sterile conditions and examined through polymerease chain reaction (PCR), among these 62.39% were male and 37.60% were female.

The median age was 23.35 years (the range, 1 year - 60 years), Where highest percentage of cases to the age group 1-40 years old and less infection rate to the age group (40-60 years).

The present study reported the diagnosis of three species of *Leishmania* by using PCR method, as in Fig (1), which include *L. tropica* (100 bp) , *L. major* (99 bp) and *L. aethiopica* (154 bp). Which is the first appearance in Iraq for *L. aethiopica*.

All patients enrolled in this study had characteristic signs and symptoms of CL, from small erythematous papules to nodules and ulcerative lesions.

Table 1: Percentage of Infection with Cutaneous Leishmaniasis According to Location, The Sexes and the Age Groups.

Location		Aldhuluiya	Balad	Aldujail	Tikrit	Sammarra	Baghdad	total	Percentage
No. sample & percentage		42 (35.89%)	24 (20.51%)	11 (9.40%)	15 (12.82)	10 (8.54%)	15 (12.82%)	117	100%
Male		27	13	7	11	6	9	73	62.39%
Female		15	11	4	4	4	6	44	37.60%
Age groups	1-10	3	11	2	5	-	3	24	20.51%
	11-20	13	7	3	1	7	1	32	27.35%
	21-30	11	4	-	5	2	7	29	24.78%
	31-40	8	-	6	3	-	4	21	17.94%
	41-50	5	2	-	-	-	-	7	5.98%
	51-60	2	-	-	1	1	-	4	3.41%

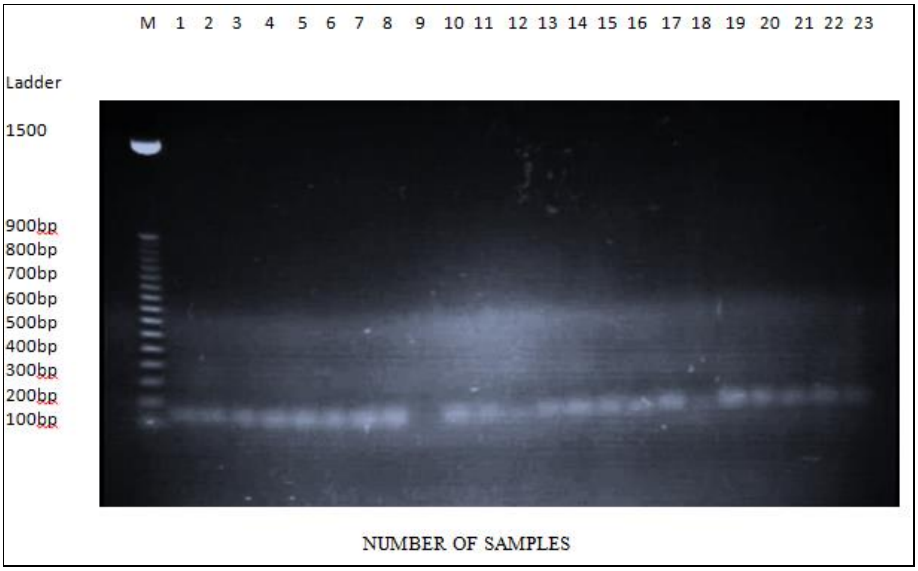


Figure (1): Identification of *Leishmania* spp. isolated of skin biopsies by conventional PCR based on specific primers for conserved sequences of DNA. Shown on 2.5% agarose gel electrophoresis stained with red safe , electrophoresis clone under 100 Volts for 90 mins, correspond to molecular weight marker, M: ladder marker (100-1500 bp), line (9, 12, 18) are negative, and Line (1,2,3,4) are positive of *L. major* (99 bp) and line (5,6,7,8,16,17,19,20,21,22,23) are *L. tropica* (100 bp) and line (10,11,13,14,15) are *L.aethiopica* (154pb)

Discussion

Cutaneous Leishmaniasis (CL) appears to be a major public health problem and endemic in Iraq, Kuwait, Iran, Afghanistan, and other places in the Middle East [13].

The present study is performed in Salah Aldeen province and Baghdad in Iraq, Where it had the highest rate of infection appeared in Aldhuluiya while lowest rate infection was in Sammaraa (Table 1), the reason might that Aldhuluiya have massive population and increased the numbers of the vectors due to found suitable condition.

In the present study was among 62.39% were male and 37.60% were female, because of the nature of the male work abroad unlike females, which resides most of the time at home and this will be the incidence in males larger.

According to Jamal et al. 2013, the percentage was significantly higher among males (70.58%) than females (29.42%). One possible cause of high male incidence may be their tendency to take off shirts while sleeping during the hot summer, which may make them more susceptible to sand fly bites. On the other hand, females are cold and keep themselves covered in a sheet that is thought to give them protection from sand fly bites [14].

In the present study, the infection were observed among all age groups. The average age was 23.35

years (the range, 1 - 60 years), with the highest rate of cases in the age group (1-40) years old and lowest infection rate in the age group (40-60 years). Due largely to the presence of this age group in the places that allow the transmission of this disease easily among people, such as schools and children's play area and the workplace for employees.

The current study has been recorded the infection caused by *L.tropica*, *L. major* and *L. aethiopica*. Several studies have shown that differences between the agents of CL in old world (*L.major* & *L.tropica*) may be related to various factors, like biological and morphological characteristics of the parasite.

In this study, *L. aethiopica* was first recorded in Iraq.

The diagnosis of CL is traditionally based on microscopic demonstration of *Leishmania* in swabs or scraped. However, this method usually lead to low sensitivity, and in atypical forms, CL may be ignored due to of similarity with other dermal diseases. Thus, it is necessary to apply specific and sensitive diagnostic methods as PCR technique [15]. In the present study, we investigated the benefits of PCR as a tool for the detection of *Leishmania* parasites in lesion aspirate specimens [16].

Diagnosis of CL although is clinically experienced of practitioner obvious in an endemic area, confirmed by using different techniques. In most CL-endemic countries, diagnosis is mainly based on epidemiological and clinical criteria, the existence of single or multiple nodular/ ulcerative skin lesions can be considered an indicator of Cutaneous Leishmaniasis causes skin sores depending on the parasite species, which can be topical in a particular place on the body (mostly on the face or limbs) or spread [17].

The best advantages of PCR-based methods are the high ability to detect the low amount of *Leishmania* DNA in a variety of clinical specimens, such as blood, skin biopsy, bone marrow puncture, or samples prepared from Giemsa-stained slides even in paraffin block. Proper diagnosis and identification of the *Leishmania* species is important for assess prognosis and appropriate treatment description [18] [19].

Cutaneous Leishmaniasis is increased in endemic and non-endemic areas due to the geographical expansion of the disease population migrations; and changing of international travel patterns [20].

The diagnostic procedure is the only remedy to determine the *Leishmania* species in hospital as well as at the community level in epidemic areas, similarity signs have occurred due to other parasites, and can cause the confusion in the diagnosis of species identification. [21].

References

[1] Al-Heany, A. R.; Sharquie, K. E.; Al-Najar, S.A.; Noaimi, A. A. (2014). Cutaneous Leishmaniasis: Comparative Techniques for Diagnosis. *IOSR J. of Dent. & Med. Sci.*; 13(4): 33- 37. Gontijo, B.; de Carvalho, M. (2003). De L. Leishmaniose tegumentar Americana. *Rev. Soc. Bras. Med. Trop.*, 36: 71-80.

[2] Dostálová, A. & Volf, P. (2012). *Leishmania* development in sand flies:parasite- vector interactions overview. Parasites & Vectors.5- 8-2.

[3] Mahmoodi, M. R.; Mohajery, M.; Afshari, J. T.; Shakeri, M. T.; Panah, M. J. Y.; Berenji, F.; Fata, A. (2010). Molecular identification of *Leishmania* species causing cutaneous Leishmaniasis in Mashhad, Iran. *JJM.*, 3(4): 195-200.

[3] Eddin, A. N.; Presber, H. W.; Warburg, A. Ignatius, R. (2010). Leishmania tropica: Molecular Epidemiology, Diagnosis and Development of an Axenic Amastigote Model. Ph.D.Universitatsmedizin Berlin.

[4] Schwenkenbecher, J. M.; Wirth, T.; Schnur, L. F.; Jaffe, C. L.; Schallig, H.; Al-Jawabreh, A.; Hamarsheh, O.; Azmi, K.; Pratlong, F.; Scho¨nian G. (2006).

[5] Goto, H., Lindoso, J. A.(2010). Current diagnosis and treatment of cutaneous and mucocutaneous Leishmaniasis. *Expert Rev Anti Infect Ther*.; 8(4): 419–33.

[6] Eslami, G.; Anvari, H.; Ebadi, M.; Nabipour. Y.; Mirzaei, F. & Gholamrezaei, M. (2013). The changing profile of cutaneous Leishmaniasis agent in a central province of Iran. *Tanzania J. of Hlth. Res.*, 15(1): 8-1.

[7] Singh, S. (2006). New developments in diagnosis of Leishmaniasis. Indian J. Med. Res., 123: 311-330.

[8] Garcia, L. S. & Brukner, D. A. (2007). Diagnostic Medical Parasitology In: *American Society for Microbiology. Washington D. C.*, 2: 760- 64.

[9] Laurent, T.; Van der Auweraa, G.; Hidec, M.; Mertensb, P.; Quispe - Tintayaa, W.; Deborggraevea, S.; De Donckera, S.; Leclipteuxb, Th.; Bañulsc, A.L.; Büschera, Ph.; Dujardina, J.C. (2009). Identification of Old World *Leishmania* spp. by specific polymerase chain reaction amplification of cysteine proteinase B genes and rapid dipstick detection. *Diag. Microb. and Infect. Dis.*, 63 : 173–181.

[10] Sambrook, J.; Fritson, E. F. (1989). Molecular cloning: A laboratory mamual. 2nd edition.

[11] Rahi, A. A.; Hraiga, B A. & Hassoni, J. J. (2014). Some Epidemiological Aspects of Cutaneous Leishmaniasis in Kut city, Iraq. *Sch. J. App. Med. Sci.*, 2(1):451-455.

[12] Jamal, Q.; Shah, A.; Ali, N.; Ashraf, M.; Awan, M. M. & Lee, C. M. (2013). Prevalence and Comparative Analysis of Cutaneous Leishmaniasis in Dargia Region in Pskstan. *Pakstan J. Zool*., 45(2):. 537-541.

[13] Kazemi-Rad, E.; Mohebali, M.; Hajjaran, H.; Rezaei, S. & Mamishi, S. (2008). Diagnosis and Characterization of *Leishmania* Species in Giemsa-Stained slides by PCR-RFLP. *Iranian J. Publ. Health*, 37(1): 54-60.

[14] Qader, A. M.; Abood, M. K.; Bakir, T. Y. (2009). Identification of Leishmania Parasites in clinical samples obtained from cutaneous

Leishmaniasis patients using PCR technique in Iraq. Iraqi *J. of Sci.*, 50(1): 32-36.
[15] Rehman, A. U.; Rehman, H.U.r.; Ayaz, S.; Gul, H.; Tahseen, M.; Ishaq, M.; Ullah, S.; Shakir, S. U. & Jehan, S. (2016). Molecular detection of cutaneous Leishmaniasis in human at district Hangu Khyber Pakhtunkhwa, *Pakistan. Journal of Entomology and Zoology Studies*, 4(3): 94-97.
[16] Bensoussan, E.; Nasereddin, A.; Jonas, F.; Schnur, F. L.; Jaffe, L. C. (2006). Comparison of PCR assays for diagnosis of cutaneous leishmaniasis. *J. Clin. Microbiol*; 44:1435–1439.
[17] Blum, J.; et al. (2004). Treatment of cutaneous Leishmaniasis among travellers. *J. Antimicrob Chemother*, 53(2): 158-66.
[18] Khosravi, S.; Hejazi, S. H.; Hashemzadeh, M.; Eslami, G. & Darani, H. Y. (2012). Molecular diagnosis of Old World leishmaniasis: Realtime PCR based on tryparedoxin peroxidase gene for the detection and identification of *Leishmania* spp. *J. Vector Borne Dis.*, 49: 15–18.
[19] Kassi, M.; Afghan, A.K.; Rehman, R.; Kasi, P.M. (2008). Marring Leishmaniasis: the stigmatization and the impact of cutaneous Leishmaniasis in Pakistan and Afghanistan. *PLoS Negl. Trop. Dis.*, 2(10):259.

إستخدام ال PCR للكشف عن أنواع اللشمانيا الجلدية في محافظتي صلاح الدين وبغداد

ميساء ابراهيم الجبوري[1] , عبدالرحمن عزيز الطائي[2] ، محمد عبد الاخوة الفحام[3]

[1]قسم علوم الحياة , كلية العلوم , جامعة تكريت ، تكريت ، العراق

[2]قسم الاحياء المجهرية , كلية الطب , الجامعة العراقية ، بغداد ، العراق

[3]قسم الاحياء المجهرية , كلية الطب , جامعة بغداد ، بغداد ، العراق

الملخص

داء الليشمانيا هو مجموعة من الأمراض الطفيلية المنتشرة عالميا والناجمة عن أنواع مختلفة من الليشمانيا، والتي تصيب مجموعة متنوعة من الثدييات بالإضافة إلى الإنسان*L. tropica, L. major, L.aethiopica* هي المسببات الشائعة لداء الليشمانيا الجلدي في العراق.

المواد وطرائق العمل: اجريت الدراسة الحالية للتحقق من انتشار داء الليشمانيا الجلدي وتحديد طفيليات الليشمانيا باستخدام تفاعل PCR في بعض المناطق المستوطنة في العراق. بلغ مجموع العينات 117 عينة والتي تم جمعها من المرضى المشتبه بإصابتهم بالليشمانيا الجلدية بمختلف الاعمار ولكلا الجنسين وتشمل 73 مريضا من الذكور و 44 من الاناث.

النتائج: أظهرت نتائج PCR إن نسبة الاصابة 62.39٪ في الذكور في حين أن النسبة 37.60٪ في الإناث. وكان متوسط العمر 23.35 سنة (المدى، سنة واحدة إلى 60 سنة)، حيث إن أعلى نسبة إصابة في الفئة العمرية (1−40) سنة وأقل معدل إصابة في الفئة العمرية (40−60 سنة). في حين تحدث أعلى نسبة إصابة من قبل *L.tropica* وتليها *L.major* وانخفاض معدل الاصابة الناجمة عن *L.aethiopica*.

الاستنتاجات: واستنتج من العمل الحالي أن الذكور أكثر إصابة باللشمانيا الجلدية من الإناث. وكشفت الدراسة الحالية أن تفاعل PCR هو أكثر الطرق فعالية وحساسية للكشف عن أنواع داء الليشمانيا الجلدي.