

DOI: <http://dx.doi.org/10.21123/bsj.2021.18.1.0029>

## Molecular Typing of Two Suspected Cutaneous Leishmaniasis Isolates in Baghdad

Duha M. Bayram<sup>1</sup>

Hayder Z. Ali<sup>2\*</sup>

<sup>1</sup>Department of Medical Laboratory Techniques, Al-Esraa University College, Baghdad, Iraq.

<sup>2</sup>Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

\* Corresponding author: [hayder.zuhair@sc.uobaghdad.edu.iq](mailto:hayder.zuhair@sc.uobaghdad.edu.iq), [duha.bayram@yahoo.com](mailto:duha.bayram@yahoo.com)

\* ORCID ID: <https://orcid.org/0000-0001-7461-7204>, <https://orcid.org/0000-0002-9265-8971>

Received 26/11/2019, Accepted 24/2/2020, Published Online First 6/12/2020, Published 1/3/2021



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

### Abstract:

Leishmaniasis is a group of parasitic diseases caused by *Leishmania* spp., an endemic infectious agent in developing countries, including Iraq. Diagnosis of cutaneous lesion by stained smears, serology or histopathology are inaccurate and unable to detect the species of *Leishmania*. Here, two molecular typing methods were examined to identify the promastigotes of suspected cutaneous leishmaniasis samples, on a species level. The first was species-specific B6-PCR and the second was ITS1-PCR followed by restriction fragment length polymorphism (RFLP) using restriction enzyme HaeIII. DNA was extracted from *in vitro* promastigote culture followed by amplification of kDNA by B6 or amplification and digestion of LITSR/L5.8S. PCR produced bands of ~359 bp and ~450 bp for B6 and ITS1, respectively. Digestion of ITS1 by RFLP revealed two distinct bands of ~150 bp and ~300 bp size. The results revealed that the two isolates belong to cutaneous Leishmaniasis, specifically *Leishmania tropica*. In conclusion, the confirmation of the studied methods will improve rapid and accurate diagnosis of *Leishmania* species of the most prevalent Iraqi strain of cutaneous leishmaniasis, *L. tropica*.

**Key words:** B6-PCR, Cutaneous leishmaniasis, Promastigotes, TS-1-PCR-RFLP.

### Introduction:

Leishmaniasis is one of the neglected diseases caused by infection with protozoan parasites belong to a member of *Leishmania* species (1). Cutaneous leishmaniasis is caused by numerous species of *Leishmania* and are able to cause human leishmaniasis counting at least twenty-one species and subspecies (2). Leishmaniasis is prevalent in tropical and subtropical countries; the disease affect about twelve million people with three hundred and fifty million people at infection risk and an estimated yearly incidence of 2 million cases (3). In Iraq, the cutaneous form is predominant and endemic; the main types responsible of ulcer formation are the anthroponotic *L. tropica* and zoonotic *L. major* (4). Visceral leishmaniasis is found but in less incidence than cutaneous, the confirmed species of visceral leishmaniasis are *L. donovani* and *L. infantum* (5). Total reported cases of leishmaniasis infections from 2008 to 2015 in Iraq were 17001 as mean of 2.9-10.5 per 100,000 individuals were officially reported by WHO, the highest occurrence cases

were reported in 2015 (4000 cases) (6). Furthermore, regional crisis resulted in outbreaks of cutaneous leishmaniasis in areas with no history of leishmaniasis and the vector-borne threat spread from endemic to non-endemic areas (7). Recent official data have declared the increasing incidence of cutaneous leishmaniasis in Baghdad and other governorates, such as Al-Khalis district/ Diyala provenance with more than 50 cases of Baghdad boil were recorded in the first two weeks of 2020 (8).

Epidemiologically, the diagnosis of cutaneous leishmaniasis, in Baghdad hospitals and suburban endemic areas generally relies on clinical presentation, microscopic examination in stained smears, rapid agglutination test and parasite culture due to the lack of developed diagnostic tools, which in most cases, cannot identify the causative species (9,10,11). Furthermore, the skin lesion appearance is similar by different species of *Leishmania* and sometimes it is problematic to be characterized from other skin infections (12).

In recent years, the polymerase chain reaction and molecular tests have developed as advanced sensitive tools rather than classical diagnosis, although, the molecular-based assays demonstrate variable outcomes depending on the target sequence determined (13, 14). Different *Leishmania* species were submitted to several molecular targets which have been evaluated for PCR application including minicircle kinetoplast DNA which is abundant in many copies per parasite cell (15,16), the miniexon gene (17), the gp63 (18), and the internal transcribed spacer (ITS-1) (19). However, few molecular and phylogenetic studies were conducted in Iraq for genotyping the local *Leishmania* strains. Moreover, new surveys confirmed the transition of foreign cutaneous *Leishmania* strains from the northern borders, in which several cases were identified as Iranian strain of *L. major* for the first time in Garmian, southeast of Kurdistan, North of Iraq; this indicates the entry of sand flies that carry this specific strain from Iran (20).

In the present study, local clinical samples were investigated by two species-specific diagnostic assays including B6-PCR amplification and ITS1-PCR-RFLP for cutaneous leishmaniasis molecular identification of parasite species as described by Altamemy (21) Kermanjani *et al.* (22) and Schönian *et al.* (23). The studied methods demonstrated rapid and sensitive assays for typing of Iraqi isolates of *Leishmania* on a species level.

## Materials and Methods:

### Collections and cultivation of isolates:

Two suspected cutaneous leishmaniasis samples were kindly provided at the Department of Biology, College of Science, University of Baghdad. The samples were previously isolated from skin ulcers of two patients attended AL-Karama Teaching hospitals in Baghdad and confirmed as cutaneous leishmaniasis by clinical presentation according to the dermatologist. Procyclic promastigotes were cultured in M199 medium supplemented with 10% heated inactivated foetal bovine serum and 1% Penicillin/Streptomycin then incubated at 26°C for 3 days to maintain log-phase harvest culture (24,25).

### DNA extraction:

DNA was extracted from log-phase of axenic promastigote culture using QIAamp DNA Mini Kit (Qiagen, USA) according to the manufacturer's protocol. Extracted DNA was stored at -20°C until polymerase chain reaction amplification.

### Species-specific primer B6:

Primer pair B6 (alpha DNA, Canada) was used for detection of kDNA by PCR, as a described by (26), which is specific for *L. tropica*. B6-Forward- (GCTCTGCCACGCACACACAG) and B6-Reverse- (CGGTGCCTGCCAAGTA). Amplification reaction was carried out in 25 µl using GoTaq® Green Master Mix (Promega, USA). Thermal Cycler program was: 1 cycle of initial denaturation at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 10 minutes.

### ITS-1-PCR-RFLP:

#### - Genus-specific PCR primer:

In order to detect *Leishmania* as a genus, the ITS1 region was amplified by LITSR/L5.8S (alpha DNA, Canada) according to (23), ITS1-forward (CTGGATCATTTTCCGATG) and ITS1-Reverse (TGATACCACTTATCGCACTT).

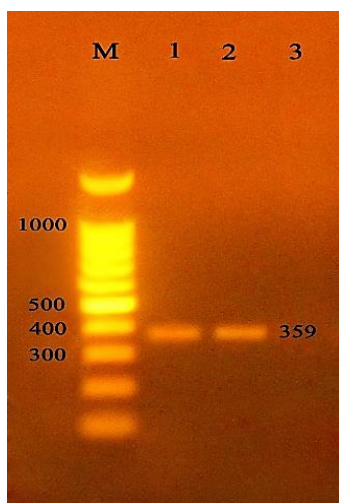
Amplification reactions were carried out in 25 µl using GoTaq® Green Master Mix (Promega, USA) according to the manufacturer's protocol. Amplification was accomplished in a thermal cycler (Eppendorf®) programmed as following: 1 cycle of an initial denaturation for 2 minute at 94°C followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 63°C and 55°C for B6 and ITS1 respectively for 1 minute, and extension at 72°C for 2 minute. At the end 1 cycle of final extension for 3 minutes at 72°C.

#### - Restriction enzyme digestion:

The amplicons of ITS1 were digested with *Hae*III enzyme (Promega, USA) according to manufacturer's protocol, 1µl (10U) of enzyme was added to 20 µl (100 ng) of PCR products and incubated at 37°C for 2 hours, according to the manufacturer's protocol and (27).

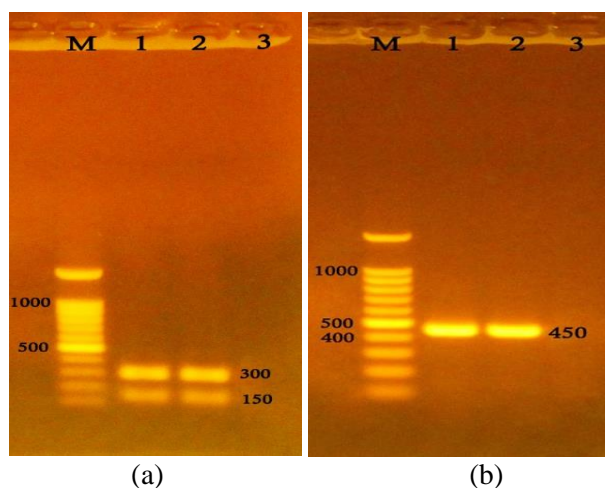
## Results and discussion:

**Species-specific B6-PCR:** This amplification of B6 primer pair was used to detect *Leishmania* sub-genus (species) of *L. tropica* in other parts of the world, k-DNA of ~359 bp was observed for both Iraqi isolates as shown in Fig.1.



**Figure 1.** Detection of cutaneous *Leishmania* isolates from culture promastigotes on 1% agarose gel electrophoresis of B6-F/B6-R, M: 100bp ladder, lane 1: first isolate, lane 2: second isolate, lane 3: negative control.

**PCR RFLP of ITS1:** Results of PCR amplification using the primer pair LITSR/L5.8S, both isolates produced a band of ~450 bp, which confirmed the presence of *Leishmania* as a genus (Fig.2a). Subsequently digestion of LITSR/L5.8S PCR products with *HaeIII* for *Leishmania* species identification, agarose gel electrophoresis yielded two distinct bands of ~300 bp and ~150 bp (Fig.2b).



**Figure 2.** Detection of *Leishmania tropica* from promastigotes culture on 1% agarose gel electrophoresis of (a) LITSR/L5.8S primer pair, (b) Digesting of ITS1 with *HaeIII* restriction enzyme profile of two *Leishmania* isolates. M: 100bp ladder, lane-1: first isolate, lane-2: second isolate, lane-3: negative control.

The development of sensitive molecular test for *Leishmania* species molecular identification in endemic areas is significant because of the existence of several *Leishmania* species with similar

clinical presentation and geographical distribution (10). Furthermore, correct diagnosis is vital, in order to prevent the progress of human leishmaniasis and to select the appropriate treatment (28). Species identification of leishmaniasis agent in Iraq is still challenging, because the country contains many areas with different environmental and ecological status; furthermore, the presence of multiple *Leishmania* sub-types of cutaneous and visceral forms including *L. tropica*, *L. major*, *L. infantum* and *L. donovani* (29,30).

Primer pair B6-plan represents an important step towards fast, affordable, and reliable diagnostics of *L. tropica*, since it is based on direct PCR amplification of a species-specific DNA fragment and can distinguish this species from other causative agents of old and new cutaneous leishmaniasis agents (26). A similar study proved that B6 specific primer verified its sensitivity to amplify the 359 bp of *L. tropica* isolates originating from diverse geographical areas with nil PCR results of 12 other cutaneous species of old and new leishmaniasis (31).

A worldwide PCR technique targeting the internal transcribed spacer 1 region was designated for identification of various clinical infections of leishmaniasis and verified the ability of parasite identification on a genus level (32). A previous study used the same primer pair to detect *Leishmania* in different biological materials of human with leishmaniasis and proved that this primer is highly species-specific and sensitive detecting around 0.2 parasites per sample (23). In this study, RFLP of the ITS1-PCR product revealed that the isolates belonged to *Leishmania tropica* because this species contains only one restriction site for *HaeIII* enzyme, which is (GG/CC), so only two bands appeared with distinct *L. tropica* standard strain base pair sizes, ~300 and ~150 bp. In contrast, *L. major* has difference sizes (~203 bp and ~132 bp). Furthermore, in case of visceral leishmaniasis *L. donovani* and *L. infantum*, both have two restriction sites, therefore, three bands should appear with different sizes (164, 75, and 54 bp) or (184, 72, and 55 bp) respectively (33).

ITS1 PCR/RFLP also can be used in a variety of sample type including direct species identification using Giemsa-stained smears or other samples without prior parasite culturing, microscopic analysis or other techniques, yet, RFLP analysis should be carefully organized to avoid cross-contamination (13, 34, 35). This method has previously been reported for species-specific identification of Old World species and New World leishmaniasis (23, 34, 36). The main feature of ITS1 digestion is that species characterization can

be accomplished by digesting the PCR product with just one restriction enzyme and this one-step typing can be used to detect the sub-genus of medically important isolates (36). Moreover, recent laboratory assessment proved that PCR-RFLP is more applicable with less complexity than multi-locus enzyme electrophoresis and less expensive from the time consuming Isoenzyme technique; it is also more accurate and sensitive than routine microscopic or Giemsa staining diagnostic methods (37, 38). Similar applications of RFLP-PCR assay is recommended for other *Leishmania* isolates from all parts of Iraqi provinces, for the detection of another local *Leishmania* species. This is to be examined for diagnosis, prevalent and epidemiology purposes.

### Conclusion:

This is one of very first trials of molecular typing of *Leishmania* in Iraq, B6-PCR and/or LITSR/L5.8S – PCR in combination with RFLP technique using *HaeIII* enzyme is a rapid and accurate method using only one primer pair for the detection and identification of Iraqi strain of cutaneous leishmaniasis, specifically *L. tropica*. It is recommended for further application of the above techniques on other isolates from different provinces in the country.

### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

### References:

1. Prajapati VK, Pandey RK. Recent Advances in the Chemotherapy of Visceral Leishmaniasis Drug Design: Principles and Applications. Springer Press. 2017. p 69-88.
2. Omidian M, Khosravi AD, Nazari M, Rashidi A. The comparison of histopathological findings and polymerase chain reaction in lesions with primary clinical diagnosis of cutaneous leishmaniasis with negative smear. Pak J Med Sci. 2008; 24(1):96.
3. Real F, Vidal RO, Carazzolle MF, Mondego JM, Costa GG, Herai RH, et al. The genome sequence of *Leishmania (Leishmania) amazonensis*: functional annotation and extended analysis of gene models. DNA Res. 2013; 20(6):567-81.
4. Al-Warid HS, Al-Saqur IM, Al-Tuwaijari SB, Zadawi KAM. The distribution of cutaneous leishmaniasis in Iraq: demographic and climate aspects. Asian Biomed. 2017; 11(3): 255-260.
5. Majeed B, Sobel J, Nawar A, Badri S, Muslim H. The persisting Burden of visceral leishmaniasis in Iraq: data of the national surveillance system, 1990-2009. Epidemiol Infect. 2013; 141(2): 443-446.
6. Al-Obaidi MJ, Al-Hussein MYA, Al-Saqur IM. Survey Study on the Prevalence of Cutaneous Leishmaniasis in Iraq. IJS. 2016; 57(3C):2181-2187.
7. Salloum T, Khalifeh I, Tokajian S. Detection, molecular typing and phylogenetic analysis of *Leishmania* isolated from cases of leishmaniasis among Syrian refugees in Lebanon. Parasite Epidemiol Control. 2016; 1(2): 159-168.
8. <https://promedmail.org/promed-post/?id=20200110.6882795> (International Society for Infectious Diseases, 2020).
9. Magill AJ. Cutaneous leishmaniasis in the returning traveler. Infect Dis Clin. 2005; 19(1):241-266.
10. Resen J, Al-Autabbi Z. Lymphocytes Subset Phenotypes in Patients with Visceral Leishmaniasis. Iraqi J Comm Med. 2011; 24(4):308-313.
11. AlSamarai AM, AlObaidi HS. Cutaneous leishmaniasis in Iraq. J Infect Develop Cntris. 2009; 3(2): 123-9.
12. Mirahmadi H, Rezaee N, Mehravaran A, Heydarian P, Raeghi S. Detection of species and molecular typing of *Leishmania* in suspected patients by targeting cytochrome b gene in Zahedan, southeast of Iran. Vet World. 2017; 11(5): 700-705.
13. Monroy-Ostria A, Nasereddin A, Monteon VM, Guzmán-Bracho C, Jaffe CL. ITS1 PCR-RFLP diagnosis and characterization of *Leishmania* in clinical samples and strains from cases of human cutaneous leishmaniasis in states of the Mexican Southeast. Interdiscip Perspect Infect Dis. 2014; 2014:607287.
14. Cruz ML, Perez A, Dominguez M, Moreno I, Garcia N, Martinez I, et al. Assessment of sensitivity and specificity of serological (IFAT) and molecular (direct-PCR) techniques for diagnosis of leishmaniasis in lagomorphs using Bayesian approach. J Vit Med Sci. 2016; 2:211-220.
15. Satow MM, Yamashiro-Kanashiro EH, Rocha MC, Oyafuso LK, Soler RC, Cotrim PC, et al. Applicability of kDNA-PCR for routine diagnosis of American tegumentary leishmaniasis in a tertiary reference hospital. Rev Inst Med Trop Sao Paulo. 2013; 55(6):393-399.
16. Galluzzi L, Ceccarelli M, Diotallevi A, Menotta M, Magnani M. Real-Time PCR applications for diagnosis of *Leishmania*. Parasit Vectors. 2018; 11(1):273-286.
17. Mauricio I, Stothard J, Miles M. *Leishmania donovani* complex: genotyping with the ribosomal internal transcribed spacer and the mini-exon. Parasitology. 2004; 128(3):263-267.
18. Victoire K, De Doncker S, Cabrera L, Alvarez E, Arevalo J, Llanos-Cuentas A, et al. Direct identification of *Leishmania* species in biopsies from

- patients with American tegumentary leishmaniasis. *Trans R Soc Trop Med Hyg.* 2003; 97(1):80-87.
19. Dávila A, Momen H. Internal-transcribed-spacer (ITS) sequences used to explore phylogenetic relationships within *Leishmania*. *Ann Trop Med Parasitol.* 2000; 94(6):651-654.
  20. Al-Bajalan MMM, Al-Jaf SM, Nijrani SS, Abdulkareem DR, Al-Kayali KK, Kato H. An outbreak of *Leishmania major* from an endemic to non endemic region posed a public health threat in Iraq from 2014-2017; epidemiological, molecular and phylogenetic studies. *PLOS Negl Trop Dis.* 2018; 12(3): 6255.
  21. Altamemy AKA. Direct diagnosis of cutaneous leishmaniasis of skin lesion specimens by PCR and evaluate the sensitivity of testing methods in Wasit Province. *JCE/ Was.* 2015; 1(20):543-558.
  22. Kermanjani A, Akhlaghi L, Oormazdi H, Hadighi R. Isolation and identification of cutaneous leishmaniasis species by PCR-RFLP in Ilam province, the west of Iran. *J Parasit Dis.* 2017; 41(1):175-179.
  23. Schönian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HDFH, Presber W, et al. PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. *Diagn Microbiol Infect Dis.* 2003; 47(1):349-358.
  24. Kamil MM, Ali HZ. Using PCR for detection of cutaneous leishmaniasis in Baghdad. *IJS.* 2016; 57(2B):1125-1130.
  25. Li J, Zheng Z-W, Natarajan G, Chen Q-W, Chen D-L, Chen J-P. The first successful report of the in vitro life cycle of Chinese *Leishmania*: the in vitro conversion of *Leishmania* amastigotes has been raised to 94% by testing 216 culture medium compound. *Acta Parasitol.* 2017; 62(1):154-163.
  26. Jirků M, Zemanová E, Al-Jawabreh A, Schönian G, Lukeš J. Development of a direct species-specific PCR assay for differential diagnosis of *Leishmania tropica*. *Diagn Microbiol Infect Dis.* 2006; 55(1):75-79.
  27. Miranda-Ortiz H, Fernandez-Lopez JC, Becker I, Rangel-Escareno. Down regulation of TLR and JAK/STAT pathway genes in association with diffuse cutaneous leishmaniasis: a gene expression analysis in NK cells from patients infected with *Leishmania meicana*. *PLOS Negl Trop Dis.* 2016; 10(3): 4570.
  28. Ben Abda I, De Monbrison F, Bousslimi N, Aoun K, Bouratbine A, Picot S. Advantages and limits of real-time PCR assay and PCR-restriction fragment length polymorphism for the identification of cutaneous *Leishmania* species in Tunisia. *Trans R Soc Trop Med Hyg.* 2011; 105(1):17-22.
  29. Rahi AA. A Cloned Antigen (Recombinant K39) of *Leishmania donovani* Diagnostic for Visceral Leishmaniasis in Human Wasit J S S. 2010; 3(1):12-18.
  30. Mahmood TA, Al-Dhalimi MA, Sultan BA, AL-Hucheimi SN. Tracking of Cutaneous Leishmaniasis by Parasitological, Molecular and Biochemical Analysis. *J N S.* 2015;5(1):65-74.
  31. Schönian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HD, Presber W, Jaffe CL. PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. *Diagn Microbiol Infect Dis.* 2003; 47: 149-358.
  32. Roelfsema JH, Nozari N, Herremans T, Kortbeek LM, Pinelli E. Evaluation and improvement of two PCR targets in molecular typing of clinical samples of *Leishmania* patients. *Exp Parasitol.* 2011; 127(1):36-41.
  33. El Tai NO, Osman OF, El Fari M, Presber W, Schönian G. Genetic heterogeneity of ribosomal internal transcribed spacer in clinical samples of *Leishmania donovani* spotted on filter paper as revealed by single-strand conformation polymorphisms and sequencing. *Trans R Soc Trop Med Hyg.* 2000; 94(5):575-579.
  34. Koarashi Y, Cáceres AG, Saca FMZ, Flores EEP, Trujillo AC, Alvares JLA, et al. *Identification of causative Leishmania species in Giemsa-stained smears prepared from patients with cutaneous leishmaniasis in Peru using PCR-RFLP.* *Acta Trop.* 2016; 158:83-87.
  35. Sagi O, Berkowitz A, Codish S, Novack V, Rashti A, Akad F, et al. Sensitive molecular diagnostics for cutaneous leishmaniasis. *Open Forum Infect Dis.* 2017; 4(2): ofx037.
  36. El-Badry AA, El-Dwibe H, Basyoni MM, Al-Antably AS, Al-Bashier WA. Molecular prevalence and estimated risk of cutaneous leishmaniasis in Libya. *J Microbiol Immunol Infect.* 2016; 50(6): 505-810.
  37. Ovalle-Bracho C, Camargo C, Diaz-Toro Y, Parra-Munoz M. Molecular typing of *Leishmania* (*Leishmania*) amazonensis and species of the subgenus *Vianna* associated with cutaneous and mucosal leishmaniasis in Colombia: A concordance study. *Biomedica.* 2018; 38(1): 86-95.
  38. Hijjawi N, Kanani KA, Rasheed M, Atoum M. Molecular diagnosis and identification of *Leishmania* species in Jordan from saved dry samples. *Biomed Res Int.* 2016; 2016: 6871739.

## التنميط الجزيئي لاثنتين من عزلات اللشمانيا الجلدية المشتبه بهما في بغداد

حيدر زهير علي<sup>2</sup>

ضحى مهند بيرم<sup>1</sup>

<sup>1</sup> قسم تقنيات المختبرات الطبية، جامعة الاسراء، بغداد، العراق

<sup>2</sup> قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

### الخلاصة:

يعتبر داء الليشمانيا من الأمراض الطفيلية التي يسببها طفيلي الليشمانيا ، وهو مرض معدي متوطن في العراق. التشخيص بالطرق التقليدية غير دقيق وغير قادر على تشخيص طفيلي الليشمانيا على مستوى النوع. في هذه الدراسة تم التشخيص بطريقتين جزيئية بالتنميط الجزيئي للاطوار المسوطة لداء الليشمانيا الجلدي ، على مستوى النوع. الأول كان تفاعل البلمرة المتسلسل (PCR) للبادئ B6 والثاني ITS1-PCR متبوعاً بتعدد الأشكال المقيدة لطول الجزء باستخدام إنزيم التقيد *HaeIII*. تم استخلاص الحمض النووي من الاطوار المسوطة خارج الجسم الحي متنوعة بتضخيم kDNA والمنطقة ITS1 باستخدام زوج البادئ B6 و LITSR / L5.8S قبل هضم ناتج تضخيم تفاعل البلمرة ITS1. نواتج تضخيم تفاعل البلمرة كانت 359bp و 450bp لـ B6 و ITS1 ، على التوالي. كشف ITS1-RFLP عن حزميتين متميزتين يتراوح حجمهما بين 150 bp و 300 bp، عل التوالي. أثبتت هذه النتائج أن العزلتين تعودان إلى داء الليشمانيا الجلدي ، تحديدا النوع الاستوائي. نوصي بالطريقتين الجزيئيتين للفحص المباشر للعينات المأخوذة من قرحة الجلد للمرضى الذين يشتبه في إصابتهم بداء الليشمانيا الجلدي للتشخيص الحساس والسريع لأكثر السلالات العراقية انتشاراً من داء الليشمانيا الجلدي وهي *Leishmania tropica*.

الكلمات المفتاحية: داء الليشمانيا الجلدي ، B6-PCR ، Promastigotes ، TS-1-PCR-RFLP.