**RESEARCH ARTICLE**

# The Genetic sequence of *Leishmania tropica* among Patients with Cuotaneous leishmaniasis in Iraqi Patients

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**ABSTRACT**

The present study was carried out on a period from 15st April 2017 to 30th Mars 2018, 150 patients; their ages ranged from 5–74 years. They were 55 females and 95 males who were inhabitants of the urban and rural areas attending to dermatology department of hospitals at Baghdad city (AL-Yarmook Hospital, AL-Karamaa Hospital and AL-Karkh Teaching Hospitals). Patients of migratory camps in and around Baghdad city Diyala, Tikrit and Kirkuk Hospitals, suffering from skin lesions either single or multiple, were located on different parts of the body and were clinically diagnosed with cutaneous leishmaniasis. Results showed that samples in this study included 95 males (63.3%) and 55 females (36.7%), their age of all individuals ranged from 5 to 74 years with a median age of 32.9 ± 15.14 years. Moreover that and were 60 (40%) were urban, while 90 (60%) were rural residents. Forty-two (35%) patients had a single lesion, while 78 (65%) patients had multiple lesions demonstrated single and multiple lesions of cutaneous leishmaniasis for some patients. The gene sequence PCR products of, *Ltropica*-was: GGTGTCCGGCAGCTGGGACAACACCA TCAAGGTGTGGAACGTGAACGGGGCAAGTGTGAGC GCACGCTCAAGGGCCACAACTA CGTGTCCACGGTG in Amplicon Length: 205. Sequence FASTA files, which are used in the phylogenetic tree, it has been submitted to the bank's General. Provide genome sequences it was presented sequence according to the guidance provided by the iInternet bank of Gene tool. The sequence analysis compares with the late transcription factor (VLTF-1) gene sequences from different pox Parra in databases using blast Online.

**Keywords:** *Leishmania tropica***,** Genetic sequence, Cuotaneous leishmaniasis.

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## INTRODUCTION

Cutaneous leishmaniasis cases are caused by three *Leishmania* species: *Leishmania major, Leishmania tropica,* and *Leishmaniainfantum*. These three species prevail under different bioclimates and differ by the nature of their vectors and reservoir hosts. They are responsible for three nosogeographical CL forms, which show different epidemiological and clinical features and need specific control measures.1 Cases of cutaneous leishmaniasis caused by *L. tropica* mostly occur in the sub urban's of big cities among large conglomerations of people where the sanitary conditions are unsatisfactory.[2] Cutaneous leishmaniasis existed sporadically in specific areas, it is endemic and has spread to disease-free regions. The existence of disease vectors in most regions of the country means that there is a risk of the disease becoming endemic in free regions.[3] Molecular technique offer one more promising tool for leishmaniasis diagnosis, employing PCR assays aiming to detect and genetically differentiate *Leishmania* species from archived microscopic slides in addition to elucidating the estimated risk factors for CL strains.[4] The kDNA minicircle is one of the best parts of the parasite genome for sequencing to identify different *Leishmania* species.[5]

### Statistical Analysis of Data

The data were analyzed using the SPSS (statistical package of the social science) package version 17; the approach to data consisted of two steps (descriptive and analytic statistic) and then analyzed using the Student t-test. A p-value ≤ 0.05 was considered statistically significant, Kestenbaum, 2009.[6]

## MATERIALS AND METHODS

Five ml of venous blood were collected from 150 serum samples were collected from cutanous leimaniasis patients; cutaneous

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scraping were done after administered local anesthesia and cleaned ulcer of crust, then dried with gauze. Scraped margin and central area of ulcer were taken from patients. The parasite cultivation was made by preparation of solid phase Novy, MacNeal-Nicolle(NNN) media. DNA Extraction from samples Preparation of *L. tropica* for PCR amplification, frozen samples were thawed and incubated at room temperature. These samples performed according to manufacturer's instruction of G-spin DNA extraction kit (intron biotechnology, South Korea). Isolation of DNA from cultured cells by a clean microcentrifuge tube, the culture cells resuspend in 200µL of PBS buffer and all steps was repeated above used in frozen samples DNA extraction. The DNA was stored at – 20°C until used in PCR (Yonggang and Pengfei, 2017).

## RESULTS

### Percentage according to age and gender in patients and control group

Table 1 showed that samples in this study included 95 males (63.3%) and 55 females (36.7%), their age of all individuals ranged from 5–74 years with a median age of 32.9 ± 15.14 years.

**Table 1:** Distribution of sample according to age and gender

| *Gender* | *No.* | *%* |
|---|---|---|
| Male | 95 | 63.3 |
| Female | 55 | 36.7 |
| Total | 150 | 100.0 |
| Age groups (years) | No. | % |
| 5-14 | 15 | 10.0 |
| 15-24 | 41 | 27.3 |
| 25-34 | 35 | 23.3 |
| 35-44 | 29 | 19.3 |
| 45-54 | 15 | 10.0 |
| 55-64 | 8 | 5.3 |
| 65-74 | 7 | 4.7 |
| Total | 150 | 100.0 |

### Prevalence Incidence of Cutaneous Leiahmaniasis According to Age and Gender Among Patients Group

Table 2 summarized the results of 120 patients studied and found that males 78(65%) were more frequently infected with cutaneous leishmaniasis than females 42(35%). There were no significant differences between male and female among almost age groups. The results showed that the higher infection rate found among the age group 25-34 years, 32 (26.7%), while the lowest infection rate found among age groups 55–64 years and 65–74 years, six (5%) in comparison with other age groups. found that males 78(65%) were more frequently infected with cutaneous leishmaniasis than females 42 (35%). There were no significant differences between males and females among almost age groups. The results showed that the higher infection rate found among the age group 25–34 years, 32 (26.7%), while the lowest infection rate was found among age groups 55–64 years and 65–74 years, six (5%) in comparison with other age groups.

### Distribution the infection according to residency

One hundred fifty samples included in this study, 60 (40%) were urban, while 90 (60%) were rural residents, as shown in Table 3.

### Number of lesions in patients

Forty-two (35%) patients had a single lesion, while 78 (65%) patients had multiple lesions demonstrated single and multiple

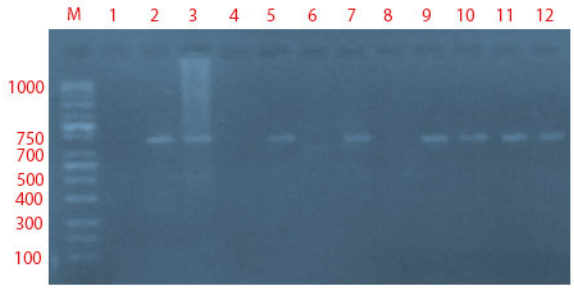


**Figure 1:** PCR product the band size 750 bp of *Lieshmania tropica*

**Table 2:** Distribution of sample according to age groups and sex of patient groups

| *Age/year* | *Male* | *Female* | *Total* | *%* |
|---|---|---|---|---|
| 5-14 | 7 | 6 | 13 | 10.8 |
| 15-24 | 17 | 10 | 27 | 22.5 |
| 25-34 | 20 | 12 | 32 | 26.7 |
| 35-44 | 18 | 6 | 24 | 20 |
| 45-54 | 7 | 5 | 12 | 10 |
| 55-64 | 5 | 1 | 6 | 5 |
| 65-74 | 4 | 2 | 6 | 5 |
| Total | 78 (65%) | 42 (35%) | 120 | 100 |

$X^2 = 3,814$, No Sig. at (p value >0.05)

**Table 3:** Distribution of individuals according to residents data

| *Residents* | *No.* | *%* |
|---|---|---|
| Urban | 60 | 40.0 |
| Rural | 90 | 60.0 |
| Total | 150 | 100.0 |

**Table 4:** The number of lesions among patients studied

| *Number of lesions* | *No.* | *%* |
|---|---|---|
| Single | 42 | 35 |
| Multiple | 78 | 65 |
| Total | 120 | 100 |

**Figure 2:** DNA sequences of *Leishmania tropica*

| | | | |
|---|---|---|---|
| 501 | GGTGTCCGGCAGCTGGGACAA-CACC | ATCAAGGTGTGGAACGT-GAA | CGGGGGCAAGTGTGAGCGCACGCTCAAGGGCCACAGCAAC-TACGTGTCCACGGTG |
| 701 | TGGAGTC-GCC | CATCAACCAGATC-GCCTTCT | CGCCCAACCGCTTCTGGATGTGCGTCGCGACGGAGAGGTCTCTGTCCGTG-TACGACCTGGAGAGCAAGGC |

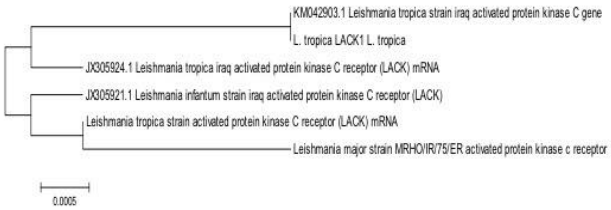


**Figure 3:** Shows the phylogenetic relationship results by applying the Night boring – Joining method.

lesions of cutaneous leishmaniasis for some patients, as shown in Table 4.

**Genetic types of *Leishmania tropica*:**

The protein kinase C (LACK) mPNA, complete cds was activated by primers of *L. tropica* strain Syrian 01. The amplicon length (205).

**The Primers of *Leishmania tropica* sequence:**

Forward: ATCAAGGTGTGGAACGTGAA.
Reverse : AGAAGGCGATCTGGTTGATG.

**Phylogeny tree analysis**

## DISCUSSION

Leishmaniasis an endemic disease especially the cutaneous type which is widely found in the central areas of Iraq. The disease incidence in Iraq increases in September and October and reaches in maximum in January and February months, while leishmaniasis incidence diseases march and reach the lowest rate in July and August months (Hassan) 2017.[8] Our study revealed that males were more susceptible leishmanasis than females, and this agreed with Abdulwahab, 2013.[9] who stated that the infection rate in males (65%) was a higher than females (35%) and also agrred with the study of Rhai, 2013 (10), who found that the disease prevalence in males was higher than female. Our study also in agreement with other studies such as Al-Mousawy 2015,[11] Al-Obaidi *et al.,* 2016[12] and Hassan, 2017.[8] spread in the central parts of the country. The incidence of the disease in Iraq was found to increase in months of September and October and reached the rate maximum in January and February. The incidence declines in March and reaches its lowest point in July and August Hassan, 2017.[8] Cutaneous leishmaniasis in the current study was found to be the highest in the age group of 25–34 years compared to the age groups (55–64) and (65–74) years, in which the incidence rate was the lowest. These results coincident with other studies carried out in Iraq, such as Radi, 2011. and Al-Qadhi *et al*., 2013,[13,14] who reported that the highest incidence of the infection occured among age group between (16-40) years, Abdulwahab, 2013,[9] while the lowest incidence rate was among age group 45–59 years, also agreed with the finding of Al-Samaria *et al*., 2016,[15] and Hassan, 2017,[8] who indicated that the highest rate of infection occurred in patients older that 15 years. In Libya, a study performed by Belal *et al*., 2012,[16] showed that (59%) of infected patients were younger than 20 years old. El-Badry *et al*., 2016,[17] also observed the highest rate of infection was with the middle age (41–59) years while the children were the last affected age group. A study in Burkina Faso, conducted by Bamba *et al*., 2017[18] found that the highest rate of leishmaniasis was among the age group 16–30 years. The present study also showed that the multiple lesions in cutaneous leishmaniasis was more predominant than single lesion, the result was in harmony with Hassan, 2017,[8] who reported that multiple sores isolates was higher than single sore isolates of leishmaniasis. This result also agreed with the study performed in Burkina Faso, Bamba, *et al.,* 2017.[18] The traditional treatment may not be useful in fighting this pathogen and modified amino acid should be manufactured and developed as the treatment in combination with DNA sequence in order to alter the protein synthesis pathway and thereby inhibit parasite growth and death.[19] The PCR product result demonstrated that L. tropica genome's molecular weight in agarose gel was 750 bp. Methods that use species-specific primers clearly separate *L. tropica* include ITS1-RFLP, high resolution melt analysis (HRM) and PCR approaches. Protein kinase of C gene is activated on a receptor from (LACK) mRNA by the phyolgenetic tree of *L. tropica.* Result of PCR products sequence of *L. tropica* were identical to the global strains, Sogand Assarnia, 2017. [20]

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