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## Study Molecular and Immunological Techniques for Detection of Leishmania Tropical, Major in Baghdad , Iraq

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## Study Molecular and Immunological Techniques for Detection of Leishmania Tropical, Major in Baghdad , Iraq

<i>Authors Names</i>	<b>ABSTRACT</b>
<p>Aliaa F. A. Al-Naimy Ali B. M. Al-Waaly</p> <p><b>Article History</b> Received on: 16/10 /2020 Revised on: 16 /12/2020 Accepted on: 1/ 2/ 2021</p> <p><b>Keywords:</b> IgM ,Leishmania, Cutaneous, l, Antigens, multiplex pcr</p> <p><b>DOI:</b> <a href="https://doi.org/10.29350/jops.2021.26. 2.1212">https://doi.org/10.29350/jops.2021.26. 2.1212</a></p>	<p>The study was conducted on 444 patients suffering from skin lesions of different ages and genders that were diagnosed by dermatologists as cutaneous leishmaniasis for people reviewing and throughout the days of the week for the department of epidemiology and transitional diseases who attended to hospitals in the city of Baghdad, namely (Al-Shaheed Dari Al Fayyad Teaching Hospital , Al-Shaheed Al Sadr Hospital , Al-Numan Teaching Hospital, Al-Karama Teaching Hospital) for the study period from October 1, 2019 until the end of March 2020.</p> <p>The methods of molecular diagnostics showed that multiplex pcr examined 40 random samples of the parasite that were collected through this technique from blood samples taken from the ulcers of infected people to reveal the DNA parts of the Leishmania parasite. Special prefixes for the Leishmania parasite species showed that 28 positive samples contain the parasite's DNA and 12 negative samples, of which 11 are for the Leishmania tropica type (27.5%) and 17 samples for the Leishmania major type(42.5%) at the molecular weight 1150 base pair.</p> <p>30 serum samples were taken from patients diagnosed with cutaneous leishmaniasis for serological tests. The current study showed a significant decrease in immunoglobulin G for patients with cutaneous leishmaniasis by <math>1205.100 \pm 210.155</math> , The current results also showed a significant decrease in the level of immunoglobulin M in patients with cutaneous leishmaniasis by <math>90.366 \pm 26.181</math></p>

### 1. Introduction

Cutaneous leishmaniasis is a widespread disease in the countries of the world, and Iraq is one of the countries where this disease is endemic, as this disease received wide attention inside Iraq (Taj-Eldin.,1954). Cutaneous leishmaniasis appears in two types: the wet type or the rural type that caused by Leishmania major . and the other is the dry

type or the urban type that is caused by *Leishmania tropica* (Al-Samaria & Al-Obaidi, 2009). The host that transmits the *Leishmania* parasite is a female sand fly mosquito that takes flagellum promastigote of the parasite while taking the blood meal from the host, and the disease is transmitted through the skin to humans as well as to other mammals (Davies et al., 2004). Molecular methods of kDNA and ribosomal DNA have been used as a template for identifying parasitic species by the method of polymerase chain reaction (PCR) is one of the best methods used to detect the parasite genome and to identify the different *Leishmaniasis* species (Aransay et al., 2000). One of the most important aspects that has been studied is the immunological aspect, due to the significant effect of the parasite on suppressing the immune system of the affected person (Sokolova, 2009). Host immunity plays an effective role in eliminating the *leishmania* parasite by a group of immune cells that include T cells, lymphocytes and phagocytic cells that have a role in eliminating the parasite within the host's body (Roitt, 2000). The production of immunoglobulins such as (IgM, IgG) are important for the primary immune response when infectious diseases are involved, including *leishmaniasis* (Rostamian, 2017). The immune response has an effective role in determining the amount of ulcers or the type of ulcers the patient has, as it mainly depends on the immune response of the host as well as on the virulence of the parasite strain (Al-Qadhi, 2013).

## 2. Methods

- Molecular diagnostics

dermal scrapings were taken from 40 patients with suspected CL who attended to hospitals in the city of Baghdad for the study period from October 1, 2019 until the end of March 2020, Parasitological diagnosis consisted of microscopic examination (magnification X1,000) of smears for amastigotes after Giemsa staining, for molecular diagnosis, Extraction of kDNA from samples taken from people with cutaneous *leishmaniasis* using a (Genomic DNA Extraction Kit) provided by the American company, Geneaid, according to the manufacturer's recommendations. PCR amplification was carried out with the *Leishmania* kinetoplast DNA (kDNA) primers, these primers were provided by the Canadian IDT Integrated DNA Technologies Company CSB2X F 5'-CGAGTAGCAGAACTCCCGTTCA - 3', CSB2X R 5'-ATTTTCGCGATTTTCGCAGAACG -3' and 13Z F 5'-ACTGGGGGTTGGTGTA - 3', R 5'-TCGCAGAACGCCCT -3', 13Z R 5'-TCGCAGAACGCCCT -3'. The reactions were done in a total volume of 25 µl containing 5 µl 1x PCR MIX, 10 picomols/µl Forward primer, 10 picomols/µl Reverse primer and 1.5 µl of the DNA was added to the mixture. The PCR amplification was done in a DNA thermocycler (Eppendorf) using 1 cycle of 95°C for 5min, 72°C for 5min and followed by 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec. Each experiment included a positive and negative control. The presence of amplification products was confirmed with 1.5% agarose gel electrophoresis analysis, and visualized by Red safe staining solution (0.5 µg/ml).

- Immunological diagnosis

3 ml of venous blood was withdrawn from 30 patients with cutaneous *leishmaniasis* and placed in anticoagulant tubes (gel tubes) and left for 15 minutes and after that, centrifugation was performed by 3000 cycles / minute for 15

minutes, and the serum was separated and placed in Eppendorf tubes size of 2 ml and on each tube patient's information is recorded and kept in the refrigerator at -4°C until the serological tests are performed.

- Measurement of IgG and IgM in serum

Immunoglobulins (IgG, IgM) levels in blood serum were measured by the RX DAYTONA+ automatic analysis device supplied by British RANDOX company, where the serum is placed in the Hitachi cup and then placed in the place designated for it inside the device and after that the device is instructed through the computer that connected to the device and after the completion of the test, results appeared on the computer screen through the device's program.

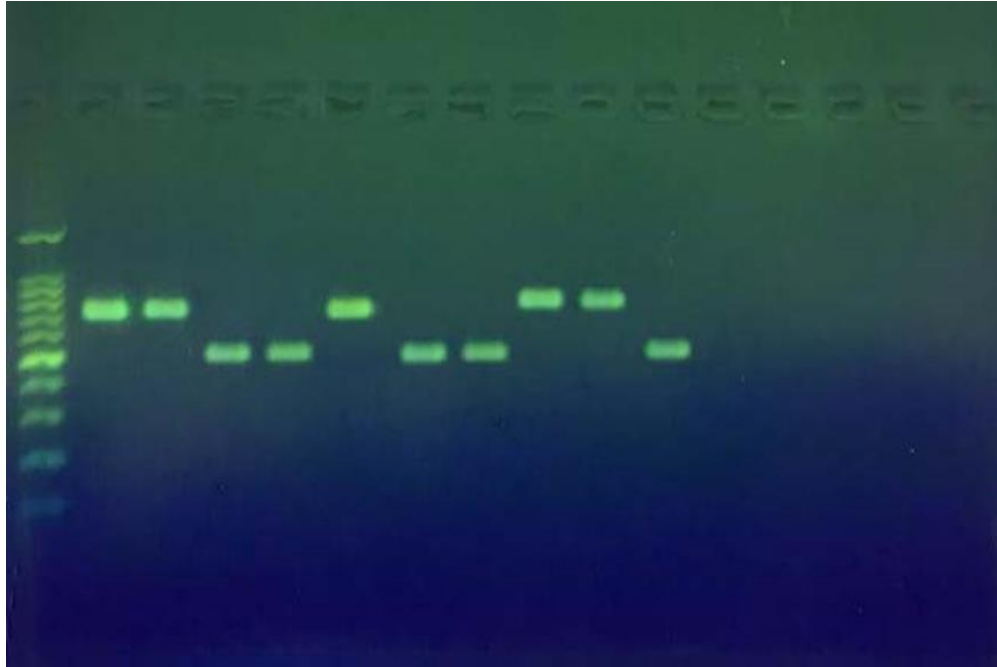
- Statistical analysis

The data was analyzed using SPSS statistic software version 25. Chi-square test was used for the assessment of association among the variables studied. The p-value of less than 0.05 was statistically significant, and highly significant for p-value of less than 0.001 (Sheskin et al., 2004).

### 3. Results

#### Multiplex PCR

The diagnosis of CL is traditionally based on microscopic demonstration of amastigote forms in tissue biopsies or smears. However, this method usually presents low sensitivity, and in atypical forms, CL may be overlooked because of similarity to other dermal diseases. Thus, it is necessary to apply specific diagnostic methods as PCR. In the present study, showed that 28 samples were clinically positive and contain the DNA of the parasite, of which 11 samples were for *Leishmania tropica* (27.5%) and 17 samples for *Leishmania major* (42.5%), and as shown in Table (4), to obtain different pieces of the DNA of leishmania. Dermatological use of Multiplex-PCR, the reaction was carried out using two types of specialized primers to obtain packages of the cutaneous *Leishmania major* with the product of length 750 and of human origin *Leishmania tropica* with the product of length 560 base pairs on gel and as shown in picture (1).



Picture (1) illustrates the electrophoresis of the polymerase chain reaction technique.

- Immunological
- IgG levels

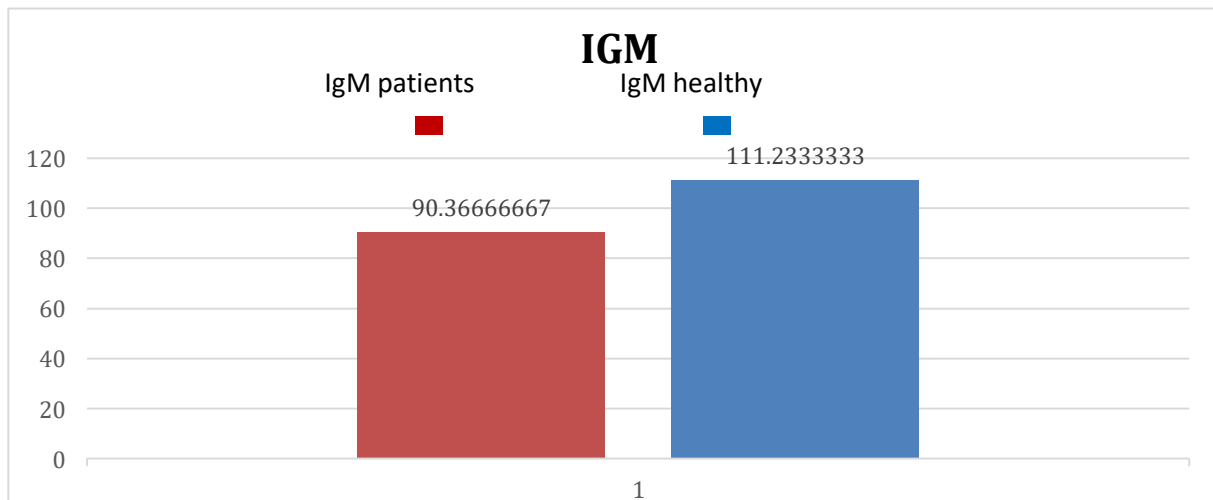


Figure (1) shows the relationship between the level of immunoglobulin G in people with cutaneous leishmaniasis ( $1205.100 \pm 210.155$ ) compared to the control group ( $933.766 \pm 133.652$ ) and at the probability level ( $p \geq 0.05$ ).

IgM levels

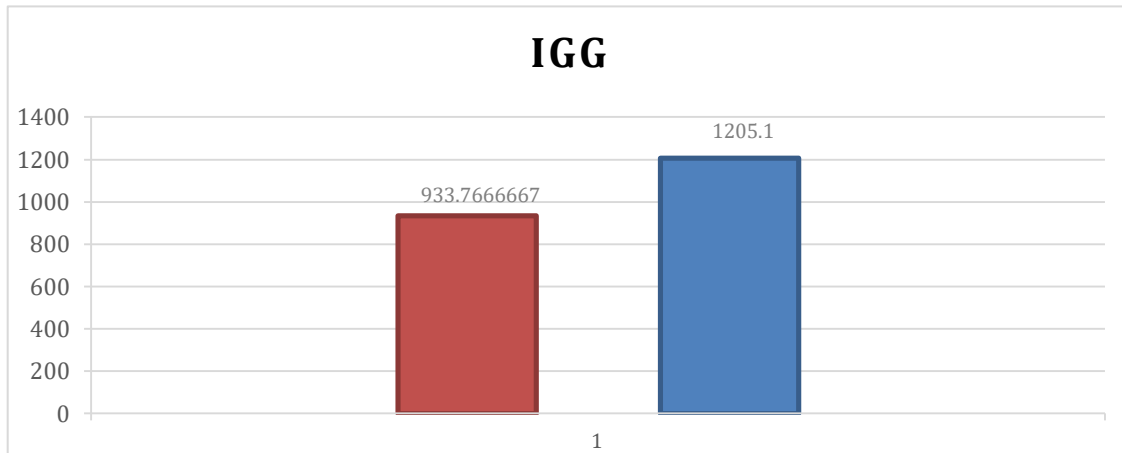


Figure (2) shows the relationship between the level of immunoglobulin M in those with cutaneous leishmaniasis ( $90.366 \pm 26.181$ ) compared to the control group ( $111. \pm 10.950$ ) and at the probability level ( $p \geq 0.05$ ).

#### 4. Discussion

##### Multiplex PCR

The reason for using the Polymerase Chain Reaction technique in diagnosing the cutaneous Leishmania parasite is due to several reasons, including that this technique is accurate and easy to investigate the types of Leishmania parasite and that this technique is a reliable method for investigating and identifying the Leishmania parasite types and can be applied in epidemiological investigations. The current study showed that, through the use of the Multiplex PCR technique, in DNA amplification by using special primers for the coding gene called kDNA of Leishmania parasite species, two types of this parasite were detected, the first: *L. major*, with a molecular weight of 750bp, and *L. trobica*, which has a partial weight of 560 bp. The results made by Abdullah et al. (2009) in Baghdad showed great similarity with the above result, in which the types of *Leishmania major* were shown, and they may be found in two models with a percentage (4.7%) of the total samples obtained in his study. Mahboudi et al. (2001) showed in his study that was conducted in the northwestern parts of Iran, especially in the city of Mashhad, that there are two types of *Leishmania*. isolated samples of *Leishmania* showed 19 *Leishmania trobica*, and two *Leishmania major* were investigated. He explained that both the ACL and ZCL foci are located in Mashhad Governorate. The results obtained from the study Mahboudi al et. (2001) coincided with the current study with the difference that *Leishmania major* is the dominant disease in the Najaf region compared to *Leishmania trobica*, which exists but in small numbers. The study agrees with what was found by Kamil & Ali (2016) in Baghdad governorate, where (80%) of samples were recorded using Nested PCR positive caused by *Leishmania major* and *Leishmania trobica*. The study also differs with Al-khayat (2020) in Baghdad governorate where (82.89%) of samples using Multiplex-PCR were positive, where (68.2%) were *Leishmania trobica* and (31.7%) *Leishmania major*. The study also

differed with Younis & Al-Thwani (2018) in Baghdad governorate, where the highest rates of infection were recorded by (61%) *Leishmania trobica* and (38%) from *Leishmania major* using Real-Time PCR technology.

#### IgG immunoglobulin levels

The current results showed a decrease in the level of immunoglobulin IgG, and the decrease in the level of IgG may be due to the immune regulation of the level of T-helper cells, as they produce the neutrophil cells in order to control the infection of the parasite, as the immune globulin decreases with the continuation of infection (Chaudhuri & Chang,1988). This study agrees with Mohamed Reda (2018) in Babil governorate, where a decrease in the level of immunoglobulin IgG was recorded. The reason for the increase in igg in the body is due to the multiple activity of B cell clones, the cutaneous leishmania and Mucocutaneous Leishmaniasis inhibit the multiple activity of The clones of the B cell due to the absence of the parasite from the spleen and the internal viscera, thus reducing the level of globulin g in the body (Ravanbod ,2000). Antibody levels are considered to be very low. This is not the case in Viseral leishmaniasis as the antibodies may reach extremely high levels resulting in hyperglobulinemia which is a feature of the diseases. In addition, seropositivity may also depend on disease duration as well as the number of cutaneous lesions (Musleh, 1995). Solomons (1998) found that Zn deficiency, albeit mild in patients with cutaneous leishmaniasis, leads to an imbalance between humoral and cellular immunity and thus reduces the production of B cells responsible for the production of immune globulin igG.

#### IgM immunoglobulin levels

The present results showed a decrease in the level of IgM immunoglobulin. The reason is the parasite's ability to actively reduce the level of IgM concentration through the activity of the virulence factor of *Leishmania gb63*. This study is consistent with Mohamed Reda (2018) in Babil governorate, where there was a decrease in the level of IgM immunoglobulin. Also consistent with Chaudhuri & Chang (1988), it was found that the ferocity factor gp63 had the ability to break down immunoglobulins, and complementary factors, proteins and lysosomes (Roitt et al ,2006) . Also, the decrease in the level of Zn in the serum leads to a decrease in the total concentration of IgM due to the immune imbalance that reduces the stimulation of the B cells to produce IgM immunoglobulins , and this is consistent with what was found by (Molder & Steward 1989; Solomons 1998).

### **5.Conclusions**

- 1- A decrease in the level of immunoglobulins (igG) and (igM) as a result of infection with the parasite.
- 2- The immune side has an important and clear role in the diagnosis of parasite infection.
- 3- The results of the study revealed the presence of two types of the cutaneous *Leishmania* parasite, *Leishmania major* and *Leishmania tropica*, as *Leishmania major* is the dominant type, and this was shown by the results of molecular diagnostics using the technique of Multiplex PCR.

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