

ARTICLE / INVESTIGACIÓN

Identification of the parasites that cause children's cutaneous leishmaniasis and several types of immune response cells

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Abstract: Cutaneous leishmaniasis has received many epidemiological, diagnostic, therapeutic, quantitative and other studies. The current study aims to determine the type of leishmaniasis parasite that causes cutaneous leishmaniasis in children in the Al-Diwaniyah governorate in central Iraq to assess the level of variations in globulins and immune cells in children with cutaneous leishmaniasis. Fifty-nine was a positive sample for the traditional laboratory examination of cutaneous leishmaniasis in children whose ages ranged between (8 months-10 and years). They were collected from the Maternity and Children Hospital from January 2020 to December 2021. The samples were subjected to molecular diagnostics to determine the type of leishmaniasis that causes the disease using Real-Time PCR. ELISA was approved to measure the levels of variation in the concentrations of IgG and IgM among the group of infected and healthy children. The results indicated that children's predominant type causing cutaneous leishmaniasis is *L. major* 52 samples (88.13%). The immunological study showed clear and statistically significant differences in the mean globulin concentrations for comparing the patient's *L. major* with the control group at a probability level ($P \leq 0.05$). The current study concluded that *L. major* is the dominant species responsible for cutaneous leishmaniasis.

Key words: Cutaneous leishmaniasis, *L. major*, Real-Time PCR.

Introduction

The introduction should briefly place the study in a broad context and highlight why it is essential. It should define the purpose of the work and its significance. The current state of the research field should be carefully reviewed, and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your particular field of research. References should be numbered in order of appearance and indicated by a numeral or numerals—e.g., (1) or (2,3), or (4–6).

Materials and methods

Fifty-nine biopsies of skin lesions of children with cutaneous leishmaniasis were approved in the current study; after all, they showed positive test results. Using the formal diagnosis by microscopic examination of biopsy smears examined with Giemsa stain in the laboratories of Al-Diwaniyah Teaching Hospital and Maternity and Children Hospital from January 2020 to December 2021. The affected children had no prior history of this disease. They did not take any treatment. 15 Skin samples were obtained from healthy individuals who underwent plastic surgery and approved as a control group⁹. Prior written consent was obtained from all study participants (patients and healthy subjects).

Molecular Study

The patients' ulcer samples underwent DNA extraction for the parasite *L. major* using (Accuprep Genomic DNA Extraction Kit) according to the recommendations of the manufacturer (Bioneer Korea), then the extracted DNA was examined for *L. major* using Real-Time PCR (Syber green) technology, based on Kinoplast DNA hetero genous minicircles, according to parasite-specific primers provided by Bioneer Korea based on the registered gene (GenBank: Km555288.1) and the NCBI database, F: TCGCGTGTCT-GACTTTTGC , R: ACTCAAGTCCCGTCCCGTCCATCAAC (95 Pb)¹⁰.

Immunological study

Venous blood samples were collected from infected children who showed positive results for the parasite *L. major* in the molecular examination. The Linked Immuno-Sorbent Assay (ELISA) was approved to investigate all immunological parameters under study. The concentrations of IgG and IgM in the serum of affected children were estimated using an ELISA kit for each assay from Elabscience.

Statistical analysis

The results were statistically analyzed using IBM-SPSS version 24 to find the significant differences between the values obtained. To determine the significant differences ($P \leq 0.05$) between two paired samples, a paired sample t-test was used.

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Results

The results of the molecular examination using the R.T-PCR technique and the use of special primers for the gene of the type *L. major* proved the presence of the parasite in 52 out of 59 (88.13%) as shown in Figure (1).

An immunostaining study to investigate cytokines indicated that the mean levels of IgG and IgM in the serum of children with *L. cutaneous* leishmaniasis were significantly increased. As shown in table (1), they reached (1201±71.3 mg/dl) (and 20.1±142.2 mg/dl) respectively, compared to the control group (602.0±14.1 mg/dl) (106.1±19.2 mg/dl) with a significant difference (P≤0.05).

Discussion

Although children are most targeted and affected by cutaneous leishmaniasis, most studies focus on the entire population, diagnostic, therapeutic, and epidemiological aspects. A few focused on the earth's relationship with children¹¹. Therefore, the current study focused on investigating the leishmaniasis that causes cutaneous leishmaniasis

in children and studying some physiological aspects of the specific body. Children and the elderly are more susceptible to cutaneous leishmaniasis, which persists for a long time due to their weak immune system¹². The ability and efficiency of diagnostic serological methods are low in investigating the parasite that causes cutaneous leishmaniasis, significantly when the number of parasites is reduced in the ulcer area in cases of chronic infection. Therefore, molecular diagnostics was relied on in the current study, characterized by its diagnostic efficiency, quantity and accuracy. The high sensitivity of molecular methods has been confirmed^{13,14}.

The results of the molecular diagnosis in the current study using the R.T.PCR technique confirmed the presence of *L. major* in 52 out of 59 (M,13%). This indicates that *L. major* is the predominant type that causes cutaneous leishmaniasis in children in Al-Diwaniyah Governorate in Iraq. This result agreed with (15) when they noted in their molecular study using Nested PCR that *L. major* species is responsible for most of the infections of cutaneous leishmaniasis in Lorestan Province in Iran.

We agree with Paz C 2003¹⁶ that *L. major* is the dominant species and is the only known type of leishmaniasis that causes cutaneous leishmaniasis in Mali. We also agree

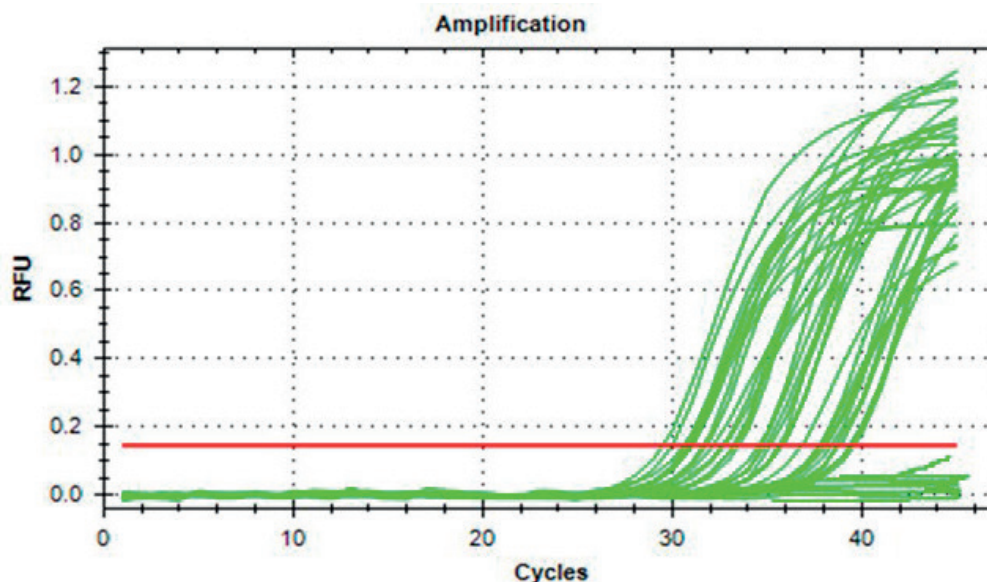


Figure 1. Shows the amplification of the R.T-PCR assay for positive results. Where the y-axis includes the RFU. The x-axis represents the thermal cycles of the reaction.

Immunoglobulins	IgG± standard error mg/del	IgM±Standerd error mg/dl
Patients (n=52)	*1201±471.3 mg/dl	*20.1±142.2 mg/dl
Control group (n=15)	602.0±14.1 mg/dl	106±19.2 mg/dl
P-value	≤0.05	≤0.05

A sign* indicates that there are significant differences in statistical significance.

Table 1. The values of variations in the concentrations of IgG and IgM in the serum of (52) patients with cutaneous leishmaniasis *L. major*.

(17) with the dominance of class *L. major* as caused by cutaneous leishmaniasis patients in Khuzestan province using the Nested-PCR technique. Our results differed from what was reported by (18) about the dominance of *L. tropica* from *L. major* as a cause of cutaneous leishmaniasis in Wasit Governorate in Iraq using PCR technique. The difference in the results may be due to the different methods of using DNA from the samples, the difference in the number of samples being studied or the target age group (19, 20). Indicated that it is necessary to use molecular methods in diagnosing the leishmaniasis parasite that causes cutaneous leishmaniasis. Due to the possibility of misdiagnosis using traditional methods, the similarity between ulcers resulting from cutaneous leishmaniasis, skin cancer and granulomatous skin necrosis, in addition to the decrease in the number of parasites in some ulcers over time.

Many researchers emphasized estimating the quantity of *L. major*, the scale of the parasite burden during the disease period in the patient's body, and its relationship to the stages of disease development. The accuracy of diagnosis and the location of disease development significantly impact the efficiency of treatment work and the correct effective management of the vital crisis without spreading the disease. There is a close relationship between the extent of the pathogenesis of Leishmania parasites and their response to drugs with the immune response of the host body^{21,22}. Investigating the details of immune cells and cytokines is very important for the difference in the exact mechanism of the immune system's work in the different stages of the disease. Leishmania infection Migration of lymphocytes, eosinophils, natural killer cells, plasma cells, dendritic cells, and Mast cells to the sites of inflammation with the appearance of the lesion; these cells subsequently activate the mediator cells in the immune response, which attract a group of distinct defense cells.

The results of the current study indicated high levels of IgG and IgM concentrations in the blood serum of children with cutaneous leishmaniasis compared to the control group. This is in agreement with the analysis of leishmaniasis cutaneous antigen immunophenotypes in the serum of adult patients with cutaneous leishmaniasis, which revealed elevated levels of IgM subclass, IgG resulting from Ploy clonal activation of leishmanial antigen-stimulated B cells, which subsequently transformed into plasma cells to secrete antibodies.

Conclusions

The current study indicates the predominance of *L. major* species as a cause of cutaneous leishmaniasis for affected children in Al-Diwaniyah Governorate in central Iraq (88.13%). This disease results in an excessive increase in some globulins and immune cells (IgG, IgM) as a defensive reaction by the body.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest

There is no conflict of interest.

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