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Detection of Leishmania Major by ITS gene and its effect on the CCR7 gene expression of the human host

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ABSTRACT
Leishmaniasis is among the most overlooked contagious illnesses, affecting around 12 million individuals and more than 350 million people at risk of infection. A total of 60 skin and mucosal lesion biopsy specimens were taken from people with clinically suspected CL in exposed part of the body mostly in the face, upper limbs, lower limbs, and before treatment. The biopsy samples were preserved in absolute ethanol and stored at room temperature from various ages, attended hospitals and health centres in the Governorate of Baghdad. The results showed out of those 60 samples 46 patients diagnosed clinically by special dermatologist as CL. And those 60 were tested by RT-PCR and 4 of the negative samples gave a positive results and only 35 samples of those samples that described as positive gave a positive results by the RT-PCR the other were negative, and the gene expression results showed an increased level of CCR7 gene expression in the positive subjects than the negative subjects.

Keywords: Real Time-PCR, Leishmania major, CCR7 gene.

INTRODUCTION

Leishmaniasis is a quality provider illness characterized by heteroxenic in developed countries, protozoan parasites of the species represent nearly 12 million people and (Gutierrez-Corbo et al., 2017). Innoculate the forms of vary in severity into the human host, the parasites that are transmitted by sandfly vectors. These are pulled in by immune cells but instead converted into the essential cytoplasmic amastigotes. Other phagocytes are then contaminated by the (Wright et al., 2015). In naive and memory T cells and APCs, CC-chemokine receptor 7 (CCR7) is strongly distributed and has significant effects on this development (Kling et al., 2013). Despite the core feature of CCR7, it has been reported that in deficient CCR7 models, defensive immune systems are triggered and that the severity of the position of CCR7, It was still necessary, however to better understand CCR7's function in the development of leishmaniasis. Polymerase chain reaction (PCR) and its derivatives are Leishmania DNA two sensors that are extremely sensitive and effective. Other methods, such as microscopy and numerous immunological studies, were shown to be superior to PCR, reducing the time from choice to test outcome, increasing sensitivity and precision, and reducing subjective evaluation (Wall et al., 2012; Jasim et al., 2023). Real-time PCR is preferable to traditional PCR because it is quicker, less labor-intensive and decreases the risk of contamination. Consequently, real-time PCR could be used for quantification by using standard curves (Alani, et al., 2022). In researching Leishmania parasites and signs for diagnosis and species detection, quantitative real-time PCR (qPCR) may be beneficial. And as part of the manufacture of vaccines, it also has broader potential, such as tracking the effects of tobacco action and assessing protection. QPCR can be used to begin therapy and classify asymptomatic carriers because of their great specificity.

MATERIALS AND METHODS

Sixty (2 mm in diameter) biopsy samples of skin and mucosal lesions were taken from persons with clinically suspected CL and prior to therapy. The biopsy samples were stored in absolute ethanol and processed at −20°C

before further processing. For diagnosis, we identified a lesion to be due to Leishmania when at least 1 of 3 tests was positive, whereby tests refer to direct smear (microscopy), culture, and qualitative PCR biopsy sample. For the 3 trials, a negative biopsy specimen. The specificity and accuracy of the qualitative and real-time dependent PCR tests have been evaluated. Biopsy specimens are minced with a sterile scalpel. The biopsy samples and pellets were then subjected to processes provided by the Intron Company for parasite DNA extraction. And afterwards the extracted amount of pellet has been used for human RNA extraction using the Zymo Quick-RNA Micro-prep Package. The extracted RNA was measured by fluorometry using Quantus- Promega. For the identification of the parasite, real-time-PCR was performed and then also used for the CC-chemokine receptor 7 (CCR7) gene expression measurement. During this analysis, the primers used for the expression of the CCR7 gene were designed: forward, CATGGACCTGGGTATGCCTGT and reverse, CAGGCTTAAAGTTCCGCACG. While the primer used to detect the parasite targeted the ITS inside the genome of the parasite, and those primer were described previously by (Toz et al., 2013)

RESULTS AND DISCUSSION

Sixty patients complained from skin lesion in exposed part of the body mostly in the face, upper limbs, and lower limbs. then out of those 60 samples 46 patients diagnosed clinically by special dermatologist as CL. And those 60 were tested by RT-PCR and 4 of the negative samples gave a positive results and only 35 samples of those samples that described as positive gave a positive results by the RT-PCR the other were negative. As seen in table (1).

Table 1: distribution of samples results by both clinical diagnosis and Real time-PCR.

Test	Result	Result by the RT-PCR		Specificity	Sensitivity
Clinically	Negative (14samples)	Negative	10	0.476	0.897
		positive	4		
	Positive (46 samples)	Negative	11		
		positive	35		

The results of the target gene amplification as it appeared by the RT-PCR instrument representing the presence of the parasite are shown in figure (1).

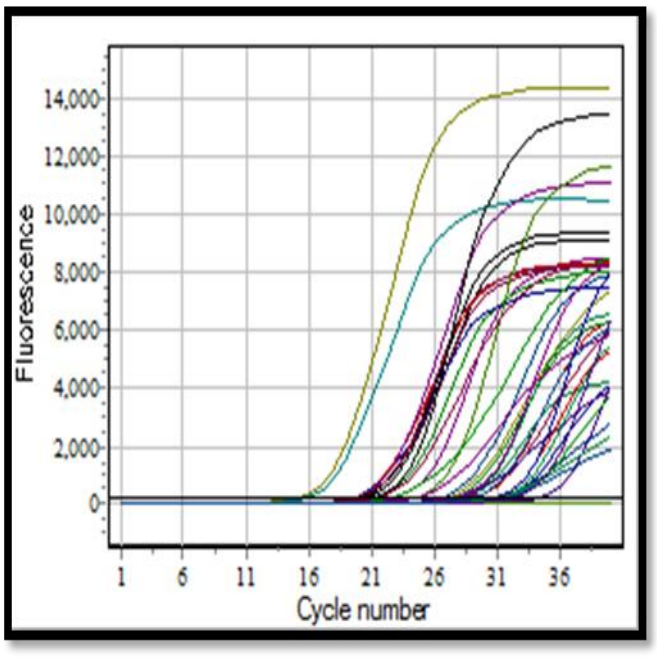

Figure 1: RT-PCR resulted curves of the target gene amplification (ITS gene).

While the results of the gene expression are also calculated by the RT-PCR by amplifying the target gene and the results of the amplification are shown in the figure (2).

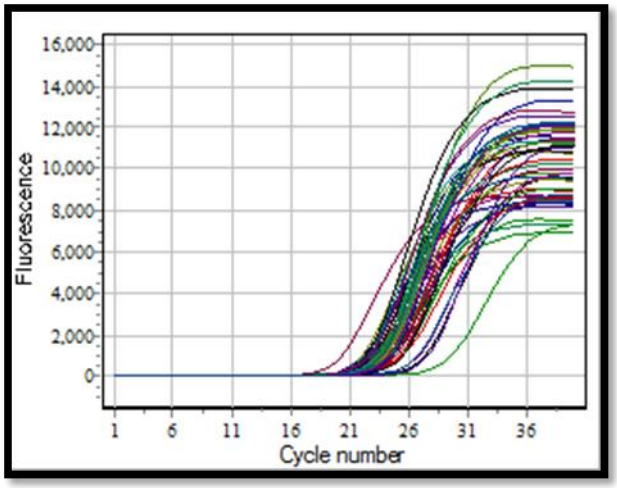

Figure 2: RT-PCR resulted curves of the target gene amplification (CCR7 gene).

The gene expression level were tested and the results represented in figure (3) and shown an increased level within the group of samples that shown a positive results while the samples that shown a negative results showed a decreased level of the CCR-7 gene expression.

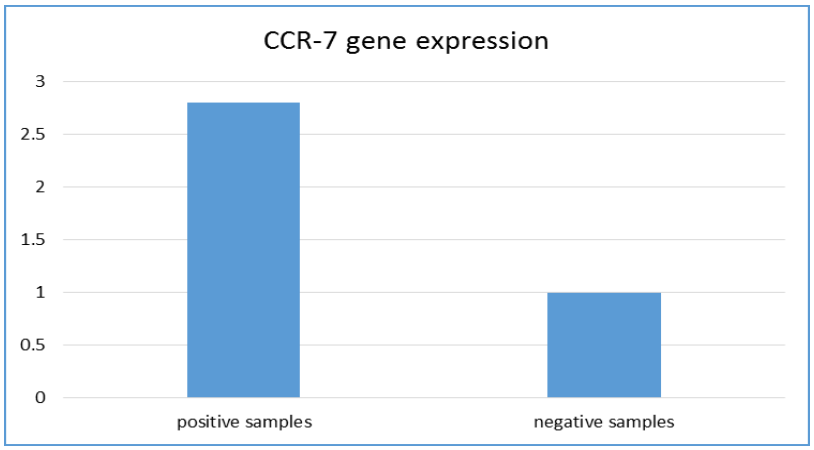

Figure 3: Compassion of CCR7 gene expression between positive and negative samples.

For Old Country Leishmania species range, the ITS1 of the ribosomal DNA functional group (rDNA-ITS1) has subsequently been manipulated using RFLP (Scho'nian et al., 2003), inverse synthesis assays (Nasereddin etal.,2008), and testing (Talmi etal.,2010). The Leishmania genome is predicted to have 20 to 200 exact copy, making it a better target for low-parasite quantity study (Odiwuor et al., 2011). A modern PCR-SSCP application technique (single-strand conformational polymorphism). The authors even said SSCP offers higher resolution, especially whenever the Pcr reaction has a poor agarose gel band and it is less costly than the RFLP process (Chargui et al., 2012). A real-time ITS1-PCR system that can detect three L species of Old World Leishmania was described in the study carried out by Toz. Complex by Donovani, L. L. With Tropica. Significant, utilizing recently designed offer fixed probes to simultaneously identify and discriminate among Turkish species (Toz et al., 2013, Abed et al, 2022). Through continuous innate immunity, proliferation, homeostasis, and development, chemokines became pivotal promoters of cell migration, and internal strategies showed that chemokine signalling also mediated the movement of neurons, neural crest cells, and germ cells throughout embryogenesis and regulated the layering (Cha et al., 2012, Lewellis & Knaut, 2012). In fact, structural changes were induced by GPCRs that triggered cell migration signalling pathwaysEarlier studies showed that the primary function of CCR7 was to guide the migration of directional cells. Consequently, CCR7-expressing cells underwent rapid shape changes and gradually polarised when exposed to their ligands (Hauser & Legler, 2016). Furthermore, reduced CCR7 expression could result in reduced cell growth potential for both mice and humans (Diaz e al., 2013, Nico et al., 2018; Jasim and Al-Amery 2023). In their study, Soong et al. (2012) stated that Leishmania parasites could change the signal transduction within mice and human DCs, which could have had an impact on human VL. During the Leishmania infection in DC, CCR7 also contributed a lot. We discovered that CCR7 was strongly expressed in L. Infection with Major-induced DC. In homeostasis and replication, CCR7 has been widely researched and has been a crucial factor in the synchronized motion of T

cells and DCs in lymphoid organs (Forster et al., 2008; Jasim and Abed (2018). An important factor in the movement of inflammatory monocytes and Tregs was CCR7, as per Kling et al (2013). Our research evaluated the effect of CCR7 in the L method, in particular. Severe disease that could be used for potential guidelines for testing and successful L treatment. In order to investigate the molecular functions of CCR7 in L, more studies have therefore been needed. It triggered a major infection.

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