Kadhim M. Haddao et al /J. Pharm. Sci. & Res. Vol. 10(3), 2018, 544-546

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Study of *Cutaneous leishmaniasis* using polymerase chain reaction technique in west of Karbala rural sites

Kadhim M. Haddao[1]; Ali – Alkubaisy [1] ; Ali Tariq Abd- Elhassan, [1]; Azhaar M. Jaffer [2]

[1]*College of Dentistry, University of Kerbala- Iraq*
[2]*College of pure science, University of Kerbala- Iraq*

Abstract:

This work was aimed to study the most common forms of leishmaniasis, *Cutaneous leishmaniasis*, and its types using Polymerase Chain Reaction(PCR) technique in west of Karbala governorate rural sites during the period between Sep. 2015 to Apr. 2016.

Leishmaniasis infection is considered as a great medical problem in most countries on the world faced the infected persons causes deformity in the manifestation site, the study included 91 infected persons by cutaneous ulcers diagnosed primarily as Leishmaniasis.

The results of the present study have revealed that the male infections (71.42%) was more than the infected female (28.57%) with a significant differences P< 0.05 . To inappreciable diagnosis of Leishmania types in west Karbala rural sites have employed PCR technique to samples. Also the results have revealed the appearance of two types of L. parasite causing the manifestation. In PCR test bundle length of about 650 base pair (bp), (77 samples) belongs *L. major* and 750 bp (12 samples) belongs *L. tropica* were obtained.

An attempts was carried out to finds suitable vaccine for this disease, in order to study the immune response using lymphocyte transformation assay. The ratio was 8.9% for L. tropica ; 19.4%, for L. major with a significant differences of p<0.05 for control sample (5.9%).

Keywords: Leishmania, *L. cutaneous, L. tropica*, PCR diagnosis,.

INTRODUCTION

The Leishmaniasis infection (Oriental Sore) is considered a great medical problem in most countries of the world facing the infected persons which causes deformity in the manifestation site[1]. Risk factors include poverty, malnutrition, deforestation, and urbanization[2]. World Health Organization (WHO) classified this disease as one of biggest six parasitic disease infect the human being(2).

Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus *Leishmania*, it spread by the bite of certain types of Phlepotomus (sandflies)[2;3]. Infections in humans are caused by more than 20 species of *Leishmania*[4;5]. It can appears in three main types: cutaneous, mucocutaneous, or visceral leishmaniasis[4]. The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies. The cutaneous form is the most common form, which causes an open sore at the bite sites, which is only healed after few months to a year and half, leaving an unpleasant-looking scar tissue[6], presents with skin ulcers, skin sores which could be erupted in weeks to months after the person is bitten by infected sandflies which transmit the infection from infected mammals reservoirs as human , rodents and animals. The parasite lived as a amastigote phase in macrophage but in sand-fly intestine as Promastigote phase)[7]. The mucocutaneous form presents with ulcers of the skin, mouth, and nose. The visceral form starts with skin ulcers and then later presents with fever, low red blood cells, and enlarged spleen and liver[8; 9]. All three types can be diagnosed by observing the parasites under the microscope[10] . Additionally, visceral form could be diagnosed by blood tests[11]. The disease can be transmitted between human beings by the vector or by blood transfusion [12;13].

L. cutaneous. can be diagnosed in the hematology laboratory by direct visualization of the amastigotes (Leishman-Donovan bodies)[10], using Buffy-coat preparations of peripheral blood or skin lesions on a slide to make a thin smear then stained with Leishman stain or Giemsa stain. Amastigotes are seen within blood and spleen monocytes or, less commonly in circulating neutrophils[11].

Taking tissue samples is often painful for the patient and identification of the infected cells can be difficult. So, other indirect immunological methods of diagnosis are used, including enzyme-linked immunosorbent assay, antigen-coated dipsticks, and direct agglutination test. Although these tests are readily available, they are not the standard diagnostic tests due to their insufficient sensitivity and specificity. So several different Polymerase Chain Reaction (PCR) tests are available for the detection of Leishmanial DNA.[14] which is a specific and sensitive diagnostic possible procedure. Till this work, no real active vaccine for humans available[6]. But researchers know that people whom recover from cutaneous Leishmaniasis are protected against future infections; for example people in the middle east have transferred fluid from lesions of infected people to non-infected people using thorns[5;6] . This process is called "leishmanization"[7]. Some experiment using genetically-modified live attenuated parasite vaccines was conducted in the 1990s and 2000s. Other experiment had used synthetic peptides and naked DNA [15].

Some attempts carried out in Iraq to produce vaccine against *L. cutaneous* by exposing the children to sand-fly bite at their buttock in order to immunized them against the disease in the future. However, another researches have been conducted to produce suitable vaccine in rodents lab-animals by using killed or attenuated vaccine. , these attempts represent the 1st generation works. Moreover there are some trials to produce the 2nd generation vaccine using parasitic DNA level [16;17;18;19;20;21].

MATERIALS AND METHODS

Samples were collected from 91 patients living in west Kerbala rural area during the period from September,2012 to April 2015 . They were having sore skin infection diagnosed by dermatologist as Cutaneous L. .The samples were preserved for DNA extraction.

Blood smear from infected persons were examined to ensure the presence of Amastigote phase, Biphasic media (Novy, McNeal – Nicolle NMN medium), were used in order to culture and growth the Promastigote phase[10] . Parasite were isolated, by injection of buffer saline from the red zone boundaries of infected skin, then cultured using semi solid medium for five days, then spreading one drop on slide to examined the Promastigote phase under light microscope.

For molecular study, nucleic acid of DNA were extracted for molecular examination followed kit license belong Geneaid company, DNA Agarose Gel Electrophoresis were done[22]. Primaries and dilutions used was supplied from Bioneer co. were CSB1xF (CGAGTAGCAGAAACTCCCGTTCA) and CSB1xR (ATTTTTCGCGATTTTCGCAGAACG).

Infected solution with antibiotic (Crystalline penicillin 1000000 IU +1 gm Streptomycin sulphate) were injected in one leg sole and phenol saline solution in the other leg sole, after twenty hrs. sole pad thickness were measured, the deference in thickness represent the cellular response[23]. The percentage of lymphoblast were calculated according to the following equation:

$$\text{Percentage of lymphoblasts formation} = \frac{\text{No.of lymphoblasts}}{\text{Total no.of lymphocytes}} \text{X } 100$$

The analysis of biostatistics was done by SPSS 10.10.

RESULTS

Table 1 explain the distribution of Leishmaniasis infection between males and females of the present study. Sixty five cases of the infected samples(71.42%) were males, while the females (26 cases) were less affected (28.57%) .

Table 1: Distribution of Leishmaniasis infection depended on age and sex.

Age categories (years)	Males	Females	Total
1≥	0	1	1
1-5	3	3	6
6-10	6	1	7
11-15	4	1	5
16-20	13	6	19
21-25	8	3	11
26-30	15	9	24
31-35	14	1	15
36-40	1	0	1
41-45	0	1	1
46-50	1	0	1
51-≤	0	0	0
	65 (71.42%)	26 (28.57%)	91

The blood smears were showing small, round bodies with indistinct cytoplasm. Occasionally, amastigotes may be seen lying free between cells(fig. 1).

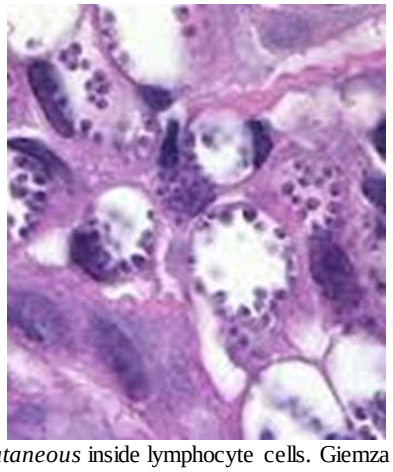

Fig. 1 : *L. cutaneous* inside lymphocyte cells. Giemza stain, X400.

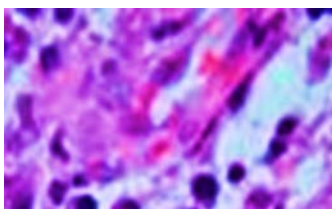

Fig.2: Primary stage of *L. cutaneous*. inside lymphocyte cell. Giemza stain, X400.

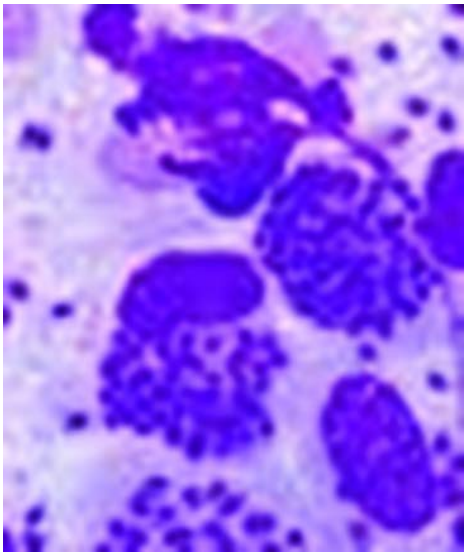

Fig. 3 : Advance stage of *L. cutaneous* inside lymphocyte cells. Giemza stain, X400.

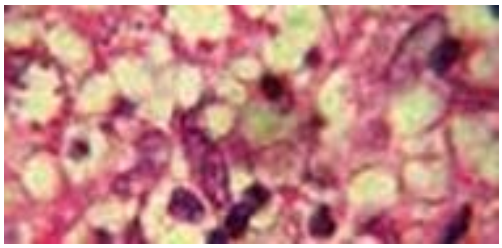

Fig.4: Chromatin granules stage, advance stage of *L. cutaneous* inside lymphocyte cell. Giemza stain, X400.

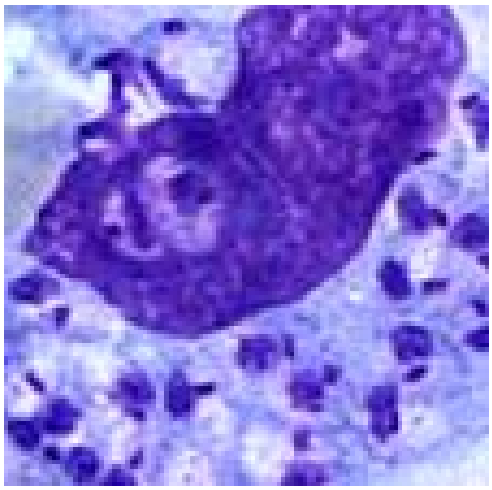

Fig.5: Granuloma phase of *L. cutaneous* inside lymphocyte cell. Giemza stain, X400.

To detect parasite type causing Leishmania, PCR technique were used, we get bundle length at 650 bp for type *L. major*,79 cases (Fig.6); and 750 bp for type *L. tropica*, 22 cases,(Fig.7).

Lymphocyte transformation assay test using putrefied Lipophosphoglycan in 10 experimental rats reveals that, the ratio was 8.9% for *L. tropica* ; 19.4%, for L. major, there is a significant differences $p<0.05$ while for control sample was (5.9%) (Table: 2). Results of late hypersensitivity test in 15 experimental rats revealed that the toe average thickness of hind limb injected by L. antigen was 1.18 mm, and to hind limb injected by saline-phenol solution was 1.24 mm, while for control gp. was 0.43mm by significant differences $p<0.05$ (table: 3).

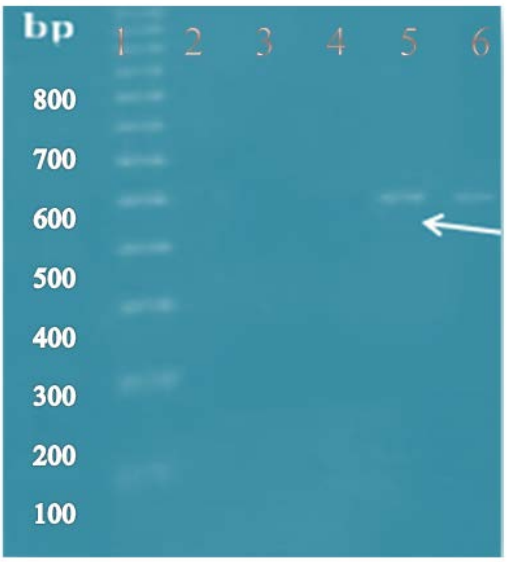

Fig 6 : Transformation detection of DNA, PCR technique in gel agarose. for parasite *L. major* type.

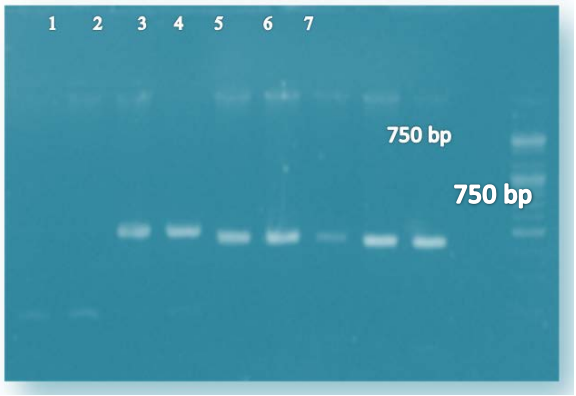

Fig 7 : Transformation detection of DNA ,PCR technique in gel agarose. for parasite *L. tropica* type.

Table 2 : Average rate of lymphocyte transformation injected by Leishmania Ag in experimental rats after 2weeks from injection.

Vaccinated gp.	Average rate of lymphocyte transformation
L. tropica gp.	9.5 %
L. majore gp.	18.2 %
Control gp.	5.1 %

Table 3: Results of late hypersensitivity test in experimental rats injected by L. Ag , deference in toe pad thickness.

Vaccinated gp.	Toe average thickness of hind limb
L. tropica	1.18 mm
L. Major	1.24 mm
Control gp.	0.43 mm

DISCUSSION

This work was aimed to the identify types of *L.cutaneous* parasite which isolated from patients admitted to the hospitals suffering from cutaneous sore lesion diagnosed by dermatologist primarily as leishmaniasis which is consider in Iraq and middle east area as endemic parasitic disease. Therefore it is importance to find suitable ways of diagnosis, treatments and best methods for vaccination against this disease and propose scientific scheme to control it[23]. Results of this work have revealed that the percentage of male infection (71.42%) was higher than female infection (28.57%); within a ranging age between16 to 30 years. These results were compatible with other works conducted by several researchers[23;24] . In agreement with Willy *et al.*[22] ,the presence of higher percentage of infection among the males was explained by higher incidence of exposure of the male to causative vector .

In this work PCR technique were used which considered the best accurate, modern, sensitive method in diagnosis and typing and serotyping, immunological reactions of the causative agent even the contaminated blood sample was very little (in nano gram) [25].

REFERENCES

1- "Leishmaniasis Fact sheet No.375". World Health Organization. January 2014. Archived from the original on 21 February 2014.
2- Azizi MH, MH; *et al.* (2016). "History of Leishmaniasis in Iran from 19th Century Onward"). Arch Iran Med. 19 (2): 153–162.
3- Aoun, K.; Bouratbine, A. (2014). "Cutaneous Leishmaniasis in North Africa: a review". Parasite. 21: 14. PMC 3952656 .
4- Barrett, MP; Croft, SL (2012). "Management of Trypanosomiasis And Leishmaniasis.". British medical bulletin 104: 175–96. PMC 3530408.
5- Ejazi, SA; Ali, N (2013). "Developments in Diagnosis and Treatment of Visceral Leishmaniasis During the Last Decade and Future Prospects.". Expert review of anti-infective therapy. 11 (1): 79–98.
6- Hepburn N C. (2017). "Cutaneous Leishmaniasis: an overview". J Postgrad Med .
7- Minodier, P; Parola, P (2007). "Cutaneous Leishmaniasis Treatment.". Travel medicine and infectious disease. 5 (3): 150–8. PMID 17448941.
8- Kennedy, Kelly (2010). "VCS Advocacy in the News: VA May Designate 9 Infectious Diseases as Related to Gulf War". Veterans for Common Sense.
9- Kahn, S; Wleklinski, M; Aruffo, A; Farr, A; Coder, D; Kahn, M (1995). "Trypanosoma Cruzi Amastigote Adhesion to Macrophages is Facilitated by the Mannose Receptor". Journal of Experimental Medicine. 182 (3): 1243–1258.
10- Visceral leishmaniasis. Archived March 23, 2010, at the Way back Machine. Institute for one World Health.
11- Barrett, MP; Croft, SL (2012). "Management of Trypanosomiasis and Leishmaniasis.". British medical bulletin. 104: 175–96.
12- Sundar, S; Chakravarty, J (2013). "Leishmaniasis: an update of current pharmacotherapy.". Expert opinion on pharmacotherapy 14 (1): 53–63.
13- Antinori S *et al.* (2009). "Is Real-Time Polymerase Chain Reaction More Useful than a Conventional PCR for the Clinical Management of Leishmaniasis?". Am J Trop Med Hyg 81:46–51.
14- Handman, E (April 2001). "Leishmaniasis: Current Status of Vaccine Development". Clinical Microbiology Reviews. 14 (2): 229–43. PMC 88972 .
15- Emanuela Handman (2001)."Leishmaniasis: Current Status of Vaccine Development"; Clin. Microbiol. Vol. 14 no. 2 229-243.
16- Srivastava, S; Shankar, P; Mishra, J; Singh, S (2016). "Possibilities and challenges for developing a successful vaccine for leishmaniasis.". Parasites & vectors. 9 (1): 277.
17- Palatnik-de-Sousa, CB (2008). "Vaccines for Leishmaniasis in the fore Coming 25 Years". Vaccine. 26 (14): 1709–24.
18- Dunning, N. (2009). "Leishmania Vaccines: from Leishmanization to the Era of DNA Technology". Bioscience Horizons. 2 (1): 73–82.
19- Elvidge, Suzanne (2012). "World's First Kala Azar Vaccine Enters the Clinic". FierceVaccines.com.
20- Myler P; Fasel Nhirf; f. (2008). "Leishmania: After the Genome". Caister Academic Press. ISBN 978-1-904455-28-8.
21- Willy, K. Tonui and Richard, G. Titus (2006). "Leishmania Major Soluble Exo-Antigens Protect Neonatal Balb /C Mice From A Subsequent Challenge With *L. Major* and Stimulate Cytokine Production by *Leishmania-Naïve* Human Peripheral Blood Mononuclear Cells". J.of Parasitology: 6, Vol. 92, No. 5, pp. 971-976.
22- Avivah Zuckerman(1975). "Current Status of the Immunology of Blood and Tissue Protozoa". I. Leishmania. Experimental Parasitology. Parasitological review, V. 38, Issue 3, December, Pages 370-400.
23- Dunning, N. (2009). "Leishmania Vaccines: from Leishmanization to the Era of DNA Technology". Bioscience Horizons. 2 (1): 73–82.
24- Khamesipour, A; Rafati, S; Davoudi, N; Maboudi, F; Modabber, F (2017). "Leishmaniasis Vaccine Candidates for Development: a Global Overview.". The Indian j of medical research. 123 (3): 423–38.