

Detection of *Visceral leishmaniosis* by RT-PCR technique in Wasit Province of Iraq.

Zahraa Ali Faieq

Department of Biology, College of Science, Wasit University, Kut,
Iraq.

الخلاصة

أجريت الدراسة الحالية في الردهة الاستشارية /قسم الباطنية في مستشفى الزهراء ومستشفيات الكرامة التعليمية في مدينة الكوت. اشتملت هذه الدراسة على تحديد مدى الإصابة بمرض داء الليشمانيات الحشوي في محافظة واسط حيث استخدم تفاعل البلمرة المتسلسل في الدراسة الحالية. تم جمع 70 عينة من الحالات المشتبه فيها المصابة بداء الليشمانيات الحشوي. كانت نتائج اختبار النتائج تفاعل البلمرة المتسلسل 70/50 (71%) كانت موجبة و 20/70 (29%) كانت سالبة. اعلى نسبة اصابة كانت في الاطفال الذين تتراوح اعمارهم اقل من سنة واحدة.

Abstract.

The current study was conducted in an advisory Pediatric in Al Zahra Hospital and Al-Karamah Teaching Hospitals in the city of Kut. This study included determining the extent of Kala-azar disease in the Wasit Province using Real-time PCR reaction in the present study. We collected 70 samples of suspected cases infected by visceral leishmaniasis. The results were 50/70 [71%] were positive and 20/70 [29%] negative in real-time PCR reaction test. The most age that have ability to infect with *donovani* was under one year.

Keywords: Real Time PCR; *leishmania donovani*.

Introduction

Protozoa are non-phototrophic, unicellular, eukaryotic microorganisms with no phone divider was numerous protozoa cause maladies in creatures and people. A few which are similar to *leishmania*, which causes Kala-azar,

most protozoa have a pimple arrange, which is torpid and high impervious to natural pressure. The trophozoite is the phase that regularly causes sickness by pathogenic protozoa tainting entire gatherings, similar to any mammal, Kala-azar is a noteworthy vector –

borne meta zoonosis which is caused by obligate intra-macrophage protozoa of the family *leishmania* [1]. There are in excess of 21 writers which have been recognized to be pathogenic to human [2]. Kala-azar is transmitted by the chomps of phlebotomies sand-fly, in the Old Word, sand-fly vector have a place with the sort phlebotomies "e.g *phebotomus papatasi* and *phlebotomies sergenti* ", which are the most vital, that encouraged on a contaminated host wherefore transmit *leishmania* species [3]. There are almost 600 types of phebtomine sand-fly are vectors, just 30% of these are imperative [4]. Kala-azar is a zoonotic sickness and significant protozoan heath issue. It is one among the six most critical vectors– borne illnesses in Worldwide, it is a gathering of ailments happen amongst human and creature, a few states of the maladies are humanities, while other zoonotic [5]. The malady is endemic in tropic and sub-tropic nations and has been accounted for more than 350 million people groups in 98 nations at peril, it is pervasive in 12 million individuals with 1.5 - 2 million new cases every year [6]. The sickness is endemic which founded in more than 60 nations, 90% of a recorded cases

happen in precisely five countries: Bangladesh, Brazil, India , Sudan and Nepal [7]. leishmaniasis happens in four principle composes in human including Cutaneous leishmaniasis (C), Mucocutaneous leishmaniasis (MC), Visceral leishmaniasis (V) and Post Kala-azar dermal leishmaniasis (PKD), the most two regular highlights influence the skin or inward organs [8]. The point of the examination was to research and location the nearness of *leishmania donovani* DNA in un healthy individual as whose living in endemic regions by Real-time PCR.

Material and Methods

Sample collection

The samples were collected from suspected cases infected by visceral leishmaniasis in Al Zahra Hospital and Al-Karamah Teaching Hospitals in the city of Kut.

RT-PCR

RT-PCR preliminaries and test were outlined in this think about by utilizing the compete arrangement of *leishmania donovani* kinetoplast DNA genome (GenBank: AF169136.1) (Bioneer company, Korea) Table [1].

Table (1): The primers of *L. donovani*

Primer	Sequence		PCR molecular weight
K-DNA Primer	F	TGGGTGGGCTAGCATTTTTG	72bp
	R	TAAAGCCGCATAACCACCAG	
K-DNA probe	FAM-TGGTTCGGGTGCCTTTGATGTGG-BHQ1		

Genomic DNA Exteraction

Genomic DNA was extracted from frozen blood samples by using AccuPrep[®] Genomic DNA extraction kit (Bioneer. Korea) and done according to company instruction.

The extracted genomic DNA was checked by utilizing Nano drop spectrophotometer (THERMO. USA), which estimated DNA focus (ng/μ) and checked the DNA immaculateness by perusing the absorbance at wave length (260/280 nm)

Genomic DNA Profile**RT-PCR materMix Preparation**

RT-PCR master mix was prepared through (AccuPower[®] DuaStar[™] RT-PCR PreMix kit

Bioneer . Korea), and done according to the company instructions Table (2)

Table (2): RT-PCR master mix components

Reaction solution	Volume
DNA template	5.μl
Forward primer [20 pmol]	1.μl

Reverse primer [20 pmol]	1.µl
Taq Manprobe [50 pmol]	1.µl
RT PCR master mix	12.µl
Total	20.µ

The parts of the RT-PCR ace blend response that mentioned in table 2 were included into RT PCR tube containing (TaqMan test premix). At that point a strip tubes vortex for blending the segments and centrifuged

for 3000rpm for 3.m in XSpin axis, after that moved into MiniOpticon RT-PCR thermocycler. Continuous RT-PCR Thermocycler conditions appeared in Table (3).

Table (3): RT-PCR thermocycler conditions

Step	Condition	Cycle
Pre-Denaturation.	95.°C 5.min	1
Denaturation.	95.°C 20.sec	50
Annealing/Extension.	60.°C 30.sec	
Detection.		

Statistical analysis

Statistical analysis: The statistical analysis was performed using SAS. (Statistical Analysis System version 9.1) [9].

Out of 70 random samples were tried ,50 (71 %) gave positive outcomes and 20(29%) gave negative outcomes of samples suspected visceral leishmaniasis by utilizing Real Time PCR method Table (4) and Figure (1).

Result

Real time PCR for detection of Kala azar

Table (4): The results of Real Time –PCR of *Leishmania donovani* patients.

Age (years)	No of samples	Positive	Negative
>1	23	18(36%)	5(25%)
1-2	22	16(32%)	6(30%)
2-3	13	8(16%)	5(25%)
3-4	0	0(0%)	0(0%)
4-5	12	8(16%)	4(20%)
Total%	70 (100%)	50 of 70 (71%)	20 of 70 (29%)

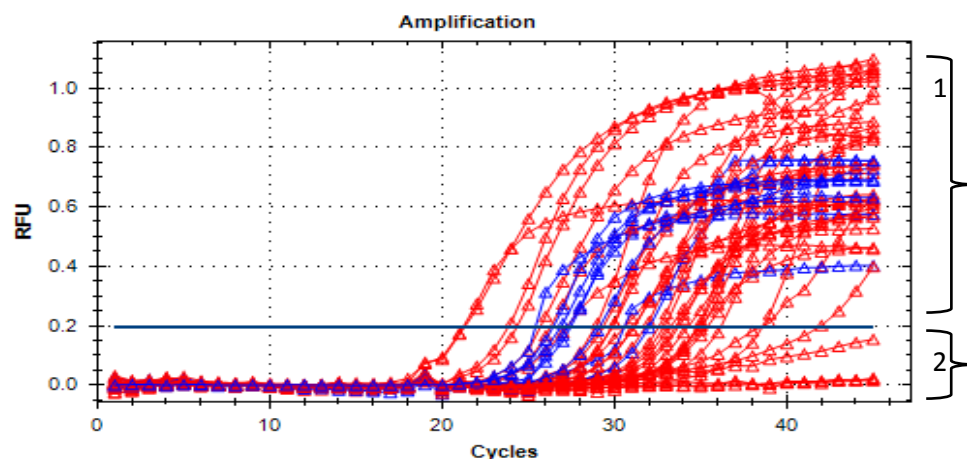


Figure (1): 1: Positive, 2: Negative, RT-PCR amplification pot of K-DNA region in *leishmania donovani* positive samples. This figure explains that where the positive samples were appeared in threshold cycle number [CT: 18] in to [CT:39] in patient samples red pot.

Discussion.

The innovation supplies RT-PCR elective process, speedy, delicate and particular in association with customary procedures, in this technique was analyzed *leishmania* parasite infections as we as species similitude in the meantime [10]. *leishmania* minicircle Kinetoplast DNA is one of the hereditary items that has been connected and prove to be helpful for disclosure of parasites in clinical examples , likewise, seclude and depiction of DNA by numerous agents [10] .Specialists illuminated that most sub-atomic symptomatic routes progressed for genotyping *leishmania* species depended on the polymorphic kDNA minicircle, that is gathered a prime select for a delicate test because of the presence of 10000 to 20000 minicircle by means of cell, large number, specificity, and redundant quality [11]. This investigation was agree with [12] in Yemen that recorded [62%] positive of the presumed visceral leishmaniasis patients were statistician contaminated. This

examination is likewise planned with the investigation by [13] in Brazil demonstrated that out of 91cases 84 (92%) were certain for *leishmania donovani*. Another examination was finished by [14] in Northern Ethiopia demonstrated that 69% positive by quantitative continuous kinetoplast kDNA PCR for distinguishing *leishmania donovani* in dry blood tests in an endemic focci . Much comparable occurrence of visceral leishmaniasis was accounted for by RT-PCR in Bangladesh by [15]. Our result which also agree with [16,17] which detected *visceral laishmania* in Iraq However a comparative outcome had been recorded by [18] who showed that 93% cases were certain in the major PCR test. Much owner occurrence of leishmaniasis were accounted for in Central and South Morocco by [19, 20, 21, 22] they were (45%) , (64%) , (58%) separately. Our outcome was in conflict with [23] which accounted for by who discovered 25% was sure; additionally couldn't help contradicting another examination who discovered 2% was certain when parasite minicircle kinetoplast DNA was increased [24] . The results were unique in relation to the outcome was accounted [25] who discovered ,20 patients with visceral

leishmaniasis positive by the variety particular PCR examine. Much lower frequency of leishmaniasis was accounted for by [26] which was 14.3%. RT-PCR that is quantitative is otherwise called RT-PCR. Conversely, traditional PCR is, best case scenario semi-quantitative. Moreover, real-time PCR information can be assessed without gel electrophoresis, bringing about decreased investigation time and expanded throughput. Finlay, in light of the fact that responses are run and information are assessed in a shut tube framework, open doors for tainting are decreased and the requirement for post intensification control is disposed of [27]. Consequently, a technique for finding that is sufficiently delicate to identify low levels of the parasite in asymptomatic or early symptomatic disease and can recognize the diverse *leishmania* species could be of huge utility in locales of endemicity and non endemicity [28,36]. Another researchers demonstrated an affectability of 96% for RT-PCR test [29, 30]. Notwithstanding, they demonstrated that contrasting and

different ways. RT-PCR was a quick test [31]. The motivation behind real-time PCR strategies are to decide presence of DNA polymerase inhibitor for a particular measurement [32]. The constant PCR methods could discover little portion of parasite DNA from *l.donovani*, the high affectability of our recognition was clear by its ability to open up parasite DNA from fringe blood of patients with Kala-azar patients in huge large part of cases [33] An atomic demonstrative measure is more quick, precise and touchy analysis technique for *leishmania* spp. Additionally it constitutes a fitting technique for phylogenic and epidemiological reconnaissance [34].

Conclusion

RT-PCR is advantageous over habitual PCR because it is quickly, less abort-intensive, decrease hazard of contamination.

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