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Comparison of Mrt-PCR Method with Microscopy for *Giardialamblia* Diagnosis

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Abstract

The current study was aimed to determine *G. lamblia*by multiplex real-time polymerase chain reaction (mRT-PCR) in faeces samples of children diagnosed by microscopic method. This study was conducted between April and November 2019 in Kirkuk, Iraq. The study population includes 283 children aged 0-12 years visiting Children's Hospital and Azadi Teaching Hospital with diarrhea. The faeceswere first analyzed by using microscopic method followed by concentration of faecessamples for the presence of *G. lamblia*. The results show that the overall positive ratio of *Giardia* infection among children below 12 years was determined by microscopy in 7.4% (21/283). After performing mReal Time-PCR analysis, the finding showed 24(8.5%) were positive for *G. lamblia*, which were negative under microscopic examination, while 21 microscopic diagnostic suspected positive cases were again diagnosed by mRT-PCR and found positive for only five samples.

Key words: ***G.*** *lamblia*;**mReal Time-PCR**;

Introduction

Giardia lamblia is defined as flagellated unicellular parasite that has the can to infect various species including man [1]. *G. lamblia* is the causative agent of infections of digestive system. The disease may happen in three kinds mild, severe and chronic [2-3]. Giardiasis disease was a universal spread and giardiasis is usual in children and adults. The spread of G. lamblia infection is high occurrence in developing countries. About 200 million patients of G. lamblia infection are yearly diagnosed global [4]. The rate of infection in an infected children with asymptomatic has been reported from 8%-30% in developing countries and 1-8% in areas with industrialize [5].The signs of *G. lamblia* infection vary between the absences of symptoms in infected patients until to various symptoms include various levels of diarrhea, abdominal pain, weight loss of infected person, nausea and vomiting [6-7]. The proper prognosis of giardiasis is significant for treatment and preventing of disorders. The laboratory prognosis of *G. lamblia* is generally based on determine and revelation of cyst in faeces samples, but immunological-based test and molecular diagnosis also are utilized for prognostic in developed countries. All diagnostic ways allow various sensitivity and specificity. This condition depends on several factors like test method, the skill of procedure and the phase that test have been done [8-9]. PCR diagnosis sensitivity is better than microscopic method, making it of better utilize to diagnosis of low numbers of cysts in patient faeces [10-11]. So, the current study was aimed to determine *G. lamblia*by qPCR in faeces samples of children diagnosed by microscopic method.

Materials & methods

Sample collection

This study was conducted between April and November 2019 in Kirkuk, Iraq. The study population includes 283 children aged 0-13 years visiting Children's Hospital and AzadiTeaching Hospital with diarrhea. 2-5 g of fresh faeces samples were collected in a clear and sterile container. After that, the faeces samples were transported to laboratory for analysis.

Microscopic diagnosis

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The macroscopic properties like color, blood presence, mucus and other abnormal conditions were observed. A part of faeces samples was taken to detect cyst stage and trophozoite stage [12]. The fecal samples were mixes with drop of normal saline followed by iodine preparation for microscopic diagnosis. Out of 283 samples, a total of 60 samples (8 positives and 52negatives cases) were sent to private laborotary in Baghdad city for further molecular analysis.

DNA extraction

DNA was extracted from faeces samples by using PowerFecal® DNA Isolation Kit (Carlsbad, CA) according to the manufacturer's procedure. The DNA samples were stored at -20°C until further molecular analysis.

Real Time-PCR

A total of randomly selected subset of 60faecessamples was analyzed. Of them 8 samples were positive, while 52 samples were negative by using microscopy examination for *G.lamblia*. Real Time-PCR assays targeting G. lamblia were performed in the private laborotary. The mixture of reaction (20 µl) contained 1x PerfeCTa qPCR ToughMix, (0.2µM) each primer and template DNA (2.5 µl). All PCR reactions were performed in duplicate using the Applied BiosystemsStepOne quantitative PCR (qPCR) system. The conditions of amplification comprised of a hold at 95°C for 15 min, than followed by 50 cycles of 95°C for 15s, 60°C for 30 s, and 72°C for 30 s. A curve of calibration with concentrations spanning the range from 10-106 copies of gene per reaction, with two replicates, was constructed.

Table (1) was show *G. lamblia*specific primers. Amplification consisted of 15 min at 95°C followed by 50 cycles of 15s at 95°C, 30s at 60°C, and 30s at 72°C and was performed with the Step One Plus (Applied BioSystems).

Table (1): *G. lamblia*specific primers

Primer	Sequence
Forward	(5′-GACGGCTCAGGACAACGGTT-3′)
Reverse	(5′-TTGCCAGCGGTGTCCG-3′)

Statistical analysis

Chi-square test was used to test the significance of attributes between the study variables. A *P* value of <0.05 was considered statistically significant.

Results and Discussion

Detection of *Giardia* spp. from microscopy in stool samples

The overall positive ratio of *Giardia* infection among children below 12 years was determined by microscopy in 7.4% (21/283) as shown in table 1.

Table (1): Microscopic diagnosis of *G. lamblia*

Name of the parasite	No. of positive samples	No. of negative samples	Positive ratio (%)
G. lamblia	21	262	7.4%

Distribution according to age and gender

Table(2) was show a total of 283feaces samples, 89 (31.4%)were females and 194 (68.6%) were males. The results showed that the positive perecentage of *Giardia* was found higher in female

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(11/283) than in male (10/47.7) ($p > 0.05$). *Giardia* infection was highest in children of age group <3 years (11/21) followed by 3-6 years (6/21) ($p>0.05$).

Table (2): Distribution according to age and gender

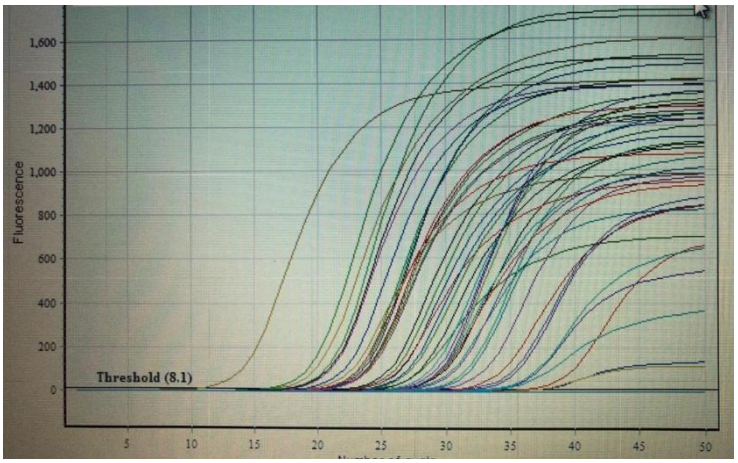
Age group (years)	Females		Males		Total of number tested	Total of *Giardia* positive number (%)
	Number tested	*Giardia* positive (%)	Number tested	*Giardia* positive number (%)		
<3	43	7 (63.6%)	106	4 (36.4%)	149	11(52.3%)
3->6	27	3 (50%)	47	3 (50%)	74	6 (28.6%)
6->9	12	1 (33.3%)	25	2 (66.7%)	37	3 (14.3)
9-12	7	0 (0)	16	1 (100%)	23	1 (4.8%)
Total	89	11 (52.3%)	194	10 (47.7%)	283	21 (100%)

Comparison of microscopy with mReal Time-PCR for *G. lamblia*

After performing **mReal Time-PCR** analysis, the finding showed 24(8.5%) were positive for *G. lamblia*, which were negative under microscopic examination, while 21 microscopic diagnostic suspected positive cases were again diagnosed by qPCR and found positive for only fivesamples.

Table (3): Comparison of microscopy with Real Time-PCR for *G. lamblia*

Microscopy (n = 283)	qPCR (n = 283)	
	Positive	Negative
Positive (21)	5	16
Negative (262)	19	243
Total	24	259



The prevalence of microscopic analysis in current study was in agreement with [13] who referred that the percentage of infected patients which were (49) positive samples with percentage (8.1%) and (554) negative samples, with the percentage was (91.9%). also the current results agree with [14] who examined (92) diarrheic stool samples and found (15.3%) infected with *G. lamblia* and the remained (84.7%) was negative results for *G. lamblia*.In present study, the prevalence of *G.*

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*lamblia*among males and females were significant (p>0.05) difference, which is in agreement with previously conducted studies [15-17]. Also, the current study was in agreement with [18-19], who reported the highest percentage of *G. lamblia*infection in females and lowest percentage of *G. lamblia*infection in males. Also, the distribution of *G. lamblia* was highest in children of age group <3years followed by 3–6 years group. the current study was in agreement with [13] who referred G. lamblia infection according to age groups of the highest percentage found 1-10 years. There is need for a sensitive and specific prognostic method for diagnosis the etiological agent of disease, with G. lamblia , techniques of molecular particularly PCR based procedures possess a greater specificity and sensitivity compare with microscopy examination [20].

Conclusions

Microscopic examination remains primary method for diagnosis digestive tract parasites because of its easy to diagnosis various parasites . This technique is relatively cheaper than PCR techniques but PCR possess more specificity and sensitivity compare with microscopy diagnosis.

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