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Article

Genetic identification of *Giardia lamblia* in children for Tikrit city, Iraq

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ABSTRACT

Giardia lamblia, also known as (*Giardia duodenalis* or *Giardia intestinalis*) is the causative agent of giardiasis, and it is the most common parasitic diarrheal disease that affects humans and more than 40 species of other mammals. The study recorded (17) positive cases of *Giardia* infection out of a total of (47) samples diagnosed microscopically for pediatric patients arriving at Tikrit General Hospital from nearby areas. The results showed that the percentage of positive cases of infection with *the Giardia* parasite amounted to (36.2%) for PCR, as significant differences appeared when compared with the microscopic examination (P value< 0.05). Also, the rates of infection with the parasite varied between males and females, and the percentage of infection in males reached (53.2%), while the percentage of infection in females was (43.14%); the results showed the relationship between infection and *Giardia* by age groups less than one year, where the percentage amounted to 44.6%, followed by the age group 1-2 years, the percentage reached 31.9%, and the lowest percentage was in the age group 3-4 years 10.6%.PCR technique diagnosed the specific region within the DNA of the parasite Giardia using special primers for the encoded gene (for Giardia2029/Gia2150c) and (for Giardia). Also, it was sequenced and aligned. The isolate in the current study was 100% similar to the globally recorded isolate. In conclusion, the methods of detection of *Giardia* showed differences in positive results for this parasite. In addition, there are more infections in males than in females aged less than one year more infected than in other ages.

Keywords: *Giardia lamblia*, Giardia2029/Gia2150c, Giardia gene

INTRODUCTION

The parasite Giardia lamblia, also known as (Giardia duodenalis or Giardia intestinalis) is the causative agent of giardiasis. It is the most common parasitic diarrheal disease that affects humans, and more than 40 species of other mammals and the World Health Organization has classified Giardia as the eleventh most important parasite. Worldwide, giardiasis is highest among children under the age of 5 years. Also, adults aged 30-40 years are at risk [1]. The people most affected by giardiasis are those who suffer from immunodeficiency and those traveling to areas with high rates of infection with this parasite [2]. The

infection may occur without symptoms, or clinical symptoms usually appear in foul-smelling diarrhea, abdominal pain, and bloating. Moreover, infection may lead to long-term problems such as irritable bowel syndrome (IBS) and chronic fatigue. Infection occurs by swallowing infectious bags through the mouth, ubiquitous in food and water (Ferguson et al., 2020). The PCR method has proven effective in detecting mixed infections, and its applicability in laboratories with basic molecular equipment and polymerase chain reaction (PCR) proved to be more sensitive for Giardia detection than microscopy [3]. Molecular diagnostics is highly sensitive because it detects infection from samples containing a low percentage of parasites, including samples from asymptomatic patients. It plays a role in the epidemiology, prevention, and treatment of parasitic diseases [4, 5]. This study aims detection of Giardia duodenalis in children by using PCR.

MATERIALS AND METHODS

Sample collection

The study included children with diarrhea; 132 samples were collected from Tikrit Hospital, including age groups from one month to 5 years, from November to January. Blood was collected for the detection blood group, Rh factor, ESR (Erythrocyte Sedimentation Rate), and C-reactive protein test.

Stool examination

All stool samples were examined microscopically, where the stool was taken on a clean glass slide, and several drops of 0.9% physiological saline were added, with the use of drops of iodine-logo solution to stain the nuclei of the cysts; the slide was examined under the power of 10 X and 40 X to diagnosis [6, 7].

DNA extraction

Extracted DNA of Giarsia by Quick-DNA™ Fecal/Soil Microbe Miniprep Kit

PCR detection

The primers were used from IDT (Integrated DNA Technologies company, Canada), as shown in table 1 and 2. Also, it was used Maxime PCR PreMix kit (i-Taq) 20µlrxn Cat. No.25025). PCR reaction with final volume 25µl included Taq Premix 5µl, Forward primer 10 picomols/µl (1 µl), Reverse primer 10 picomols/µl (1 µl), DNA template *2*µl, Distill water *16*µl and the program cycles for each gene as shown in tables 3 and 4, In addition, it was electrophoresis by using Safe red stain and SiZer DNA Markers Ladder 1000bp (intron/Korea) to detect PCR product for each gene. It was sequencing PCR products by the Sanger technique, which was sent to Macrogen/Korea

Primer	Sequence	Tm (°C)	G.C. (%)	Product size
Forward	5′-AAGTGTGGTGCAGACGGACTC-3′	56.31	57	497bp [20]
Reverse	5'-CTGCTGCCGTCCTTGGATGT -3′	55.88	60	

Table 1. Gia2150c/Gia2029 primers

Primer	Sequence	Tm (°C)	G.C. (%)	Product size
Forward	5′-CGCCGTACACCTGTC-3′	52.6	66.7	322bp [21]
Reverse	5'-AGCAATGACAACCTCCTTCC -3′	56.8	50.0	

Table 2. Specific primer of genus *Giardria*

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	30sec	35 cycle
3-	Annealing	54°C	30sec	
4-	Extension-1	72°C	30sec	
5-	Extension -2	72°C	10 min.	1 cycle

Table 3. Program cycles for Gia2150c/Gia2029

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	10 min.	1 cycle
2-	Denaturation -2	95°C	45sec	35 cycle
3-	Annealing	62°C	45sec	
4-	Extension-1	72°C	45min	
5-	Extension -2	72°C	10min.	1 cycle

Table 4. Program cycles for Specific primer of gene Giardia

RESULTS

The current study recorded (17) positive cases of Giardia infection out of a total of (47) samples diagnosed microscopically for children patients arriving at Tikrit General Hospital from nearby areas. The results showed that the percentage of positive cases of infection with the Giardia parasite amounted to (36.2%), and significant differences appeared at ($P<0.05$) as shown in Table 5.

PCR	Microscopic examination				Total
	Positive	Percentage	Negative	Percentage	
Positive	17	36.2	0	0	17
Negative	30	63.8	85	100	115
Total	47	100	85	100	132
	Chi-Square = 60.07 P-Value = 0.001				

Table 5. Comparison of microscopy and polymerase chain reaction for the number of positive cases in *Giardia lamblia*

The parasite infection rate has likely increased as a result of the country's poor economic and living situation in some areas due to housing in some unqualified areas, the lack of health services, population density and lack of access to unclean drinking water, and in addition to several Tikrit city areas It suffers from a shortage in the availability of services due to the resulting increase in population and as a result of the lack of adequate health and educational guidelines for citizens.

Distribution of the percentages of people infected with Giardia by gender

The current study shows that the infection rates with the parasite varied between males and females, and the percentage of infection in males reached (53.2%), while the percentage of infection in females was (43.14%), as shown in Table 6. There were no significant differences at the probability of 0.05.

Gender	Positive	Percentage
Males	25	53.2
Females	22	43.14
Total	47	100.0
	Chi-square=54 P- value=0.001	

Table 6. The percentages of the number of infected samples by gender

Distribution of Giardia infections by age

The current study showed the relationship between those infected with the parasite Giardia by age groups less than one year, where the percentage amounted to 44.6%, followed by the age group 1-2 years, the percentage reached 31.9%, and the lowest percentage was in the age group 3-4 years 10.6%, as shown in Table 7.

Gender	Positive	Percentage
Less than one year	21	44.6
1 - 2	15	31.9
2 – 3	6	12.8
3-4	5	10.6
Total	47	100.0
	Chi-Square = 5.8 P-Value = 0.12	

Table 7. The percentage of age groups infected with Giardia

The results of the statistical analysis showed that there were no significant differences at the level of probability (0.05), as most injuries occur in children

under the age of one and a half years due to the lack of commitment by some mothers to the rules and system of basic general hygiene, and in addition to that the contamination of breastfeeding tools, and in addition to that.

Genetic analysis

When using polymerase chain reaction (PCR) technique to duplicate a specific region within the DNA of the parasite Giardia using special primers for the encoded gene (for Giardia2029/Gia2150c) and (for Giardia), and after the amplified DNA products into the electrophoresis device, the result showed that The molecular size of the DNA (the coding gene (Gia2029/Gia2150c) is about 497 bp. The coding gene (Giadria) is about 322 bp, compared to the standard size guide for Ladder DNA, as shown in Figures 1,2,3,4.

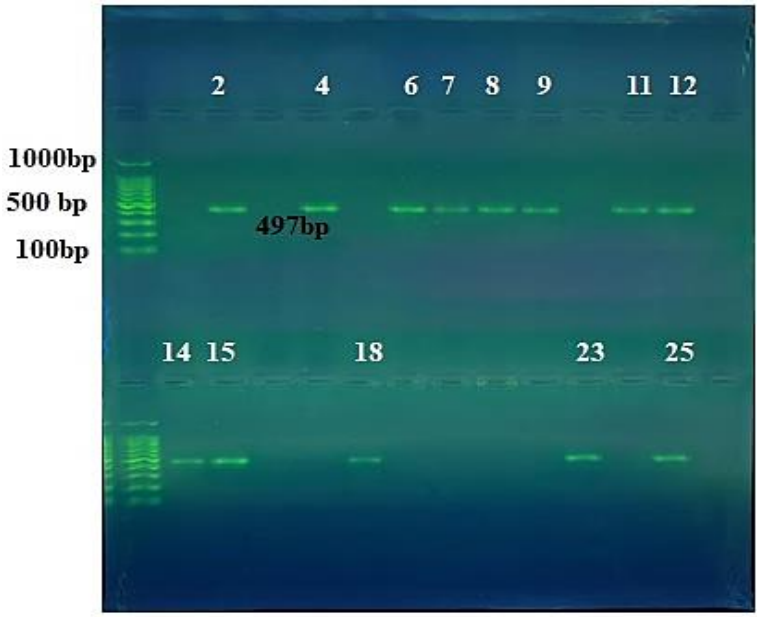

Figure 1. PCR product of the gene (Gia2029/Gia2150c) for samples (2, 4, 6, 7, 8, 9, 11, 12, 14, 15, 18, 23, 25) and the gene size was 497 bp. Electrophoresis was carried out in a 2% agarose gel at 75 V/15 cm^2 for 1 hour and Ladder 1000 bp.

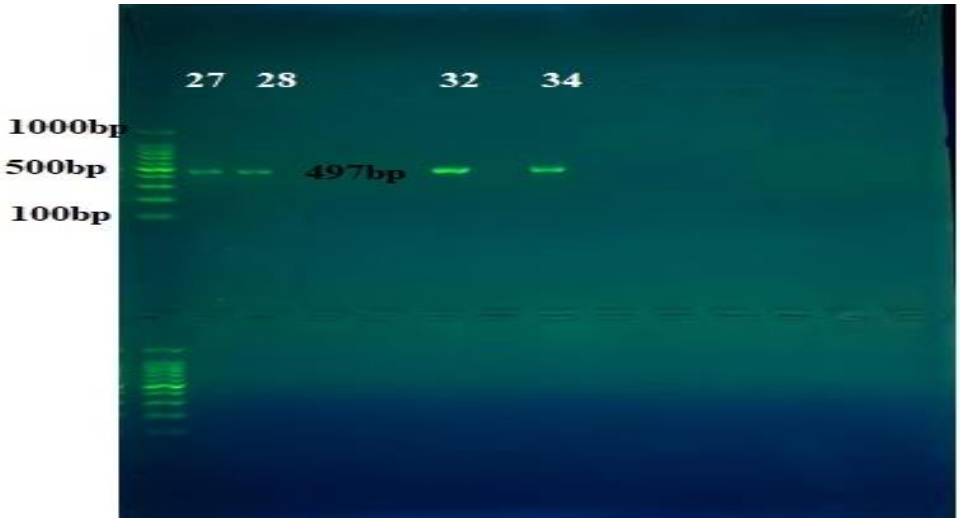

Figure 2. PCR product of the gene (Gia2029/Gia2150c) for samples (27, 28, 32, 34), and the gene size was 497 bp. Electrophoresis was carried out in a 2% agarose gel at 75 V/15 cm^2 for 1 hour and Ladder 1000 pb.

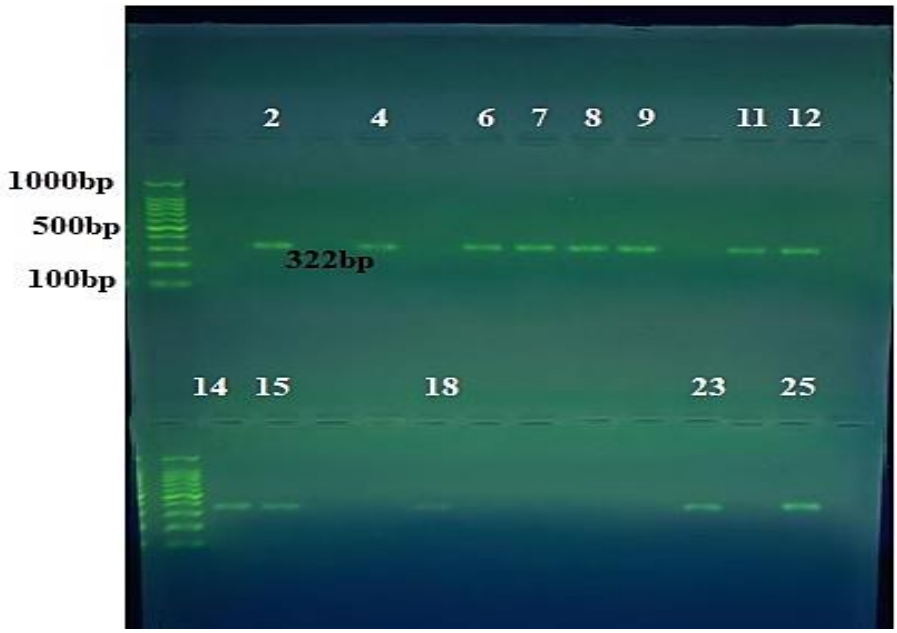

Figure 3. The PCR product for the Specific *Giardia* gene for samples (2, 4, 6, 7, 8, 9, 11, 12, 14, 15, 18, 23, 25) and the size of the gene was 322 bp. Electrophoresis was carried out in a 2% agarose gel at 75 V/15 cm^2 for 1 hour and Ladder 1000 bp.

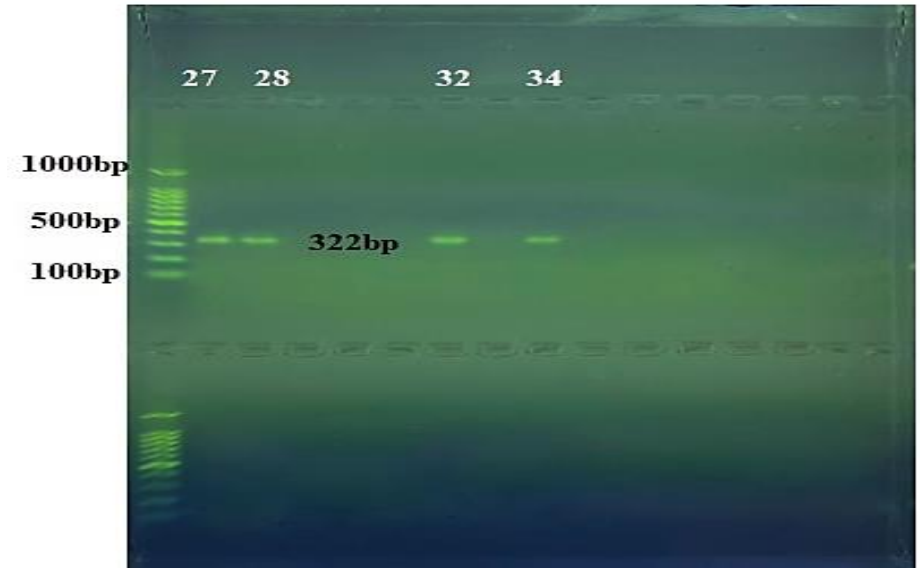

Figure 4. PCR product of the Specific *Giardia* gene for samples (27, 28, 32, 34). The gene size was 322 pb. Electrophoresis was carried out in a 2% agarose gel at 75 V/15 cm^2 for 1 hour and Ladder 1000 bp.

After sequencing the nitrogenous bases of the genes used in the study, the samples were identical to the globally identified isolates after performing the matching process through nucleotide sequence analysis using the Bioedit alignment program to compare with the globally registered isolates in GenBank. Relevant sequences with samples were obtained from the NCBI database (www.ncbi.nlm.gov/nucleotide). The isolates in the current study were 100% similar to the globally recorded isolates *(*Giardia intestinalis isolates 737 triose-phosphate isomerase gene ID: MN844148.1), as shown in Figure 5.

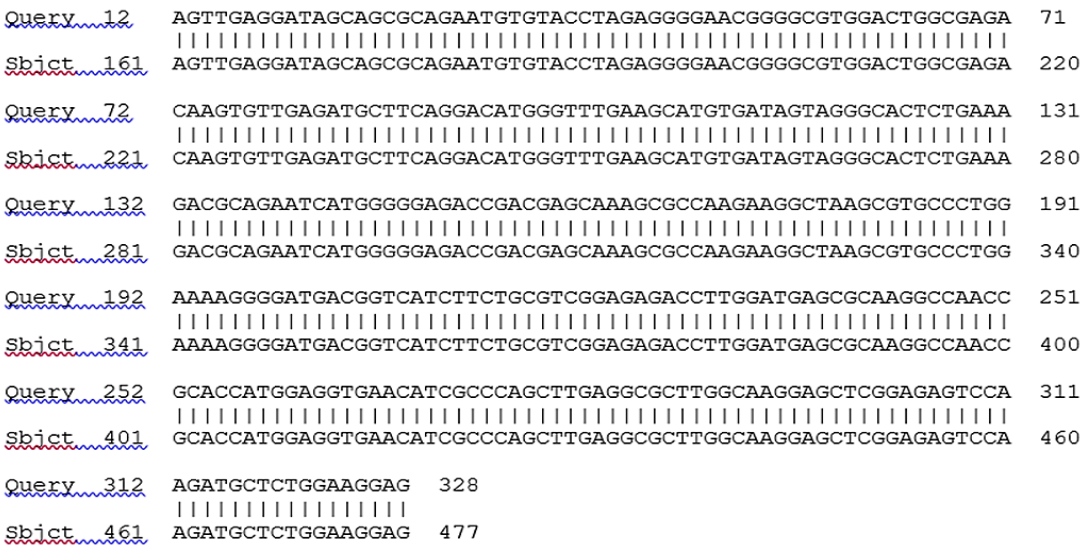

Figure 5. The concordance with the current study isolates, and 100% matching with the globally registered isolate with I.D. number MN844148.1 (Giardia intestinalis isolate 737 triose-phosphate isomerase gene)

Other studies showed the importance of detecting the Giardia parasite, as the polymerase reaction technique was used to diagnose and estimate the spread of the Giardia parasite in fresh vegetables using a direct wet swab with iodine stain. DNA was extracted from all samples and extracted from 102 samples from all samples with a percentage of 44.3%. Moreover, the results of the PCR test for the 16SrRNA gene of the Giardia parasite were detected in an average of 5.9% of the samples [16]. One of the studies showed the efficiency of the PCR technique in detecting the Giardia parasite using 16S rRNA. The primers for the first amplification were (Gia2029 and Gia2150c).

DISCUSSION

Among (47) samples included (17) infected samples and a percentage of (36.2%) infected with the Giardia parasite of the total infected samples, which matched or did not match this result with what was stated [8] in the city of Tikrit, where it reached 14.30 %, and it coincided or did not match the result of the current study with what was stated by [9] in Kirkuk and [10] in Mosul, where the infection rate of Giardia parasite was recorded 57.89%, 1.04%, respectively**.** The result of the current study agreed with the result of [11] in the city of Najaf, and the results of this study agreed with what was stated in Baqubah [12]. At the same time, the results of this study did not coincide with what was stated [13]. The infection rate in the current study among females was lower than the rate of infection among males. No significant differences were found at P>0.05. Children under two years old have low immunity. They are susceptible to infection easily, and repeated exposure to the parasite for young age groups (children) may stimulate their immune system and be more developed, which leads to a decrease in the infection rate [14]. The PCR technique was used to detect DNA for rapid diagnosis and to avoid the low sensitivity of other traditional tests. By using molecular methods, parasites can be diagnosed and genotyped. It also allows us to overcome the difficulties faced by direct examination that does not allow us to determine the type of parasite that causes infection [15]**.** The results showed that 40 isolates from humans and 66 from cows were 100% positive for this technique [17]. In addition, a study identified oth-

er Giardia parasite genes in stool samples by amplifying the gdh gene using polymerase chain reaction technology for isolated isolates from humans and cattle. The gene was diagnosed in 9 samples from humans and 17 from cattle [18]**.** The polymerase chain reaction was also used to detect other parasites, such as Cryptosporidium, by detecting the HSP-70 gene using polymerase chain reaction (PCR), where it was found in 10 samples out of 102 samples that were diagnosed microscopically [19].

CONCLUSION

The methods of detection of Giardia showed differences in positive results for this parasite. In addition, there are more infections in males than in females aged less than one year more infected than in other ages. The isolate in this study was 100% similar to the globally recorded isolate.

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