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Molecular detection of glutamate dehydrogenase gene of *Giardia lamblia* isolated from food handlers in Erbil city

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Abstract

Background and objective*:* *Giardia lamblia* is the intestinal flagellated protozoan parasite that infects vertebrates, including humans. Giardiasis is the major diarrheal disease found worldwide. It can be symptomatic or may be an asymptomatic carrier that led to chronic disease. This study aimed to determine the proportion of giardiasis among food handlers and evaluate the correlation between two laboratory methods for identifying the *Giardia lamblia*.

Methods: A total of 308 stool samples were collected from food handlers that annually attend the central laboratory in Erbil City. Wetmount microscopic examination was performed for the diagnosis of cysts and trophozoites of *the Giardia* parasite. Molecular analysis done for positive samples, DNA extraction performed using the QIAamp Fast DNA Stool Mini Kit (Qiagen Company, Germany). Nested PCR analysis was done targeting the Glutamate dehydrogenase gene using two sets of primers for amplification of 734bp fragment. Gel electrophoresis was performed for visualizing the amplified DNA by Ultraviolet light.

Results: The mean age of food handler participants was 29 years. Most (93.8%) of the food handlers were males, and the majority (98.6%) of the participants did not have any signs and symptoms. Four (7.4%) microscopy positive sample participants were highly educated. There was no association between educational level and positive rate by microscopy (P = 0.066). The majority of participants did not receive treatments, particularly most of the microscopic positive samples. The food handlers did not take any antiparasitic treatments 9 (3.4%) (P = 0.676). From 11 (3.6%) microscopically positive samples, 10 (90.9%) *Giardia lamblia* gdh gene 734 bp fragments were amplified by nested PCR.

Conclusion: Amplification of 734bp of gdh gene by the nested PCR is the most specific and sensitive method for identifying *Giardia lamblia*. Food handlers were important people to care about sanitation and preparing food, particularly for avoiding diseases transmitted by food.

Keywords: Giardiasis; Genetic characterization; gdh gene; Nested PCR.

Introduction

Giardia lamblia is the parasite that belongs to the protozoan parasite. It infects the upper part of the small intestine of vertebrates animal including humans.[1] It is the only flagellated parasite that causes diarrhea worldwide.[2] The parasite has a public health problem generally in both developing and also in non-developing nations, it is thought that 280 a million persons get this parasite globally.[3,4] Fecal-oral road transmission of *Giardia* initiated giardiasis by the ingestion of cyst through non-filtrated water and contaminated food. Binucleate trophozoites forms after the excystation of the cyst stage in the small intestine. The parasite colonizes and adheres to the microvilli of the intestine, which leads to infection.[5] The medical characterization of infections caused by *Giardia lamblia* parasite begins with asymptomatic to acute diarrheal

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infection, abdominal pain, lactase deficiency, malaise, and malabsorption in chronic cases. It is the most predominant parasite that causes diarrhea from other parasites that infect the intestinal tract.[6] *Giardia lamblia* has different genotypes that morphologically the same, based on genetic characterizations classified into eight genotypes or assemblages (A-H), which is host-specific.7 Strains that are defined in humans are categorized to assemblages A and B considered zoonotic, infecting humans and a wide range of animals. Other assemblages are specialized for specific hosts.[8] In recent years, the assemblage E was defined in animals and humans. *Giardia lamblia* infecting humans can be commonly categorized into assemblages A and B.[9] Various diagnostic tools are available to detect intestinal parasites, including *Giardia lamblia*. Stool microscopy is a manual procedure used in all laboratories. The test sensitivity is low; it depends on factors that affect the diagnosis of the parasite in a stool sample. Three stool samples are recommended for adequate identification that depends on the density of stool and personal interpretations. Another diagnosis tool is immunological assays that are determined by antigen-antibody complex assays and include enzyme-linked immune sorbent assay (ELISA) and immunochromatographic test.[10,11] Polymerase chain reaction (PCR) has been developed, which is a more sensitive method than other assays for the detection of microorganisms, including parasites.[12] In Kurdistan region, especially in Erbil city, there is a program for screening food handlers for parasitic infections and other microbes every year for controlling and reducing the transmission of infection. The program is performed by the health authority. The current study aimed to determine the proportion of *giardiasis* among food handlers and to molecular detection of glutamate dehydrogenase gene of *Giardia lamblia*.

Methods

Study design and setting

This cross-sectional study was carried out from September 2018 to May 2019 on food handlers that annually attend the central laboratory in Erbil city.

The questionnaire

A questionnaire was designed that included socio-demographic information like age, gender, and history of previous parasitic diseases and was filled out by all participants.

Stool samples collection and population

A single stool sample was collected in a sterile stool cup from 308food handlers that were selected randomly. Macroscopic and microscopic examination of all stool samples were done immediately after collection. The negative and positive samples were collected in special tubes without adding any preservatives to samples and were stored at −20 °C until the molecular analysis was performed.

Laboratory methods

A) Macroscopic examination

The test was done by naked eyes, and the stool samples were checked for consistency (formed, soft, and liquid), color (eg. brown, yellow, or other abnormal), odor, and the presence or absence of the mucus and blood in stool samples.

B) Microscopic examination

A small amount of stool was placed on a microscope slide and was mixed with a drop of normal saline, then putting cover slide and examined at 10X and 40X magnifications for detection of cysts and trophozoites of the *Giardia lamblia* parasite.

C) Molecular method

DNA extraction

The genomic DNA was extracted from stool samples by using the QIAamp Fast DNA Stool Mini Kit (QIAgen Company, Germany) and by following the instruction of the protocol of the manufacturer. The first step was weighing the frozen sample by analytical balance. The sample could not thaw until adding the lysis buffer (Inhibit EX Buffer) to the stool sample to

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avoid the degradation of the DNA. The extracted DNA was stored at −20 °C for less than six months until used for running the PCR. The purity of the extracted DNA was measured by the Nanodrop .

Amplification of *Giardia lamblia* DNA by nested PCR and primers

To identify and knowing molecular characterization of Giardia lamblia loci: glutamate dehydrogenase gene (gdh) by nested PCR. The gdh gene is short length gene. Most research studies use the same designed primer. Primers were used for amplification of (gdh) gene; two sets of oligonucleotides were used, as shown in Table 1.

Nested PCR condition

Nested is a type of PCR composed of two rounds. The first round of PCR needs initial denaturation. The thermocycler was set up at 94 °C for 5 minutes that need a high temperature to break DNA bonds. Then, denaturation was done at 94 °C for 30 seconds. The best annealing temperature of the primer is at 60 °C for 30 seconds for the gdh gene. The final step was the extension of genomic DNA at 72 °C for 30 seconds for 25 cycles. At last needs a final extension at 72 °C for 5 minutes. For the second round of nested PCR, all the phases were the same. Nonetheless, the cycles differ from the first-round 35 cycles for the second round of nested PCR.

Gel electrophoresis

For separation of amplified DNA, 2% agarose gel was used, and the DNA was stained by DNA safe dye, DNA ladder was used on 100 voltages for 25 minutes and visualized under the UV light.

Ethical considerations

The study was approved by the Research Ethical Committee of the College of Medicine, Hawler Medical University. Written informed consent was taken from each participant before sample collection.

Statistical analysis

Data were analyzed by using the statistical package for the social sciences (SPSS, version 23). Simple descriptive analysis and frequency have been done for the variables. Chi-square and Fischer's exact test were used to assess the significance of the responses, and a *P* value ≤0.05 was considered statistically significant.

Results

Among 308 food handlers that participated in this study, the majority were males (93.8 %). The age range of the participants was between 14-64 years. The mean±SD age of the participants was 29±9.70 years, as shown in Table 2. Microscopically wet mount examination was done for 308 stool samples, and 11 (3.6%) samples were positive for *Giardia lamblia*. The parasite was identified more frequently in

Table 1: Two sets of primers used in this study.

Gene	*Primer*	*Sequence*	Fragment size	Reference
GDH First round	*GDHeF (Forward)*	*TCAACGTYAAYCGYGGYTTCCGT*		
	GDH2 (Reverse)	*ACCTCGTTCTGRGTGGCGCA*	772 base pair	[13]
GDH Second round	*GDHiF (Forward)*	*CAGTACAACTCYGCTCTCGG*	734 base pair	[13]
	GDH4 (Reverse)	*GTGGCGCARGGCATGATGCA*		

Each PCR reaction tubes contain a total volume (26µl), 0.5 µl of each forward and reverse primers with 20 µl of Taq 2x Master Mix (AMPLIQON, Denmark), 5µl of extracted DNA.

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graduated participants (4 cases, 7.4%) and from participants with high school education (6 cases, 4.3%), with only one case (0.9%) positive sample from participants with primary school education. The association between education level and positive examination was statistically insignificant (*P* >0.05), as shown in Table 3.

Table 2: Frequency of socio demographic characteristics of the food handlers participants.

Variable	No. of participants	(%)
Gender		
Male	289	93.8
Female	19	6.20
Age group		
13-18 years	17	5.50
More than 18 years	291	94.5
Residency		
Urban	263	85.4
Rural	45	14.6
Educational level		
Primary school	114	37.0
Secondary	140	45.5
University	54	17.5
Did you have any gastrointestinal symptoms?		
Yes	5	1.60
No	303	98.4
Total	**308**	**100**

Table 3: Identification of *Giardia lamblia* by microscopic examination according to the educational level of participants.

Educational Level	No. examined	No. positive (%)	***P value**
Primary school	114	1 (0.9)	
High school	140	6 (4.3)	0.066
University	54	4 (7.4)	
Total	**308**	**11 (3.6)**	

* Fisher's Exact Test

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Most participants with microscopically positive stool samples for *Giardia lamblia* did not receive any treatment (9 cases, 3.4%), while 2 cases (4.3%) of positive samples of participants received treatment. The association between positive microscopic examination and receiving treatment was not significant (*P* = 0.678), as shown in Table 4. Nested PCR (Figure 1) was done for all microscopically positive stool samples (11 cases, 3.6%). The *gdh gene* was amplified from 10 (90.1%) samples and 1(9.1%) not amplified sample, as shown in Table 5.

Table 4: History of taking treatments and association with microscopically positive with *Giardia lamblia*

Variable	Microscopic Examination +ve No. (%)	Microscopic Examination -ve No. (%)	Total No.	*P value
Did you receive treatment?				
Yes	2 (4.3)	45 (95.7)	47	0.678
No	9 (3.4)	252 (96.6)	261	
Total	**11 (3.6)**	**297 (96.4)**	**308 (100)**	

* Fisher's Exact Test

Table 5: Frequency of detection of *Giardia lamblia* by direct and PCR methods.

Method	Positive No. (%)	Negative No. (%)	Total
Direct microscopic Examination	11 (3.6)	297 (96.4)	308 (100)
Polymerase chain reaction (PCR)	10 (90.9)	1 (9.1)	11 (100)

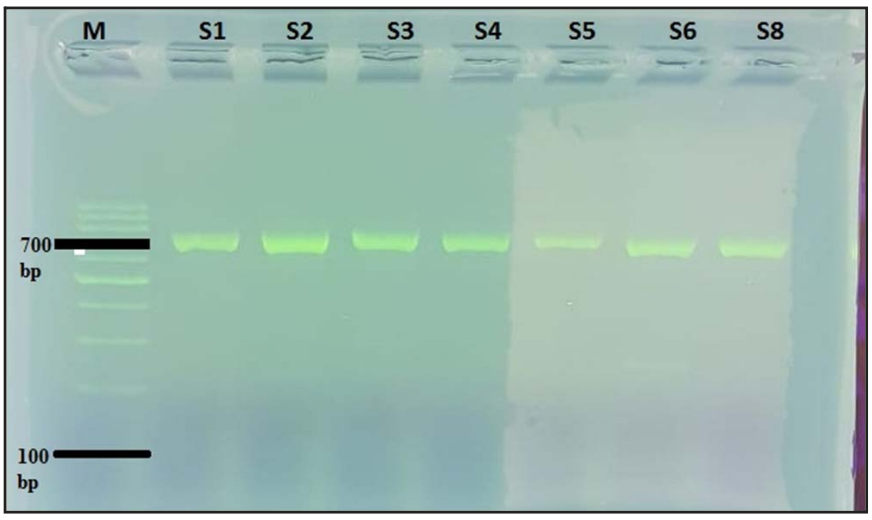

Figure 1: Nested PCR second round (2%) gel electrophoresis showed the amplification of 734bp of *gdh gene* of *Giardia lamblia*, M (100bp DNA ladder, numbers are the amplified samples.

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Discussion

Giardia lamblia is the most predominant intestinal protozoa which cause diarrhea in human. The parasite till now is considered a major enteric parasite worldwide, especially in food handlers who have an important role in spreading the parasite.[14,15] The majority of food handler participants were males (93.8%), particularly in Kurdistan Region. The opportunity of working in restaurants is more for men. A study conducted in Sudan food handlers showed that approximately all screened persons were males (92.9%).[16] Giardiasis may not always show signs and symptoms. Asymptomatic carriers act as healthy people who pass the infection to others without suspecting it, and they are considered the main source of infection. The parasite transmission road has an effect on spreading the infection,[17] especially by food handlers that deal with foods. The present study shows that most participants did not have clinical symptoms (98.6%), and all microscopically positives samples did not show symptoms. The other investigator also noticed this problem.[18] This study showed that the education level of participants did not show any significant effect on infection rate by *Giardia parasite*. The parasite transmits by food and water. People might ingest it in a place that is not clean from outside, or they become infected by communication with some people that are not educated. Four (7.4%) of positive samples were graduates (P = 0.678). Antiparasitic treatments have the effects of eradication and cure from disease and affect the diagnosis of the parasite microscopically.[19] In this study, approximately most of the food handlers were not taking treatment, and 9(3.4%) of *Giardia lamblia* positive samples examined by microscope had not received antiparasitic drugs. There are different methods for identifying intestinal parasites. Although the microscopic examination is not sensitive as other techniques for the diagnosis of *Giardia lamblia* in the stool sample till now, it is a golden standard method that is used almost in all laboratories.[20] Recently, genetic characterizations are used to analyze microorganisms with very high sensitivity and provide information on all genes.[21] This study showed that (90.9%) of positive samples by the microscope were amplified by nested PCR for *gdh* gene. Only 1 (9.1%) case was not amplified by PCR; this result agrees with a study done by Bertrand *et al.*[22] Monitoring food handlers' health is important for preventing from a source of disseminating infection, particularly *Giardia lamblia* that is one of the most prevalent intestinal parasites which causes diarrhea. Another factor in the current study is that all participants were asymptomatic, and they may act as a source of infection for the environment.

Conclusion

PCR technique now a day is the more specific and sensitive method for detection and identification of microorganisms, especially in protozoan parasites such as *Giardia lamblia*, by amplification of 734bp of *gdh* gene. It also has a negative side that is more expensive than other assays, and it needs time. In food handles, the asymptomatic carriers of the *Giardia* parasite are the risk for transmission and infecting people that eating the food which they prepare.

Competing interests

The authors declare no competing interests.

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