RESEARCH ARTICLE

The Molecular Sequence Of Giardia Lamblia By Using (tpiA) and (tpiB) Genes

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Received: 08th July, 19; Revised: 14th August, 19, Accepted: 05th September, 19; Available Online: 12th September, 2019

ABSTRACT

Gairdia lamblia is one of the parasites that cause intestinal problems within the human body, particularly private travelers and children. In this study, a total of (100) diarrheal patients, 20 patients with Giardiasis were identified by fecal antigen. A total of 9 out of 20(20%) of them were infected by fecal antigen, while 9(9%) of them were infected by using the screening general stool examination (GSE). The stool samples were collected from patient how vested the Medical City/ Baghdad and Tikrit Teaching Hospital during the period from 01 May 2018 to 01 February 2019. The result is revealing a significant difference ($p < 0.05$) between the two methods of detection for G. lamblia (Fecal antigen method and GSE). IT has been shown that out of 20 infected individuals, 12(12%) were males and 8(8%) were females, indicating regarding no significant difference in the distribution of Giardiasis among genders. In regard the age, our results showed that highest infection rate 8(3.2%) was recorded in the age group (10-19) years, followed by the age group (20-2) years which was 692.4%). In this study, five mutations were recorded at position (926, 1094, 1202and 1304), by using tpiA gene sequence method, and tpiB gene was on point mutation change (G254A), in the position (85) of triosephosphate isomerase.

Keywords: Giardia lamblia; Molecular sequence; (tpiA) gene and (tpiB) gene.

International Journal of Pharmaceutical Quality Assurance (2019); DOI: 10.25258/ijddt.v9i3.8

How to cite this article: Al-doury, S.M., Al -Nasrawi, M.A. and AL-Samarraie, M.Q. (2019). The molecular sequence of Giardia lamblia by using (tpiA) and (tpiB) genes. International Journal of Pharmaceutical Quality Assurance, 09(03):374-377.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

It has been estimated that about 200 million people had been infected with Giardisis each year in Africa, Asia, and Latin America. In industrialized countries, the overall prevalence rate of giardiasis was 2-5%.[1] Furthermore, Giardiasis was a well-recognized cause of enteric disease among international travelers in the United States, Canada, and Europe. In the United States in 2012, 15,223 cases had been reported.[2-4] In Iraq, *G. lamblia* had been recognized as the most common intestinal protozoan parasite infecting humans[5] particularly in Kirkuk and other Iraqi Provinces.[6] Assemblages A and B infect humans and other mammals, while assemblages C through H were more host-specific. Many data suggested that *Giardia* assemblage A and B could be two different species and several studies had recently shown associations between assemblage type and specific symptoms.[7,8] Other *G. lamblia* assemblages, including C and D which infects dogs, E infect livestock and F infects cats, have been sporadically documented in a number of human isolates, providing molecular evidence of potential zoonotic transmission.[9,10] The study aimed to Molecular detection of Giardia lamblia by nested and semi-nested PCR and Genotyping of *G. lamblia*, sequencing.

MATERIAL AND METHODS

The stool specimens were collected in sterile dry plastic cups with tight lids specially made for this purpose; each cup was given a unique name representing the patient. Each fresh fecal sample was divided into two parts by using a sterile container, and one of them was used for the detection of parasite. While the other part was transported into a cooled box (temperature approximately 10°C). Then, the samples were transported to the laboratory and stored immediately at-20°C for molecular analysis (Multiplex -PCR). A nitrocellulose membrane strip contains a test band (T band) and control band (C band). The T band has been pre-coated with other monoclonal anti-bacterial Ab, and the C band has pre-coated with goat anti-mouse IgG Ab. The oligonucleotide primers supplied by (Bioneer company, Korea) used in this study for PCR amplification of the following genes *ßg* (Lalle *et al*., 2005), *gdh* (Read *et al* ., 2004) and *tpi* (Elbakri *et al*., 2014; Turki

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et al., 2016) which were listed in tables(3-4 and 3-5). Primers were *tpi-A* F1: CCAAGAAGGCTAAGCGTGC 140bp and R1: GGTCAAGAGCTTACAACACG208bp.

tpi-B F1: GCACAGAACGTGTATCTGG476bp and R1: CTCTGCTCATTGGTCTCGC576bp.

STATISTICAL ANALYZING

Preceded data has been entered to the computer with the use of "Statistical Package of Social Science" Software program, v. 18 (SPSS).

RESULTS

A total of 100 patients with diarrhea were investigated for Giardiasis, and 20 (20%) of them showed positive results when examined by fecal Ag test, while 9(9%) of them showed positive results on general stool examination (GSE). There was a significant difference ($p < 0.05$) between these two examinations, as shown in Table 1.

Table 2 represents the distribution of *G. lamblia* infection according to patients gender. The results showed that 12(12%) of them were males, and the 18(8%) samples were females. There was no significant difference ($p > 0.05$) between the distribution of *G. lamblia* infection among gender.

Table 3 showed the highest number and percentage 8(3.2%) of *G. lamblia* infection within the was age group (10-19) years followed by 6 (2,4%) within the age group (20-29) years.

The molecular diagnosis for *tpi*A gene of *G. lamblia* isolates by using PCR showed that products in lanes 2,3 and 4 were positive results, and showed the mutation of DNA bands according to molecular weight at 476 bp PCR product size.

*The tpi*A gene sequence

The comprising of *G. lamblia* in both study samples and reference samples for genotype (A) There are five mutations were shown in table 4 and recorded at positions (926, 1094, 1202, 1284 and 1304).

Reference sample (L02120.1). Identity was 99% and E-value was 0.0. Amplified region located between nucleotides 921 to 1321 of (tpiA) gene.

Nucleotide substitutions were seen in five sites of (*tpi*A) gene for *G. lamblia* (2 *tpi*A.ab1, 12 *tpi*A.ab1 and 18 *tpiA*.ab1). Three mutations of them were (point mutations) transitional changes C926T, A1284G, and A1304G) and two was (silent mutations) transversional and transitional changes C1094A and T1202C.

The alterations in nucleotide sequence of gene cause four mutations of *tpi*A gene that changed amino acids (Threonine at 309 changed to Methionine, Serine at 365 changed to Tyrosine, Isoleucine at 401changed to Serine and Histidine at 435 changed to Arginine) and one silent mutation Leucine at 428 remained Leucine.

The tpiB gene sequence

In *(tpiB*), gene of sequence is isolated. This substitution (point mutation) was a transitional change (G254A). This alteration in the nucleotide sequence of gene caused mutation at position (85) of triosephosphate isomerase (*tpi*B) gene that changed the amino acid.

DISCUSSION

Giardiasis is a disorder of the intestinal tract with the occurrence of gases and the release of fatty droplets with stool.[11] The infections with G. lamblia among (10-29) years were more than other age groups, and these findings did not agree with Gebretsadik, D. et al., 2018 who found that the prevalence of intestinal parasites and associated factors was among under 5 years children in Dessie Referral Hospital.[12] Nucleotide was shown in five positions of (tpiA) gene for

Table 1: The positive and negative GSE and fecal Ags of G. lamblia infections

	G. lamblia (GSE)		
G. lamlia F. Ags.	*Negative H. C.*	*Positive*	*Total*
	80	0	80
Negative	80%	0.0%	80%
	11	9	20
Positive	11%	9.0%	20%
	91	9	100
Total	91%	9.0%	100%
p = 0.01 >0.05 (S)			

Table 2: Distribution of *G. lamblia* infection according to gender

G. L. F. A.			
Gender	*Positive*	*Negative*	*Total*
Female	32	8	40
	32%	8%	40%
Male	48	12	60
	60%		60%
	60.0%		
Total	80	20	100
	80%	20%	100%
p = >0.05 (NS)			

Table 3: Distribution of *G. lamblia* infection according to patient's age

	G. L F.A.		
Age	*Negative*	*Positive*	*Total*
(10-19)	17	8	25
	6.8%	3.2%	10.0%
(20 - 29)	44	6	50
	17.6%	2.4%	20.0%
(30 - 39)	46	3	49
	18.4%	1.2%	19.6%
(40 - 49)	64	3	67
	25.6%	1.2%	26.8%
(50 - 59)	32	0	32
	12.8%	0%	12.8%
(60-69)	27	0	27
	10.8%	0%	10.8%
Total	230	20	250
	92.0%	8.0%	100%
p = 0.01 < 0.05 (S)			

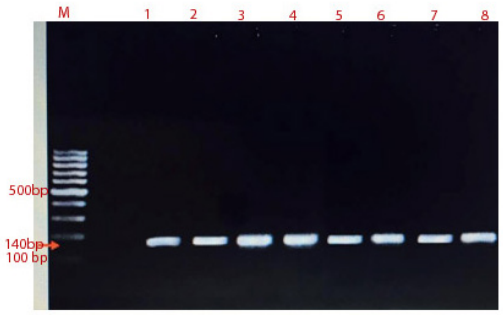
Table 4: Nucleotide alteration, amino acid substitution, and type of mutation in *tpi A* genotype

Sample No.	*Nucleotide change*	*Location of substitute*	*Type of substitute*	*Amino acid change*	*Predicted effect*
2,12,18	ATG>ACG	926	Transition	Thr-309-Net	Missee mutation
2,12,18	TAC>TCC	1094	Transversion	Leu-128-Leu	Sileut mutatiou
2,12,18	TCG>TTG	1202	Transition	Te-101-Ser	Sileut mutatiou
2,12,18	TTG>TTA	1234	Transition	Ser-36 Tyr	Misseuse mutatiou
2,12,18	CGT>CAT	1304	Transition	His-335-Arg	Misseuse mutatiou

Table (): Nucleotide alteration, amino acid substitution and type of mutation in *tpi B* genotype

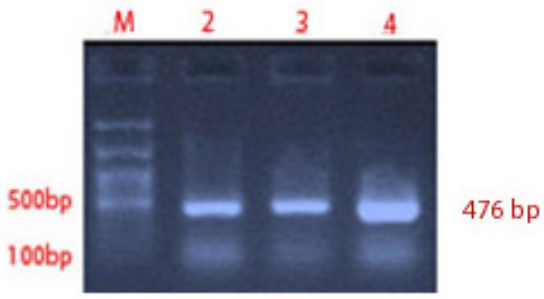
Sample No.	*Nucletide change*	*Location of substitute*	*Type of substitute*	*Amino acid change*	*Predicted effect*
1, 2, 3	AAC>AGC	254	Transition	Ser-85-Asn	Missense mutation

Giardia lambilia isolate 3 TPI genes, sequence length 530 No.1: Range 1to 229 to 288



A

Figure 1 A: Agarose gel electrophoresis image of (tpi-A) gene of *G. lamblia*. Lane M marker DNA ladder 100bp molecular weight marker. lanes 2,3 and 4 were positive results which shows the migration of DNA bands according to molecular weight at 476 bp.



B

Figure 1 B: Agarose gel electrophoresis image of (*tpi*B)gene of *G. lamblia*. Lane M marker; DNA ladder 100bp molecular weight marker. lanes 1,2,3,4,5,6,7and 8 were positive results which shows the migration of DNA bands according to molecular weight at 140 bp

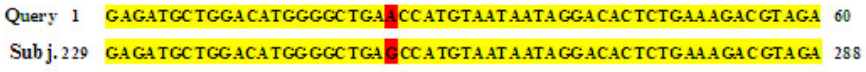


Figure (): The reference sample (KY613606.1). Identity was 99%. Amplified region located between nucleotides 229 to 288 of (tpiB) gene.

all G. lamblia, but three mutations were (point mutation) transitional changes C926T, A1284G and A1304G) and two were (silent mutations) transversional and transitional changes C1094A and T1202C. These results agreed with Ozkara, HA. and Sandhoff, K. (2003), who reported the sixth mutations associated, the mutation is a single nucleotide transition (G to A) at the last nucleotide of exon 3 of hexosaminidase A (HEX A), and the 14 exons and their flanking sequences of the HEX A gene of exon 3 showed a homozygous mutation. The point mutation was transitional changes (G254A).[13] This alteration in the nucleotide sequence of gene caused mutation at position (85) of the triosephosphate isomerase (tpiB) gene that changed the amino acid. This report was in harmony with t Gloria Hernández, A., et al.,(2013) who indicated that triosephosphate isomerase of *Giardia lamblia* (GlTIM) as a target for rational drug design against giardiasis, one of the most common parasitic infections in humans. Since the enzyme exists in the parasite and the host, selective inhibition is a major challenge because essential regions that could be considered molecular targets are highly conserved. Previous biochemical evidence showed that chemical modification of the non-conserved non-catalytic cysteine 222 (C222) inactivates specifically GlTIM.[14] The occurrence within exons does not result in a change to the amino acid sequence of a protein, in which there is no change in the phenotype.[15,16] In addition, the silent mutation which found in the present study was classified as transversion mutations due to this type of mutation occurred when a purine base substituted for a pyrimidine base, or vice versa.[17] The change in RNA was not serious because several RNA copies were synthesized for each RNA. On the contrary, alteration in DNA sequence affects all copies of the encoded protein, resulting in structural and functional changes or decrease or complete loss of expression of the encoded protein.[18] The DNA sequences were assembled and aligned using the software MEGA6[19], to build the phylogenetic tree to assess the extent of genetic diversity within G. lamblia isolates, as well as their evolutionary relationship with other Giardia spp.[20] Based on our findings, we conclude that the sequencing of tpiA gene showed five recorded mutations were recorded, the occurrence of point mutation transitional changes, and

silent mutation transversional changes with alterations in the nucleotide sequence of gene tpiA causing four mutations that change the sequence of amino acids and one silent mutation. Sequencing of tpiB gene showed replacement of Guanine nucleotide to Adenine nucleotide at a locus (254). Nucleotide substitution caused one point mutation and changed amino acid from Serine to Asparagine, and the parasite may give more ferocity within the human body.

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