

Molecular Detection and Genotyping of *Giardia lamblia* from Human Samples in Wasit Province, Iraq

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Giardia lamblia is considered one of the most common intestinal pathogens worldwide which cause gastroenteritis in human and animal. The purpose of the present study was to determine the prevalence and the most genotype that common of *G. lamblia* in the Wasit province, Iraq. A total of 50 stools samples have been collected from patient's that suffering from severe diarrhea from hospitals Al-Zahraa and Al-Karama in Wasit province, Iraq during the period from May 2016 to September 2016. The results showed 36 (72%) was positive, while 14 (28%) was negative by Nested PCR technique. Then amplification by Nested PCR has been made to observe the most genotype frequent which common, the results showed 8 (22%) contained genotyping A genotyping and B 28 (78%) contained genotype B. In this study molecular techniques played a significant role in the detection, epidemiological reconnaissance and outbreak studies.

Keywords: *Giardia lamblia*, Genotyping, Giardiasis.

Giardia lamblia is an intestinal protozoan parasite which causing diarrheal disease in human worldwide (Adam, 2001; Berkman *et al.*, 2001; Fricker *et al.*, 2001; Karanis *et al.*, 2006) about 280 million are infected with *Giradia* each year in the world (Ortega YR *et al.*, 2013). This parasite has been observed firstly by van Leeuwenhoek in 1681 through checked his own stool sample under microscope (Ankarklev J *et al.*, 2010), Giardiasis which causing by *Giardia lamblia* is a wide spreading in Asia, Africa and Latin America whereas, about 200 million persons have been reported (Mohammed *et al.*, 2009) The contaminated food or water with oocysts, as well as fecal-oral route from person to person (Balcioglu *et al.*, 2003), among children in schools, (Duffy T *et al.*, 2013) and by sexual practices of adults (Pakianathan *et al.*, 1999 ; Escobedo *et al.*, 2014) are the main ways to transmission *Giardia lamblia*.

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The oocysts have the ability to be infectious for few months and they can acclimatize to or resist critical environmental conditions (Duffy T *et al.*, 2013). The clinical manifestation of Giardiasis is absorption, watery stools, dehydration and abdominal cramping (Adam, 2001; Amar *et al.*, 2002; Bertrand *et al.*, 2005). Giardiasis in generally is self-limiting in healthy individuals and in most cases being asymptomatic (Amar *et al.*, 2002; Bertrand *et al.*, 2005). However, the prolonged of giardiasis which causing chronic malnutrition in children has been correlated with poor cognitive functions later in life (Berkman *et al.*, 2001). The earlier molecular studies of *Giardia* intestinal have been showed eight major genotypes (Berkman *et al.*, 2001). These genotype of *Giardia lamblia* are infected a wide range of mammalian and others appears to be restricted to groups such as cats, dogs and some infected only one host. The genotypes are including A, B, C, D, E, F, G and H. The A and B genotype are infected many host which include human, cats, dogs, beavers and guinea pigs (Feng Y *et al.*, 2011). Genotype C and D infected dogs

and cats, while genotype E infected cattle, sheep and goats, genotype F infected only cats, genotype G infected rats and genotype H infected seals. Only genotypes A and B infected humans and others mammals, while other genotypes have so far not been detected in human Feng Y *et al.*, 2011). Genotyping characterization of *G. lamblia* have been shown a useful tool in epidemiological studies or outbreak investigation (Van der Giessen J *et al.*, 2006; Palmer CS *et al.*, 2008) In view of this, the present study was designed for the detection of *Giardia lamblia* by Nested PCR and then genotypes on the basis of Nested PCR.

MATERIALS AND METHODS

Sampling

50 stool samples were collected from patient with severe diarrhea during period between May to September 2016 from Al-Zahraa and Al-Karama hospitals, Wasit, Iraq. The fecal sample was placed to a clean, dry plastic container and transported to the laboratory for analysis.

DNA Extraction

The extraction of genomic DNA from stool samples was done according to company instructions by using stool lysis protocol method

with Proteinase K (Stool DNA extraction Kit, Bioneer. Korea) (Haque R *et al.*, 1998; Minvielle M *et al.*, 2008) After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored at -20C at refrigerator until used in PCR amplification. Nested PCR assay was performed for detection and genotyping of *Giardia lamblia* from human stool samples.

Nested PCR

The Nested PCR assay was carried out according to (Yason, J *et al.*, 2007) based on two amplifications nested PCR runs by using primers for triosephosphate isomerase (tpi) gene that specific for genotyping A and B. Primers were provided by (Bioneer company. Korea) Table1.

The PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions (Haque R *et al.*, 1998) in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into

Table 1. List of primers that used in Nested PCR amplification

NestedPCR	Primer		Sequence	Amplicon
First round	TPIA	F	CGAGACAAGTGTGAGATG	576 bp
		R	GGTCAAGAGCTTACAACACG	
Second round	TPIB	F	GTTGCTCCCTCCTTGTGC	208 bp
		R	CTCTGCTCATTGGTCTCGC	
	N-TPIA	F	CCAAGAAGGCTAAGCGTGC	476bp
		R	GGTCAAGAGCTTACAACACG	
N-TPIB	F	GCACAGAACGTGTATCTGG	140bp	
	R	CTCTGCTCATTGGTCTCGC		

Table 2. The Nested PCR cycles

Repeat cycle	Time	Temperature	PCR step
1	5 min	95 °C	Initial Denaturation
30	30 sec	95 °C	Denaturation
	30 sec	52 °C	Annealing
	1 min	72 °C	Extension
	5 min	72 °C	Final Extension

Table 3. Show the positive and negative result by using Nested PCR

Samples	Nested PCR detection	Percentage (%)
Positives samples	36	72 %
Negatives samples	14	28 %

20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Techne TC-3000. USA) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 52 °C for 30 s, and extension 72 °C for 1 minute and then final extension at 72 °C for 7 min. The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained

with ethidium bromide, and visualized under UV transilluminator Table 2.

RESULTS

The Nested PCR was performed in a total of 50 stool samples for detection of *Giardia lamblia*, the results showed 36(72%) stool samples were positive, where 14(28%) were negative for *Giardia lamblia*. Table 3, Figure 1 and 3). The Nested PCR was performed for these samples which were positive for *Giardia lamblia* for genotyping purpose. The results were 8 (22%) samples Genotype A, 28 (78%) samples Genotype B. Nested Multiplex PCR developed and evaluated in the present study which showed the size of diagnostic fragments of PCR products Table 4, Figure 2 and 4.

Table 4. Show the Genotyping for the positive PCR samples

Nested PCR genotyping	Total	Positive	Percentage (%)
Genotype A	36	8	22%
Genotype B	36	28	78%

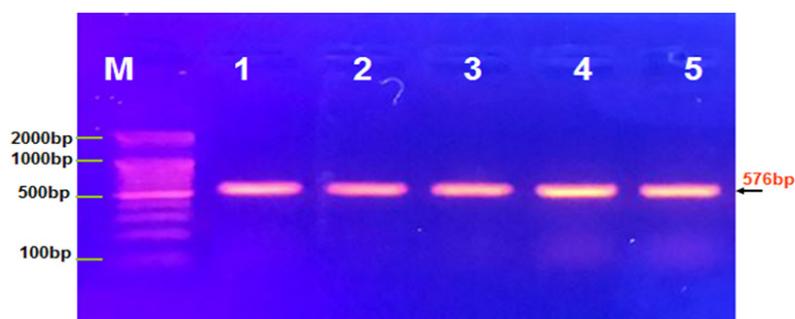


Fig. 1. Agarose gel electrophoresis image that show first round PCR product analysis of *Giardia lamblia* genotype A from human stool samples. Where, Lane (M) DNA marker (100-2000bp), Lane (1-5) some positive at PCR product size 576bp

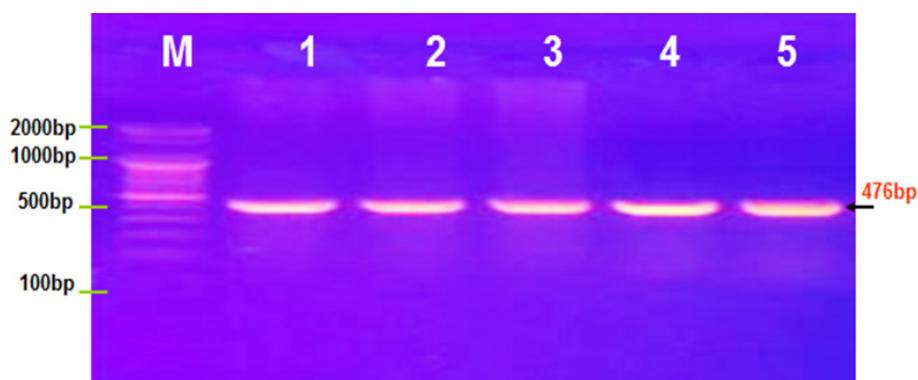


Fig. 2. Agarose gel electrophoresis image that show second round Nested PCR product analysis of *Giardia lamblia* genotype A from human stool samples. Where, Lane (M) DNA marker (100-2000bp), Lane (1-5) some positive at PCR product size 476bp *Giardia lamblia* genotype A

DISCUSSION

Giardia lamblia is one of the widest spread parasites in human's intestine and animals which causing giardiasis (Roberts and Janovy 2009). This disease is traditionally considered an epidemic and zoonosis disease which infected all ages (Caccio *et al.*, 2005). Genotyping A and B have been finding in human and many other different mammals (Guy *et al.*, 2004). The present study, describes a Nested PCR strategy for species-specific detection and differentiation of *Giardia lamblia* DNA directly in the stool samples of patients. In currently study, the detection of genotyping of *Giardia lamblia* isolated from human by nested PCR amplification were 8 (22%) genotyping A and 28 (78%) genotyping B. our study has shown the presence of *Giardia lamblia* in Wasit, whereas 36 (72%) stool samples were

positive out of 50 stool samples by Nested PCR. Nested PCR was used in the present study because it increases sensitivity. (Foronda *et al.*, 2008). Clinical specimens such as stool often contain PCR inhibitors even after purification steps. The two rounds of Nested PCR might have assisted in compensating the effects of inhibitors present in clinical specimens (Mahdy *et al.*, 2009). The product of first PCR may be just enough to provide adequate templates for the synthesis of second PCR product in the nested reaction to be detected by ethidium bromide staining (Ririe K *et al.*, 1997). The nested multiplex PCR was negative in 14 out of 50 stool specimens. The negative PCR result in these 20 stool samples may be due to the presence of other pathogenic parasite.

This study agrees with (Guy *et al.*, 2004), which observed 17(68%) were genotype B and 3(12%) were genotype A. There is

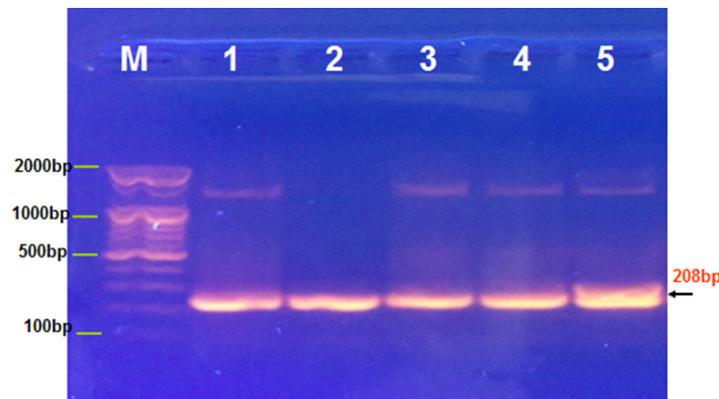


Fig. 3. Agarose gel electrophoresis image that show first round PCR product analysis of *Giardia lamblia* genotype B from human stool samples. Where, Lane (M) DNA marker (100-2000bp), Lane (1-5) positive at PCR product size 208bp

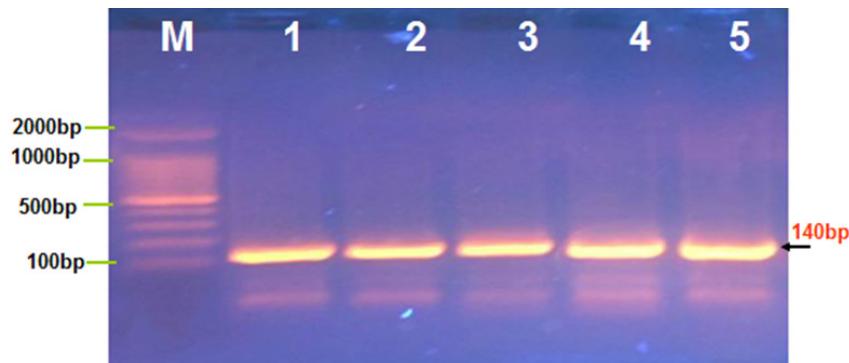


Fig. 4. Agarose gel electrophoresis image that show second round PCR product analysis of *Giardia lamblia* genotype B from human stool samples. Where, Lane (M) DNA marker (100-2000bp), Lane (1-5) positive at PCR product size 140bp as *Giardia lamblia* genotype B

agreement between our study and other studies in England (Amar et al., 2002) which found that genotype B was (64%) and genotype A was (27%). Also the results of the present study agreed with our results obtained by Marta C et al., (2008). In India, Read et al., (2002) reported that genotype A and B infectious in peoples were (39%) and (61%) respectively. Another study was detected a higher prevalence in genotype B (93.02%) and genotype A (6.98%) (Traub et al., 2004). Also our study confirmed the results of several studies (Amar et al., 2002; Read et al., 2002; Haque et al., 2005; Foronda et al., 2008; Mahdy et al., 2009; Sarkari et al., 2012) which showed that infections with genotype B was more prevalent than infections with genotype A. However, the present study disagreed with the finding of other researchers (Sambrook et al., 2000; Pestehchian et al., 2012) who observed that the infection associated with genotype A was higher than the infection associated with genotype B.

CONCLUSION

Nested PCR is considered as an alternative tool in epidemiological studies and the diagnosis of Giardiasis and for genotyping of *Giardia lamblia*. The results showed that Genotype B was the more frequent which common in Wasit, Iraq, while the lowest frequent was Genotype A.

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