Nat. Volatiles & Essent. Oils, 2021; 8(5): 11280-11286

NVEO
Natural Volatiles & Essential Oils

Molecular diagnosis of co-infection between *Giardia lamblia* parasite and *Helicobacter pylori* bacteria by Nested-PCR in patients from Wasit Province, Iraq

[1]Prof. Dr. Abdulsada A. Rahi , [2]Raheeq F. Kadhim

[1,2]Wasit University/ College of Science

Abstract

The present study was performed to identify the mixed infection of *Giardia lamblia* parasite in correlation with *Helicobacter pylori* bacteria in patients from Wasit Province, Iraq. The study included 72 stool samples ; 24 healthy individuals as control , 24 patients with *G*. *lamblia* (GL group), and 24 patients with *H*. *pylori* (HP group), collected during January to July, 2021 at Al-Kut General Teaching Hospital, Kut-Iraq. All previously samples were tested using stool antigen test and microscopy. In the present work, the GL and HP groups were examined using a nested polymerase chain reaction (NPCR) method for the detection of *G*. *lamblia* and *H*. *pylori* in the patient stool samples. According to the NPCR test, the results showed that 14 (58.3%) patients were infected with *G*. *lamblia*, and 21 (87.5%) patients were infected with *H*. *pylori*. For the detection of *G*. *lamblia*-*H*. *pylori* co-infection, the findings revealed that 16 (66.7%) of the GL patients also were infected with *H*. *pylori*, and 22 (91.7%) of the HP patients also were infected with *G*. *lamblia*. There was significant ($p \leq 0.05$) positive correlation for the co-infection of *G*. *lamblia* and *H*. *pylori*. The study suggests that *H*. *pylori* infection may highly indicated the mixed infection of *G*. *lamblia* in clinical cases presented to the Department of Gastrointestinal Diseases, Al-Kut General Teaching Hospital (Kut City, Wasit Province, Iraq).

Keywords: *Giardia lamblia*, *Helicobacter pylori*, intestinal infections, mixed infection, stomach infections.

Introduction

As the most prevalent parasite infections in children, and the leading reason of diarrhea in travelers, *Giardia lamblia* (also referred as *G. duodenalis* or *G. intestinalis*) is the highest frequently encountered parasitic illness. Since an approximated 280 million infections occur annually, the World Health Organization (WHO) acknowledges *Giardia* as an ignored tropical disease (1,2). This is due to the prevalence of *Giardia* among youngsters in economy-deficient areas, the possibility for repeated and continuous infections, and the shortage of budget-friendly and efficient treatment interventions (3–5). In resource-rich environments, *Giardia* infections may happen due to the parasite's ability to stay in the ecosystem for lengthened periods of time as tough cysts, its ability to propagate via both anthroponotic and zoonotic reservoirs, and its infectious dosage, which is as small as 10 cysts. For instance, infections in the United States are associated with waterborne dissemination throughout the summer months and concentrated outbreaks in daycares. Vegetables and culinary bivalves containing *G. lamblia* excreted from coastal and marine life may also serve as means of dissemination (6–9).

When Giulio Bizzozero isolated *H. pylori* for the first time in 1892, he found a spiral microbe. *Campylobacter pyloridis* was introduced by Barry Marshall and Robin Warren in 1983 because of its similarities to *Campylobacter*. As it possesses helical shape and is located in the pyloric area of the stomach, Goodwin et al. termed it "*Helicobacter pylori*" in 1989 (10). Most of the world's people are infected with the Gram-negative bacteria, *H. pylori*, which is about two to four-micrometer long and a half to one-micrometer wide (11).

After a two-year investigation from 1991 to 1994, the WHO-related International Agency for Research on Cancer concluded that *H. pylori* causes stomach cancer. This conclusion was validated in 2009 based on epidemiological evidence. Peptic ulcer disease may be caused by the bacterium *H. pylori*, according to a study from the National Institute of Health (NIH) in 1994. When Marshall and Warren discovered the function of H. pylori bacteria in gastritis and ulcer illness, they won the 2005 Nobel Prize in Physiology for their research on *H. pylori* (12,13). The present study was aimed to identify the presence of mixed infection of *Giardia lamblia* in correlation with *Helicobacter pylori* in patients from Wasit Province, Iraq.

Materials and methods :

Patients and samples :

The study included 24 healthy individuals, 24 patients with *G*. *lamblia* (GL group), and 24 patients with *H*. *pylori* (HP group), in which all previously were tested by using stool antigen test and microscopy. The stool samples were collected from the patients, who attended the Department of Gastrointestinal Diseases, Al-Kut General Teaching Hospital (Kut City, Wasit Province, Iraq). The samples were placed in sterile plastic containers that were submitted to the NPCR test. The collection of the specimens was constructed between January and July, 2021.

Nested polymerase chain reaction :

DNA extraction :

The DNA from the stool samples was extracted using Presto™ Stool DNA Extraction Kit (Geneaid, Taiwan) and was performed utilizing the extraction steps provided with the kit by the manufacturer. The DNA obtained from the extraction process was evaluated for the determination of its quality and quantity using a NanoDrop (Thermo Scientific, UK). The DNA was deep-freeze-stored for doing the next NPCR test.

NPCR master mix :

The primers used in the current NPCR test were designed using the NCBI-based websites and Primer 3 Plus software, and they were GenBank-deposited under the accession numbers of AF473852.1 and KC311711.1. These primer-sets were purchased via (Scientific Researcher Co. Ltd, Iraq). These primers are displayed in Table 1 and 2.

Table 1: Nested PCR primers for *Giardia lamblia*

Primers	Sequence 5'-3'		Product size (bp)
ssu-rRNA gene PCR	F	CTGCTGCAGTTAAAACGCCC	575
	R	GTTGTCGCAATGGAGCAGAC	
	F	ACCGCCTCTGTCAATCAAGG	227

ssu-rRNA gene Nested PCR	**R**	GTCAGATTGAGCCGCAGACT	

Table 2: Nested PCR primers for *Helicobacter pylori*

Primers	**Sequence 5'-3'**		**Product size (bp)**
16S rRNA gene PCR	**F**	GAAACCCTGAAGCAGCAACG	692
	R	CCCAACATCTCACGACACGA	
16S rRNA gene Nested PCR	**F**	GGCGACCTGCTGGAACATTA	335
	R	CGTGCAGCACCTGTTTTCAA	

The master mix was generated via the use of the manufactural-instruction-guided GoTaq® Green Master Mix kit (Promega, USA).

Statistical analysis :

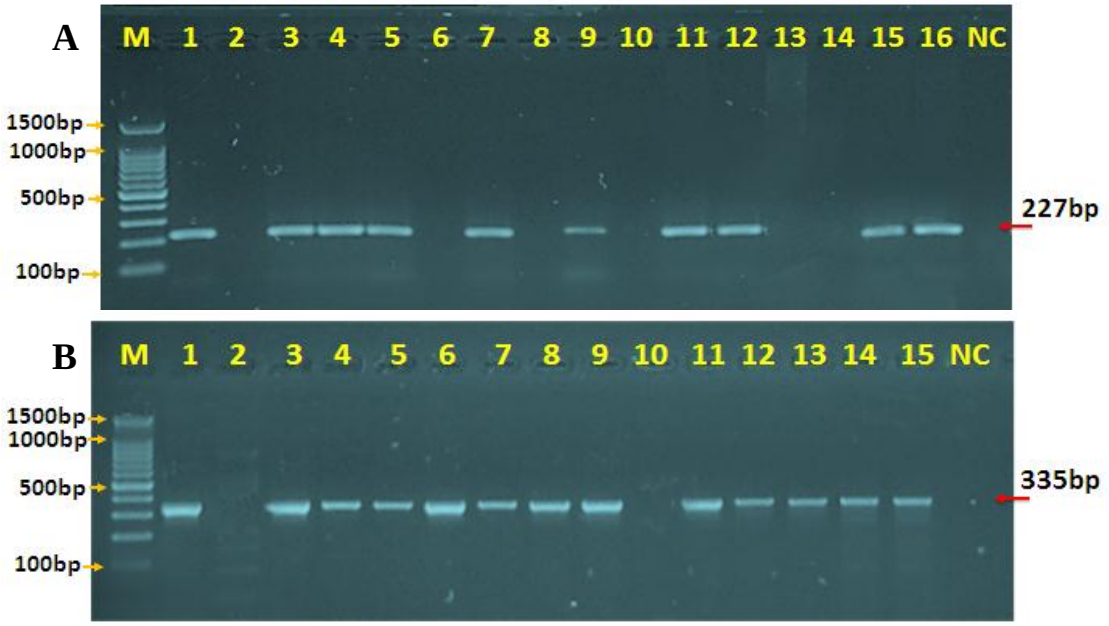
Chi-square and *t*-test were used to analyze the data. Mean±SE was utilized to analyze and graph the data. GraphPad Prism (California, USA) was used to analyze and generate graphs. If the *p* value was equal or higher than 5%, the test was not significant.

Results :

According to the NPCR test, the results showed that 14 (58.3%) patients were infected with *G*. *lamblia*, and 21 (87.5%) patients were infected with *H*. *pylori* (Table 3 and Figure 1 and 2).

Table 3: Incidence of *G. lamblia* and *H. pylori* in patients according to nested PCR test.

Group	**Incidence**		***P* value**	**R squared**
	No	**%**		
G. lamblia	14	58.3	<0.0001	1
H. pylori	21	87.5		



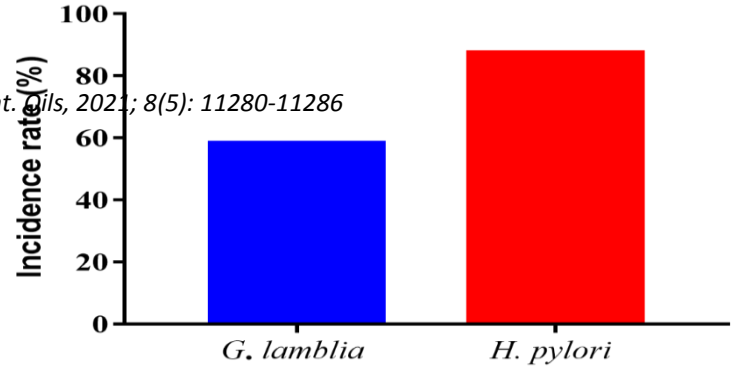


Figure 2: Incidence rate of *Giardia lamblia* and *H. pylori* from human stool samples examined via the use of nested PCR.

For the detection of *G*. *lamblia-H*. *pylori* mixed infection, the findings revealed that 16 (66.7%) of the GL patients also were infected with *H*. *pylori*, and 22 (91.7%) of the HP patients also were infected with *G*. *lamblia*. There was significant ($p \leq 0.05$) positive correlation for the co-presence of *G*. *lamblia* in a mixed infection with *H*. *pylori* (Table 4 and Figure 3).

Table 3: **Correlation between *G. lamblia* and *H. pylori* infection in patients according to nested PCR test.**

Incidence of mixed infection				*P* value	R squared	Relative risk
G. lamblia* in HP		***H. pylori* in GL****				
No	%	No	%			
22	91.7	16	66.7	<0.0001	0.9931	4.3

***HP**: Single *H*. *pylori* infection detected group.

****GL**: Single *G*. *lamblia* infection detected group.

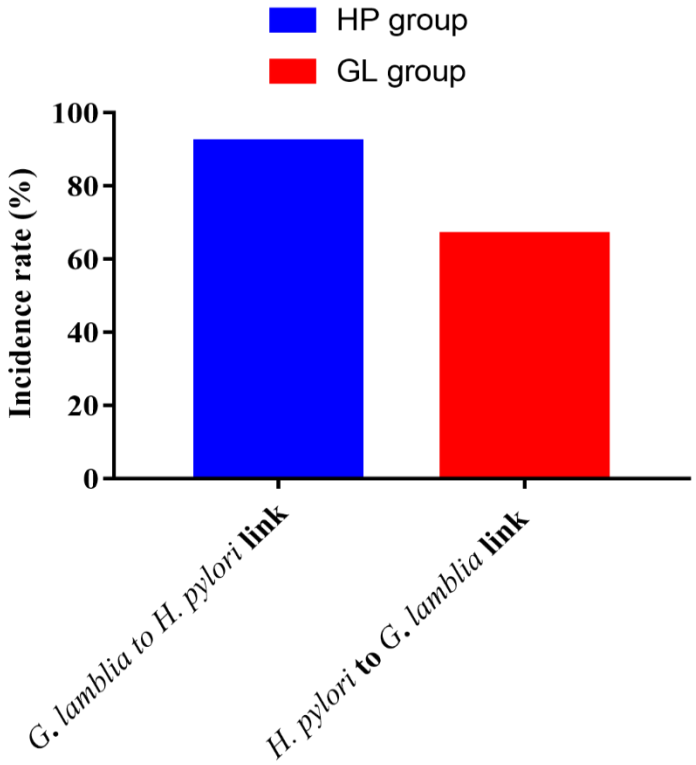


Figure 3: Incidence rate of *Giardia lamblia* and *H. pylori* mixed infection from human stool samples examined via the use of nested PCR.

Discussion :

Internal parasites, such as *G*. *lamblia*, and bacterial microorganisms, including *H*. *pylori*, are considered the leading cause of significant gastrointestinal tract infections that are recognized by the presence of gastric related problems and diarrheal illnesses (15,16).

The present study showed via its findings that *G*. *lamblia* infection was associated with the occurrence of *H*. *pylori* infection. The present study recorded 91.7% of *G*. *lamblia* infection which was higher than Elbagi *et al*. (2019) (17) from Sudan who found that 4% of *G*. *lamblia* and *H. pylori* infection. In addition, our result also was higher than that 7.61% from Turkey by Uğraş and Miman , (2013) (18). The reason for our high rate might be due to the high spread of both microorganisms in the current tested city and evolution of *G*. *lamblia* to establish its infection more frequently with the presence of *H*. *pylori* infection. Moreover, Pomari *et al* . (2020) (19) reported that 50% of *H. pylori* infected travelers and immigrants came to Italy from different world countries had *G*. *lamblia* infection.

The co-infection of internal parasite was more frequently detected by protozoa (19). In a research performed on 115 Egyptian patients with irritable bowel syndrome (IBS) revealed that 27% of *H. pylori* patients had *Blastocystis* indicating the coexistence of internal parasite infection with *H. pylori* infection (20). This was also reported in Pakistan 67% of patients had *H*. *pylori* infection (21). An Ethiopian study that included 363 patients indicated that the *G. lamblia* incidence rate was 22.3% in *H. pylori* individuals (22). Furthermore, Ankarklev *et al*. (2012) (22) found that 20.1% of the *H. pylori* asymptomatic in Ugandan children had *G. lamblia* as a concomitant microorganism. Ibrahim *et al*. (2019) (23) revealed that NPCR-detected *H. pylori* was in 36.8% of their tested children, of which 43.9% of those patients had *G. lamblia* co-infection with *H. pylori*.

Conclusion : The present study concluded that *H*. *pylori* infection may highly indicated the co-presence of *G*. *lamblia* infection in clinical cases presented to the Department of Gastrointestinal Diseases, Al-Kut General Teaching Hospital (Kut City, Wasit Province, Iraq).

References :

1. Muhsen, K., & Levine, M. M. (2012). A Systematic Review and Meta-analysis of the Association Between *Giardia lamblia* and Endemic Pediatric Diarrhea in Developing Countries. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *55*(Suppl 4), S271–S293.
2. Ross, A. G. P., Olds, G. R., Cripps, A. W., Farrar, J. J., & McManus, D. P. (2013). Enteropathogens and chronic illness in returning travelers. The New England Journal of Medicine, 368(19), 1817–1825.
3. Bartelt, L. A., Lima, A. A. M., Kosek, M., Peñataro Yori, P., Lee, G., & Guerrant, R. L. (2013). "Barriers" to child development and human potential: the case for including the "neglected enteric protozoa" (NEP) and other enteropathy-associated pathogens in the NTDs. PLoS Neglected Tropical Diseases, 7(4), e2125.

4. Solaymani-Mohammadi, S., Genkinger, J. M., Loffredo, C. A., & Singer, S. M. (2010). A meta-analysis of the effectiveness of albendazole compared with metronidazole as treatments for infections with Giardia duodenalis. PLoS Neglected Tropical Diseases, 4(5), e682.
5. Rossignol, J. F. (2010). *Cryptosporidium* and *Giardia*: treatment options and prospects for new drugs. Experimental Parasitology, 124(1), 45–53.
6. Thompson, R. C. A., & Monis, P. (2012). *Giardia*—From Genome to Proteome. Advances in Parasitology, 78, 57–95.
7. Elbakri, A., Samie, A., Bessong, P., Potgieter, N., & Abu Odeh, R. (2014). Detection and molecular characterisation of *Giardia lamblia* genotypes in Sharjah, United Arab Emirates. Transactions of the Royal Society of Tropical Medicine and Hygiene, 108(8), 466–473.
8. Mahmoudi, M. R., Kazemi, B., Mohammadiha, A., Mirzaei, A., & Karanis, P. (2013). Detection of *Cryptosporidium* and *Giardia* (oo)cysts by IFA, PCR and LAMP in surface water from Rasht, Iran. Transactions of the Royal Society of Tropical Medicine and Hygiene, 107(8), 511–517.
9. Lipoldová, M. (2014). *Giardia* and Vilém Dušan Lambl. PLoS Neglected Tropical Diseases, 8(5), e2686.
10. Bartelt LA, Sartor RB.(2021). Advances in understanding *Giardia*: determinants and mechanisms of chronic sequelae. 12; 7 (5): 62–75.
11. Öztekin, M., Yılmaz, B., Ağagündüz, D., & Capasso, R. (2021). Overview of *Helicobacter pylori* Infection: Clinical Features, Treatment, and Nutritional Aspects. Diseases, 9(4), 66–84.
12. Ansari, S., & Yamaoka, Y. (2017). Survival of *Helicobacter pylori* in gastric acidic territory. Helicobacter, 22(4), 1–25.
13. Łaszewicz, W., Iwańczak, F., & Iwańczak, B. (2014). Seroprevalence of *Helicobacter pylori* infection in Polish children and adults depending on socioeconomic status and living conditions. Advances in Medical Sciences, 59(1), 147–150.
14. Fink, M. Y., Shapiro, D., & Singer, S. M. (2020). *Giardia lamblia*: Laboratory Maintenance, Lifecycle Induction, and Infection of Murine Models. Current Protocols in Microbiology, 57(1), e102.
15. Reshetnyak, V. I., Burmistrov, A. I., & Maev, I. V. (2021). *Helicobacter pylori*: Commensal, symbiont or pathogen? World Journal of Gastroenterology, 27(7), 545–560.
16. Elbagi, Y. Y. A., Alla, A. B. A., & Saad, M. B. E. (2019). The relationship between *Helicobacter pylori* infection and intestinal parasites in individuals from Khartoum state, Sudan: a case-control study. F1000Research, 8(1), 2094.
17. Uğraş, M., & Miman, O. (2013). [The prevalence of intestinal parasites in children with *Helicobacter pylori* gastritis evaluated retrospectively]. Turkiye Parazitolojii Dergisi, 37(4), 245–248.
18. Pomari, E., Ursini, T., Silva, R., Leonardi, M., Ligozzi, M., & Angheben, A. (2020). Concomitant Infection of *Helicobacter pylori* and Intestinal Parasites in Adults Attending a Referral Centre for Parasitic Infections in North Eastern Italy. Journal of Clinical Medicine, 9(8), 1–10.
19. El-Badry, A. A., Abd El Wahab, W. M., Hamdy, D. A., & Aboud, A. (2018). *Blastocystis* subtypes isolated from irritable bowel syndrome patients and co-infection with Helicobacter pylori. Parasitology Research, 117(1), 127–137.
20. Yakoob, J., Abbas, Z., Khan, R., Tariq, K., Awan, S., & Beg, M. A. (2018). Association of *Helicobacter*

pylori and protozoal parasites in patients with chronic diarrhoea. British Journal of Biomedical Science, 75(3), 105–109.

21. Seid, A., Tamir, Z., Kasanew, B., & Senbetay, M. (2018). Co-infection of intestinal parasites and *Helicobacter pylori* among upper gastrointestinal symptomatic adult patients attending Mekanesalem Hospital, northeast Ethiopia. BMC Research Notes, 11(1), 144.
22. Ankarklev, J., Hestvik, E., Lebbad, M., Lindh, J., Kaddu-Mulindwa, D. H., Andersson, J. O., Tylleskär, T., Tumwine, J. K., & Svärd, S. G. (2012). Common coinfections of Giardia intestinalis and *Helicobacter pylori* in non-symptomatic Ugandan children. PLoS Neglected Tropical Diseases, 6(8), e1780.
23. Ibrahim, A., Ali, Y. B. M., Abdel-Aziz, A., & El-Badry, A. A. (2019). *Helicobacter pylori* and enteric parasites co-infection among diarrheic and non-diarrheic Egyptian children: seasonality, estimated risks, and predictive factors. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*, *43*(2), 198–208.