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Association of *Helicobacter pylori* and intestinal parasites in the stool samples of patients with dyspeptic complaints

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Abstract

Aim: *Helicobacter pylori* and infections due to intestinal parasites are quite common in developing countries around the world, in societies with low socioeconomic levels, and in regions with inadequate and unhealthy drinking water and food. Our study aimed to reveal the association *Helicobacter pylori* positivity and intestinal parasites (IP) in the stool patients who came to the hospital due to dyspeptic complaints.

Materials and Methods: Between 01.2018 and 04.2024, 953 patients admitted to the hospital with dyspepsia and whose stool samples were taken were examined. The data of patients for whom stool *Helicobacter pylori* rapid antigen test, IP test and demographic variables were requested were evaluated retrospectively.

Results: Of the 953 patients, 396 (41.6%) were male and 557 (58.4%) were female. Patient ages ranged from 18 to 85 years and the average was 38.65∓15,15.64 years. The patients were divided into two age groups. There were 882 (92.5%) patients between the ages of 18-65, and 71 (7.5%) patients between the ages of 66-85. *Entamoeba histolytica (E. histolytica)/Entamoeba dispar (E. dispar)* in 12 (1.3%) of the patients, *Giardia intestinalis (G. intestinalis)* in 24 (2.5%), *Blastocystis hominis (B. Hominis)* in 3 (0.3%) and *H. pylori* was detected in 128 (13.4%). The relationship between *H. pylori* and IP was found to be statistically significant. IP was detected in 20.3% of cases in which *H. pylori* was detected. No relationship was found between gender and *H. pylori* and IP. No relationship was found between age groups and *H. pylori* and IP.

Conclusion: *H. pylori* and IP are found quite frequently in the world. To better understand the association between them, more comprehensive multi-center studies are needed, in which high-sensitivity methods are used and data can be compared with healthy control groups.

Introduction

Intestinal parasite (IP) infections are a significant problem in regions with low socioeconomic levels. Factors such as education level, access to clean food and water, and personal hygiene play a crucial role in the incidence of IP infections [1]. *Helicobacter pylori* is an important pathogen that can cause gastroenteritis, duodenal ulcers, stomach ulcers, and stomach cancer [2]. It is estimated that 50% of the world's population, particularly in developing countries, is infected with *H. pylori*.

There is a common transmission route and similar risk factors between *H. pylori* and IP. *H. pylori* and IP have been reported to cause gastrointestinal complications [3]. The combination of *H. pylori* and IP has been shown to weaken the immune system and increase gastric mucosal damage [4] However, the relationship between *H. pylori* and IP is not yet fully understood.

Gastrointestinal symptoms are quite common, with dyspepsia being the most frequent [5]. Dyspeptic symptoms are present in 25% of the population [6]. While the factors causing dyspepsia are not fully understood, the most commonly known are *H. pylori*, IP, and celiac disease [7, 8]. In this study, we retrospectively evaluated the data of patients who presented with gastrointestinal dyspeptic complaints and were tested for stool *H. pylori* rapid antigen and IP. This study aims to determine the association between *H. pylori* and IP in patients with dyspeptic complaints and to assess the relationship with gender and age groups.

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Materials and Methods

The study was started after receiving ethics committee approval from Siirt University Non-Interventional Clinical Research Ethics Committee dated 24.05.2024 and numbered 108756. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. An a priori power analysis was conducted using G*Power version 3.1.9.7 (Faul et al., 2007) to determine the minimum sample size required to test the study hypothesis. Results indicated the required sample size to achieve 80% power for detecting a medium effect, at a significance criterion of $\alpha = .05$, was N = # for [insert statistical test you are using to test your hypothesis]. Thus, the obtained sample size of N = # is adequate to test the study hypothesis".

The association of IP and *H. pylori* in 953 patients who were examined with complaints of gastrointestinal system (GIS) dyspepsia and whose stool samples were sent at Siirt Training and Research Hospital between 01.2018 and 04.2024 was retrospectively examined. The relationship between gender and age groups and *H. pylori* and IP was evaluated.

Stool samples were evaluated for intestinal parasites by macroscopic examination within an hour after collection, and the preparations prepared by the native-Lugol method were examined under a direct light microscope.

Entamoeba Antigen Cassette Test (True Line China) was used to detect *Entamoeba histolytica/Entamoeba dispar* antigen in stool samples. A stool rapid antigen test for *H. pylori* (*H. pylori* Antigen Rapid Test Cassette, DiagnoCheck) was used.

Statitstical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, version 29.0 (IBM, Armonk, NY, USA). Descriptive statistics were reported as frequencies, percentages, means, and standard deviations for continuous variables. Categorical data were compared using the Chi-Square test, Fisher's Exact Test and Yates' Continuity Correction test.

The relationships between categorical variables, such as the presence of *H. pylori*, IP, gender, and age groups, were analyzed using the aforementioned tests. Statistical significance was set at a p-value of less than 0.05 for all tests. Post-hoc analyses were conducted when significant associations were identified, to determine the nature of the relationships between specific groups.

Results

Nine hundred and fifty-three people were evaluated in the research. 396 (41.6%) of the patients were male and 557 (58.4%) were female. Patient ages ranged from 18 to 85 years and the average was 38.65∓15,15.64 years. 882 (92.5%) of the patients are in the 18-65 age group, and 71 (7.5%) are in the 66-85 age group. *Entamoeba histolytica* (*E. histolytica*)/*Entamoeba dispar* (*E. dispar*) in 12 (1.3%) of the patients, *Giardia intestinalis* (*G. intestinalis*) in 24 (2.5%), *Blastocystis hominis* (*B. hominis*)) in 3 (0.3%) and *H. pylori* was detected in 128 (13.4%).

Table 1. Relationship between IP and *H. pylori* according to gender (n=953).

	Sex		p
	Male	Female	
E. histolytica/E. dispar			
Negative	392(99.0%)	549(98.6%)	0.770&
Positive	4(1.0%)	8(1.4%)	
G. intestinalis			
Negative	390(98.5%)	539(96.8%)	0.145$
Positive	6(1.5%)	18(3.2%)	
B. hominis			
Negative	394(99.5%)	556(99.8%)	0.574&
Positive	2(0.5%)	1(0.2%)	
H. pylori			
Negative	352(88.9%)	473(84.9%)	0.077*
Positive	44(11.1%)	84(15.1%)	

*: Chi-Square Test, &: Fisher Exact test, $: Continuity Correction.

Table 2. Relationship between IP and H. pylori according to age groups (n=953).

	Age		p
	18-65	66-85	
E. histolytica / E. dispar			
Negative	870(98.6%)	71(100.0%)	1.000&
Positive	12(1.4%)	0(0.0%)	
G. intestinalis			
Negative	861(97.6%)	68(95.8%)	0.415&
Positive	21(2.4%)	3(4.2%)	
B. hominis			
Negative	879(99.7%)	71(100.0%)	1.000&
Positive	3(0.3%)	0(0.0%)	
H. pylori			
Negative	758(85.9%)	67(94.4%)	0.068$
Positive	124(14.1%)	4(5.6%)	

&: Fisher Exact test, $: Continuity Correction.

The relationship between IP and *H. pylori* according to gender is summarized in Table 1. No significant relationship was detected between gender and *E. histolytica/E. dispar* (p = 0.770), *G. intestinalis* (p = 0.145), *B. hominis* (p = 0.574) and *H. pylori* (p = 0.077). Distributions of IP and *H. pylori* according to age groups are given in Table 2. No significant relationship was detected between age groups and the presence of *E.histolytica/E. dispar* (p=1.000), *G. intestinalis* (p = 0.145), *B. hominis* (p=1.000) and *H. pylori* (p = 0.068).

The gender and age group distributions of patients with IP and *H. pylori* are summarized in Table 3. According to these results, 4 (33.3%) of the 12 *E. histolytica/E. dispar* detected patients were male and 8 (66.7%) were female (p

Table 3. Gender and age group distributions of patients with IP and *H. pylori* detected.

	E. histolytica / *E. dispar* (n=12)	*G. intestinalis* (n=24)	*B. hominis* (n=3)	*H. pylori* (n=128)
Sex				
Male	4(33.3%)	6(25.0%)	2(66.7%)	44(34.4%)
Female	8(66.7%)	18(75.0%)	1(33.3%)	84(65.6%)
*p**	*0.248*	*0.014*	*0.564*	*0.000*
Age Groups				
18-65	12(100.0%)	21(87.5%)	3(100.0%)	124(96.9%)
66-85	0(0.0%)	3(12.5%)	0(0.0%)	4(3.1%)
*p**	-	*<0.001*	-	*<0.001*

*: Chi-Square Test.

Table 4. Relationship between *H. pylori* and IP.

	H. pylori		p
	Negative	Positive	
Intestinal Parasite			
Negative	812(98.4%)	102(79.7%)	<0.001$
Positive	13(1.6%)	26(20.3%)	
E. histolytica/E. dispar			
Negative	821(99.5%)	120(12.8%)	<0.001&
Positive	4(10.4%)	8(6.3%)	
G. intestinalis			
Negative	819(99.3%)	110(85.9%)	<0.001&
Positive	6(0.7%)	18(14.1%)	
B. hominis			
Negative	822(99.6%)	128(100.0%)	1.000&
Positive	3(0.4%)	0(0.0%)	

&: Fisher Exact test, $: Continuity Correction.

= 0.248). Of the 24 patients in whom *G. intestinalis* was detected, 6 (25.0%) were male and 18 (75.0%) were female (p = 0.014). Of the 3 patients in whom *B. hominis* was detected, 2 (66.7%) were male and 1 (33.3%) was female (p = 0.564). Of the 128 patients in whom *H. pylori* was detected, 44 (34.4%) were male and 84 (65.4%) were female ($p < 0.001$).

When we examined the presence of IP and *H. pylori* according to age groups, it was determined that all 12 patients with *E.histolytica* / *E.dispar* were between the ages of 18-65. Of the 24 patients with *G. intestinalis* detected, 21 (87.5%) were in the 18-65 age group and 3 (12.5%) were in the 66-85 age group (p<0.001). All 3 patients with *B. hominis* were between the ages of 18-65. Of the patients with *H. pylori* detected, 124 (96.9%) were in the 18-65 age group and 4 (3.1%) were in the 66-85 age group (p <0.001).

There are 102 patients with only *H. pylori*. 37 (36.3%) of these patients were male and 65 (63.7%) were female (p = 0.006). Of the patients with only *H. pylori*, 101 (99.0%) were between the ages of 18-65 and 1 (1.0%) patient was in the 66-85 age group (p<0.001).

The number of patients with only parasites was 13. Of the patients with only parasites, 5 (38.5%) were male and 8 (61.5%) were female (p = 0.405). All patients with only parasites are between the ages of 18-65.

The number of patients with both IP and *H. pylori* is 26. 7 (26.9%) of these patients were male and 19 (73.1%) were female (p = 0.019). Of the patients with both the parasite and *H. pylori* together, 23 (88.5%) were in the 18-65 age group and 3 (11.5%) were in the 66-85 age group (p<0.001).

The relationship between *H. pylori* and IP is shown in Table 4. A statistically significant relationship was detected between the presence of *H. pylori* and the presence of IP (p <0.001). IP was detected in 20.3% of the patients in whom *H. pylori* was detected. The presence of *H. pylori* and the presence of *E. histolytica*/*E. dispar* was 6.3% and a statistically significant relationship was detected between them (p = 0.000). *E. histolytica*/*E.dispar* was detected in 10.4% of patients in whom *H. pylori* was not detected. A statistically significant relationship was detected between the presence of *H. pylori* and the presence of *G. intestinalis* with a rate of 14.1% (p <0.001). *G. intestinalis* was detected in 0.7% of patients in whom *H. pylori* was not detected. No statistically significant relationship was detected between the presence of *H. pylori* and the presence of *B.hominis* (p = 1.000).

Discussion

Helicobacter pylori and IP are more common in developing countries, but socioeconomic level and geographical region affect this. The transmission route of *H. pylori* has not been fully explained, but it is thought that it may be transmitted from person to person or from the environment through water and food [9, 10]. *H. pylori* and IP may boost the body's susceptibility to other infections [11].

In studies conducted on *H. pylori* and IP, it is thought that co-infections may be related to each other. When looking at the correlation between *H. pylori* prevalence and gender and age, it has been indicated that the prevalence is different in various regions.

The most prevalent enteric protozoa are *Giardia lamblia*, *E. histolytica* and *B. Hominis* [12]. The prevalence of giardiasis in developed countries is around 2% [13]. The incidence of *E. histolytica* is approximately 12% in the world, and this rate is higher in tropical regions. It is thought that around one billion people in the world are infected with *B. hominis.* The prevalence is in developing countries (30.0%-100.0%) compared to developed countries (1.5%-20.0%) [14].

In a study in Turkey, the prevalence of *H. pylori* was found to be 82.5% and was found to be statistically significant in those with low socioeconomic status, but its relationship with gender was not examined [15]. In a study conducted in Cameroon, where the prevalence of *H. pylori* was 64.39% *H. pylori* was found to be statistically significant with increasing age and low socioeconomic level [16]. In a study in the Czech Republic, *H. pylori* was detected at a rate of 39.8% among those aged 55> years, and no significant relationship was found with gender, but it was shown to increase with age and to be associated with low socioeconomic conditions [17]. Although the prevalence of *H. pylori* infection in Brazil varies between 36% and 90% depending on the region, low socioeconomic status and inadequate hygiene conditions are effective [18]. A study in the United States found that the prevalence of *H. pylori* was 50%, while the overall prevalence of *H. pylori* infection in children in Europe was approximately 25%. Portugal had the highest prevalence of 66.2% in Europe, and the risk of *H. pylori* was found to be significant in low socioeconomic status [19].

In our study, patients were classified as 18-65 years old and 66-85 years old. The relationship between *H. pylori* and IP positivity and age groups was examined, but it was not found to be statistically significant. The effect of gender on *H. pylori* and IP positivity was examined and the relationship between them was not statistically significant. IP and its association with *H. pylori* were found to be statistically significant. IP was found in 20.3% of *H. pylori* positive patients. *E. histolytica/E.dispar* was found in 6.3% of *H. pylori* patients and *G.intestinalis* was found in 14.1%. The association of *H. pylori* and *B. hominis* was found to be statistically insignificant.

In two studies conducted by H. Spotts et al. in Ethiopia in 2020, the coexistence of *H. pylori* and IP was found in 23% of 434 children, and *G.intestinalis* was detected most frequently [11]. In a study published by A. Seid et al. in 2018, they investigated *H. pylori* IgG in stool samples and serum samples of 363 patients. IP was found in 44.3% of 225 patients with *H. pylori* IgG positivity. The most frequently detected IP (22.3%) was *G.intestinalis* and it was statistically significant [3].

Y. Yousif Abd Elbagi et al showed that 23% of patients with *H. pylori* infection in Sudan in 2019 had IP. *E. histolytica* and *G. intestinalis* were found the most in IP and their association was shown to be statistically significant [20].

In a study conducted in Egypt in 2019, it was reported that the most common co-infection was *H. pylori* and *G. intestinalis* [21].

In the study published by J. Yakoob et al. in Pakistan in 2018, it was reported that the association of *H. pylori* infection with *Blastocystis sp.* and *E. histolytica* was higher [22]. In our study, the parasite most associated with *H.pylori* was *G.intestinalis.*

It has been observed that *H. pylori* and IP infections are more common in underdeveloped and developing countries. There is not much data showing the amount of coinfection in developed countries.

In the study published by E. Pomari et al. in Italy in 2020, the co-existence of *H. pylori* and IP was found to be 74%, and among these, *Blastocystis sp* co-infection was the most common [23]. A study published in China showed that there is a significant relationship between *H. pylori* and *Blastocystis sp.* infection [24]. In our study, B. hominis was seen in 3 patients, but its association with *H. pylori* was not significant.

Our study was single-center and included a limited region in Turkey. Molecular methods with high sensitivity were not used for diagnosis. Since our study was retrospective, data in the patient group could not be compared with healthy controls. These reasons showed the limitations of our study.

Conclusion

This study is limited to one region in Turkey and is a retrospective study that does not use high-sensitivity molecular methods for diagnosis. To better understand the association between *H. pylori* and IP, more comprehensive, multicenter, more epidemiological and experimental studies should be conducted using high-sensitivity methods and comparing data with healthy control groups.

Ethical approval

The study protocol was approved by the Siirt University Non-Interventional Clinical Research Ethics Committee (Date: May 24, 2024, No; 108756).

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