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Helicobacter pylori and Intestinal Parasites Co-infection: Estimation of Risk Factors among Dyspeptic Patients in Mukalla city, Hadhramout, Yemen

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Abstract :, Patients with dyspepsia often have Helicobacter pylori (H. pylori) infections, which are more common in the countries of the third world. H. pylori has ability to colonizing the gastric mucosa, and the infections often lead to development of various gastrointestinal complications. In addition, H. pylori infection and intestinal parasite colonization in humans is a regular occurrence. The purpose of the current research was to determine the prevalence of intestinal parasites and H. pylori co-infection, as well as related risk factors, in dyspeptic patients who were sent to hospitals in Mukalla City, Hadhramout governorate, Yemen. This research used a cross-sectional design to collect and analyze 100 stool samples. The purpose was to detect H. pylori infection using a quick immunochromatographic stool-based antigen test, and also to identify any presence of parasite infection. To obtain data, we used a structured questionnaire. The data were examined using SPSS version 24, and logistic regression analysis was utilized to identify the independent predictive risk variables, and P-value considered significant at 0.05. The total prevalence of H. pylori infection among dyspeptic patients was 30%. Males had a higher prevalence infection of H. pylori (COR=0.386, 95%CI=0.160-0.934, P=0.035). Furthermore, positive H. pylori results were significantly linked with unfiltered drinking water (COR=3.67; 95%CI=1.436-9.363; P=0.007), regurgitation and heartburn symptoms (COR=0.865, 95%CI=0.034-0.536, P=0.004), and antibiotic use (COR=0.312, 95%CI=0.125-0.780, P=0.013). This study indicates that intestinal protozoa Entamoeba histolytica and Giardia lamblia were the commonest co-infected parasites with H. pylori in dyspeptic patients with no significant association. Finally, gender, unfiltered water source, regurgitation and heartburn symptoms, and antibiotics used were risk variables associated with co-infection with the intestinal parasites *E. histolytica* and *G. lamblia* and H. pylori.

Keywords: Co-infection, Dyspeptic patients, Entamoeba histolytica, Giardia lamblia, Helicobacter pylori, Hadhramout, Prevalence, Yemen

1. Introduction:

H. pylori colonization of the human gastrointestinal mucosa leads to the development of gastric cancer, chronic gastritis, peptic ulcers, gastric mucosa associated lymphoid tissue (MALT) lymphoma, and peptic ulcers [1]. Some developing countries have 90%, while developed countries have values ranging from 25 to 40% prevalence of H. pylori infection [2]. Dyspepsia is a prevalent health issue that is seen globally [3], and is the most common symptoms of the upper gastrointestinal [4]. The incidence rate of dyspepsia associated with H. pylori infection is 25% [3,5]. Uncertain factors, including viral and non-infectious agents, may contribute to dyspepsia [6]. The major causes include gastroduodenitis, peptic ulcer, malignancies, esophagitis, parasitic infection and functional dyspepsia [7].

The poly-microbial that causes the gastrointestinal disturbances have clinical significance [8]. Co-infection of H. pylori and intestinal parasites are commonly causing of gastrointestinal symptoms and disturbances [9]. Intestinal parasites have a global distribution that affects millions of people worldwide [10]. Intestinal parasites and co-infection with H. pylori are both prevalent causes of gastrointestinal illnesses and important infectious pathogens for global public health. Intestinal parasite infection is common in Uganda [11], Pakistan [12], Ethiopia [13], Sudan [14], Nigeria [15], and Yemen [16], and some studies have indicated a high frequency of H. pylori infection among these individuals.

In Yemen, some studies reported the prevalent infection of H. pylori in dyspepsia, gastritis, duodenal and gastric ulcers patients in Mukalla city, Hadhramout [17], Sana'a city [18], Taiz city [19], and Dhamar [20-21]. However, there are little data available on the prevalence of co-infection with intestinal parasites and H. pylori. In addition to H. pylori infection, dyspeptic patient's clinical outcomes must be evaluated for intestinal parasite infection. The aim of this research is to look at intestinal parasites and H. pylori co-

infections, along with the risk factors associated with these co-infections, in patients in Mukalla City, Hadhramout Governorate, Yemen who have dyspeptic symptoms.

2. Materials and methods:

2.1 Sample size, population, and study design:

This research was planned as a cross-sectional study and done in Mukalla city, which is located in Hadhramout Governorate, Yemen. One hundred stool samples were taken from patients who were referred to several major hospitals in Mukalla city, Hadhramout with probable dyspepsia.

2.2 Inclusion and exclusions criteria:

In this study, patients who showed symptoms of dyspepsia (heartburn and acid regurgitation) were included. Patients when they were without symptoms of dyspepsia were excluded.

2.3 Collection of data:

For the purpose of gathering information about the risk factors of the infection of H. pylori, a standard structured questionnaire was utilized. It was composed of an interview with the participant and a set of systematic questions about the study's variables.

2.4 Stool antigen H. pylori test:

The InTec Products, INC, USA offers the H. pylori one-step antigen test, a quick immunochromatographic assay for the identification of H. pylori antigen in samples of human feces. Based on the manufacturer's criteria, this test was utilized to generate qualitative and visual results with a high degree of specificity (98.6%) and sensitivity (98.4%).. Feces sample passes across the conjugate pad when it is introduced to the sample pad, mobilizing the gold anti-H. pylori conjugate that is placed on the conjugation pad. The combination undergoes a reaction with the anti-H. pylori that is present on the test section of the membrane as it moves downwards due to capillary action [17].

2.5 Detection technique for intestinal parasites:

Direct saline wet-mounting preparation was used. A little amount of stool sample was located on a glass slide microscope. Using a light microscope at Olympus, Philippines, a drop of Lugol iodine was applied, covered with a glass coverslip, and the specimen was examined at 40× and 100× magnification to look for parasite cysts and eggs [22].

2.6 Participant consent and ethical research permission:

Faculty of Science at Hadhramout University granted this project ethical permission. Before beginning the study, written consent was obtained. The hospital's management provided letters of authorization. The participants' information was collected after they verbally consented to it in accordance with the informed consent with confidentially the findings of each participant.

2.7 Analysis of Data:

Version 24 of SPSS was utilized for the analysis of the data, a statistical software package for social sciences. The characteristics of the participants were described using descriptive statistics of frequencies and percentages. Logistic regression analysis was used so that a determination could be made about the nature of the connection between the dependent and independent variables. The 95% confidence intervals (CI) and crude odds ratio/adjusted odds ratio (OR) were used to show the relationship between the variables. At 0.05, the P-value has been described as significant.

3. Results

3.1 .Risk factors and H. pylori prevalence in dyspeptic patients.

In this examination, it was discovered that H. pylori infection had a prevalence of 30%. A higher prevalence of H. pylori infection was seen among men (63% vs. 37%; COR=0.386, 95%CI=0.160-0.934, P=0.035), although this figure shows a statistically significant difference between males and females. Age groups 19 to 32 years show the highest rate 40.0% of H. pylori infections, followed by age group 33 to 46 years that show 33.0% of H. pylori infections without significant related factors. Unfiltered water sources increased the incidence of H. pylori infection in positive patients by 3.667 times (95% CI: 1.436–9.363, P=0.007). Food habits and H. pylori infection were not substantially correlated (P > 0.05). Clinically, there is a strong correlation between heartburn and regurgitation (COR=0.865, 95%CI=0.034-0.536, P=0.004). Additionally, as indicated in Table 1, there is a significant association between using antibiotics and H. pylori infection (COR=0.312, 95%CI=0.125-0.780, P=0.013).

Table 1. H. pylori infection prevalence and risk factors in individuals with dyspepsia.

Variable		No. of cases	Positive fecal Ag(%)	COR	CI(95%)	P-value
Gender	Male	47	19(63.0)	0.386	0.160-0.934	0.035*
	Female	53	11(37.0)	1		
Age group (years)	5 – 18	3	1(3.0)	0.067	0.072-12.105	0.958
	19 – 32	38	12(40.0)	1.011	0.327-3.124	0.985
	33 – 46	37	10(33.0)	1.260	0.397-3.995	0.695
	47 – 60	22	7(24.0)	1		
Food patterns	Fatty, citrus and spicy	93	27(90.0)	1.833	0.384-.8.746	0.447
	Nothing	7	3(10.0)	1		
Water sources	Unfiltered	48	8(27.0)	3.667	1.436-9.363	0.007*
	Filtered	52	22(73.0)	1		
Clinical symptoms	Regurgitation	4	0(0.0)	269245810.7	0.000	0.999
	Heartburn	37	6(20.0)	0.139	0.258-5.219	0.845
	Heartburn and regurgitation	38	21(70)	0.865	0.034-0.536	0.004*
	No symptoms	21	3(10.0)	1		
Antibiotics used	Used	29	14(47.0)	0.312	0.125-0.780	0.013*
	Non-used	71	16(53.0)	1		

*Statistically significant P-value <0.05, Abbreviations: CI, confidence interval, Crude Odds Ratio; COR

3.2 Intestinal protozoa co-infection with H. pylori:
The intestinal protozoa Entamoeba histolytica (*E. histolytica*) cyst and the trophozoite and Giardia lamblia (*G. lamblia*) trophozoite were detected 6(20%) in dyspeptic patients infected with H. pylori without statistically significant association (P = 0.890) as presented in Table 2.

Table 2. Co-infection of H. pylori and intestinal protozoa among dyspeptic patients

Type of intestinal protozoa infection	No. of cases	Positive fecal Ag(%)	COR	CI(95%)	P-value
***E. histolytica* cyst**	9	2(7.0)	3.500	0.284-43.161	0.328
***E. histolytica* trophozoite**	5	2(7.0)	1.500	0.106-21.312	0.765
***G. lamblia* trophozoite**	2	1(3.0)	1		
***E. histolytica* cyst and trophozoite**	1	0(0.0)	1615474864	0.000	1.000
***E. histolytica* cyst and *G. lamblia* trophozoite**	2	1(3.0)	1		
No parasitic infection	81	24(80.0)	2.375	0.316-17.853	0.401
Total	100	30(100.0)			

For those related risk factors that demonstrated significance in the estimation of the crude odds ratio, the logistic regression of multivariate analysis was carried out. Table 3 demonstrates that there is still a significant association between the incidence of H. pylori infection, sex, unfiltered water, regurgitation and heartburn symptoms, and antibiotic usage.

Table 3. Analysis of multivariate logistic regression for significant associated risk factors with H. pylori infection among dyspeptic patients

Variable		Fecal antigen test		
		AOR	CI(95%)	P-value
Gender	Male	2.591	1.071-6.267	0.035*
	Female	1		
Water source	Non filtered	0.727	0.107-0.696	0.007*
	Filtered	1		
Clinical symptoms	Regurgitation	-	-	-
	Heartburn	0.161	0.258-5.219	0.845
	Heartburn and regurgitation	7.412	1.866-29.444	0.004*
	No symptoms	1		
Antibiotics used	Used	3.208	1.283-8.024	0.013*
	Non-used	1		

*Statistically significant P-value <0.05
Abbreviations: CI, confidence interval; AOR Adjusted odds ratio

4. Discussion:
The prevalence of H. pylori infection among patients with dyspepsia was estimated to be 30% in the present research. Other investigations exhibited similar findings regarding the frequency of H. pylori infection within varying ranges: 23.5% [23], 24.3% [24], 29.6% [25], 37.8% [26], and 80.3% [27]. The variance in the prevalence of H. pylori infections may be attributed to lifestyle factors or different levels of exposure to risk factors.
Our results revealed a high prevalent infection of H. pylori observed among males than among females with significant association (P=0.032). Similar findings revealed that infection of H. pylori was more prevalent among men [28], other more prevalent H. pylori infection showed in males 78% [29]. On the other hand, 35.7% of females found to be positive for H. pylori infection [23]. Another study showed that in general 62% of women were infected with H. pylori [30], while a study showed 63.6% infected females with H. pylori [31]. According to a study, women are more likely than men to have H. pylori infection [25].
The present investigation revealed that the largest prevalence of H. pylori infections was seen among individuals aged 19–32 and 33–46 years. Furthermore, there was no significant association between infection frequency and age among those aged 47 to 60 years (P = 0.969). Similar research revealed that 61.6% of H. pylori-positive individuals were in the 31–60 age range [29]. Infection of H. pylori starts early in childhood and adolescence and peaks in adults between the ages of 35 and 44 [30]. A peak prevalence was seen, according to another study in the group of people aged 40 to 49 years [32].

The prevalent infection of H. pylori was substantially greater in patients aged 5-50 years [33]. In the age range of 46 to 55 years, the highest prevalence of H. pylori was noted [34]. Another study found that the age range of 18 to 30 years had a higher frequency of H. pylori infection [28], while a third study showed that the age range of 30-39 years had a 90.8% prevalence of H. pylori infection [35].

Our research revealed a lack of substantial link between the consumption of food meals and the occurrence of H. pylori infection (P=0.349). However, we did observe a statistically significant link between the source of water and the frequency of H. pylori infection (P=0.012). Some studies showed H. pylori infection prevalence was associated with some foods [35-36]. Another study carried out in Yemen revealed a statistically significant correlation between H. pylori positivity and fat rich meals and Qat chewing [18]. Other study results showed prevalence of H. pylori infection were high significantly among individuals with consumption of unboiling and unclean water sources [24,33,37-38].

Participants who reported regurgitation and heartburn showed substantial clinical evidence of H. pylori infection (P = 0.001). Additionally, the prevalence of H. pylori infection was substantially correlated with the usage of antibiotics (P=0.011). Similar findings demonstrated that H. pylori infection affected every patient with upper stomach pain and frequent burping [30]. Other investigations revealed that the most typical symptoms of H. pylori infection were dyspepsia, heartburn, and epigastric discomfort [18,28,37].

According to a different study, symptomatic participants frequently experienced heartburn, followed by nausea, vomiting, abdominal pain, and black, unpleasant-smelling stools [23]. According to a study, esophagitis, peptic ulcers, and gastritis are all substantially correlated with H. pylori infection [38]. Consistent with previous research, the antibiotics applied were shown to be a significant contributing factor to the probability of H. pylori infection [18,39].

The most prevalent parasites found to be co-infected with H. pylori in dyspeptic patients in the current investigation were intestinal protozoa, with no evidence of a significant correlation, which is consistent with the findings of prior studies [40]. Intestinal protozoa and H. pylori infection may be related, according to several research [6,41]. In Yemen, patients claiming to have gastric giardiasis had H. pylori infection rate of 75% [16].

Another study has shown a high H. pylori infection prevalence associated with intestinal parasitic infections; protozoal, helminthic and both [42], while other study showed the patients with H. pylori infection have *G. lamblia* and *E. histolytica*/dispar [43]. Another study found that *E. histolytica* was the most common parasite co-infected with H. pylori infection [28]. In addition to the infection of H. pylori, *G. lamblia* was reported among patients with dyspepsia [6,44]. There is a high association between concurrent intestinal parasites *G. lamblia* and H. pylori infections in rates of 38.3–70.2% [13].

Furthermore, intestinal parasites *E. histolytica*, Entamoeba coli, and *G. lamblia* were more common among patients infected with H. pylori [14]. Other studies showed involvement of H. pylori infection and intestinal parasites *E. histolytica*, *G. lamblia*, and other parasites with significant differences as probable causes of duodenal, gastric, and peptic ulcer [15]. Other study concluded a high of H. pylori prevalence and *G. lamblia* [45]. The prevalence of *G. lamblia* was significantly high among patients infected with H. pylori [46]. The prevalence of H. pylori infection was associated risk factor for G. intestinalis infection [47]. A study showed 69.4% H. pylori, 51.4% co-infected with *G. lamblia* or *E. histolytica* [48]. H. pylori co-infected with *E. histolytica*/E. dispar [39]. Therefore, H. pylori infection may provide appropriate conditions for infection of giardiasis [49].

In our study, the rate of co-infections of H. pylori and *G. lamblia* or *E. histolytica* could be due to the role of H. pylori-produce urease enzyme that converts the stomach wall urea to ammonia resulting in increased in pH of the stomach [50]. It is found a significant increased urease enzyme activity among co-infected Giardiasis and H. pylori compared to single *G. lamblia* infected individuals [51]. Additionally, possible transmission routes similar to the fecal–oral route could explain the observed the incidence of intestinal parasites among infected participants with H. pylori in this study.

This research has numerous limitations: (1) the cross-sectional study design limits the ability to reveal a true association between dyspepsia and H. pylori infection, (2) H. pylori testing based on stool antigen may exaggerate the prevalence of H. pylori infection, (3) The approach of direct wet mount preparation may underestimate the prevalence of intestinal parasites, (4) the prevalence of co-infection H. pylori with intestinal parasites could not be representative the general population since only dyspeptic symptomatic individuals were included in the study.

5. Conclusion

The present study showed 30% of dyspepsia and H. pylori infection. Sex, unfiltered drinking water, heartburn and regurgitation symptoms and antibiotics drug used were significantly associated with intestinal parasites and H. pylori accompaniment co-infection. The reported findings need more research to study the observed correlation in greater depth, as well as reasons for this association. Screening for *E. histolytica* and *G. lamblia* infection in the intestine is advised for effective care of symptomatic dyspeptic patients.

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العدوى المختلطة لبكتيريا الحلزونية البوابية والطفيليات المعوية: تحديد عوامل الخطورة لدى مرضى عسر الهضم في مدينة المكلا، حضرموت، اليمن

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الملخص: غالباً ما يصاب المرضى الذين يعانون من عسر الهضم بعدوى بكتيريا الحلزونية البوابية، وهي أكثر شيوعاً في دول العالم الثالث. تمتلك بكتيريا الحلزونية البوابية القدرة على استعمار الغشاء المخاطي في المعدة، وغالباً ما تؤدي العدوى إلى تطور مضاعفات الجهاز الهضمي المختلفة. بالإضافة إلى ذلك، تُعد عدوى بكتيريا الحلزونية البوابية والطفيليات المعوية لدى البشر أمراً شائعاً. هدفت هذه الدراسة إلى تحديد مدى انتشار الطفيليات المعوية والعدوى المصاحبة للبكتيريا الحلزونية البوابية، بالإضافة إلى دراسة عوامل الخطورة المتعلقة بالعدوى لدى مرضى عسر الهضم في المستشفيات الرئيسية في مدينة المكلا، محافظة حضرموت، اليمن. في هذه الدراسة المقطعية تم جمع وتحليل 100 عينة من البراز. وكان الغرض هو الكشف عن عدوى بكتيريا الحلزونية البوابية باستخدام اختبار مستضد البراز المناعي السريع، وكذلك تحديد وجود عدوى طفيلية مترافقة مع عدوى بكتيريا الحلزونية البوابية. تم الحصول على بيانات المستهدفين باستخدام أداة الاستبيان، وتم فحص البيانات باستخدام نظام التحليل الإحصائي SPSS الإصدار 24، وتم استخدام تحليل الانحدار اللوجستي لتحديد متغيرات الخطورة التنبؤية المستقلة، واعتمدت القيمة المعنوية عند 0.05. كان إجمالي معدل انتشار عدوى بكتيريا الحلزونية البوابية بين مرضى عسر الهضم 30٪. كان لدى الذكور معدل انتشار أعلى للعدوى الحلزونية (COR = 0.386، 95٪ CI = 0.160–0.934، P = 0.035). علاوة على ذلك، ارتبطت النتائج الإيجابية لعدوى بكتيريا الحلزونية البوابية بشكل كبير بمياه الشرب غير المفلترة (COR = 3.67؛ 95% CI = 1.436–9.363؛ P = 0.007)، وأعراض الارتجاع والحرقة (COR = 0.865، 95% CI = 0.034–0.536، P =0.004)، واستخدام المضادات الحيوية (COR = 0.312، 95٪ CI = 0.125– 0.780، P = 0.013). تشير هذه الدراسة إلى أن الأوليات المعوية المتحولة الحالة للنسيج والجيارديا اللامبلية كانت أكثر الطفيليات المرتبطة بعدوى بكتيريا الحلزونية البوابية شيوعاً في المرضى الذين يعانون من عسر الهضم دون وجود ارتباط معنوي. كانت متغيرات الجنس ومصادر المياه غير المفلترة وأعراض الارتجاع والحرقة واستخدام المضادات الحيوية هي أبرز عوامل الخطورة المرتبطة بعدوى بكتيريا الحلزونية البوابية المصاحبة لعدوى الطفيليات المعوية المتحولة الحالة للنسيج والجيارديا اللامبلية.

الكلمات المفتاحية: العدوى المشتركة، مرضى عسر الهضم، المتحولة الحالة للنسيج، الجيارديا اللامبلية، الحلزونية البوابية، حضرموت، الانتشار، اليمن