## ORIGINAL ARTICLE

# Association between intestinal Parasites and Helicobacter Pylori infections in patients with gastrointestinal complaints attending Aswan University hospital

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## ABSTRACT

**Keywords**: H.pylori, Intestinal parsasite, - Gastrointestinal Complaints

Introduction

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**Background:** Intestinal parasitic infections (IPIs) caused by parasitic protozoa and helminthes have been known to compromise the quality of human life since prehistoric times. **Aim:** The aim of the study was to determine the relation between *H. pylori* infection and intestinal parasitic infections in patients with gastrointestinal complains attending Aswan university hospital. **Materials and methods:** A cross-sectional study was carried out in four areas in Aswan Governorate: Eltaamen, Elsadaqa, Elseel, Elnafaq. Parasitological examination of 100 stool samples were collected from people who are suffering from gastrointestinal symptoms , and performed using *H. pylori* stool antigen test to detect *H. pylori* infection then the samples will be microscopically examined to detect parasitic infections either helminthes or protozoa. **Results :** *H. pylori* positivity showed a statistically significant change compared to *E. histolytica*, *Entrobius vermicularis*, *Giardia lamblia*, *H.nana*, and *Ascaris* (p=0.021, 0.04, 0.003, 0.02, and 0.016 respectively), where there's more *H. pylori* positivity detected among those patients with positive all parasite species **Conclusion:** Gastrointestinal parasites are more common among H. pylori patients compared to individuals without H. pylori; there was high prevalenceof co-infection of H. pylori with intestinal parasites in Aswan university hospital. G. lamblia, Cryptosporidium and B. hominis were prevalent in H. pylori patients than H. pylori free ones with significant differences. But this infection rate was not affected by gender.

## INTRODUCTION

In developing countries, co-infections with different pathogens are common, and are attributable to the feco-oral transmission of bacterial and parasite pathogens brought about by the poor quality of the water consumed by the population at large living in unhygienic

conditions, i.e., overcrowding, poor toilet facilities, and absence of quality health care **(Zaidi et al., 2004**) **(Alum et al., 2010).**

Intestinal parasite infections are among the most prevalent diseases in the world; it is estimated that 3.5 billion people are impacted and 450 million individuals are infected. In addition to intestinal parasites, Helicobacter pylori infection is widespread in underdeveloped nations. These illnesses occur because of poor personal hygiene and socioeconomic conditions **(Jayalakshmi and Dharanidevi, 2016)**

## PATIENTS AND METHODS

This study was carried out in tropical outpatient clinics, Aswan University Hospital, Aswan, Upper Egypt

### Ethical Consideration

After approval of the protocol from ethics committee of Faculty of Medicine, Aswan University. Informed consents were taken from all patients before sample collection and after approval of the Ethical Health Committee by the Egyptian Ministry of Health and Population

### Study population:

This study was conducted on 100 child and adult attending tropical outpatient clinics Aswan University hospital aged (18-65) after getting their approval in period from November 2021 to October 2022 , A questionnaire was administraited and history was taken from them regarding name, age, gender, residence and gastrointestinal complaines will be employed to analyze the prevalence of Helicobacter pylori and various intestinal parasites, and the relevant co-infection status among study participants. Various social and demographic risk factors will be analyzed in association with the co-infection prevalence rates among participants.

The stool samples were examined microscopically to detect intestinal parasites and using H. pylori stool antigen test to detect H. pylori infection .

### Laboratory testing

### Stool examination:

Two fresh stool samples will be collected from each patient at the time of enrollment in the study.

**The first sample** diagnose *H. pylori* by detecting antigen using *H. pylori* stool antigen test (One step *H. pylori* antigen test device; ACON Laboratories Inc., San Diego, CA, USA) according to manufacturer's directions **(Silva et al., 2010).** So, patients will be divided into 2 groups: *H. pylori*-positive cases (GI) and *H. pylori*-negative cases (GII).

**The second stool sample** will be transferred to the parasitology laboratory, Faculty of Medicine to be examined immediately by different techniques for parasitic infections. In the laboratory, slides will then be prepared directly for wet mount in saline as well as in iodine and then were microscopically examined initially under low power then under high power. Finally, the sample will be concentrated by applying the formalin-ethyl acetate technique **(Dubey et al., 1990**). The iodine-stained slides will be prepared and examined microscopically. Fixed smears will be stained with Modified Ziehl-Neelsen stain **(El-Shazly et al., 2006)** and Giemsa stain (Garcia,

2004). Also, stool culture for nematode larvae on agar plates will be done for the detection of *S. stercoralis* or hookworm larvae **(Knopp et al., 2008).**

**1. *H. pylori* status**

*H. pylori* will be detected using Human *Helicobacter Pylori* Rapid Test (Cassette) Antibody Rapi Card Insta Test stool antigen test (Diagnostic Automation Inc., USA). Monoclonal anti-*H. Pylori* antibodies will be used to capture antibodies while peroxidase-conjugated monoclonal antibodies will be used for detection. A small portion of the stool sample will be homogenized with buffer solution and two drops will be added to the test well for the immunoassay. Following a 15-min wait period, the test will be ready for analysis. The presence of both control and test lines will be defined as a positive result, even in the case of the control line being much darker than the test. However, if the only line present is the control, this test is deemed negative. The rapid antibody test (Diagnostic Automation Inc. USA) will be used to detect either past or current infection, but cannot distinguish the history of old versus active infection. Approximately 1 to 2 drops of blood serum will be added to the test well, in which a double antigen chromatographic lateral flow immunoassay will be performed, similarly to the rapid antigen test. After 15 min the test will be read and the presence or absence of test lines will be interpreted the same way as the antigen test 2 H. **(Spotts et al., 2020).**

**RESULTS**

. Our study included a hundred participants, where males comprised 48 percent (48/100), and females 52 percent (52/100), also, they were distributed on the following areas: 36 percent from Elseel (36/100), 28 percent from Elnafaq (28/100), 20 percent from eltaamen (20/100), and 16 percent from elsadaqa (16/100). The mean age was 24.14±13.65 years, as shown in **table (1).**

**Table (1): socio-demographic characteristics of study participants. (N=50)**

| | Frequency (N.) | Percentage (%) |
|---|---|---|
| **Sex** | | |
| Male | 48 | 48 |
| Female | 52 | 52 |
| **Locality** | | |
| Elnafaq | 28 | 28 |
| Elsadaqa | 16 | 16 |
| Elseel | 36 | 36 |
| Eltaamen | 20 | 20 |
| **Age in years**: (mean±SD), Median | (24.14±13.65), 23.5 | |
| $25^{th}$ percentile, $75^{th}$ percentile, IQR | 12.75, 34, 21.25 | |

**Table (2): Complaint of study participants. (N=100)**

| | Frequency (N.) | Percentage (%) |
|---|---|---|
| **Diarrhea** | | |
| Yes | 16 | 16 |
| No | 84 | 84 |
| **Vomiting** | | |
| Yes | 38 | 38 |
| No | 62 | 62 |
| **Distension** | | |
| Yes | 28 | 28 |
| No | 72 | 72 |
| **Abdominal pain** | | |
| Yes | 46 | 46 |
| No | 54 | 54 |
| **Perianal itching** | | |
| Yes | 20 | 20 |
| No | 80 | 80 |
| **General examination (pallor)** | | |
| Yes | 30 | 30 |
| No | 70 | 70 |

Among study participants, the following symptoms were reported: 16 percent had diarrhea (16/100), 38 percent had vomiting (38/100), 28 percent had distension (28/100), 46 percent had abdominal pain (46/100), 20 percent had perianal itching (20/100), and 30 percent showed pallor on general examination (30/100), as shown in **table (2).**

**Table (3): Intestinal parasites and *H. pylori* detected among study participants. (N=100)**

| | Frequency (N.) | Percentage (%) |
|---|---|---|
| ***H. pylori*** | | |
| Positive | 30 | 30 |
| Negative | 70 | 70 |
| ***E. histolytica*** | | |
| Positive | 70 | 70 |
| Negative | 30 | 30 |
| ***Entrobius vermicularis*** | | |
| Positive | 26 | 26 |
| Negative | 74 | 74 |
| ***Giardia lamblia*** | | |
| Positive | 24 | 24 |
| Negative | 76 | 76 |
| ***H. nana*** | | |
| Positive | 14 | 14 |
| Negative | 86 | 86 |
| ***Ascaris*** | | |
| Positive | 28 | 28 |
| Negative | 72 | 72 |

Stool examination of study participants revealed the following results: *H. pylori* was found positive in 30 percent (30/100), *E.histolytica* was found positive in 70 percent (70/100), *Entrobius vermicularis* was found positive in 26 percent (26/100), *Giardia lamblia* was found positive in 24 percent (24/100), *H.nana* was found positive in 14 percent (14/100), and Ascaris was found positive in 28 percent (28/100), as illustrated in **table (3)**

**Table (4*): H. pylori* distribution by demographic data.**

| | *H. pylori* | | p-value |
|---|---|---|---|
| | Positive | Negative | |
| **Sex** | | | |
| Male | 8 (26.7%) | 40 (57.1%) | **0.047 *** |
| Female | 22(73.3%) | 30 (42.9%) | |
| **Locality** | | | |
| Elnafaq | 6 (20%) | 22 (31.4%) | 0.372 * |
| elsadaqa | 6 (20%) | 10(14.3%) | |
| Elseel | 8 (26.7%) | 28 (40%) | |
| Eltaamen | 10 (33.3%) | 510(14.3%) | |
| **Age** (mean±SD) | 25.26±12.46 | 23.65±14.27 | 0.707 ** |

*: Chi-square test. **: Independent samples t-test.

The change of H. pylori status according to the demographic characteristics of the patients was analyzed using chi-square test **(Table 4)**. It was observed that the presence or absence of *H. pylori* did not differ according to the locality or age of the patients (p=0.372, and 0.707 respectively). However, there was a statistically significant difference between sex (p=0.047); where *H. pylori* was more positive among females (73.3%) than males (26.7%).

**Table (5): *H. Pylori* distribution by symptoms observed.**

| | *H. pylori* | | p-value* |
|---|---|---|---|
| | Positive | Negative | |
| **Diarrhea** | | | |
| Yes | 6 (20%) | 10 (14.3%) | 0.614 |
| No | 24(80%) | 60 (85.7%) | |
| **Vomiting** | | | |
| Yes | 16(53.3%) | 22 (31.4%) | 0.144 |
| No | 14(46.7%) | 48 (68.6%) | |
| **Distension** | | | |
| Yes | 22 (73.3%) | 6(8.6%) | **<0.001 ** ** |
| No | 8(26.7%) | 64 (91.4%) | |
| **Abdominal pain** | | | |
| Yes | 24 (80%) | 22 (31.4%) | **0.002** |

| | | | |
|---|---|---|---|
| No | 6(20%) | 48 (68.6%) | |
| **Perianal itching** | | | |
| Yes | 4 (13.3%) | 16(22.9%) | 0.702 ** |
| No | 26 (86.7%) | 54 (77.1%) | |
| **General examination (pallor)** | | | |
| Yes | 10(33.6%) | 20 (28.6%) | 0.747 ** |
| No | 20 (66.7%) | 50 (71.4%) | |

*: Chi-square test. **: Fisher's exact test.

According to some symptoms observed in the patients, the variation of the presence or absence of *H. pylori* was analyzed using the chi-square test **(Table 5)**
*H. pylori* positivity didn't show any significant relationship with all symptoms observed; however, there was a statistically significant difference between rates of distension, and abdominal pain (p<0.001, and 0.002 respectively). On the other hand, there was no significant difference between rates of diarrhea, vomiting, perianal itching or pallor upon general examination (p=0.614, 0.144, 0.702, and 0.747 respectively).

**Table (6): The relationship between *H. pylori* positivity and parasite species.**

| | ***H.pylori*** | | p-value* |
|---|---|---|---|
| | Positive | Negative | |
| ***E. histolytica*** | | | |
| Positive | 28 (93.3%) | 42 (60%) | **0.021** |
| Negative | 2(6.7%) | 28 (40%) | |
| ***Entrobius vermicularis*** | | | |
| Positive | 14(46.7%) 16(53.3%) | 12 (17.1%) | **0.04** |
| Negative | | 58 (82.9%) | |
| ***Giardia lamblia*** | | | |
| Positive | 16 (53.3%) | 8 (11.4%) | **0.003** |
| Negative | 14(46.7%) | 62 (88.6%) | |
| ***H.nana*** | | | |
| Positive | 10 (33.3%) | 4 (5.7%) | **0.02** |
| Negative | 20 (66.7%) | 66 (94.3%) | |
| ***Ascaris*** | | | |
| Positive | 16 (53.3%) | 12 (17.1%) | **0.016** |
| Negative | 14 (46.7%) | 58 (82.9%) | |

*: Chi-square test. **: Fisher's exact test.

Relationship between *H. pylori* positivity and parasite species positivity was examined **(Table 6).** The results indicate that *H. pylori* positivity showed a statistically significant change compared to *E.histolytica, Entrobius vermicularis, Giardia lamblia, H.nana,* and *Ascaris* (p=0.021, 0.04, 0.003, 0.02, and 0.016 respectively) as shown in **table (6)** where there's more *H.pylori* positivity detected among those patients with positive all parasite species

## DISCUSSION

This study aimed to examine the prevalence of different intestinal parasitic infections and *H. pylori* among patients with gastrointestinal symptoms. This cross-sectional study included a hundred patients with different gastrointestinal symptoms, with mean age was 24.14±13.65 years. Males comprised 48 % and females 52 %.

In the present study, the most frequent intestinal parasite associated with *H. pylori* infection was *E. histolytica*. The other parasites that showed an association with *H. pylori* infection were *Entrobius vermicularis, Giardia lamblia, H. nana and Ascaris.*

Stool examination of this study participants revealed that; 70% had positive *E. histolytica*, which is the most commonly detected. *H. pylori* was found positive in 30% of the participants. *Ascaris* was found positive in 28 %, *Entrobius vermicularis* was found positive in 26 %, *Giardia lamblia* was found positive in 24 % and *H. nana* was found positive in 14 %.

This comes agreeing with an Egyptian study by **(AHMED et al. 2018**) who reported more parasitic infection among H. pylori positive patients

In **Pomari and colleague's** cohort, a higher rate of *H. pylori* infection was observed among intestinal parasites infected individuals compared to uninfected subjects **(Pomari et al. 2020).** Similarly, **Abd Elbagi et al.** results showed that *Entamoeba histolytica* was seen in 12% of *H. pylori* cases followed by *Entamoeba coli* in 7% and *G. lamblia* in 4% of *H pylori* positive cases. Among the *H pylori* negative group *E. histolytica* was reported at 5%, followed by *G. lamblia* at 3% and *E. coli* at 2% **(Abd Elbagi et al. 2019). ) While Dyab et.al.2016** who was observed in Aswan the commonest helminthic infection was *E. vermicularis* 6.6% followed by *H. nana* 3% *Ascaris lumbricoides*1%. The commonest protozoa infection *was E. histolytica* 8.3% followed *by Giardia lamblia* 3.7% and
*Cryptosporidium parvum* 1.7%. Mixed infection was *E. vermicularis* plus *E. histolytica* (23.4%),*E vermicularis* plus *G. lamblia* (17.6%), *E. vermicularis* plus *C. parvum* (11.8%), *E. histolytica* plus *H. nana* (11.85%), *A. lumbricoides* plus *E. histolytica* (17.6%) and *G. lamblia* plus *E. histolytica*
in (11.8%).

Also, according to **(Yakoob et al.2018)** patients with *H pylori* infection were more likely to be co-infected with *Blastocystis sp* and *E histolytica* **.**This is consistent with a study of patients with duodenal biopsies who also had a gastric biopsy found that those with *giardiasis* were more likely to be colonized with *H. pylori* **(Zylberberg et al. 2017).**

**Ankarklev and colleagues** found a significantly higher frequency (3 times) of giardial infection in cases where infected children also harbored the bacterial pathogen *H. pylori* **(Ankarklev et al. 2012).** This is comparable with findings from recently published cross-sectional surveys **(Zeyrek et al. 2008; GSh and Efimova 2010).** Similarly,( **Abd Elbagi et al.2019)** found that *Entamoeba histolytica* was found in 12% of *H. pylori* cases followed by *Entamoeba coli* (7%) and *Giardia lamblia* (4%). There was a significant difference in the prevalence of intestinal parasites between *H. pylori* patients group and healthy control group **.**

Conversely,**( Moreira et al.2005)** did not find significant association between *E. histolytica* infection and *H. pylori* seropositivity **.**However,**( Torres et al.2003)** showed a significantly lower prevalence of *H. pylori* infection among adults carrying *E. histolytica* compared to those who were negative to the parasite **.**

Our finding was in total disagreement with the finding of **(Seid et al. 2018)**in Ethiopia, who reported no significant association between *H. pylori* and *E. histolytica* but there was significant association with *G. lamblia* **.**This may be due to differences in the study areas.

***Helicobacter pylori*** is a gram-negative, helical-shaped, motile bacillus bacterium, which colonizes the gastric mucosa. *H. pylori* bacterium secretes urease, a special enzyme that converts urea to ammonia. Ammonia reduces the stomach's acidity. This risk factor allows pathogenic intestinal protozoa such as *G. lamblia* to take the opportunity to cross through the stomach's increased pH and cause disease **(Tanih et al., 2008).**

The current study observed significant high prevalence of parasitic infection among *H. pylori* positive patients (P<0.005), as compared to *H. pylori* negative patients. This finding can be explained by the fact that *H. pylori* escaped the destructive effect of stomach acidity by neutralization, as it produces large amounts of urease enzyme which breaks down plasma urea in the stomach wall to ammonium ion. *H. pylori* proved risky infection by destroying stomach acidity **(Smoot 1997)** an important body defense against ingested pathogens **(Smith 2003).**

Among whole study patients; abdominal pain was the most commonly detected symptom (46%), followed by vomiting, pallor and distension (38%, 30% and 20%, respectively). The least commonly detected symptom was perianal itching and diarrhea (20% and 16%, respectively). Among *H. pylori* positive patients in this study, 80% had abdominal pain and 73.3% had abdominal distension, which significantly higher than *H. pylori* negative patients. Diarrhea, vomiting, perianal itching and pallor showed insignificant association with *H pylori* positivity.

Agreeing with this study, **Sabah et al.** reported that epigastric pain and heart burn were commonest symptoms associated with *H. Pylori* Ag positive patients **(Sabah et al. 2015),** which also, agreed with **Yang et al. (YANG et al. 2005).**

Similarly, **Abd El Hameed and colleagues** reported that among 150 children of both sexes having gastrointestinal complaints, the most common clinical presentation was abdominal pain (85.3%), abdominal distension (52%) **(Abd El Hameed et al. 2021).**

Also, among 93 adults with concomitant infection of helicobacter pylori and intestinal parasites included in **(Pomari et al.2020)** study 34% reported GI symptoms, of which 59% had upper abdominal pain, 31% epigastric pain, 14% diarrhea, and 13% gastritis/duodenitis **.** Similarly, diarrhea wasn't reported to be a prominent symptom in group with *H. pylori* infection (29.1%) in a study of**(Abd El Hameed et al. 2021).**

This study showed no association between infection with *H. pylori* and intestinal parasites and sex, it was observed that there was a statistically significant difference between *H pylori* positive and negative patients as regards sex (p=0.047); where *H. pylori* was more positive among females (73.3%) than males (26.7%).

The meta-analysis by de **Martel and Parsonnet** based on 18 studies with at least 500 adult participants, found a 16% increased odds of *H. pylori* infection for men **(De Martel and Parsonnet 2006).** In a systematic review performed by **Zamani et al.** the worldwide

prevalence of *H. pylori* infection was estimated at 46.3% among men and 42.7% in women **(Zamani et al. 2018).**

**Lim and colleagues** observed that seropositivity of *H. pylori* was significantly lower in females than in males **(Lim et al. 2013).** In contrast **Şeyda et al** detected that among 1680 patients with variable gastrointestinal complaints, *H. pylori* positivity was 67.7% in males and 68.2% in females (p = 0.865), with statistically insignificant difference between both sex **(Şeyda et al. 2007).**

A statistically insignificant relation was detected between age and *H pylori* positivity among this tudy participants. A contradicting finding was reported in **Robertson and colleagues** who investigated 500 consecutive blood donors from Australia; overall *H. pylori* seroprevalence was 32% and ranged from less than 20% in subjects 21–30 years up to 54% in subjects aged > 60 years **(Robertson et al. 2003).**

**Graham et al.** found that the prevalence of *H. pylori* infection increased rapidly with age at 1% for every year for the overall population **(Graham et al. 1991).** The difference of the current study from the previous studies may be due to the lower sensitivity of the present study blood testing procedure.

## CONCLUSION

Gastrointestinal parasites are more common among *H. pylori* patients compared to individuals without *H. pylori*; there was high prevalence of co-infection of *H. pylori* with intestinal parasites in Aswan university hospital. *G. lamblia*, *Cryptosporidium* and *B. hominis* were prevalent in *H. pylori* patients than *H. pylori* free ones with significant differences. But this infection rate was not affected by gender.

Co-infection remains a complex biological problem that must be approached from broad epidemiological as well as molecular-level studies to maximally decrease the impact. *H. pylori* may support the colonization by intestinal parasites or vice versa. The interaction between *H. pylori* and intestinal parasites may have serious health consequences.

Interactions between *H. pylori*, parasites, and the host are under the influence of multiple factors including immune responses, gut barrier function and gut microbiome and hence subject to variable clinical consequences. Co-infection with protozoa may worsen gastric pathology but co-infection with helminths may be potentially beneficial. The gastric outcomes are largely mediated by the balance between Th1 and Th2 responses.

Our findings indicate the relatively high burden of H pylori and parasitic co-infection especially *E. histolytica*, the most commonly detected pathogen, among patients with gastrointestinal complaints attending Aswan University Hospital.

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