Journal of the Egyptian Society of Parasitology, Vol.50, No.2, August 2020
J. Egypt. Soc. Parasitol. (JESP), 50(2), 2020: 390 - 393

# *HELICOBACTER PYLORI* CO-INFECTED WITH COMMON INTESTINAL PROTOZOA IN GASTROINTESTINAL SYMPTOMATIC PATIENTS

By
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**Abstract**

The zoonotic intestinal protozoan parasites and *Helicobacter pylori)* are worldwide distributed human pathogens. This study evaluated the co-infection between *H. pylori* and the commonest intestinal protozoan parasites. A total of 240 stool samples were collected over one year from patients attending outpatients' clinics of Kafrelsheikh University Hospitals, after verbal consent for participation. Samples were subjected to microscopic examination directly before and after formol ether concentration. Samples were examined using merthiolate iodine formalin (MIF) direct smears to detect *G. intestinelis* and permanent modified trichrome and modified acid-fast cold kinyoun's stained smears to detect *E. histolytica/dispar* and *Cryptosporidium* spp. respectively. Screening with immune-chromatographic test (ICT) detected *H. pylori* infection. The results showed that *H. pylori* were in 160/240 (66.7%). Univariate analysis showed significance association between *H. pylori* and protozoan causing diarrhea ($P$ = 0.015). These protozoan parasites were with *G. intestinalis* ((P=0.002)*, E. histolytica/dispar* (P= 0.041)*, Cryptosporidium parvum* (P =0.018) and *Blastocystis hominis* (P= 0.040).

**Key words:** Patients, *Heliobacter pylori*, Intestinal protozoan parasites

## Introduction

Among the commonest prevalent diseases worldwide were intestinal parasitosis (Jayalakshmi and Dharanidevi, 2016). These infections are a serious public health infection causing physical and mental health problems (Tandukar *et al*, 2013). These infections included intestinal protozoa; *Giardia lamblia* and *Cryptosporidium* spp. that proved to have a serious impact on children and *Entamoeba histolytica* with a higher morbidity in all aged patients (Mortimer and Chadee, 2010).

*Helicobacter pylori (H. pylori)* are gram-negative bacteria that colonize human stomach and considered the most common chronic human bacterial infection worldwide infecting more than 50% of the population in both developed and developing countries (Dane and Gurbuz, 2016) with much higher prevalence in developing countries (Eldash *et al*, 2013). *H. pylori* colonize the stomach's mucus layer inducing chronic gastric inflammation (Konturek, 2003). It is considered to be a major cause of gastric and duodenal ulcers and a risk factor for the gastric malignancies (Mutaz and Carmen, 2015). They produced some enzymes that could cause destructive effect on the epithelial lining of the stomach and more importantly, active urease enzyme (Cameron *et al*, 2001).

Urease produced by *H. pylori* can transform the urea of stomach wall to ammonia resulting in the increment of gastric environment's pH (Isaeva and Efimova, 2010). Acidity of the stomach is considered an important barrier of the innate immune system that protect against invasion by pathogens, therefore the diminished acidity will give pathogens the opportunity to break this barrier and go across it invading the gastric mucosa (Kazemian *et al*, 2016). *H. pylori* infection shared intestinal *Parasites* in multiple clinical presentations as diarrhea, dysentery, vomiting, lack of appetite and abdominal distention (Ahmed *et al,* 2018). *H. pylori* can be identified by using simple techniques (Guerrant *et al*, 2011) and Copro immunoassays as immune-chromatographic test (ICT) (Abd Elbagi *et al*, 2019).

This study aimed to evaluate co-infection prevalence of *Heliobacter pylori* in sympto-

matic patients with intestinal protozoa causing diarrhea *Giardia intestinalis, Entamaeba histolytica/dispar, Cryptosporidium pa-rvum* and *Blastocystis hominis* among the outpatients of Kafrelsheikh University Hospitals.

## Materials and Methods

Study design: A cross sectional study was conducted over 240 patients of both sexes, aged from 1 to 45 years old attending Outpatient Clinics of Kafrelsheikh University Hospitals all over the year 2019, suffering from gastrointestinal manifestations including abdominal pain, nausea, vomiting, diarrhea, abdominal bloating. Patients on anti-diarrheal medications were excluded. Patients included were divided into 3 age groups; G1:1-15 years, G2:16-30 & G3: 31-45 years. A single fecal sample was obtained from each patient after verbal consent and recording demographic, clinical and environmental data by using a designed questionnaire.

All samples were microscopically examined to detect protozoa by wet mount smear & formol ether concentration. Merthiolate iodine formalin (MIF) direct smear were used for *G. intestinelis* (El-Taweel and Abou Holw, 2008), modified acid fast cold Kinyoun's stain for *C. parvum* (El-Shazly *et al*, 2006). and modified Trichrome (Garcia, 2007) for *E. histolytica/dispar*

*H. pylori* copro-antigen was detected using ICT (ACON Laboratories Inc., San Diego, USA) after the manufacturer's instructions.

Statistical analysis: Data were tabulated and analyzed by using the package SPSS version 21 (Chicago, IL, USA) for the statistical analysis. Data were described using frequency and percentage with $P < 0.05$ was considered significant.

## Results

In the present study, *H. pylori* were detected in 160/240(66.7%) samples. Infection in G1, G2 & G3 was 55%, 25% & 20% in respectively among 54.4% males & 45.6% females, without significance between age nor sex. As to gastrointestinal pictures, 128/160 (80%) *H. pylori* patients were presented by abdominal pain, 72(45%) nausea, 64 (40%) vomiting and 96 (60%) diarrhea. Bloating was 96(60%) patients, significant association (P= 0.015) between *H. pylori* infection and diarrhea only. Of *H. pylori* positive patients, 134 (83.8%) were co-infected with protozoa; 31 (19.3%), 27 (16.8%), 32 (20%) and 68 (42.5%) with *G. intestinali, E. histolytica*, *C. parvum* & *B. honinis* respectively. Significant association was between *H. pylori* and *G. intestinali* (P= 0.002), *E. histolytica* (P= 0.041), *C. parvum* (P=0.018) & *B. hominis* (P= 0.040). Also, 45/80 (56.2%) patients were *H. pylori* free but with *G. intestinalis* 4 (5%), *E. histolytica/dispar* 22 (27.5%), *C. parvum* 7 (8.7%) and *B. honinis* 24 (30%).

The details were given in tables (1 &2).

Table 1: Prevalence of *H. pylori* among population according to age, sex and clinical data

| Variants | | Positive cases (n=160) | Negative cases (n=80) | P value |
|---|---|---|---|---|
| Age | G1 (1-15 years) | 88 (55%) | 44 (55%) | 1.000 |
| | G2 (16-30 years) | 41 (25.7%) | 20 (25%) | |
| | G3 (31-45 years) | 31 (19.3%) | 16 (20%) | |
| Sex | Male | 87 (54.4%) | 44 (55%) | 0.519 |
| | Female | 73 (45.6%) | 36 (45%) | |
| Associated GIT symptoms | Abdominal pain | 128 (80%) | 60 (75%) | 0.234 |
| | Nausea | 72 (45%) | 40 (50%) | 0.276 |
| | Vomiting | 64 (40%) | 28 (35%) | 0.272 |
| | Diarrhea | 96 (60%) | 60 (75%) | 0.015 |
| | Bloating | 96 (60%) | 44 (55%) | 0.273 |
| Over all percentage | | 66.7 | 33.3 | |

Table 2: Co-infection between *H. pylori* and intestinal protozoa significance

| Associated Variants | +ve (n=160) (66.7%) | -ve (n=80) (33.3%) | P value |
|---|---|---|---|
| *Giardia intestinalis* | 31 (19.3%) | 4(5%) | 0.002 |
| *Entamoeba histolytica/dispar* | 27 (16.8%) | 22 (27.5%) | 0.041 |
| *Cryptosporidium parvum* | 32 (20%) | 7 (8.7%) | 0.018 |
| *Blastocystis hominis* | 68 (42.5%) | 24 (30%) | 0.040 |
| Free from intestinal protozoa | 26 (16.2%) | 35 (43.8%) | |

## Discussion

Generally speaking, *H. pylori* is one the principle cause of chronic gastric inflammation, peptic and duodenal ulcers, non-ulcerative dyspepsia, gastric carcinoma and gastric mucosa associated lymphoid tissue lymphoma (Khalifa *et al*, 2010). The reduction of stomach acidity caused by urease activity of *H. pylori* was as a risk factor for parasitic infections (Sanad *et al*, 1996).

This study showed higher prevalence of *H. pylori* among 1-15 years age group followed by 16-30 years age group was 55% & 25% respectively. This agreed with Abd Elbagi *et al*. (2019) who found that high prevalence of *H. pylori* was in 1-15 years age group (40%) and second high prevalence was in ages older than 16-30 years (38%), but disagreed with Fadul *et al.* (2016) who found a high rate (50%) in patients >66 years old.

The present results showed prevalence of *H. pylori* in males and females was 54.4% & 45.6% respectively without s significant difference. This agreed with El-Massry *et al*. (2003) and Kazemian *et al*. (2014). But, this disagreed with El-Badry *et al*. (2017) who reported that sex was significantly in *H. pylori* co-infected with *Giardia*. Also, Seid *et al*. (2018) who reported significant association between the male sex and *H. pylori*-co-infected with intestinal parasites

In the present study, gastrointestinal manifestations were abdominal pain, nausea, vomiting, diarrhea and bloating with significant association between *H. pylori* and diarrhea (P = 0.015). This agreed with Castillo-Montoyaa *et al.* (2017) who reported significant association between *H. pylori* and protozoa causing diarrhea (P = 0.0389).

The prevalence of G. intestinalis (19.3%) was lower than that reported by Kazemian *et al.* (2016) and Isaeva *et al.* (2010) who showed that *G. intestinalis* co-infected with *H. pylori* was 42% & 100% respectively. Also, Moreira *et al*. (2005) and Ankarklev *et al*. (2012) reported significant relation between *H. pylori* and *G. intestinalis*.

The present rate of *E. histolytica/dispar* (16.8%) was higher than that found by Abd Elbagi *et al*. (2019) who reported 12% for *E. histolytica/dispar* among *H. pylori* patients but lower than (21.5%) reported by either Torres *et al*. (2003) or (55.5%) reported by Ahmed *et al*. (2018).

In the present study, *B. hominis* (42.5%) was lower than 67.2% reported by Nghaimesh *et al*. (2018). Concerning *C. parvum* (20%), Ibrahim *et al.* (2019) found a rate of 5.3% with a significant statistical association between *H. pylori* infection and *Cryptosporidium* spp. (p =0.02).

## Conclusion

There was high prevalence (66.7%) of *H. pylori* in Kafrelsheikh. Also, *G. intestinalis, E. histolytica/dispar, C. parvum* and *B. hominis* were prevalent in *H. pylori* patients than *H. pylori* free ones with significant differences. Undoubtedly, treating of the gastrointestinal parasites is a must when treating *H. pylori*

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