RESEARCH ARTICLE

The relationship between Helicobacter pylori infection and intestinal parasites in individuals from Khartoum state, Sudan: a case-control study [version 1; peer review: 1 approved, 3 approved with reservations]

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Abstract

Background: In developing countries, Helicobacter pylori infection is common, as are intestinal parasites. Socioeconomic circumstances and low personal hygiene lead to the spread of these infections. This research aimed to evaluate the relationship between intestinal parasites and H. pylori in Khartoum, Sudan.

Methods: This study was conducted in various hospitals in Khartoum between June and October 2018. The study involved 200 individuals: 100 patients with H. pylori as a case group and 100 healthy individuals as a control group. A stool sample was taken from each individual, and wet preparation, saturated sodium chloride flotation and formal ether concentration were used to detect intestinal parasites.

Results: The results showed that 23% of H. pylori patients and 10% of healthy individuals had gastrointestinal parasites; Entamoeba histolytica was found in 12% of H. pylori cases followed by Entamoeba coli (7%) and Giardia lamblia (4%). Control group: Entamoeba histolytica in 5% followed by G. lamblia in 3% and E. coli in 2% of individuals. There was a significant difference in the prevalence of intestinal parasites between groups (P = 0.013).

The prevalence rate of intestinal parasites among men and women was 24% and 22%, respectively, in the case group, and 9% and 11%, respectively, in the control group. In the case group, the highest prevalence rates (40% and 38%) were found among the age groups 1-15 and 46-60 years old, respectively, while the lowest rate (10.7%) was found among the 31-45 age group. In the control group, the highest prevalence rate (15%) was among the 31-45 age group, the lowest...
prevalence rate (8%) was found among the 16-30 age group. **Conclusion:** Together, we found that intestinal parasites are more common in patients with *H. pylori*. We also noticed that the rate of infection was not affected by gender while the age group was affected.

**Keywords**
H.pylori, Intestinal parasite, Khartoum, Alsaha, Yastabsheron.

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**Author roles:** Yousif Abd Elbagi Y: Formal Analysis, Investigation; Abd Alla AB: Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; Saad MBE: Supervision

**Competing interests:** No competing interests were disclosed.

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Introduction

Among the most common diseases in the world are intestinal parasite infections; an estimated 3.5 billion people are affected, and 450 million people are infected (Jayalakshmi & Dharanidevi, 2016). These infections are considered a serious public health problem as they cause anaemia with iron deficiency, retardation of growth in children, and other physical and mental health problems (Okyay et al., 2004; Tandukar et al., 2013; Wongstitwilaoroong et al., 2007). One example is a pathogenic intestinal protozoon that infects the small and/or large intestine (Farthing & Kelly, 2005), or an intestinal worm, such as Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, and hookworms, which affect people in tropical countries (Smyth, 1990).

Health impacts differ with age: the small intestinal protozoa Giardia lamblia and Cryptosporidium spp. have a serious impact on children (Harhay et al., 2010), while the large intestine pathogen Entamoeba histolytica has a higher morbidity among adults of all ages (Mortimer & Chadee, 2010). Some protozoa, in particular, Cryptosporidium and Isospora belli, cause significant morbidity in individuals with immunodeficiency (Bachur et al., 2008), for example giardiasis and amoebiasis are opportunistic parasites (Biggs & Brown, 2001). Helicobacter pylori is the most common chronic human bacterial infection, infecting 70–90% of the population of developing countries and 25–50% of the people of developed countries (Gillespie & Hawkey, 2006). H. pylori colonizes the stomach’s mucus layer and induces chronic active gastritis inflammation (Konturek, 2003). It is a major cause of peptic ulcers and a risk factor for gastric malignancies (Lesbros-Pantollickova et al., 2007).

H. pylori can be easily identified in all microbiology laboratories using simple techniques (Guerrant et al., 2011). Numerous serological diagnostic tests used for the detection of H. pylori include bacterial agglutination, complement fixation, indirect immunofluorescence test, enzyme immunoassay, and enzyme-linked immunosorbent assay (Kim, 2016). Since H. pylori and intestinal parasites are prevalent in developing countries, this study aimed to determine the prevalence of intestinal parasites in patients with H. pylori in Khartoum state.

Methods

Study design

This was a case-control study carried out in Khartoum state, Sudan, at Alsaaha Specialized Hospital and Yastabshiren Hospital between 1 June and 27 October 2018. The study was conducted in 100 patients who were H. pylori positive as a case group and 100 individuals without H. pylori as a control group. The H. pylori test had already been performed in hospitals using immuno-chromatographic test (ICT) for identification of H. pylori Ag in stool sample. Participants were divided into groups according to gender and age (see below).

Ethical considerations

Approval for the study was obtained from the Ethical Authorization Committee (number, MLS-IEC-10-17) of the College of Medical Laboratory Sciences, Sudan University of Science and Technology. Written informed consent for participation and disclosure of data was obtained from each participant in the study and in the case of children (<18 years) written informed consent was obtained from their guardians.

Participants

Individuals in this study who were already being screened for presence of H. pylori using ICT for antigen in stool were asked to participate. Individuals with a positive ICT for H. pylori antigen were included in the case group after they agreed to participate in the study, while the control group were those with negative H. pylori antigen, who were also only included in the study after they agreed to participate.

Sampling collection

In total, 200 stool samples were collected from the participants in the study. Samples were collected immediately after participants agreed to partake in the study. Each participant was provided with a labelled stool container (transparent and clean) and was instructed to collect a faecal sample.

Method of stool examination

Every stool sample was examined for the detection of the intestinal parasite by wet preparation, saturated sodium chloride floatation and formal ether concentration. If one detection method was positive, then the sample was counted as positive for intestinal parasites, even if other methods were negative.

Wet preparation. A small portion of stool was mixed with a drop of normal saline with a wooden applicator stick and deposited on a slide. This was covered with a cover slip and routinely examined under a microscope using 10X and a high magnification 40X to detect more parasites, as per the World Health Organization protocol (WHO, 2001).

Formal ether concentration. Approximately 1 g of faeces from various parts of the stool was collected and emulsified in a glass beaker in 5 ml of formal saline. There a further 5 ml of saline was added and mixed. The resulting suspension was strained using a sieve with small pores. The filtered sample was poured into a centrifugal tube, and an equal volume of ether was added. For one minute, the tube was mixed and then centrifuged at 2000 rpm for 5 minutes. The upper three layers were discarded, and the sediment was moved to a slide, covered with a cover slip and analysed under a microscope using magnifications 10X and 40X. This was as per the protocol by Smith & Mank (2011).

Saturated sodium chloride floatation. Approximately 0.5 g of faeces was collected from different parts of the stool and emulsified in a long glass tube half-filled with saturated sodium chloride solution. Then the container was filled with sodium chloride until the top of tube. Carefully, a cover glass was put on the top of the tube avoiding air bubbles. After 30 to 45 minutes, the cover glass was removed from the top of the tube and put on a clean and dry slide and examined under the microscope using 10X and 40X magnifications. This was as per the protocol by Dryden et al. (2005).
Data analysis
Statistical analysis was performed using SPSS version 20.0. The Chi-square method was used to compare variables. P values < 0.05 were considered statistically significant.

Results
The results showed that 23 of the 100 patients with H. pylori were infected with gastrointestinal parasites (23%). Of the 100 control individuals, 10 were found to be infected with gastrointestinal parasites (10%). Between the case and control groups, there was a statistically significant difference in prevalence of intestinal parasites (P = 0.013).

Among H. pylori patients, the occurrence of intestinal parasites in men and women was similar (24% and 22%, respectively; P = 0.841; Table 1). On the other hand, the prevalence of gastrointestinal parasites in men and women in the control group was found to be 9% and 11%, respectively, but this difference was not statistically significant (P = 0.789; Table 1).

In the case group, the highest occurrence rates (40% and 38%) were reported among the 1–15 and 46–60 age groups, while the lowest rate (10.7%) was reported among the 31–45 age group. These differences were not statistically significant (P value= 0.132; Table 2). For the control group, the highest prevalence rate (15%) was reported among the 31–45 age group, while the lowest prevalence rate was among the 16–30 age group (8%). This difference was not statistically significant (P = 0.528; Table 2).

The 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 cases (Table 3). Among the control group E. histolytica was reported at 5%, followed by G. lamblia at 3% and E. coli at 2% (Table 3).

Discussion
From the study, it is evident that the gastrointestinal parasite overall occurrence among H. pylori patients is relatively high (23%). It was found that this rate was higher than the published rate by Uğraş & Miman (2014) in Turkey (7.61%). As far as the control group is concerned, the overall occurrence rate reported was 10%. This rate is lower than the rate among H. pylori patients and higher than the rate reported by Uğraş & Miman (2014). The difference in rates between the control group and patient with H. pylori was significant. This, in our opinion, might mean that there is an association between the establishment of gastrointestinal parasites and H. pylori.

The difference in prevalence rates between men and women in H. pylori patients and control individuals was not statistical difference. This finding did not agree with Yakoob et al. (2005), who considered G. lamblia occurrence in Pakistan. That study found a higher rate in men (72%) than in women (28%).

In our study, the highest occurrences (40% and 38%) were reported among the 1–15 and 46–60 year age groups, respectively, in the H. pylori patients, and the 31–45 year age group (15%) for the control group. Our finding disagreed with the finding of Fadul et al. (2016), who reported the highest occurrence rate (50%) in the age group >66 years old. Our results also showed that E. histolytica was seen in 12% of the H. pylori cases followed by E. coli in 7% of cases and G. lamblia in 4%. Lower rates were reported among the control group where E. histolytica was seen in 5% followed by G. lamblia in 3% and E. coli in 2%. Our result are not in line with the findings of Gökşen et al. (2016) who reported 14.8% for G. lamblia in the H. pylori-positive group, which was in agreement with Escobar-Pardo et al. (2011) who also found a significant association between H. pylori and G. lamblia. However, our conclusion was in total disagreement with the finding of Uğraş & Miman (2014), who reported no significant association

| Table 1. Occurrence of gastrointestinal parasites among H.pylori patients and control individuals according to gender. |
|----------------------------------|----------------|----------------|----------------|----------------|
| Gender             | N examined | N positive (%) | Gender             | N examined | N positive (%) |
|                    | Case      | Control       | Case           | Control       |
| Male               | 46        | 54            | 11 (24)        | 5 (9)         |
| Female             | 54        | 46            | 12 (22)        | 5 (11)        |
| Total              | 100       | 100           | 23 (23)        | 10 (10)       |

Case difference between groups: P = 0.841; Control difference between groups: P = 0.789.

| Table 2. Occurrence of gastrointestinal parasites among H. pylori patients and control individuals according age group. |
|----------------------------------|----------------|----------------|----------------|----------------|
| Age group, years | N examined | N positive (%) | Age group, years | N examined | N positive (%) |
|                    | Case      | Control       | Case           | Control       |
| 1–15               | 10        | 14            | 4 (40)         | 2 (14.3)      |
| 16–30              | 37        | 37            | 7 (19)         | 3 (8)         |
| 31–45              | 28        | 33            | 3 (10.7)       | 5 (15)        |
| 46–60              | 21        | 12            | 8 (38)         | 0 (0)         |
| 61–75              | 4         | 4             | 1 (25)         | 0 (0)         |
| Total              | 100       | 100           | 23 (23)        | 10 (10)       |

Case difference between age groups: P = 0.132; Control difference between age groups: P = 0.528.

| Table 3. Occurrence of different gastrointestinal parasites in H. pylori patients and control individuals. |
|----------------------------------|----------------|----------------|----------------|----------------|
| Parasite                  | N examined | N positive (%) | Parasite                  | N examined | N positive (%) |
| Entamoeba histolytica      | 100        | 12 (12)        | G. lamblia              | 100        | 4 (4)         |
| Giardia lamblia            | 100        | 7 (7)          | Entamoeba coli          | 100        | 2 (2)         |

| Parasite                  | N examined | N positive (%) | Parasite                  | N examined | N positive (%) |
| Entamoeba histolytica      | 100        | 12 (12)        | G. lamblia              | 100        | 4 (4)         |
| Giardia lamblia            | 100        | 7 (7)          | Entamoeba coli          | 100        | 2 (2)         |
between \textit{H. pylori} and intestinal parasites in Turkey. This may be due to differences in the study areas with different food and life style of individuals.

**Conclusions**

Gastrointestinal parasites are more common among \textit{H. pylori} patients compared to individuals without \textit{H. pylori}; but this infection rate was not affected by gender. The highest infection rate was reported in the 1–15 and 46–60 age group among \textit{H. pylori} patients and 31–45 years of age group among the control patients.

**Data availability**

**Underlying data**


This project contains the following underlying data:
- Raw data file.sav
- Data dictionary

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

**References**


Publisher Full Text


Published Abstract | Publisher Full Text


Published Abstract | Publisher Full Text


Publisher Full Text


Open Peer Review

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Version 1

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Yeong Yeh Lee

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The study adds to a body of literature supporting the common presence of *H. pylori* and intestinal parasites co-infection. There are several concerns as below:

1. Best if the study design involved age and gender-matched cohorts. Case-control might be biased especially selection bias.
2. Sample size calculation - how 100 samples each arm is decided?
3. Ideally, two serological tests for *H. pylori* are needed to confirm infection.
4. Only limited parasites were detected. Please provide explanations.
5. Please discuss reasons for co-infection of detected parasites with *H. pylori*, the significance, and discuss the age group differences found between current study and others.

References


Is the work clearly and accurately presented and does it cite the current literature? 
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Helicobacter pylori, gut microbiota, gastrointestinal cancers

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
4. The current study associated with the "developing countries" especially Sudan, while the discussion is focused on studies conducted a specific country "Turkey", which mainly not considered as a developing country. I prefer to compare the present study with other studies in Egypt (10.1007/s12639-018-1075-y), Iran (10.5812/ped Infect.15294) and Ethiopia (10.1186/s13104-018-3246-4). These countries more suitable for that topic.

References

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious diseases, mostly focused on H. pylori and Cryptosporidium

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
The article is a short report which aims to show the higher frequency of selected parasites in \textit{H. pylori}-positive patients. I believe the manuscript is worth indexing, however, I suggest to apply two corrections:

- I think that it would be easier for readers to give p-values in tables as an independent column (this way it will be easy to read which results are statistically different from each other).

- I believe that the discussion should be extended to the immunological part, even though it was not the aim of this article. The results obtained by the Authors indicate a higher frequency of parasites in \textit{H. pylori}-positive patients aged 1-15 and 46-60. These results indicate that an immature/weakened immune system may influence the parasite-\textit{H. pylori} relationship in the host. Thus, I suggest that the Authors should discuss this topic and add relevant literature references.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Microbiology, mostly focused on \textit{H. pylori}

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
Elena Pomari
Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Verona, Italy

Authors describe the occurrence of *H. pylori* and intestinal parasites concomitant infections in individuals from a specific state in Sudan.

**Major revision:**
1. The rationale of the study is focused on the "in developing countries" and the discussion is mostly on studies conducted in Turkey. To the best of my knowledge, the current (or recent) Turkey GDP is not so low. Thus, authors should compare their findings with other available data reported in studies conducted for example in Egypt, Ethiopia ... (doi: 10.12816/0010855 1; 10.1186/s13104-018-3246-4 2, etc).

2. In the discussion, authors conclude suggesting that different food and life style of individuals among different geo regions might determine various occurrence of infections. Did authors have any information about diet, life style of the included subjects? These variables could be added in tables and analysis.

**Minor revision:**
1. Keywords: check typing for "Intestinal parasite" and "Yastabsheron"

**References**

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes
If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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