RESEARCH ARTICLE

REVISED The relationship between Helicobacter pylori infection and intestinal parasites in individuals from Khartoum state, Sudan: a case-control study [version 2; peer review: 2 approved, 2 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

Open Peer Review

Reviewer Status ✔✔??

	Invited Reviewers			
	1	**2**	**3**	**4**
version 2 (revision) 18 Jan 2021	✔ report	✔ report		
	↑	↑		
version 1 12 Dec 2019	? report	✔ report	? report	? report

1. **Elena Pomari** [ID], IRCCS Sacro Cuore Don Calabria Hospital, Verona, Italy

2. **Paweł Krzyżek** [ID], Wroclaw Medical University, Wroclaw, Poland

3. **Asmaa Ibrahim** [ID], University of Sadat city, Sadat, Egypt

4. **Yeong Yeh Lee** [ID], School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Malaysia

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.

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

H.pylori, Intestinal parsasite, Khartoum, Alsaha, Yastabsheron

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REVISED   **Amendments from Version 1**

In this version, we changed the reference from Turkey and substituted with another one from Ethiopia as the reviewer said that Turkey does not belong to a developing country. Another change was spelling mistake in Keyword (intestinal parasite and Yastabsheron). Also we deleted the life style and feeding habits from last paragraph in discussion because in Sudan and Ethiopia, the life style and feeding habits may be the same.

Any further responses from the reviewers can be found at the end of the article

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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; Wongstitwilairoong *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-Pantoflickova *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 Yastabshiron 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 a 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

Table 1. Occurrence of gastrointestinal parasites among *H. pylori* patients and control individuals according to gender.

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 (%)	
	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

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 (%)	
	Case	*Control*	*Case*	*Control*
Entamoeba histolytica	100	100	12 (12)	5 (5)
Giardia lamblia	100	100	4 (4)	3 (3)
Entamoeba coli	100	100	7 (7)	2 (2)

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 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.

Conclusions

Gastrointestinal parasites are more common among *H. pylori* patients compared to individuals without *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 *H. pylori* patients and 31–45 years of age group among the control patients.

Data availability
Underlying data
Figshare: yasir and ahmed.sav, https://doi.org/10.6084/m9.figshare.10315769.v2 (Abd Alla & Yousif, 2019).

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).

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Open Peer Review

Current Peer Review Status: ✓ ✓ ? ?

Version 2

Reviewer Report 19 January 2021

https://doi.org/10.5256/f1000research.47350.r77618

✓ **Paweł Krzyżek** (iD)

Department of Microbiology, Wroclaw Medical University, Wroclaw, Poland

The quality of the article has improved. I still think that in the discussion it would be beneficial to include the part about the influence of the host immune system on the observed results (which was not included), but I leave it to the Authors' choice.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology, mostly focused on 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.

Reviewer Report 19 January 2021

https://doi.org/10.5256/f1000research.47350.r77620

✓ **Elena Pomari** (iD)

Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Verona, Italy

No further comments to make.

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.

Version 1

Reviewer Report 10 August 2020

https://doi.org/10.5256/f1000research.23567.r67155

Yeong Yeh Lee 🆔

Department of Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Malaysia

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.

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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.

Reviewer Report 27 July 2020

https://doi.org/10.5256/f1000research.23567.r67158

? **Asmaa Ibrahim** (iD)

Department of Molecular biology, Genetic Engineering and Biotechnology Research Institute, University of Sadat city, Sadat, Egypt

The article aims to evaluate the prevalence rate of intestinal parasites in *H. pylori* positive patients in a specific state in Sudan.

Major revision:
1. The introduction needs to be more updated (ex. Haque (2007) and Nyantekyi et al., (2010) instead of (Smyth, 1990), both articles associated with helminthic infections in developing countries.

2. In the method of stool examination part, I prefer to make also examination with acid-fast stain (AF), in case of diarrhea or immunodeficiency to detect coccidian parasites.

3. In data analysis part, if there are any data about socio-demographic characteristics such as (residency in rural or urban areas, animal contact, and water source) and clinical symptoms such as (diarrhea, vomiting and fever) were collected, I prefer to analyze its association with

the infection as estimated risk factors (multivariate logistic regression, 95% CI and OR).

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[1]), Iran (10.5812/pedinfect.15294[2]) and Ethiopia (10.1186/s13104-018-3246-4[3]). These countries more suitable for that topic.

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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.

Reviewer Report 21 July 2020

✔

Paweł Krzyżek [iD]

Department of Microbiology, Wroclaw Medical University, Wroclaw, Poland

The article is a short report which aims to show the higher frequency of selected parasites in *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 *H. pylori*-positive patients aged 1-15 and 46-60. These results indicate that an immature/weakened immune system may influence the parasite-*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 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.

Reviewer Report 15 July 2020

?

Elena Pomari iD

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 knowlegde, 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 parsaite" and "Yastabsheron"

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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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