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ORIGINAL ARTICLE

Co-Infection of *Helicobacter Pylori* with *Entamoeba Histolytica* and *Giardia Lamblia* Among Patients with Dyspeptic Symptoms Attending Bugando Medical Center, Tanzania

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ABSTRACT

Background: *Helicobacter pylori (H. pylori)* and i*ntestinal protozoa* are common infections that pose a serious threat to public health because they frequently cause gastrointestinal discomfort. There is a lack of information regarding the co-infection in Tanzanian tertiary hospitals, despite the high global prevalence of these infections. Due to the fact that both *H. pylori* and intestinal protozoa share similar transmission routes and are influenced by socioeconomic factors, such as poor sanitation and inadequate hygiene practices, co-infection is most likely. The aim of this study was to determine the prevalence of co-infection between *Helicobacter pylori* and intestinal protozoa (*Entamoeba histolytica* and *Giardia lamblia*) and assess factors associated with *H. pylori* infection among dyspeptic patients.

Methodology: A cross-sectional study was conducted with 277 participants who met the inclusion criteria between April and July 2023 at BMC Mwanza. Stool samples from patients with dyspeptic symptoms were screened for *H. pylori* using stool antigen test and examined for intestinal protozoa using formalin-ether concentration and wet preparation methods. Data were analyzed using STATA version 13.0. Variables with $P < .2$ in univariate analysis were included in multivariate logistic regression analysis. Statistical significance was considered at $P < .05$

Results: The prevalence of *H. pylori* was found to be 50.5%, while intestinal protozoa had a prevalence of 9.3%. Female participants constituted 58.8% and the mean age was 37.9 ± 21.6 years. Co-infection with both pathogens was observed in 5.1% of the participants. Water treatment, hand washing practices and consuming uncooked diet were significantly associated with *H. pylori* infection

Conclusions: The study reveals a high prevalence of *H. pylori* infection and a notable co-infection rate with intestinal protozoa (*E. histolytica* and *G. lamblia*). Factors associated with H. pylori infection included poor hand hygiene, consumption of untreated water, and consumption of uncooked food. Due to the notable presence of co-infection between *H. pylori* and intestinal protozoa. we suggest improvement of preventive measures and further researches on this topic.

BACKGROUND

Dyspepsia, is a prevalent upper gastrointestinal symptom affecting about 25% of people worldwide.[1] The etiology of dyspepsia is multifactorial, involving both infectious and non-infectious causes. *Helicobacter pylori* (*H. pylori*), a gram-negative bacterium, is a well-established cause of dyspepsia and peptic ulcer disease. [2] *Giardia lamblia* (*G. lamblia*) and *Entamoeba histolytica* (*E. histolytica*) are protozoan parasites that also contribute to gastrointestinal symptoms.[3]

H. pylori are highly prevalent worldwide, with infection rates exceeding 50% in developing countries.[4,22] *G. lamblia* affects approximately 200 million people globally, with an annual incidence of 500,000 new cases.[5] *Entamoeba histolyitica* is one of the major causes of diarrhea illness especially in children. Globally, approximately 500 million people are infected with *Entamoeba histolytica* annually and it causes around 75000 deaths annually.[6]

Several studies reported the co-infection between *H. pylori* and intestinal protozoa especially in resource limited areas due to the similarities in transmission routes and are influenced by socioeconomic factors, such as poor sanitation and inadequate hygiene practices.[7] Apart from H. pylori infection, intestinal protozoa have showed to trigger several symptoms gastrointestinal diseases such as dyspepsia has been reported to be the common symptom of giardiasis. [8]

G. lamblia and *H. pylori* are common cause of dyspepsia symptom, studies have reported varying rates of *H. pylori* and *G. lamblia* co-infection.[9] The study which was done in Ethiopia assessing co-infection of H. pylori and intestinal parasites showed co-infection with G. lamblia was 26.3% and co-infection with E. histolytica was 13.7%. Co-infections of *H. pylori* with intestinal protozoa like *G. lamblia* and *E. histolytica* is very common in children nearly Co-infection between H. pylori and intestinal protozoa has been reported among children with gastrointestinal symptoms.[10]

Chronic *H. pylori* infection is a significant risk factor for gastric adenocarcinoma, with varying seroprevalence and cancer incidence rates worldwide.[11] In Japan, seroprevalence reaches 40%-70%, correlating with higher gastric cancer rates compared to the U.S., where seroprevalence is 10%-20%.[12] Apart of testing *H. pylori* alone there is a need of assessing intestinal protozoa in patients presenting with dyspeptic symptoms so as to improve patient outcome because of the remarkable presence of co-infection. Therefore, the aim of this study was to assess co-infection rate between *H. pylori* and intestinal protozoa (*E. histolytica* and *G. lamblia*) and the associated factors.

METHODOLOGY

Study Design

This was a hospital-based cross-sectional study conducted at Bugando Medical Centre (BMC) in Mwanza, Tanzania between April and July 2023.

Study Area

Bugando Medical Centre (BMC) is a tertiary referral and teaching hospital located in Mwanza region in northwestern Tanzania. The hospital serves approximately 16 million people from the Lake Zone regions and provides specialized medical and diagnostic services including gastroenterology services. The Hospital offers super specialized medical services and interventions.

Study Population, Inclusion and Exclusion Criteria

The study included adult patients aged 18 years and above presenting with dyspeptic symptoms at the Internal Medicine Outpatient Clinic at BMC. Dyspepsia was defined by ROME IV criteria. All the patients who used antibiotics and anti-parasite one week prior the study involvement were excluded. Adults aged 18 years and above presenting with dyspeptic symptoms who consented to participate in the study. Patients who had used antibiotics, antiparasitic drugs, proton pump inhibitors, or bismuth compounds within one week prior to recruitment were excluded because these medications may affect stool antigen test results.

Sample Size and Sampling Technique

The Kish Leslie formula was used for sample size. Consecutive enrollment was used as a sampling technique until the required sample size was reached a total of 277.

Data Collection and Analysis

A structured questionnaire designed specific for this study was used in data collection.

The questionnaire included section of age, sex, area of residence, occupational, education level, hand washing. Each participant who met the inclusion criteria was given a pre-labeled clean and dry plastic container to collect fresh stool specimen for checking *H. pylori* infection and intestinal parasites.

Specimen Analysis

All collected stool sample was screened for intestinal protozoa by direct wet mount using 0.85% sodium chloride solution as recommended by standard guideline. Both cysts and trophozoites were examined using direct wet mount and formalin-ether concentration techniques from fresh stool samples, *H. pylori* stool antigen test was used in testing for *H. pylori* infection

Data Analysis

Data analysis was done using STATA version 13.0. Categorical variables were presented by percentage while mean and median was used for continuous variables. Variables with $P < .2$ in univariate analysis were included in multivariate logistic regression analysis.

Ethics Approval

Ethical clearance for conducting this study was sought from CUHAS/BMC research ethics and review committee (CREC/1043/2021). Confidentiality and privacy of the study participants was maintained. Informed consent to participate in this study was obtained from all the participants. This study adhered to the declaration of Helsink.

RESULTS

Baseline Characteristics

A total of 277 participants were included in this study, 58.8% were females, and the mean age was 37.9 years. The majority had higher education 37.6%, and 40.4% were unemployed. Majority 63.5% were using tap water for consumption. Table 1

Prevalence of H. Pylori Infection and Intestinal Protozoa Co-infection

The prevalence of *H. pylori* infection was 50.5%, while intestinal protozoa had a prevalence of 9.3%, with *E. histolytica* being the most common at 7.9%. Co-infection of both *H. pylori* and intestinal protozoa was observed in 5.1% of participants detailed in Table 2.

Factors Associated with H. Pylori Infection

Significant associations were identified between *H. pylori* infection and the following factors; inadequate washing hand behavior was associated with higher rates of *H. pylori* infection (P=.003), consuming uncooked (P=.048), using untreated drinking water (P=.031), presence of blood in stool (P= .001). Factors associated with H. pylori infection are detailed in Table 3.

TABLE 1: Baseline Socio-demographic and Clinical Characteristics of Study Participants N=277

Variables	Frequency (%)
Age of Respondents	
18-19	73 (26.4)
20-39	69 (24.9)
40-59	85(30.7)
60 +	50 (18.0)
Mean (SD)	37.9 (21.6)
Sex of Respondents	
Female	163 (58.8)
Male	114 (41.2)
Occupation of Respondents	
Agriculture	23 (8.3)
Business	79 (28.5)
Employed	57 (20.6)
Fishing	6 (2.2)
None	116 (40.4)
Education Level of Respondent	
No Formal Education	33 (11.9)
Primary Education	48 (17.3)
Secondary Education	92 (33.2)
College And Above	104 (37.6)
How Often Do You Wash Hands	
Yes	57 (20.6)
No	220(79.4)
How Often Do You Wash Fruits and Vegetables	
Yes	55 (19.9)
No	222 (80.1)
Have You Ever Consumed Uncooked Food	
Yes	51 (18.4)
No	226 (81.6)
Source of Drinking Water	
Tap	176 (63.5)
Well Or Lake	101 (36.5)
Do You Treat or Boil Drinking Water	
No	133 (48.0)
Yes	144 (52.0)
Type of Latrine	
Flash Toilet	234 (84.5)
Pit Toilet	43 (15.5)
Symptoms Experienced in Past One Month	
Bloating	39 (14.1)
Epigastric Pain	94 (33.9)
Gastrointestinal Disturbance	144 (52.0)
Ever Noticed Blood in Stool	
No	230 (83.0)
Yes	47 (17.0)
Helicobacter Pylori	
Negative	137 (49.5)
Positive	140 (50.5)
Type of Protozoa	
Entamoeba Histolytica Cysts	22 (7.9)
Giardia Lambria Cysts	4 (1.5)
None	251 (90.6)

TABLE 2: Prevalence of H. pylori Infection and Intestinal Protozoa Co-infection

Protozoa Observed	Negative	Positive
E. histolytica	9 (40.9)	13(59.1)
Giardia lamblia Cysts	3 (75.0)	1(25.0)
None	125 (49.8)	126(50.2)

*Among participants with *Entamoeba histolytica*, 59.1% were co-infected with *H. pylori*.

TABLE 3: Factors Associated with *H. pylori* Infection

Variables	COR [95% CI]		*P Value*	AOR [95% CI]		*P Value*
Age of Respondents						
1-19	1					
20-39	0.57 (0.293	1.107)				
40-59	0.75 (0.398	1.407)				
60 +	0.55 (0.265	1.135)				
Age As Continuous Variable	0.99 (0.982	1.003)				
Sex of Respondents						
Female	1					
Male	1.15 (0.714	1.861)	.561			
Occupation of Respondents						
Agriculture	1					
Business	1.20 (0.473	3.069)	.696			
Employed	1.35 (0.508	3.567)	.55			
Fishing	2.6 (0.394	17.159)	.321			
None	1.15 (0.586	3.574)	.423			
Education Level of Respondent						
No Formal Education	1					
Primary Education	0.80 (.328	1.956)	.626			
Secondary Education	0.68 (0.303	1.507)	.338			
College And Above	0.74 (0.334	1.624)	.338			
How Often Do You Wash Hands						
Yes	1					
No	2.02 (1.109	3.680)	.022	2.44 (1.674	5.051)	.003
How Often Do You Wash Fruits and Vegetables						
Yes	1					
No	0.98 (0.544	1.772)	.951			
Have You Ever Consumed Uncooked Food						
Yes	1	1				
No	1.84 (1.685	3.437)	.05	1.95 (1.005	3.787)	.048
Source of Drinking Water						
Tap	1					
Well Or Lake	1.36 (0.834	2.228)	.217			
Do You Treat or Boil Drinking Water						
Yes	1					
No	1.67 (1.036	2.681)	.035	0.58(0.348	0.951)	.031
Type of Latrine						
Flash Toilet	1					
Pit Toilet	0.67 (0.342	1.276)	.217			
Symptoms Experienced in Past One Month						
Bloating	1			1		
Epigastric Pain	0.92 (0.428	1.980)	.833	0.92(0.417	2.022)	.832
Gastrointestinal Disturbance	0.45 (0.216	0.922)	.029	0.41 (0.193	0.872)	.02
Ever Noticed Blood in Stool						
No	1					
Yes	3.06 (1.535	6.098)	.001	3.44 (1.674	7.051)	.001

DISCUSSION

This study assessed the prevalence of co-infection between *H. pylori* and intestinal protozoa and factors associated with *H. pylori* infection among dyspeptic patients at Bugando Medical Centre. The prevalence of *H. pylori* was 50.5%, intestinal protozoa 9.3%, and co-infection 5.1%.

Prevalence of *H. pylori*, Intestinal Protozoa, and Co-infection

This study investigated the prevalence and co-infection of *H. pylori* and intestinal protozoa (*E. histolytica* and *G. lamblia*) among patients presenting with dyspeptic symptoms at Bugando Medical Centre. The prevalence of *H. pylori* infection in this study was 50.5%, which is consistent with global estimates showing infection rates exceeding 50% in developing countries.[13] This finding underscores the significant role of *H. pylori* as a major etiological agent of dyspepsia and peptic ulcer disease, particularly in low- and middle-income settings characterized by inadequate sanitation and overcrowding. [4,13]

The prevalence of intestinal protozoa in this study was 9.3%, with *E. histolytica* (7.9%) being the most predominant. This result is comparable to studies conducted in Ghana and Ethiopia, which reported similar prevalence of intestinal protozoan infections among patients with gastrointestinal symptoms. [14,15] The relatively lower detection rate of *G. lamblia* (1.5%) in our study may be attributed to regional differences, seasonal variations, and differences in diagnostic methods used, such as stool microscopy versus antigen detection techniques. [15,16] The low prevalence of *G. lamblia* observed may have been influenced by the limited sensitivity of direct wet mount microscopy compared to antigen-based assays or molecular methods.

The co-infection rate between *H. pylori* and intestinal protozoa was found to be 5.1%. Although this is lower than previously reported rates ranging between 13% and 44% in other African and Asian studies, [17,18] it still reflects a meaningful coexistence of these pathogens in patients with dyspeptic symptoms. The variation may result from differences in study population, sanitation practices, and laboratory diagnostic sensitivity.[19,10] Co-infection suggests that both pathogens may share similar transmission routes mainly fecal oral and may potentiate each other's pathogenic effects on the gastrointestinal tract.[7]

Factors Associated with Infection and Public Health Implications

Our study revealed that untreated or unboiled drinking water was significantly associated with *H. pylori* infection. This finding aligns with evidence from previous studies linking contaminated water sources to the fecal–oral transmission of *H. pylori*.[20] Waterborne transmission remains a key risk factor in low-resource settings, emphasizing the urgent need to promote safe water practices in Mwanza and similar regions. Consumption of treated or boiled water appeared protective against *H. pylori* infection.

Poor hand hygiene was also independently associated with *H. pylori* infection. Similar observations were made in previous research where lack of handwashing after defecation or before eating increased the risk of gastrointestinal infections.[21] Hand hygiene interventions have proven to reduce diarrheal diseases by up to 40%, which suggests that improving domestic hygiene could significantly reduce *H. pylori* and protozoal transmission.[21] Additionally, participants who consumed uncooked food had a higher prevalence of infection, possibly due to ingestion of contaminated or poorly handled food, reinforcing the need for proper food hygiene practices.

The association between *H. pylori* infection and the presence of blood in stool observed in this study may indicate underlying mucosal damage or peptic ulceration, consistent with previous literature describing *H. pylori* as a leading cause of gastrointestinal bleeding.[17] Collectively, these findings highlight the necessity of integrated public health approaches that combine health education, improved sanitation, and routine screening for both *H. pylori* and intestinal protozoa in dyspeptic patients.

Furthermore, the use of stool antigen testing for *H. pylori* and microscopy for protozoa, although cost-effective, may underestimate infection prevalence due to limitations in sensitivity. Future studies should employ molecular or antigen-based diagnostic methods for improved accuracy.[16,19]

Study Limitation

This study was limited by its cross-sectional design, which restricts causal inference between identified risk factors and infection. The relatively small sample size of 277 patients may affect the generalizability of the findings. Additionally, reliance on stool microscopy for protozoa detection and stool antigen testing for *H. pylori* may have underestimated the true prevalence due to diagnostic limitations. The study was also conducted at a single tertiary hospital, which may not fully represent the community-level infection burden across Tanzania. Recall bias may have occurred because some information depended on participant self-reporting. Some participants attending this tertiary hospital may have received prior treatment before presentation. Use of stool microscopy may have underestimated protozoal prevalence because of lower sensitivity compared to molecular techniques.

Recommendations

Based on these findings, we recommend strengthening public health education on personal hygiene, safe water treatment, and proper food handling to minimize *H. pylori* and protozoal transmission. Routine screening for co-infection should be considered in patients presenting with dyspeptic symptoms, especially in resource-limited settings. Health authorities should ensure access to clean water and promote handwashing campaigns at the community level. Further longitudinal and multi-centre studies using molecular diagnostic methods are needed to better understand the epidemiology and clinical impact of *H. pylori*–protozoa co-infection.

CONCLUSION

The study reveals a high prevalence of *H. pylori* and a notable rate of co-infection with intestinal protozoa among patients with dyspeptic symptoms at Bugando Medical Center. The associations with untreated water and poor hand hygiene underscore the need for improved public health measures and diagnostic strategies. Further research is warranted to explore the implications of co-infection and to develop targeted interventions to address these common gastrointestinal diseases.

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