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ORIGINAL RESEARCH

Association Between *Helicobacter pylori* Infection and Anemia Among Adult Dyspeptic Patients Attending Kiryandongo General Hospital, Uganda

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Purpose: To determine the prevalence of anemia and its association with *Helicobacter pylori* infection among adult dyspeptic patients.

Patients and Methods: A cross-sectional study was conducted among 283 dyspeptic patients at Kiryandongo General Hospital, in Uganda. A structured questionnaire was administered to capture demographic and clinical characteristics of study participants. Four milliliters of blood were then collected into an EDTA vacutainer for Complete Blood Count (CBC) and analyzed using HUMA COUNT 30^{TS}, and peripheral blood smears were made and stained using Giemsa stain. Anemia was defined as hemoglobin levels <12g/dl in females and <13g/dl in men according to the World Health Organization (WHO). *Helicobacter pylori (H. pylori)* stool antigen test was performed using Whole power *H. pylori* Ag rapid test device, and saline stool preparation was examined for intestinal parasites. Chi-squared test and Logistic regression were performed to determine association, and a p-value of ≤0.05 was considered statistically significant.

Results: The overall prevalence of *Helicobacter pylori* infection was 42.4% (120/283). The prevalence of anemia among *H. pylori*-infected patients was 25.8% (31/120) and 15.3% (25/163) among *H. pylori*-negative counterparts. *H. pylori* infection was significantly associated with anemia (p-value 0.042), age (p-value 0.02, 0.009), water sources (p-value 0.0049,) and intestinal parasitic infestation (p-value 0.02), respectively.

Conclusion: This study has shown that the prevalence of *H. pylori* infection and anemia is high among dyspeptic patients at Kiryandongo General Hospital. *H. pylori* infection was found associated with anemia, age, water sources, and intestinal parasitic infestation. Routine screening of anemia in *H. pylori*-infected individuals and further studies to explore the relationship between anemia and *H. pylori* disease is highly recommended.

Keywords: anemia, prevalence, *Helicobacter pylori* infection

Introduction

Helicobacter pylori (*H. pylori*) infection is a global public health problem affecting both developed and developing countries[1,2] with a higher burden of 50.8% reported in developing countries compared to 34.7% in developed countries.[3] *Helicobacter pylori* is a helix-shaped, curved rod and gram-negative bacteria. It causes gastritis, peptic ulcer disease, ggastroduodenal ulcer, atrophic gastritis, gastric cancers, and dyspeptic symptom.[4,5] However, more than 80% of persons who become infected with *H. pylori* are usually asymptomatic. *H. pylori* plays a vital role in the natural stomach ecology.[6]

H. pylori infection affects about 4.4 billion people worldwide.[1] Africa has the highest prevalence of the infection, 70.1%, and the prevalence ranged from 18.9% in Switzerland to 87.7% in Nigeria.[1] In Southern Asia, Pakistan and India

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showed the highest prevalence and in Western Asia, Turkey reported the leading prevalence, 77.2%.[6] In Uganda, the prevalence of *H. pylori* among dyspeptic patients was 74%.[7]

H. pylori infection has been implicated in hematological manifestations such as anemia and micronutrient deficiency (iron and vitamin B12).[8] It has also been related to extra-gastric manifestations, eg thrombocytopenic purpura, reduction in growth velocity, iron deficiency, and/or anemia.[9,10] Several studies described that by eliminating *H. pylori* bacteria, the iron nutritional status becomes normal without the necessity for iron supplementation.[11–14]

The mechanism or mechanisms through which *H. pylori* infection may cause iron deficiency and/or anemia are not fully understood but probable mechanisms comprise an upsurge in intragastric pH; reduced concentration of ascorbic acid in gastric juices, which disturbs iron absorption from the diet; chronic bleeding caused by the increase of micro-erosions in gastric mucous; production of lactoferrins by neutrophils; and capture of iron by the bacteria.[15] Another way could be the rise in the synthesis of hepcidin, an essential regulator of iron metabolism that inhibits iron absorption in the small intestine.[16]

Therefore, we aimed to determine the prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients.

Materials and Methods

Study Area

The study was carried out at Kiryandongo General Hospital which is located in Kiryandongo District. The hospital is approximately 225 km from Kampala along the Kampala-Gulu highway. It is the biggest public hospital along the Kampala-Gulu highway serving Kiryandongo and parts of Masindi, Nakasongola, Apac, Amuru, and Oyam districts with an estimated catchment population of 47,155 people. Kiryandongo General Hospital laboratory is accredited by the South African National Accreditation System (SANAS) with the capacity to diagnose *H. pylori* infection and anemia.

Study Design and Period

This was a prospective cross-sectional study conducted from November 2021 to February 2022.

Sample Size Calculation

This was done according to a similar study carried out in Southwest Ethiopia where the prevalence of anemia was 24.3%[17] using the Kish and Leslie formula.

$n = Z^2 P(1-P)/d^2$ (1)

Where.

n = the desired sample size.

Z = critical values of normal distribution at 95%, which corresponds to 1.96

P = the proportion of the target population estimated to have *H. pylori* infection and anemia 24.3%

Hence n = 283 study participants.

Sampling Technique

A random sampling procedure was done where every adult dyspeptic patient identified was considered for the study. Study participants who met the inclusion criteria were allowed to pick numbers from the box and those that picked odd numbers were enrolled in the study.

Selection Criteria

All adult dyspeptic patients aged 18 years and above attending Kiryandongo General Hospital who consented to participate in the study were included. Patients who had undergone gastrectomy and iron supplements were excluded from the study. Adults who had a history of chronic disease or severely ill patients were also excluded from the study.

Data Collection

Demographic data (age, sex, occupation, household income, marital status, level of education) and risk factors associated with anemia, dietary supplements, history of alcohol consumption, deworming status and drinking boiled or treated water were collected from the participants using a questionnaire.

Dyspepsia was defined as upper abdominal discomfort, often chronic or persistent indigestion, and symptoms include fullness, bloating, nausea, loss of appetite or upper abdominal pain. All study participants with the above symptoms were enrolled in the study.

Four mL (4mL) of the venous blood sample was then collected from each dyspeptic patient into ethylenediamine tetraacetic acid (EDTA) vacutainers. Complete blood count (CBC) was determined using an automated hematology analyzer (HUMA COUNT 30^{TS}, Germany). Thin blood films were made on all blood samples with anemia (Hb concentration <12g/dl for females and <13g/dl for males). Thin and thick blood films were made, thin films were fixed with absolute methanol, and both were stained using Giemsa stain for 20 minutes. Thick films were examined at ×1000 magnification for malaria parasites, and thin films were examined for morphological classification of anemia. Sterile stool containers were given to the participants with an explanation on how to collect approximately 1 gram of stool specimen and tested for the presence of *H. pylori* by stool antigen rapid test strips (Whole Power *H. pylori* antigen rapid test device, Zhejiang Orient Gene Biotech Co, LTD, China) with the sensitivity and specificity of >95 and 95.7%, respectively. Saline stool preparations were also done and examined for the presence of intestinal parasites.

Data Analysis

Data collected were entered into an Excel spreadsheet, cleaned and checked fo,r completeness and then exported to STATA software version 14 for analysis. Demographic data were analyzed and presented in the form of percentages and frequencies. The association between *H. pylori* infection and anemia was studied using Pearson's chi-squared test. Association between *H. pylori* infection and risk factors was studied using logistic regression. Odds ratio >1 and p-value ≤0.05 were considered statistically significant.

Ethical Consideration

This study was approved by the Faculty Research Committee (FRC) of Mbarara University of Science and Technology with approval number (MUST/MLS/30). Ethical clearance was also got from the management of Kiryandongo General Hospital before conducting the study. Informed consent was obtained from all study participants before their involvement in the study and was confirmed by their signature or thumbprint on the consent form. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (1964). Confidentiality of the study participants was observed by giving each participant a study code that was not traceable to them. Participants were also informed that their participation is free and voluntary and that they had a right to withdraw from the study at any time and that their withdrawal would not affect their access to medical care.

Results

Socio-Demographic Characteristics of Study Participants

We recruited 283 dyspeptic patients, and 193 (68.2%) were females. The mean age of the study participants was 31.9 years and a standard deviation of 12.7 years. The majority of them were aged 18–22 years (26.8%), had primary education (53.7%), were living in the rural area (73.5%), were not smoking (97.2%), were not taking alcohol (88%) and were drinking piped water (52.3%) as indicated in Table 1.

Prevalence of *H. pylori* Infection Among Dyspeptic Patients

The overall prevalence of *H. pylori* infection was 42.4% (120/283) (Figure 1).

Table 1 Baseline Characteristics of the Study Participants

Variable	Category	Frequency (n)	Percentage (%)
Gender			
	Male	90	31.8
	Female	193	68.2
Age (years)			
	18–22	76	26.8
	23–27	51	18.0
	28–32	52	18.4
	33–37	32	11.3
	38–42	22	7.8
	≥42	50	17.7
Education level			
	None	30	10.6
	Primary	152	53.7
	Secondary	87	30.7
	Tertiary	14	5.0
Residence			
	Rural	208	73.5
	Urban	75	26.5
Smoking			
	No	275	97.2
	Yes	8	2.8
Alcohol intake			
	No	249	88.0
	Yes	34	12.0
Water source			
	Piped	148	52.3
	Spring	107	37.8
	Open water	28	9.9

Prevalence of Anemia Among Dyspeptic Patients and Its Association with *H. pylori* Infection

The prevalence of anemia among dyspeptic patients was 19.8% (56/283). The mean hemoglobin was 13.9 g/dL with a Standard Deviation (SD) of 2.7 g/dL. Mean hemoglobin among males and females was 14.9 g/dL and 13.5 g/dL with SD of 2.7 g/dL and 2.6 g/dL, respectively. However, the prevalence of anemia among *H. pylori*-infected individuals was higher at 25.8% (31/120) compared to *H. pylori*-negative patient counterparts at 15.3% (25/163). The difference in the prevalence of anemia among *H. pylori* patients and uninfected patients was statistically significant with a Pearson chi-squared value of 4.8 and a p-value of 0.029 (Table 2).

Morphological Classification of Anemia Among *H. pylori*-Infected Dyspeptic Patients

The majority of the *H. pylori*-infected patients who were anemic had microcytic hypochromic anemia 19 (61.3%), followed by normocytic normochromic anemia 5 (16.1%), macrocytic normochromic anemia 4 (12.9%) and macrocytic hypochromic anemia 3 (9.7%) as shown in Figure 2.

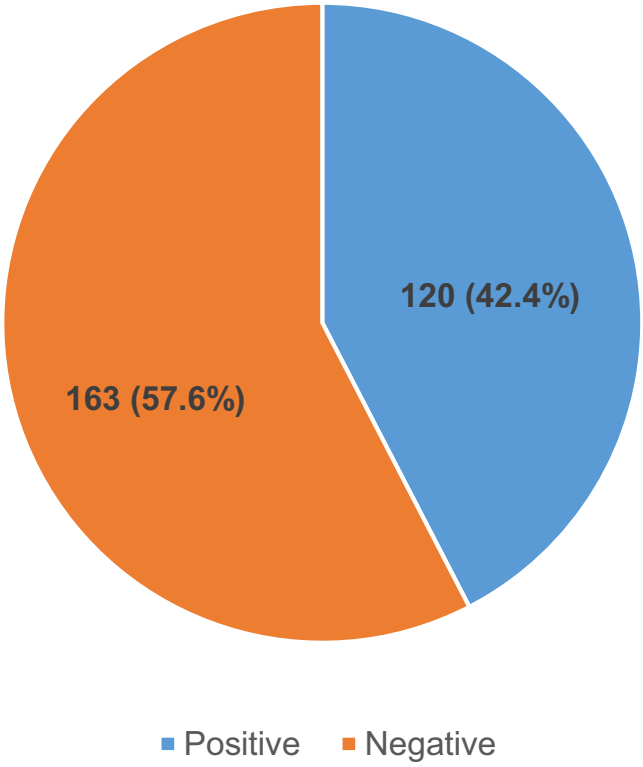

Figure 1 Pie chart showing prevalence of *H. pylori* infection among adult dyspeptic patients at Kiryandongo General Hospital.

Factors Associated with *H. pylori* Infection Among Dyspeptic Patients

Bivariate Analysis of Factors Associated with *H. pylori* Infection

On bivariate analysis, age (p = 0.08 and 0.009), and stool Parasitemia (p = 0.027) were associated with *H. pylori* infection (Table 3).

Multivariate Analysis of Factors Associated with *H. pylori* Infection

On multivariate analysis, age (p = 0.020 and 0.009), water source (p = 0.049) and stool Parasitemia (p = 0.020) were found associated with *H. pylori* infection as shown in Table 4.

Discussion

Prevalence of Anemia and *H. pylori* Infection Among Dyspeptic Patients

In our study, the overall prevalence of anemia was 19.8% and *H. pylori* infection among dyspeptic patients was 42.4%. However, the prevalence of anemia among *H. pylori*-infected individuals was higher at 11% compared to *H. pylori*-negative patient counterparts at 8.8%. This prevalence is higher than the reported prevalence of *H. pylori* infection in Bwera Hospital in Kasese, Uganda, by Tsongo et al at 29.9%,[18] in Kampala 35.7%,[19] 27.3% in eastern Uganda[20] and 29.2% in Butembe Health Centre III Kyankwanzi district, Uganda.

Table 2 A 2 × 2 Dyspeptic Patients with *H. pylori* Infection in Association with Anemia

	***H. pylori*-Negative**	***H. pylori*-Positive**	**Total**	**X^2 (p-value)**
Non-anemic	138	89	227	
Anemic	25	31	56	4.8 (0.029)
Total	163	120	283	

Note: Chi-square 4.8 and p-value 0.029.

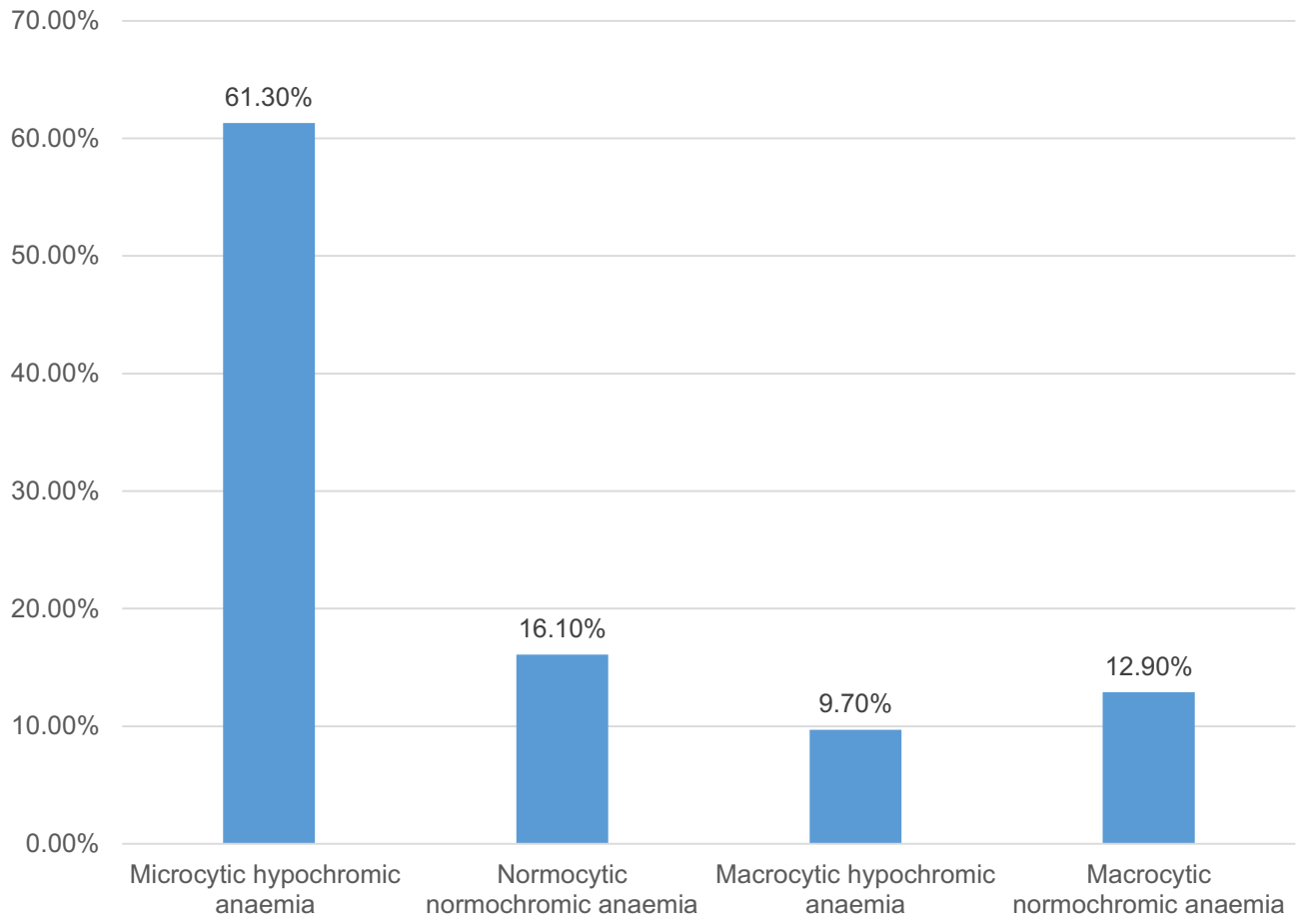

Figure 2 *H. pylori* infected patients with different morphological types of anemia.

Our study finding is not comparable with a study finding from China that found that the burden of *H. pylori* infection was 35.3%[21] but consistent with a study by Baingana et al in Uganda 45.2%[7] in Nairobi 46.2%[22] 43.9% reported in China[23] and 41% observed in the United Arab Emirates.[6] Our study finding is lower than the reported prevalence of *H. pylori* infection among dyspeptic patients from Ethiopia 52.4%,[24] Nigeria 67.4%,[25] and Ibadan 63.5%.[26] The observed variation in the burden of *H. pylori* infection is probably attributed to the lack of clear cut-off for dyspepsia, different diagnostic methods used, sample size, and socioeconomic factors.

Association of Anemia and *H. pylori* Infection

Anemia was significantly associated with *H. pylori* with a Chi-squared value of 4.8 and a p-value of 0.029. It also showed at a multivariate level that those who were infected with *H. pylori* were 1.92 times more likely to be anemic than the *H. pylori*-negative group. This finding is in line with findings from Ethiopia,[24] and China[23] that observed an association of *H. pylori* infection with anemia. Another study from China showed that *H. pylori* patients had a 2.53 times increased risk of being anemic compared to the *H. pylori*-negative group.[21] Our finding is however not in line with other scholars' findings from Latin America[27] and the rural Haitian population[28] that reported no association between *H. pylori* and anemia. This could be due to the sample size studied, geographical variation of the disease, and methods used in the diagnosis of *H. pylori* and anemia.

This study observed that *H. pylori* infection was significantly associated with middle age (33–42 years old) and intestinal parasites. This finding is consistent with the findings in studies in Ethiopia[24,29] Kuwait,[30] South Africa,[31] Brazil and in the United Arab Emirates where *H. Pylori* infection was observed to Increase with age[32] but it differs with the

Table 3 Bivariate Analysis of Factors Associated with *H. pylori* Infection

Variables		Odds ratio	95% Confidence Interval	P value
Gender				
	Male	Ref		
	Female	0.87	0.49–1.54	0.634
Age (years)				
	18–22	Ref		
	23–27	1.44	0.66–3.15	0.366
	28–32	1.96	0.89–4.30	0.094
	33–37	3.48	1.38–8.76	0.008*
	38–42	4.18	1.42–12.26	0.009*
	>42	1.02	0.41–2.53	0.961
Education level				
	None	Ref		
	Primary	0.40	0.16–1.04	0.060
	Secondary	0.50	0.18–1.41	0.188
	Tertiary	0.34	0.07–1.57	0.167
Boil drinking water				
	Yes	Ref		
	No	0.75	0.43–1.34	0.334
Washing hands before eating				
	Yes	Ref		
	No	3.93	0.87–17.72	0.075
Water sources				
	Piped	Ref		
	Spring	0.61	0.34–1.09	0.097
	Open well	0.50	0.19–1.27	0.144
Washing facility at the toilet				
	Yes	Ref		
	No	1.33	0.39–4.56	0.641
Residence				
	Rural	Ref		
	Urban	1.52	0.83–2.80	0.169
Smoking				
	No	Ref		
	Yes	0.86	0.17–4.25	0.853
Alcohol				
	No	Ref		
	Yes	0.93	0.40–2.17	0.871
Anemia				
	Non-Anemic	Ref		
	Anemic	1.53	0.78–3.00	0.212
Stool Parasitemia				
	No	Ref		
	Yes	11.30	1.31–97.46	0.027*

Note: *Significant factor associated with *H. pylori*.

study by Ahmed et al, in Khartoum[33] that reported *H. pylori* infection and its association in individuals >60 years old and a study in eastern Uganda where age was not significantly associated with *H. pylori* infection.[20]

A case–control study in Sudan reported a relationship between intestinal parasites and *H. pylori* infection.[34] Another study in Venezuela also showed evidence of an association between *H. pylori* infection and intestinal parasitic infections,[35] and similar finding had been reported in Ethiopia.[36] A study by Zylberberg et al in the US reported that

Table 4 Multivariate Analysis of Factors Associated with *H. pylori* Infection

Variable		Adjusted Odds Ratio	95% Confidence Interval	P value
Age (years)				
	18–22	Ref		
	23–27	1.37	0.64–2.95	0.416
	28–32	1.91	0.88–4.15	0.100
	33–37	2.85	1.18–6.90	0.020*
	38–42	4.06	1.42–11.63	0.009*
	>42	0.85	0.36–2.05	0.725
Education level				
	None	Ref		
	Primary	0.42	0.17–1.05	0.064
	Secondary	0.54	0.20–1.48	0.231
	Tertiary	0.42	0.10–1.82	0.247
Water sources				
	Piped	Ref		
	Spring	0.57	0.33–1.00	0.049*
	Open well	0.55	0.22–1.39	0.207
Washing hands before eating				
	Yes	Ref		
	No	3.60	0.82–15.72	0.090
Stool Parasitemia				
	No	Ref		
	Yes	12.34	1.48–103.11	0.020*

Note: *Significant factor associated with *H. pylori*.

H. pylori infected study participants are independently associated with giardiasis,[37] and similar finding was reported in Germany.[38]

This current study observed association between *H. pylori* infection and different water sources. The risk of acquiring *H. pylori* infection seems to be multifactorial and potentially contaminated environmental sources, such as local drinking water, swimming in rivers, or the ingestion of fecally contaminated vegetables have been reported as risk factors for *H. pylori* infection.[39,40] A study by Khoder et al similarly demonstrated the association between *H. pylori* infection and source of drinking water.[6] Baingana et al in Uganda demonstrated that *H. pylori* infection was independently associated with using water from public wells, boreholes or springs and from rivers, lakes or streams.[7] However, the association found in this study does not imply causality but it predicts the risk of the occurrence of the disease among the population with the associated variables.

Morphological Types of Anemia Among *H. pylori* Patients

The major morphological form of anemia in *H. pylori*-infected patients was microcytic hypochromic anemia followed by normocytic normochromic anemia, macrocytic normochromic anemia, and macrocytic hypochromic anemia. This highly suggests that *H. pylori* infection causes iron deficiency by reducing iron absorption from the intestine and causes majorly microcytic hypochromic red blood cells with low hemoglobin concentration due to iron deficiency.[24] This is not in line with findings from Ethiopia.[17] They found that the major morphological type of anemia among *H. pylori*-infected patients was normocytic normochromic anemia. Another study found that the major morphological class of anemia among *H. pylori* patients was macrocytic normochromic anemia.[23]

Limitations

The cross-sectional design coupled with lack of control population for comparison limited the opportunity to establish the association of *H. pylori* infection and anemia.

Conclusion

The prevalence of *H. pylori* infection reported among dyspeptic patients in the Kiryandongo district in this study was high. *H. pylori* infection was independently associated with anemia, increasing age, water sources and intestinal parasitaemia among dyspeptic patients. Microcytic hypochromic anemia was the commonest morphological type of anemia among dyspeptic patients with *H. pylori* infection. Routine screening of dyspeptic patients for *H. pylori* infection, hemoglobin estimation of the infected patients and improvement of water sources is advised.

Data Sharing Statement

The datasets used in the analysis of this research study are available from the corresponding author upon reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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