Association between *Helicobacter Pylori* Infection and Iron Deficiency in Sudanese Population in Khartoum state

Abstract

Background: *Helicobacter pylori* infection is a major gastric infection worldwide and has been associated with many gastrointestinal and non-gastrointestinal diseases including hematological disorders.

Objective: Aimed to study the association between *H. pylori* infection and iron deficiency among Sudanese population.

Materials and methods: This is analytical case control study, conducted in Sudan, Khartoum state in Omdurman locality, during the period from May 2018 to April 2019. Include 100 samples, the stool and blood samples were collected from 100 Sudanese subjects (50 were infected with *H. pylori* as cases and 50 were apparently healthy subjects as controls). Stool samples were tested for *H. pylori* Ag by commercially available kits (HanzouAllTest Biotech Co., Ltd, Germany), all blood samples were analyzed for complete blood count using (SYSMEX KX21N) automated analyzer and serum iron profile (iron, ferritin, and TIBC) using spectrophotometery and turbidimetry. The obtained results is analyzed by SPSS versions 16.0, significant level was set at p-value equal or less than 0.05 and the results were presented in form of tables and figures.

Results: The results of *H. pylori* antigen were positive in all cases and negative in control samples. Serum iron level mean was significantly lower among *H. pylori* positive patient (62±18.1) than control group (91.3±16.7) (p-value 0.001), serum ferritin level mean was significantly lower in *H. pylori* infected patients (36.8±16.5) than control group (64±16.4) (p-value 0.003), hemoglobin level mean was significantly lower in *H. pylori* patients (12.5 ±1.1) than control group (13.8±1.0) (p-value 0.009), PCV level mean was lower in *H. pylori* patients (37.6±3.1) than control group (41±3.0) (p-value .036), TIBC mean was 313 in cases and 308 in control samples. All other parameters showed no significant difference between *H. pylori* positive patients and control subjects.

Conclusion: This study concluded that *H. pylori* infection is associated with iron deficiency in Sudanese patients.

Keyword: *Helicobacter pylori* Infection, Iron Deficiency, Sudanese

1. Introduction

Iron is one of the most common elements in the Earth's crust, yet iron deficiency is the most common cause of anemia, affecting about 500 million people worldwide. Organic dietary iron is partly absorbed as heme and partly broken down in the gut to inorganic iron. Absorption occurs through the duodenum. In developed countries, chronic blood loss, especially uterine or from the gastrointestinal tract, is the dominant cause of iron deficiency and dietary deficiency is rarely a cause on its own (1).

Gluten induced enteropathy, partial or total gastrectomy and atrophic gastritis (often autoimmune and with *Helicobacter pylori* infection) may predispose to iron deficiency. The cause of iron deficiency is done according to the patient case. In premenopausal women the most cause is menorhagia or pregnancy, while in postmenopausal women and men the most cause of iron deficiency is gastrointestinal loss in these cases the deficiency may be investigated either by: occult blood test, endoscopy for GIT, tests for parietal cell antibodies and detection of *H. pylori* infection (1).

When gastroenterological evaluation fails to disclose a likely cause of IDA, or in patients refractory to oral iron treatment, screening for celiac disease, autoimmune gastritis, and *H. pylori* is recommended (2).

Epidemiological studies of *H. pylori* show acquisition in early childhood. However, infection often remains asymptomatic in children and, except for peptic ulcer disease (which is rare in childhood), a relationship between abdominal pain and *H. pylori* infection is not demonstrated. At the same time several gastrointestinal and non-gastrointestinal diseases has been associated with this infection (3). There is a strong association between the presence of *H. pylori* infection and duodenal ulceration (4).

H. pylori infection is a major gastric infection in the world. Approximately more than 50% of the adult population in the developed countries and 90% of those in the developing countries are infected with this bacterium. *H. pylori* associated gastritis can result in many extra gastric complications like vitamin B12 and iron deficiency, megaloblastic anemia, and iron deficiency anemia respectively and other hematological changes (5).

H. pylori associated chronic gastritis has emerged as a potential cause of iron deficiency anemia that is unresponsive to iron therapy. Knowledge into the pathogenesis of the anemia is still lacking. The refractoriness to iron treatment and the finding that the eradication of the bacterium may reverse anemia and normalize the iron profile, have been demonstrated in a few studies (6).

There are several literature studies found that *H. pylori* infection can lead to diminished iron in the body which ends by IDA. Gastric *H. pylori* infection cause of IDA of previously unknown origin in adult patients (7).

Any previous *H. pylori* infection can be associated with higher prevalence of anemia and reduction of hemoglobin level and red cell indices in school-age children independent of socioeconomic variables (8). Infection with *H. pylori* has a role in iron deficiency and the subsequent IDA in infected patient also in puberty and childhood (9 & 10).

2. Materials and methods

2.1 Study design: analytical case control study.

2.2 Study area: This study was conducted in Sudan, Khartoum state in Omdurman locality, this area characterized with high prevalence of *H. pylori* infection and IDA.

2.3 Study population and sample size: A total of 100 samples from Sudanese population with different gender and age were included in this study; 50 of them were positive for *H. pylori* antigen as cases; the remaining 50 were negative for *H. pylori* antigen used as controls. From each we requested an stool sample for detection of *H. pylori* Ag by commercially available kits (HanzouAllTest Biotech Co., Ltd, Germany), and 5 ml of blood collected (2.5 ml in EDTA, and 2.5ml in plain tube) for analysis of CBC parameters and iron profile.

2.4 Selection criteria:

2.4.1 Inclusion criteria: The criteria to be included in this study as cases is that the person should be positive for *H. pylori* by detection of its antigen in the stool sample. Controls criteria should be negative for *H. pylori* antigen.

2.4.2 Exclusion criteria: The exclusion criteria were patient under treatment within 10 days prior to the study with drugs have known effect on iron metabolism, iron drugs, or blood transfused patients. Subjects with any history of GI surgery, peptic ulcer, systemic disease, hematological disease, diabetes mellitus and smokers were eliminated from the study.

2.5 Ethical approval: Ethical approval was obtained from college of graduate studies (SUST) and from Saad Rashwan laboratory management. Verbal consent was obtained from all subjects of the study.

2.6 Tests performed:

2.6.1 Detection of *H. pylori* Ag in stool sample: This was done by the commercially available kits from (HanzouAllTest Biotech Co., Ltd, Germany) in which we followed the manufacturer instructions.

2.6.2 Complete Blood Count: CBC parameters were analyzed using the automated hematology analyzer Sysmex KX21N.

2.6.3 Iron profile: The analyses of serum iron, serum ferritin and TIBC we used Biosystems reagents (Spain).

2.7 Statistical analysis: All data was analyzed using Statistical Package for Social Science (SPSS, version 16.0) computer software. Significant level was set at p-value equal or less than 0.05 and the results were presented in form of tables and figures.

3. Results

3.1 Demographic data: A total of one hundred Sudanese individuals with different ages (between 10-75 years) and sex were included in this study. Of them, 50 were infected with *H. pylori* as cases (50%), and 50 were healthy individuals negative for H. pylori antigen as controls (50%). 58% of the cases were females (29 subjects) and 42% of them were males (21 subjects). 58% of the controls were females (29 subjects) and 42% of them were males (21 subjects). The age of cases was ranged 10-75 (mean 31.8 years), while controls between 10-65 years with (mean 32.3 years).

We used the following sample size equation to calculate the sample size and we selected 100 samples due to the high cost of reagents and devices.

$$n0 = \frac{z2\ pq}{d2}$$

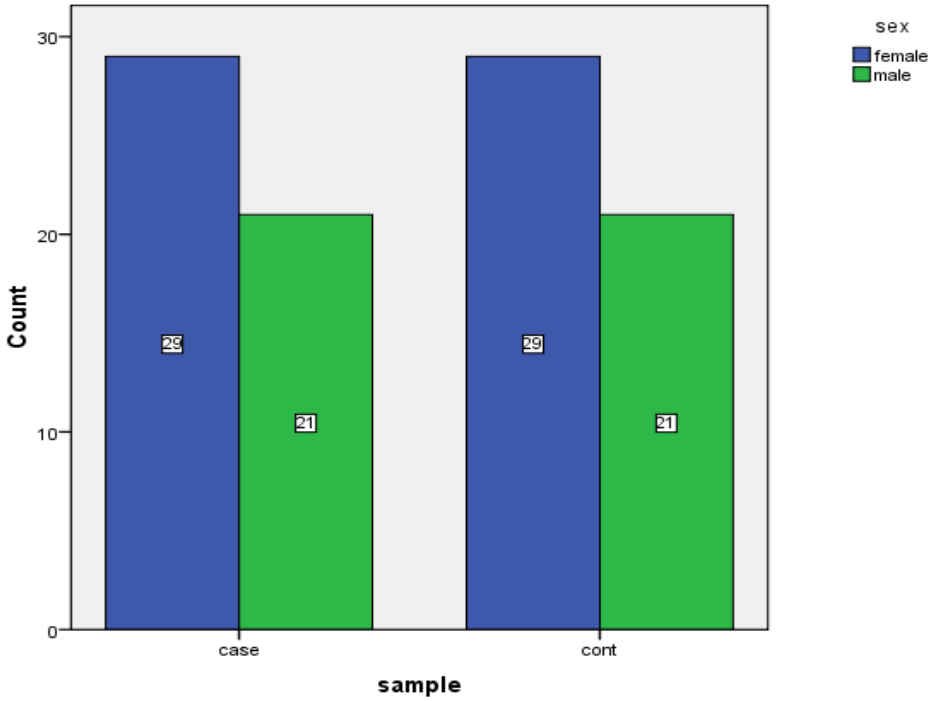

Figure 1: Distribution of the study population according to gender.

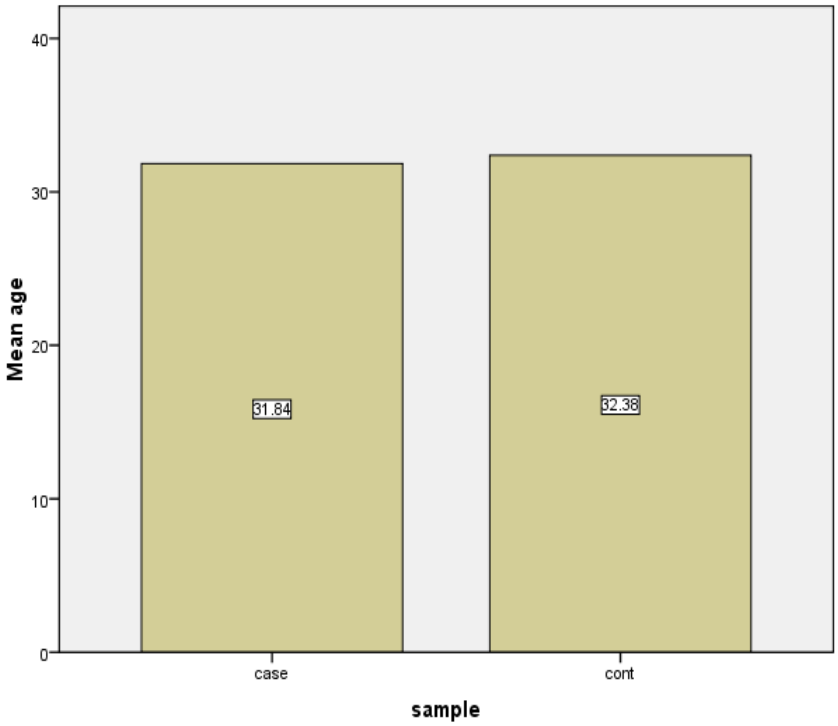

Figure 2: Frequency of age (years) among cases and controls.

3.2 Complete Blood Count: The mean hemoglobin concentration and PCV of cases was lower than that of controls which was (12.5 ±1.1, 13.8±1.0) for hemoglobin and (37.6±3.1, 41±3.0) for PCV. No difference detected on the other CBC parameters between cases and controls.

Table 1: Means and standard deviations of CBC among cases and controls.

Population	Mean ± Std.D						
	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)	WBC (cell/ul)	Plts (cell/ul)
Cases	12.5 ±1.1	37.6±3.1	81.6±6.3	27±2.4	32±2.1	6.8±2.8	298±76
Controls	13.8±1.0	41±3.0	82±2.7	29±1.9	34±1.5	6.4±1.8	318±73

3.3 Iron study: The concentration of serum iron and serum ferritin was lower in cases than controls (62±18.1, 91.3±16.7) for serum iron and (36.8±16.5, 64±16.4) for ferritin. Total iron binding capacity was not affected in cases and controls.

Table 2: Means and standard deviations of serum iron profile among cases and controls.

Population	Mean ± Std.D		
	S. Iron	S. ferritin	TIBC
Cases	62±18.1	36.9±16.5	313±67
Controls	91.3±16.7	63.8±16.4	308±20.7

3.4 Comparison of CBC between infected and non-infected persons: There was significant difference in hemoglobin and PCV in the infected persons with *H. pylori* and non-infected persons (p-value less than 0.05) while all other parameters not affected by the infection with *H. pylori* (p-value more than 0.05).

Table 3: CBC parameter in relation to *H. pylori* infection.

Parameter	*H. pylori*	No.	Mean ± SD	*P*.value
Hb	Positive	50	12.5 ±1.1	0.009
	Negative	50	13.8±1.0	
RBCs	Positive	50	4.6±0.013	0.220
	Negative	50	4.8±0.01	
PCV	Positive	50	37.6±3.1	0.036
	Negative	50	41±3.0	
MCV	Positive	50	81.6±6.3	0.400
	Negative	50	82±2.7	
MCH	Positive	50	27±2.4	0.600
	Negative	50	29±1.9	
MCHC	Positive	50	32±2.1	0.070
	Negative	50	34±1.5	
WBCs	Positive	50	6.8±2.8	0.500
	Negative	50	6.4±1.8	
Plts	Positive	50	298±76	0.054
	Negative	50	318±73	

Independent sample T test, P. value ≤ 0.05 is significant

3.5 The association between *H. pylori* and iron profile: There was a significant difference in serum iron and serum ferritin between infected people with *H. pylori* and healthy people (p-value less than 0.05). While there was no difference in TIBC between them.

Table 4: Iron profile in relation to *H. pylori* infection.

Parameter	*H. pylori*	No.	Mean ± SD	*p*. value
Serum iron	Positive	50	62±18.1	0.001
	Negative	50	91.3±16.7	
Serum ferritin	Positive	50	36.8±16.5	0.003
	Negative	50	64±16.4	
TIBC	Positive	50	313±67	0.094
	Negative	50	308±20.7	

Independent sample T test, P. value ≤ 0.05 is significant

4. Discussion

Helicobacter pylori infection is considered a worldwide problem and it is the most common cause of chronic gastritis, and has been strongly linked to peptic ulcer disease and gastric cancer. Several gastro-intestinal and non-gastrointestinal diseases have been reported to have a significant association with *H. pylori* infection. *H. pylori* associated gastritis has emerged as a potential cause of iron deficiency anemia that is unresponsive to iron therapy (3).

In Baghdad a study conducted by Jasem *et al. (2011)* (11) found that *H. pylori* infection has a role in iron deficiency and subsequently IDA. Another study in India done by Umakiran *et al.*(2011) (12) found that there is an association between *H. pylori* infection and IDA, and they also suggested that even asymptomatic infection can impair iron absorption, and treatment of the infection along with iron supplements can improve IDA. In Iran, Qujeq *et al. (2011)* (13) concluded that *H. pylori* may lower iron profile and it may impair iron metabolism, it competes the host in iron uptake. On the

other hand, there are some studies did not support that, for example in Iran, a study done by Keramati *et al*. (2007) (14) found that there is no correlation between *H. pylori* infection and iron deficiency.

We enrolled this research to study this hypothesis in Sudan, since *H. pylori* and iron deficiency both were commonly distributed.

Our results showed that hemoglobin, PCV, serum iron and serum ferritin were significantly lower in patients infected with *H. pylori* than control group whom were negative for *H. pylori* (p-value less than 0.05) which confirm that theory there is an association between *H. pylori* infection and iron deficiency and subsequently iron deficiency anemia. All other parameters showed no difference between *H. pylori* infected patients and healthy subjects. This may be due to some factors, it may be due to that most patient were newly diagnosed cases and the change in these parameters is time dependent according to the phases of IDA.

In this study most parameters values were near or in the permissible limits, this also may be due to the time of diagnosis or also may be due to the type of *H. pylori* strain, some strains have a virulence factor cause iron deficiency and other strains lack that factor.

Several possible mechanisms for the association between *H. pylori* infection and iron deficiency must be considered. Chronic bleeding that may result from the peptic ulcer can be a cause of deficiency. *H. pylori* may act as an iron-acquisition mechanism in vivo; it competes with the host for iron.

H. pylori infection may progress into diffuse corpus gastritis. These conditions may play an important role in gastric hypoacidity. On the other hand, as high gastric acidity facilitates the solubilization of non-heme iron, iron uptake may be impaired in subjects with *H. pylori* infection due to loss of iron. Despite all of that, the mechanisms by which *H. pylori* infection may lead to iron deficiency and anemia remains unclear and need more work.

5. Conclusion

This study found that the hemoglobin, PCV, serum iron and ferritin were lower in *H. pylori* patients than healthy subjects (p-value < 0.05). The study concluded that there is an association between *H. pylori* infection and iron deficiency and subsequent iron deficiency anemia (Odds ratio 4.4).

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