Role of *Helicobacter pylori* in refractory iron deficiency anaemia

M. T. AFIFI[*], H. K. ABD EL-AZIZ[†], N. A. M. HAMED[‡], N. A. BARGHASH[†], A. ABDO[§] and M. GAMAL[#]

Departments of [*]Internal Medicine (Gastroenterology Unit), [†]Medical Biochemistry, [‡]Internal Medicine (Haematology Unit), [§]Tropical Medicine and [#]Microbiology and Immunology, Faculty of Medicine, Alexandria University, Egypt

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ABSTRACT

The role of *Helicobacter pylori* infection in the development of iron deficiency anaemia has been the focus of attention over the past decade. However, confirmation of a relationship has not confirmed the pathophysiological mechanisms involved in the phenomenon. The aim of the present work is to study the levels of fasting gastric acidity (free and total) as well as the level of tumour necrosis factor-α (TNFα) in male refractory iron deficiency anaemia patients seropositive for *H. pylori* infection versus those who are seronegative. Thirty adult patients with iron deficiency anaemia and gastroduodenitis were subdivided into two groups of matched age and haemoglobin value. Group 1 was *H. pylori*-seropositive for infection and these patients did not receive prior treatment for eradication of *H. pylori* infection. Group 2 comprised patients seronegative for *H. pylori* infection (control group). Patients with active bleeding or previous medical problems were excluded from the study. All patients and controls were subjected to the following at presentation: history taking and thorough clinical examination, complete blood picture, reticulocytes (%), assessment of serum iron, total iron binding capacity, serum ferritin, IgG anti-*Helicobacter* antibody and TNFα, stool for occult blood and measurement of gastric acidity (total and free). Upper endoscopy was performed and multiple biopsies were taken and tested for expression of cytotoxin-associated gene A (*cag*A) by the polymerase chain reaction (PCR). Results showed significantly higher values of free and total gastric acidity as well as TNFα levels in Group 1 compared to controls (Group 2). Among those in Group 1, higher TNFα levels were seen in seven *H. pylori cag*A-positive patients than in eight *cag*A-negative patients. Haemoglobin values were inversely correlated with TNFα levels. Thus, elevated serum TNFα in the *H. pylori*-seropositive group may be one of the underlying pathophysiological mechanism for iron deficiency anaemia observed in these patients.

KEY WORDS: Anemia, iron deficiency.
Ferritins.
Helicobacter pylori.
Iron-binding proteins.
Tumor necrosis factor-alpha.

Introduction

The role of *Helicobacter pylori* infection in the development of iron deficiency anaemia has been the focus of attention over the past decade. Epidemiological studies indicate that *H. pylori* seropositivity is associated with low serum ferritin and haemoglobin levels in adults and children.[1] These findings are supported by case reports in which eradication of *H. pylori* resulted in improvement of iron deficiency anaemia in patients resistant to iron replacement therapy. It has also been reported that eradication of *H. pylori* may result in improvement of anaemia even in the absence of iron supplementation.[1]

Confirmation of the relationship between *H. pylori* infection and iron deficiency anaemia has not explained the pathophysiological mechanisms involved in the phenomenon.[1] However, two hypotheses have been proposed to explain the association. The first is sequestration of iron by antral *H. pylori* infection. A previous study showed that iron was diverted from the bone marrow in patients with *H. pylori* infection and iron deficiency anaemia.[1] The second suggests that *H. pylori*-related changes in gastric physiology result in iron deficiency anaemia.

H. pylori gastritis decreases gastric acidity and the ascorbic acid content of gastric juice, both of which may decrease non-heme iron absorption. It has been demonstrated that both pan-gastritis and pan-gastritis-induced hypochlorohydria are more prevalent in adult *H. pylori*-infected patients with anaemia than in those who do not have anaemia.[1]

The aim of the present study is to compare the levels of fasting gastric acidity (total and free) and level of tumour necrosis factor-α (TNFα) in refractory iron deficiency in male anaemic patients seropositive for *H. pylori* infection, and in a group seronegative for *H. pylori* infection. In addition, an attempt was made to find the underlying pathophysiological mechanism for the iron deficiency anaemia seen in these patients.

Correspondence to: Dr H. K. Abd El-Aziz
Email: hanankam60@yahoo.com

Materials and methods

Thirty adult male patients with iron deficiency anaemia and gastroduodenitis were recruited. All patients presented with dyspeptic symptoms or epigastic pain of at least two months' duration. They were subdivided into two groups of matched age (Group l: 28±7.64 years; Group 2: 26.4±7.73; t=0.57, P=0.573) and haemoglobin value. Patients in

Group 1 were seropositive for *H. pylori* infection, and they did not receive prior treatment for eradication of *H. pylori* infection. Those in Group 2 were seronegative for *H. pylori* infection (control group). Patients with active bleeding or with other medical problems (e.g., cardiac, hepatic or renal disease) were excluded from the study.

All patients in Group 2 received treatment with a proton-pump inhibitors (PPI) for four to six weeks, followed by oral iron therapy for at least three weeks. Minimum rate of response of a rise of 20 g/L in haemoglobin every three weeks[2] was not attained in all patients. Thus, triple therapy for *H. pylori* eradication using a PPI (omeprazole 20 mg) with amoxicillin (1 g) and clarithromycin (500 mg) was then initiated twice daily for two weeks, followed by oral iron therapy, and showed good therapeutic effect.

All patients and controls were subjected to the following at presentation:

- history taking and thorough clinical examination
- complete blood picture and brilliant cresyl blue smears for reticulocytes (%)
- colorimetric determination of serum iron (bioMérieux) and total iron binding capacity (Biodiagnostics, Egypt)[4]
- serum ferritin by enzyme-linked immunosorbent assay (ELISA, DRG International)[5]
- occult blood test (Acon Laboratories, USA)[6]
- gastric acidity (total and free) by a chemical method[7]
- quantitative determination of plasma TNFα by an ELISA method[8]
- hepatic and renal function tests[9]
- quantitative determination of serum IgG anti-*Helicobacter* antibody using an enzyme immunoassay technique (Biocheck, Foster City, USA[10]
- upper gastrointestinal endoscopy performed after overnight fast.[11]

Detection of cytotoxin-associated gene A (*cag*A) by a polymerase chain reaction (PCR) was performed as follows. Two biopsy specimens were dipped into a small screw-capped tube containing 1 mL brain heart infusion broth supplemented with 10% human serum and three sterile glass beads.[12] The tube was vortex-mixed at high speed for two minutes. Then, 200-mL quantities of the tissue suspension were inoculated on two plates: selective Dent's agar and Columbia blood agar. The inoculated plates were incubated at 37°C under microaerophilic conditions using a Campy gas pack (Oxoid) for four to six days. Colonies that exhibited characteristic morphology were identified as *H. pylori* if they rapidly hydrolysed urea, produced catalase and oxidase and were Gram-negative spiral rods.[12,13] Positive cultures were subjected to PCR for *cag*A detection.

To extract DNA,[14] *H. pylori* colonies were digested by proteinase K (25 ng/mL) and incubated at 70°C for 2 h. DNA was extracted with 1 vol phenol-chloroform isoamyl alcohol (25:24:1; Sigma) and then precipitated with 2.5 vol absolute ethanol at –20°C for 2 h. The DNA was centrifuged at 4°C for 15 min and the pellet was resuspended in 20-mL distilled water.

DNA amplification[15] was performed in a final volume of 50 µL of PCR mixture containing 50 mmol/L KCl, 10 mmol/L Tris, 200 mmol/L deoxynucleotide triphosphate, 30 pmol each primer, 0.1 µg bovine serum albumin, 2.5 units AmpliTaq (Promega) and 20 ng template DNA. Amplification was performed using the following cycling profile: 94°C for 45 sec, 50°C for 45 sec and 72°C for 45 sec for 35 cycles, and then an extension at 72°C for 10 min. The *H. pylori cag*A primers DZ3 (5`-AGTAAGGAGAAACAATGA) and Roo9 (5`-AATAAGCCTTAGAGTCTTTTT-GCAAATC) were derived from the sequenced *cag*A gene, giving an amplified product of 135 bp.

The amplified PCR products[16] were purified by electrophoresis on 1.4% agarose gel (Sigma) at 80 V and then visualised using ethidium bromide (0.5 µg/mL) staining.

Results

Table 1 shows the laboratory parameters of the two groups. Both showed comparable values of haemoglobin, serum ferritin, total iron binding capacity and serum ferritin levels. The mean values for serum iron and total iron binding capacity were 43.53±11.45 µg/dL and 478.73±39.48 µg/dL, respectively, for Group 1, and 42.4±10.75 µg/dL and 456.33±29.39 µg/dL, respectively, for Group 2 (serum iron: t=0.279, P=0.782; total iron binding t=1.76, P=0.089). Significantly higher values for free and total gastric acidity, as well as TNFα levels, were seen in *H. pylori*-seropositive individuals compared with the control group. Among those in Group 1, higher TNFα levels were seen in seven *H. pylori cag*A-positive patients (44.5±4.25) than in eight *cag*A-negative patients (35±5.11).

Table 2 shows the correlation between the different clinical and laboratory parameters in Group 2 (seropositive for *H. pylori* infection). Haemoglobin values were inversely correlated with TNFα levels (r=–0.644, P=0.01).

Table 1. Laboratory parameters of the studied groups.

Parameter		Group 1	Group 2	*t*-test
Haemoglobin (g/dL)		10.74±0.99	10.29±1.15	1.14
Serum ferritin (ng/mL)		24.8±13.22	30.8±12.44	1.28
Gastric acidity (mL 0.1 N NaOH %)	Total	80.13±31.21	29.93±10.75	5.89*
	Free	33.4±7.41	14.33±4.73	8.396*
TNFα (pg/mL)		39.43±6.699	22.2±4.89	8.049*

Group 1: *H. pylori*-seropositive patients
Group 2: *H. pylori*-seronegative patients
Results presented as mean±SD
*Statistically significant, P<0.01

Table 2. Correlation coefficient (*r*) between clinical and laboratory parameters in group 1 (*H. pylori*-seropositive group)

		Haemoglobin	Serum ferritin	Total gastric acidity	Free gastric acidity	TNFα
Age (years)	*r*	0.435	0.702*	0.004	–0.028	–0.036
	P	0.105	0.004	0.988	0.922	0.898
Haemoglobin	*r*		0.288	0.048	–0.034	–0.464*
	P		0.298	0.864	0.903	0.01
Serum ferritin	*r*	0.288		–0.408	–0.385	–0.097
	P	0.298		0.131	0.157	0.731
Total gastric acidity	*r*	0.048	–0.408		0.763*	0.333
	P	0.864	0.131		0.001	0.225
Free gastric acidity	*r*	–0.034	–0.385	0.763*		0.351
	P	0.903	0.157	0.001		0.199
TNFα	*r*	–0.644*	–0.097	0.333	0.351	
	P	0.010	0.731	0.225	0.199	

*Statistically significant, $P<0.05$

Discussion

H. pylori gastritis decreases gastric acidity.[1] Gastric colonisation by *H. pylori* also induces a transient period of achlorohydria. Factors contributing to altered gastric secretion during *H. pylori* infection include urease activity, a soluble protein of 46 kDa and certain fatty acid components of *H. pylori* lipopolysaccharide. *H. pylori*-induced achlorohydria may also be mediated by interleukin-1, which has been shown to inhibit gastric acid secretion in rats.[17] Gastric acidity increased after *H. pylori* pan-gastritis has been cured.[18]

In the present study, the level of total and free gastric acid was significantly higher in the *H. pylori*-seropositive group, compared to the *H. pylori*-seronegative group. This may be explained by the presence of duodenitis in the studied patients.

H. pylori infection that follows a predominantly antral pattern leads to inflammation in which higher levels of TNFα and other cytokines are produced. These stimulate gastric acid production directly by increasing gastrin release from G cells and inhibiting somatostatin production by D cells. This leads to a net increase in gastric acid secretion, which leads to increased acid load in the duodenum, overwhelming the mucosal defence.[19]

Following elimination of *H. pylori* gastritis in duodenal ulcer patients, acid secretion does not decrease to normal levels. Thus, increased gastric acid secretion rates in duodenal ulcer patients cannot be attributed to *H. pylori* gastritis alone. It is likely that cytokines derived from inflammatory cells in the gastric antrum are responsible for hypergastrinaemia, either by augmenting antral G-cell function or by suppressing antral D-cell function.[20]

Recent reports suggest that *H. pylori* infection can affect iron homeostasis,[21,22] although the mechanisms by which *H. pylori* causes iron deficiency remains unclear.[21] The present study has confirmed the results of other studies which have shown that iron deficiency anaemia refractory to oral iron therapy improved following *H. pylori* treatment alone and was hastened by iron supplementation.[21] This has established a causal role for *H. pylori* infection in the development of iron deficiency anaemia.[21] However, it is not clear if this beneficial effect results from the removal of *H. pylori*-specific effects, the elimination of other infectious pathogens, or through the reduction in total infectious burden.[23]

Tumour necrosis factor-α is an inflammatory cytokine implicated in the suppression of erythropoiesis.[24] *In vitro* studies suggest that TNFα can inhibit the production of erythropoietin.[25] In the present study, there was a statistically significant increase in TNFα in the seropositive group when compared with the seronegative group. In addition, there was an inverse correlation of significant value between serum TNFα and haemoglobin level in the *H. pylori*-seropositive group.

H. pylori infection leads to mucosal increase in many pro-inflammatory and immunoregulatory cytokines, and also increases in members of the chemokine group of peptides. The stomach has a large surface area and continuous over-production of locally produced cytokines into the bloodstream is possible; however, there are conflicting data on circulatory pro-inflammatory cytokine levels in patients with *H. pylori* infection.[26]

In one study, no difference in the mean circulatory levels of TNFα was seen in *H. pylori*-positive cases without systemic diseases and in *H. pylori*-negative groups. Other workers report elevated serum TNFα levels in patients with *cag*A gene-positive *H. pylori* infection.[27] There is also evidence of a role for *cag*A-positive *H. pylori* infection in iron deficiency anaemia.[28]

This study can conclude that elevated serum TNFα levels in *H. pylori*-seropositive patients may be one of the underlying pathophysiological mechanisms responsible for iron deficiency anaemia observed in this group. □

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