The Impact of *H. pylori* Eradication on Response to Oral Iron Therapy in Patients with Iron Deficiency Anemia

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Infection with *Helicobacter pylori* has been associated with Iron deficiency anemia (IDA). We assessed the effect of eradication of *H. pylori* infection on response to oral iron treatment. Twenty patients with IDA of no obvious cause, unresponsive to oral iron therapy with positive gastric biopsy for *H. pylori* infection received sequential eradication therapy for 10 days followed by oral iron therapy. Treated patients were followed up at 6 weeks and 12 weeks post eradication to assess dynamic changes in hemoglobin, serum ferritin and transferrin saturation. While 65% of anemic *H. pylori* infected cases had pangastritis, 35% had antral gastritis. In the antrum severe and moderate gastritis were found in 40% and 45% of cases, respectively. Hemoglobin and serum ferritin level correlated inversely with grade of gastritis (*P* <0.001). Improvement in hematological parameters and serum iron profile was observed 6 weeks and 12 weeks post successful *H.* eradication oral iron therapy. In conclusion, eradication of *H. pylori* infection enhances the response to oral iron supplementation in patients with refractory IDA. Screening and eradication of *H. pylori* should be a standard procedure for patients with IDA.

Iron deficiency anemia (IDA) is the most common nutritional deficiency worldwide. It can cause reduced work capacity in adults (Hass *et al*., 2001) and impact motor and mental development in children and adolescent (Halterman *et al*., 2001). There is some evidence that iron deficiency without anemia affects cognition in adolescent girls and cause fatigue in adult women (Algarin *et al*., 2003).

Chronic gastritis is an inflammatory condition of the gastric mucosa characterized by elementary lesions whose extent and distribution are related to their etiology and host responses. Infection with *Helicobacter pylori* is by far the most common cause of chronic active gastritis worldwide. Chronic gastritis is epidemiologically and biologically linked to the development of gastric cancer (Correa, 1998).

Helicobacter pylori are unique bacteria ideally suited in the acidic environment of the human stomach. Their spiral shape and multiple flagella allow them to move freely through the gastric mucosa layer, where they remain protected from low gastric PH. Moreover, organisms produce large amount of urease, that permits the bacteria to further control the PH of their microenvironment and also helps in its diagnosis (Kusters *et al*., 2006).

The organism chronically colonize the mucosa of the stomach leading to intense local inflammatory and systemic immune response and altering acid secretory physiology (Schubert *et al*., 2008). The ultimate clinical manifestation of *H. pylori* infection include gastric and duodenal ulcer, gastric mucosa associated lymphoid tissue (MALT) lymphoma and adenocarcinoma; yet most infected individuals remain asymptomatic for life despite developing chronic histologic gastritis (Amieva *et al*., 2008).

The present study was undertaken to assess the effect of *H. pylori* infection eradication on response to oral iron treatment.

Patients and Methods

Our study included 20 patients with iron deficiency anemia of no obvious cause who were referred to Tanta University hospital, Internal Medicine, Endoscopy unit as a part of investigations for IDA because of refractoriness to oral iron supplementation therapy despite accepted period of treatment and who were proved to be positive for *H. pylori* infection by gastric biopsy ' histopathological examination. Anemia was defined as hemoglobin level less than 13g/dl for male and less than 12g/dl for female with MCV less than 80 fl with at least one of the following: a) Serum ferritin ≤25 ng/ ml. b) Transferrin saturation <20.

Exclusion criteria were: obvious causes of blood loss, such as active gastrointestinal bleeding, heavy menstrual loss, history of intake of NSAID and also intake of antacids or antibiotics in the past one month as it interfere with test results. Patients who were lactating or pregnant and those with malnutrition or cancer were also excluded. All study patients confirmed their participation by signing a written informed consent form. The protocol of the study was approved by the ethical committee of the Faculty of Medicine, Tanta University

All patients included in the study were subjected to, full history taking and thorough clinical examination. Laboratory investigations included (Complete blood count, Serum ferritin, transferrin saturation, Occult blood in stool. Abdomen and pelvis ultrasound, Upper GIT endoscopy with gastric biopsy and histopathological examination, Lower GIT colonoscopy for some of the patients were also done.

Our Study included patients received sequential therapy regimen for *H. pylori* eradication. The sequential therapy is a two step, 10 days therapy, consisting of PPI(20mg) and amoxicillin(1gm) (both twice daily) given for the first 5 days followed by a triple therapy including PPI(20mg), clarithromycin (500mg) and tinidazole (500mg) (all twice daily) for the remaining 5 days. Following eradication therapy, patients received oral iron supplementation therapy in the form of 200 mg iron sulphate/day. Stool antigen test was done for all participants for confirmation of successful eradication of *H. pylori* 6 weeks post its therapy. Laboratory investigations including complete blood count and iron study parameters were re-evaluated at 6 weeks post therapy for all patients and only for cases with successful eradication at 12 weeks to monitor changes.

Methods

1- Laboratory investigations

Hemoglobin was determined by SYSMEX XT 1800i (Roche Diagnostics, Basel, Switzerland). Serum ferritin was estimated by ferritin ELISA coated microtiter strips. Serum iron and total iron binding capacity were tested (Siedel 1984, Ramasy 1958) and transferrin saturation was calculated by dividing serum iron/total iron binding capacity. Occult blood in stool was measured by a rapid chromatographic immunoassay technique. Stool antigen testing for *H. pylori* (premium platinum HpSA kit, Meridian biosciences Inc, USA). This is a rapid enzyme immunoassay, which uses monoclonal anti *H. pylori* capture antibodies adsorbed to plastic wells of an ELISA tray.

2- Endoscopic examination

The patients were instructed to fast for 10 hours before the procedure then sedation was done using midazolam and the patient lies on his left lateral position then endoscopic evaluation was done using Olympus fibroptic version up to the second part of the duodenum. Multiple biopsy specimens from antum and fundus were taken.

3-Histopathological examination

All gastric mucosal biopsy specimens were fixed in 10% buffered formalin processed for routine histological preparations and stained with hematoxylin and eosin for routine histopathological examination. Gastritis was evaluated according to the updated Sydney system in corpus and antral samples. (Dixon *et al*., 1996) Tissue sections from corpus and antrum were stained with Giemsa stains for detection of *H. pylori* (Gray *et al*., 1986).

Statistical Analysis

All statistical analyses were carried out using SPSS software (version 17; IBM Corporation, Armonk, NY, USA). Data are presented as mean ± SD. The differences of the follow up values were determined using the student t test for paired analysis. A *P* value of < 0.05 was considered significant. Spearman's correlation (linear correlation) was used to correlate between variables.

Results

Table 1 shows the main demographic and clinical data of the studied patients. Our study included 20 patients with refractory IDA of

unknown cause with positive gastric biopsy for *H. pylori* infection. They were, 12 female and 8 male with mean age 35±18.34. They were all refractory to oral iron supplementation therapy, mean duration 9±4.9 months. Positive occult blood was found in 5 patients and lower GIT colonoscopy was done for 9 patients including patients with positive occult blood with no abnormal findings for all of them.

Upper endoscopic examination done for our 20 patients has revealed normal upper endoscopy in 11 out of 20 (55%) patients with minimal endoscopic findings in the rest of the studied patients.

Table 1. Demographic and clinical data of the studied patients.

Patients		N (%)
Sex	Female	12 (60)
	Male	8 (40)
Age	Range	25-55
	Mean±SD	35±18.34
History of GIT symptoms	Positive	9 (45)
	Negative	11 (55)
Occult blood	Positive	5 (25)
	Negative	15 (75)
Duration of iron therapy (month)	Range	(6-18)
	Mean±SD	9±4.9
Hemoglobin (gm/dl)	Range	8.4-10.5
	Mean±SD	9.51±0.72
Transferrin Saturation (%)	Range	12-19
	Mean±SD	15.58±2.24
Serum ferritin (ng/ml)	Range	15-24
	Mean± SD	19.45±3.28

Endoscopic biopsy specimens from all studied patients were evaluated using routine hematoxylin, eosin and stained with Giemsa stain for detection of *H. pylori.*

H. pylori were tiny spiral shaped coil within the mucous layer covering the surface epithelium and within foveola. Infection by *H. pylori* was mainly in both corpus and antrum (65%). There was no recorded cases in the corpus alone. In total anemic *H. pylori* positive patients, there has been an evidence of associated gastritis with pangastritis predominant in (65%) of our patients and antral gastritis in (35%) of them. In all studied patients there was no corpus only predominant gastritis (table 2).

Table 2. Topography of *H. pylori* infection and associated gastritis.

Site	*H. pylori*		Gastritis	
	N	%	N	%
Antral	7	35	7	35
Corpus	0	0.00	0	0.00
Antral & corpus	13	65	13	65

The intensity of inflammatory cells within the lamina propria was scored according to the visual analogue scales of the updated Sydney system (13) into (Absent 0, mild I, moderate II and severe III). In the antrum severe gastritis was found in 45% of patients while moderate gastritis was found in 40% of cases. In the corpus, severe gastritis was found in 25% of cases and moderate gastritis was found in 25% of cases (table 3).

Grade of inflammation results from combination of the degree of the inflammatory lesions in addition to its location in antrum and corpus mucosa. Grades range from 0 (absence of inflammatory cells in any of the specimens) to grade IV (very dense infiltrate in all biopsy samples) according to the visual analogue scale of the Updated Sydney System. Grade III and grade IV gastritis were found in 70% of our patients, (table 3).

Gastric atrophy was present in 15 patients (75%). 4 cases were in the antrum, 3 cases in the corpus and 8 cases in both antrum and corpus (table 4). Atrophy detected in our cases was of focal atrophic gastritis that is characterized by damage of the glands by inflammation or loss of glands and replacement of the lamina propria by extracellular matrix and fibroblast.

In our patients, grade of gastritis correlated inversely with hemoglobin level, serum ferritin and transferrin saturation (P<0.001), (Table 5).

Table 3. Scoring and grading of *H. pylori* associated gastritis

		Scoring	N (%)
Site:	Corpus	Absent	7 (35)
		Mild	3 (15)
		Moderate	5 (25)
		Severe	5 (25)
	Antrum	Absent	0 (0.00)
		Mild	3 (15)
		Moderate	8 (40)
		Severe	9 (45)
	Grading		Number (%)
	Grade 0		0 (0.00)
	Grade I		2 (10)
	Grade II		4 (20)
	Grade III		6 (30)
	Grade IV		8 (40)

Table 4. Topography and number of cases with gastric atrophy.

Site	(%) Number
Antrum	(20) 4
Corpus	(15) 3
Antrum & corpus	(40) 8

Table 5.Correlation between grade of gastritis and iron studied parameters.

Grade Of gastritis	r	*P-value
Serum ferritin (ng/ml)	-0.922	<0.001
Transferrin Saturation (%)	-0.927	<0.001
HB(g/dl)	-0.906	<0.001

* significant at *P*<0.05

Stool antigen test done 6 weeks post sequential therapy, confirmed successful eradication in 18 out of 20 patients. Laboratory investigations done 6 weeks post therapy (eradication + iron) showed, significant increase of hemoglobin (10.35±0.79 vs. 9.51±0.72), serum ferritin (29.8±7.61 vs. 19.45±3.28) and transferrin saturation (19.35±3.57 vs. 15.58±2.24) in 18 out of 20 patients compared to their levels at the start of the study (*P*<0.001 for each of them). Regarding the 2 cases with unsuccessful eradication, there was no change of their hemoglobin or iron parameters at 6 weeks follow up investigations. The two cases received another 14 days therapy with Amoxicillin (1gm), clarithromycin (500 mg) and PPI (20 mg) twice daily, but they were excluded from the study at this stage. At 12 weeks post therapy, laboratory investigations done for the 18 patients who completed the study, showed significant increase of hemoglobin, serum ferritin and transferrin saturation compared to their level at the start of the study and to their values at 6 weeks post therapy, (*P*<0.001) (Table 6).

Table 6. Serial changes of serum ferritin, transferrin saturation and hemoglobin level

Variables		Range	Mean±SD	Paired t-test *P*-value	
Serum ferritin (ng/ml)	Baseline	15-24	19.45±3.28	*P*1	<0.001*
	After 6ws.	14-45	29.80±7.61	*P*2	<0.001*
	After 12ws	34-65	44.67±9.37	*P*3	<0.001*
Transferrin Saturation (%)	Baseline	12-19	15.58±2.24	*P*1	<0.001*
	After 6ws.	12-25	19.36±3.57	*P*2	<0.001*
	After 12ws	21-30	25.07±2.81	*P*3	<0.001*
HB(g/dl)	Baseline	8.4-10.	9.51±0.72	*P*1	<0.001*
	After 6ws.	8.4-11.2	10.35±0.78	*P*2	<0.001*
	After 12ws	10.7-11.8	11.26±0.33	*P*3	<0.001*

P1 values at 6 weeks compared to base line. *P*2: values at 12 weeks compared to base line. *P*3: values at 12 weeks compared to values at 6 weeks * significant at *P*<0.05

Discussion

Iron deficiency anemia is one of the most common organic disorders in clinical practice (Lee *et a*l., 1999), therefore WHO suggested that, researchers should investigate its etiology and then develop the best therapeutic strategies for dealing with it (Ramakrishnan, 2002). Gastroenterologists who investigate patients with iron deficiency anemia and no gastrointestinal symptoms justifiably direct most effort at identifying an occult bleeding source. However, upper and lower gastrointestinal investigations will identify lesions of this type in only around half of the patients (Rockey *et al*., 1993), raising the question whether there are yet unrecognized causes of iron deficiency anemia (David 2005).

Chronic *H. pylori* infection is the main cause of chronic gastritis leading to atrophy of gastric glands with resultant gastric acid hyposecretion. This occurs mainly when infection by *H. pylori* involves the corpus with related increase in Intragastric PH (Zhang *et al*., 1998). This may be the case in our study as 65% of our patients had pangastritis and 75% of the patients had gastric atrophy. The association between *H. pylori* infection, chronic gastritis and gastric atrophy in patients with IDA was observed by Nahon *et al*., (2003) who reported a significantly higher incidence of gastric atrophy in the iron deficiency anemia group than matched controls.

In this study we had found inverse correlation between grade of gastritis and the degree of anemia, this was against an old study in 1964 by Ikkala *et al*., they evaluated gastric histology and iron studies in 100 patients with iron deficiency anemia. They found that 75% of their patients had histological evidence of gastritis, but the authors noted no difference in serum iron or hemoglobin according to severity of gastritis. This difference between their observations and findings of our study may be due to the more advances that occurred in histopathological examination and the more recent grading and classification systems for gastritis that enabled us to assess gastritis more accurately and to do better relation with degree of anemia.

Raia *et al*., (2011) studied 40 antenatal women between 14-16 week gestation, with mild to moderate IDA and having *H. pylori* infection as detected by stool antigen test. These women were randomly divided into group I (n=20): *H. pylori* treatment group (amoxicillin, clarithromycin, omeprazol) for two weeks and group II (n=20): placebo group. Both groups received therapeutic doses of iron and folic acid. Eradication therapy resulted in significantly better response to oral iron supplementation among *H. pylori* infected pregnant women with IDA.

In a study by Chen *et al.,* (2007), a total of 86 IDA adult patients with *H. pylori* positive gastritis were enrolled. They were divided into two groups: group A, receiving ferrous succinate combined with triple therapy for *H. pylori,* and group B, treated with ferrous succinate only. During treatment of IDA, dynamic changes in hemoglobin level and iron parameters were compared between the groups. They concluded that treatment of *H. pylori* can enhance the efficacy of ferrous succinate therapy in IDA patients with *H. pylori* positive chronic gastritis.

Annibale *et al*., (1999), have studied 30 patients with long standing iron deficiency and no gastrointestinal pathology except *H. pylori* gastritis. Six months after eradication of *H. pylori* and discontinuation of iron replacement therapy, there has been noticeable recovery from anemia in 75% with corresponding increase in ferritin level.

Twelve months later 91.7% have also been recovered.

An explanation for a relationship between *H. pylori* infection and IDA involves the possible effect of *H. pylori* gastritis on gastric acid secretion and hence iron absorption.(Lombard *et al*.,1997) It is also thought that the bacteria exerts a negative effect on iron balance by chronic blood loss from the gastrointestinal tract (Konno *et al.,* 2000). Similar to many bacteria, *H. pylori* requires iron as a growth factor and it has been found that it possess an iron binding protein resembling ferritin morphologically and biochemically. This ferritin like protein has an important role in storage of iron sequestered by the bacteria as well in protection against toxicity in conditions of iron excess. (Doig *et al*., 1993)

H. pylori can uptake iron from gastric mucosa through a receptor mediated lactoferrin acquisition method. Lactoferrin is an iron binding protein found in body fluids including milk, lacrimal secretions, saliva and urine and its secretion in the gastric mucosa seems to be influenced by some signals from *H. pylori*. It appears that when *H. pylori* was grown in iron restricted environment it leads to signals that increase lactoferrin secretion from the gastric mucosa then absorbs the iron from lactoferrin by specific receptors that leads to iron depletion and IDA (Choe *et al*., 2003).

From the results of our study we can conclude that good assessment of patients with IDA of no obvious cause should put in consideration *H. pylori* infection. Eradication of *H. pylori* may be followed by an improved response to oral iron therapy in previously refractory IDA patients.

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