The role of endoscopic gastric biopsy in assessment of patients with unexplained iron deficiency anemia

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Abstract: Background: Anemia is a world wide problem and iron deficiency is the most prevalent cause especially in developing countries. There is an increased importance of gastrointestinal tract (GIT) as a cause for Iron Deficiency Anemia (IDA) resulting from iron malabsorption due to gastric mucosal changes or iron loss via bleeding gastrointestinal tract lesions. Nowadays, there has been an increased attention towards gastric mucosa in iron malabsorption and IDA through atrophic gastritis and *Helicobacter pylori* related gastritis. **Objective:** The aim of the present study is to evaluate the diagnostic value of gastric biopsy in patients with IDA. **Patients & Methods:** twenty patients with IDA were included in our study, they were subdivided into two groups; ***Group I:*** 13 patients newly diagnosed as IDA with no obvious cause. ***Group II*****:** 7 patients with probable cause for IDA refractory to oral iron supplementation therapy. ***Group III:*** ten patients without anemia, matching for age and sex as control group and have had upper GI endoscopy for any cause rather than anemia. All patients included in the study were subjected to full history taking, complete clinical assessment,laboratory investigations (complete blood count, complete iron study and occult blood in stool), abdominal and pelvic ultrasound and upper GI endoscopy with multiple fundal and antral gastric biopsies for histopathological evaluation of the biopsies as regard grading, topography and staging of gastritis,then detection of *H.pylori* infection by Giemsa and immunoperoxidase stain. **Results:** There was significant difference between group I and control group as regard hemoglobin level, serum iron, transferrin saturation and serum ferritin. Also, there was significant difference between group II and control group as regard the same parameters. While, in comparing group I and group II there was only significant difference as regard hemoglobin level. There was significant increase in percentage of *H.pylori* infection in anemic group than the control group with percentage ratio of (95.00%) versus (60.00%) respectively. Moreover, infection by *H.pylori* in anemic group was mainly in both corpus and antrum (65.00%) in comparison to the control group that had infection mainly in antrum (40.00%). There was no recorded cases in corpus alone in neither patient nor control group. There was an increased (but not significant) percentage of infection by *H.pylori* in group I than group II with percentage ratio of (100.00%) versus (85.72%) respectively. Also, as regard topography of infection there was insignificant difference between the two groups (*P*-value=0.520%). There was inverse relation between Hb level and grade of gastritis, and there was significant decrease of Hb level in cases of combined atrophy in both corpus and antrum (*P*-value<0.028*), the same was for *H.pylori* infection (*P*-value<0.001*). There was significant decrease in serum ferritin level in patients with grade IV gastritis (*P*-value= 0.033*). **Conclusion:** IDA was highly associated with severe grades of gastritis, atrophy of gastric mucosa and intestinal metaplasia that may be a cause for anemia even in patients with other probable cause. Also, *H.pylori* infection had been noticed to be more prevalent in patients with IDA than control.

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1. Introduction

Anemia is defined as a decrease in red blood cell (RBC) mass. The function of the RBC is to deliver oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. This is accomplished by using hemoglobin (Hb). Anemia impairs the body's ability for gas exchange by decreasing the number of RBCs transporting oxygen and carbon dioxide [(1)]. Iron Deficiency Anemia (IDA) is a decrease in the total hemoglobin levels caused by a lack of sufficient iron [(2)]. IDA is the most common nutritional deficiency worldwide. It can cause reduced work capacity in adults and impact motor and mental development in children and adolescents [(3, 4)].

Gastrointestinal (GI) diseases are among the most common etiologies of IDA because the GI tract is a common site of blood loss and GI diseases may cause malabsorption of iron [(5)]. IDA is usually due to chronic GI blood loss when there is no obvious source of bleeding. The standard of care for these patients with IDA includes evaluation of the GI tract for bleeding lesions [(6)]. IDA is considered as an alarm sign for the presence of possible GI malignancies, and inadequate evaluation of patients with IDA may delay

the diagnosis of GI tumors especially colorectal cancer [7].

Conventional upper GI endoscopy and colonoscopy are the most important routes for the recognition of IDA. Endoscopic findings revealed at least one likely cause for IDA in 86.7% of patients; causes included: bleeding, colon cancer, peptic ulcer, non bleeding related, atrophic gastritis, helicobacter pylori and celiac disease [6]. Studies have shown that increasing age, male gender, decreased ferritin level, prior NSAIDs use, positive fecal occult blood test were factors predictors of endoscopic lesions in patients with IDA with and without GI symptom [8].

Routine gastric and duodenal biopsies should be considered in all patients undergoing upper endoscopy for IDA in order to determine the cause of anemia [9]. There is strong evidence supporting the role of the gastric mucosa in unexplained or refractory IDA in two clinical entities which are not yet widely recognized as common causes of IDA; autoimmune atrophic gastritis manifested by hypergastrinemia in combination with antiparietal cell antibodies (APCA) and *H.pylori* gastritis, with considerable overlap between these two conditions [10].

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* (*H.pylori*) is by far the most common cause of chronic active gastritis worldwide [11].

There is no universally accepted classification for gastritis; however the most two popularly used classifications are both the original Sydney System (1990) [12] and its updated version (1994) [13]. The updated Sydney Systems took into account the topographical distribution of the elementary lesions in the different gastric compartments and recommended that multiple endoscopic biopsy samples to be taken from predefined sites of the stomach [13]. Five main sites were considered necessary: greater and lesser curvature of the distal antrum (mucus-secreting mucosa), greater and lesser curvature of the proximal corpus (oxyntic mucosa), and lesser curvature at the incisura angularis, where the earliest atrophic-metaplastic changes tend to occur [14].

The topography of histological gastritis is an important risk factor for gastric cancer. Imagawa *et al.* (2007), had studied *H.pylori* -positive patients undergone diagnostic upper GI endoscopy. Biopsy specimens were obtained from the gastric antrum and body to assess the grade of gastritis. Three types of active gastritis had been known topographically; antrum predominant gastritis (AP), pan-gastritis (PAN) and corpus predominant gastritis (CP) [15].

Using the framework provided by the Sydney System's (1994) and the Atrophy Club's analytic approach (2000), Rugge and Genta (2005) described a proposal for grading and staging scheme that integrates the relevant histopathological data gathered and interpreted by the pathologist [16]. Grading is considered a measure of the severity of the inflammatory lesions. It represents the semiquantitative assessment of the combined severity of mononuclear and granulocytic inflammation scored in both antral and oxyntic biopsy samples. Grades range from 0 (absence of inflammatory cells in any of the specimens) to 4 (a very dense infiltrate in all the biopsy samples) [17]. Staging refers to the extent of atrophy with or without intestinal metaplasia (IM). The stage of chronic gastritis is related to both its duration and to the host's response to the etiological agent(s) and may have implications for the prognosis and management [18].

Atrophic gastritis is a histopathologic entity characterized by chronic inflammation of the gastric mucosa with loss of gastric glandular cells and replacement by intestinal-type epithelium, pyloric-type glands, and fibrous tissue. Atrophy of the gastric mucosa is the endpoint of chronic processes, such as chronic gastritis associated with *H. pylori* infection, other unidentified environmental factors, and autoimmunity directed against gastric glandular cells [19].

Environmental metaplastic atrophic gastritis (EMAG), also called multifocal atrophic gastritis (MAG), is characterized by the involvement of the antrum and body with mucosal atrophy and intestinal metaplasia. The pathogenesis of EMAG is multifactorial [20]. Recent studies suggest a role for *H.pylori* in the early pathogenesis of autoimmune gastritis; evidence of infection early in the course of the disease in individuals with parietal cell antibodies is frequent [21].

H.pylori are unique bacteria ideally suited to live in the acidic environment of the human stomach. Their spiral shape and multiple unipolar flagella allow them to move freely through the gastric mucous layer, where they remain protected from low gastric pH [22]. The ultimate clinical manifestations 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 [23].

Histologically, there is diffuse, chronic inflammatory infiltrate which includes numerous lymphocytes and plasma cells that expand the lamina propria and epithelium with injury to the surface with loss of apical mucin and reactive nuclear changes and erosions. Also lymphoid follicles with germinal

centers are characteristic of an infection with *H.pylori* [24].

It was a matter of debate how can chronic gastritis with underlying *H.pylori* infection cause IDA. Several mechanisms have been proposed to explain this possible causal relationship. The most accepted possible pathogenic mechanisms are: ***I)*** decreased iron absorption secondary to chronic gastritis and hypo or achlorhydria [25], ***II)*** occult blood loss secondary to chronic erosive gastritis : there have been reports of *H.pylori* -associated hemorrhagic gastritis associated with iron deficiency that may add a view of evidence to this theory [26, 27]), ***III)*** increased iron uptake and utilization by bacteria[28, 29] and ***IV)*** sequestration of iron in lactoferrin in the gastric mucosa and its uptake [30].

2.Patients and Methods

This study was conducted on twenty patients with IDA who were referred to endoscopy unit of internal medicine department of Tanta University Hospitals for upper GI endoscopy as a part of the investigations for IDA and ten patients without IDA as a control group. Written informed consent was taken from patients and the protocol was approved by local ethical committee of Tanta Faculty of Medicine.

IDA was defined as hemoglobin level less than 13g/dl for males and 12 g/dl for females with MCV less than 80 fl with at least one of the following: a) serum ferritin ≤25ug/l ; b)iron concentration ≤8umol/l or with transferrin saturation <20%.

Our patients were classified into 3 groups; *group I:* 13 Patients newly diagnosed as IDA with no obvious cause; *group II:* 7 Patients with probable cause for IDA who were referred to our endoscopy unit because of refractoriness to oral iron supplementation despite accepted period of therapy & *group III (Control group):* 10 patients, without IDA, matching for age and sex, who were undergoing upper GI endoscopy (with biopsies from gastric fundus and antrum) for any other cause rather than anemia and in whom there was no gross endoscopic lesion.

All patients included in the study were subjected to full history taking, complete clinical examination and laboratory investigations including complete blood count, iron study (serum ferrtin, transferrin saturation, serum iron & total iron binding capacity), occult blood in stool, abdomen and pelvis ultrasound and upper GI endoscopy with gastric biopsy.

For endoscopic examination; patients were instructed to fast for 10 hours before the procedure then sedation was done using midazolam and the patient lied on left lateral position then endoscopic evaluation was done using Olympus fibroptic version up to the second part of the duodenum. Multiple fundal and antral biopsies were taken (at least two biopsies from each site).

All gastric mucosal biopsy specimens were fixed in 10% buffered formalin, processed for routine histological preparations, cut at 4 μm, and stained with hematoxylin and eosin for routine histopathological diagnosis. Gastritis was evaluated according to the updated Sydney system (Dixon *et al.,*1996) in corpus and antral samples [13].

The different parameters scored included: **chronic inflammation;** (mononuclear inflammatory cells e.g. lymphocytes, plasmocytes), **activity**; (polymorph nuclear inflammatory cells), **atrophy;** metaplastic epithelial **transformation;** (intestinal metaplasia in both antral and/or oxyntic biopsy samples and pseudopyloric metaplasia in oxyntic biopsy samples and the presence of lymphoid aggregates and lymphoid follicles). These parameters were graded from absent (0), mild (1), moderate(2) and severe (3). Active gastritis was scored on the basis of the topographic locations according to Imagawa *et al.* [15], (2007) into: (i) antrum-predominant gastritis (AP), (ii) corpus-predominant gastritis (CP) and (iii) pangastritis (PAN). Mucosal atrophy and metaplasia was classified according to Nahon *et al.*, (2003) into: (i) antrum-alone (ii) corpus-alone and (iii) combined antrum and corpus [31].

Tissue sections from corpus and antrum were stained with Giemsa stains (Gray *et al.,*1986) [32] and immunohistochemical stain against polyclonal antibody directed against the whole *H. pylori* organism (Rabbit polyclonal antibody) using Thermo scientific ready to use staining to maximize the diagnostic accuracy.

Statistical presentation and analysis of the present study was conducted, using the mean, standard error, unpaired student t-test, chi-square, Analysis of variance [ANOVA] tests, Mann-Whitney and Kruskal-Wallis by SPSS V17 [33].

3.Results

I-Demographic and clinical results:

there was no significant statistical difference between patient and control group as regards age, sex, history of non steroidal anti inflammatory drugs (NSAIDs) and smoking. GIT symptoms were significantly elevated in the control group (*P*-value =0.002) (Table 1). There was significant increase in duration of iron therapy in group II (the mean was 6.357±1.282 months) than group I (the mean was 0.813±0.239 months) (*P*-value<0.001) (Table 2).

II-Laboratory results:

There was significant difference between group I and control group as regard hemoglobin level, serum iron, transferrin saturation and serum ferritin (*P*-value =0.000, 0.000, 0.001 and 0.000 respectively). Also, there was significant difference between group II and control group as regard the same parameters (*P*-value =0.001, 0.000, 0.001 and 0.000 respectively). While, in comparing group I and group II there was only significant difference as regard hemoglobin level (*P*-value =0.027*) (Table 3).

III-Endoscopic results:

Upper endoscopic examination done for our patient and control groups has revealed normal upper endoscopy in 7 out of 20cases (35.00)% of patient groups in comparison to 6 out of 10cases (60.00%) in control group with minimal endoscopic finding in rest of studied patient and control groups (Table 4). Patients in whom upper endoscopic examination has revealed gross endoscopic lesion were excluded from the study.

IV-Histopathological results:

As regards the topography of gastritis; all the cases were classified into three groups on the basis of the topographic locations (corpus alone, antral alone or present in both). In total anemic group (20 cases) pangastritis was more predominant (85%) in comparison to antral predominant gastritis (15%), which was the same in control group with percentage ratio of (60.00%) versus (40.00%) respectively. In all studied groups there was no corpus predominant gastritis (Table 5). Pangastritis was more evident in group I than group II with percentage ratio of (92.31%) versus (71.43%) respectively. While, antral gastritis was more evident in group II than group I with percentage ratio of (28.57%) versus (7.69%) respectively (Table 6). As regards the scoring of inflammation; for antrum; there was significant increase in percentage of severe gastritis in anemic patient groups (40.00%) in comparison to control group (20.00%) (*P*-value = 0.025). For corpus; there was no significant difference between anemic and control group concerning severe gastritis (*P*-value = 0.130) (Table 7). As regard the antrum; severe gastritis was more evident (but not significant) in group I than group II with percentage ratio of (46.15%) versus (28.57%) respectively. But, as regard the corpus; severe gastritis was more evident (but not significant) in group II than group I with percentage ratio of (42.84%) versus (15.39%) respectively table (8). As regards grading of gastritis; there was significant increase in percentage of grade IV gastritis (45.00%) in patient group in comparison to the control group that didn't exhibit any case with grade IV gastritis (00.00%) (*P*-value = 0.036). (Table 9). There was insignificant difference between groups I and II as regard grade IV gastritis (*P* -value =0.537). (Table 10). As regards gastric atrophy; in total anemic group (20 cases); there was non significant increased percentage of gastric atrophy (85.00%) in comparison to the control group (60.00%). Moreover, gastric atrophy in anemic groups was mainly combined in both corpus and antrum (55.00%) in comparison to the control group that had similar percentage of atrophy in corpus, antrum and both (20.0%) for each and this was also statistically non significant (Table 11). There was an increased (but not significant) percentage of gastric atrophy in group I than group II with percentage ratio of (92.31%) versus (71.43%) respectively. As regard topography of atrophy there was also insignificant difference between the two groups (*P* -value = 0.700%) (Table 12). As regards intestinal metaplasia; there was significant increase in percentage of intestinal metaplasia in patient group (65.00%) in comparison to control group (20.00%) (*P* -value =0.05*). Moreover, intestinal metaplasia in anemic group was mainly in both corpus and antrum (30.00%) in comparison to the control group that had metaplasia only in antrum (20.00%). (Table 13). There was an increased (but non significant) percentage of intestinal metaplasia in group I than group II with percentage ratio of (76.92%) versus (42.86%) respectively Also, as regard topography of intestinal metaplasia there was insignificant difference between the two groups apart from absence of intestinal metaplasia in antrum of group II (*P* -value=0.531%) (Table 14). As regards presence of *H.pylori*; the overall *H.pylori* positive cases were 19 out of 20 cases (95%) in the anemic group. There was significant increase in percentage of *H.pylori* infection in anemic group than the control group with percentage ratio of (95.00%) versus (60.00%) respectively (*P*-value =0.003). Moreover, infection by *H.pylori* in anemic group was mainly in both corpus and antrum (65.00%) in comparison to the control group that had infection mainly in antrum (40.00%). There was no recorded cases in corpus alone in neither patient nor control group. (Table 15). There was an increased (but not significant) percentage of infection by *H.pylori* in group I than group II with percentage ratio of (100.00%) versus (85.72%) respectively. Also, as regard topography of infection there was insignificant difference between the two groups (*P*-value=0.520%) (Table 16). There was inverse relation between Hb level and grade of gastritis, the more severe the degree of gastritis, the less Hb level with the least Hb level with grade IV gastritis.(*P*-value<0.001*). Concerning topography of atrophy there was significant decrease of Hb level in cases of combined atrophy in both corpus and antrum (*P*-value<0.028), the same was for *H.pylori* infection (*P*-value<0.001) (Table 17). There was significant decrease in serum ferritin level in patients

with grade IV gastritis (*P*-value= 0.033*). Also, as regard topography of *H.pylori* infection there was significant decrease in serum ferritin level in patients with infection in both corpus and antrum (pangastritis) (*P*-value = 0.03) (Table 18).

Table (1): Demographic and clinical data of anemic patient groups and control group.

Demographic and Clinical data		Patient group		Control group		Chi-square	
		N	%	N	%	X^2	*P*-value
Sex	Female	15	75.00	7	70.00	0.021	0.883
	Male	5	25.00	3	30.00		
Age (Years)	Range	17-65		22-57		T=1.055	0.327
	Mean+SD	40±15.169		45.6±12.903			
Smoking	Non smoker	17	85.00	8	80.00	0.120	0.729
	Smoker	3	15.00	2	20.00		
History of NSAIDs	Negative	11	55.00	6	60.00	0.068	0.794
	Positive	9	45.00	4	40.00		
History of GIT symptoms	Negative	12	60.00	0	0.00	10.000	0.002*
	Positive	8	40.00	10	100.00		

P-value <0.05(significant)

Table (2): Comparison between group I and group II as regard duration of iron therapy.

Iron therapy	Duration of iron therapy(month)		T-test	
	Range	Mean ± SD	t	*P* -value
Group I	0.500 - 1.000	0.813 ± 0.239	-8.380	<0.001*
Group II	4.500 - 8.000	6.357 ± 1.282		

Table (3): Comparison between all studied groups as regard laboratory data.

Laboratory data.				ANOVA		Tuky's test	
		Range	Mean ± SD	F	*P*-value		
Hb % (gm/dL).	Group I	4.500 - 11.200	7.385 ± 1.957	31.055	0.000	P1	0.027*
	Group II	5.200 - 11.700	9.400 ± 1.739			P2	0.000*
	Control Group	12.000 - 13.100	12.560 ± 0.455			P3	0.001*
Serum Iron (umol/L).	Group I	5.000 - 7.210	5.757 ± 1.452	29.598	0.000	P1	0.140
	Group II	4.100 - 7.900	6.797 ± 0.824			P2	0.000*
	Control Group	8.560 - 10.570	9.372 ± 0.745			P3	0.000*
Trans. Satur (%).	Group I	9.200 - 17.300	13.527 ± 3.020	14.655	0.000	P1	0.993
	Group II	10.000 - 19.000	14.919 ± 3.781			P2	0.001*
	Control Group	20.540 - 66.000	38.674 ± 19.853			P3	0.001*
S.ferritin (ug/L).	Group I	0.100 - 23.100	7.409 ± 7.240	19.342	0.000	P1	0.999
	Group II	1.100 - 20.120	7.769 ± 9.585			P2	0.000*
	Control Group	25.650 - 82.900	49.484 ± 27.905			P3	0.000*

*P*1=Comparison between group I and group II *P* 2=Comparison between group I and control group
P 3=Comparison between group II and control group

Table (4): The main abnormal findings revealed by upper endoscopy in all studied groups.

Abnormal Upper Endoscopic Findings	Anemic patient groups (20)		Control group(10)	
	N	%	N	%
Incompetent cardia with reflux esophagitis	**3**	**15.00**	**1**	**10.00**
Sliding hiatal hernia	**1**	**5.00**	**2**	**20.00**
Fundal gastritis	**1**	**5.00**	**0**	**0.00**
Biliary reflux	**2**	**10.00**	**1**	**10.00**
Antral erythema	**3**	**15.00**	**0**	**0.00**
Prepyloric erosions	**1**	**5.00**	**0**	**0.00**
Bulb duodenitis	**2**	**10.00**	**0**	**0.00**
Total	**13**	**65.00**	**4**	**40.00**

Table (5): Comparison between total anemic group and control group on the basis of the topographic locations of gastritis.

Topography		Total patient groups(20)		Control Group(10)	
		N	%	N	%
Antral predominant		3	15.00	4	40.00
Corpus predominant		0	0.00	0	0.00
Pangastritis		17	85.00	6	60.00
Chi-square	X^2	1.141			
	P-value	0.2854			

Table (6): Comparison between group I and group II on the basis of the topographic locations of gastritis.

Topography		Group I		Group II	
		N	%	N	%
Antral predominant		1	7.69	2	28.57
Corpus predominant		0	0.00	0	0.00
Pangastritis		12	92.31	5	71.43
Total		13	100.00	7	100.00
Chi-square	X^2	0.349			
	P-value	0.5546			

Table (7): Comparison between total anemic groups and control group as regard scoring of gastritis.

Scoring		Total anemic group(20)		Control group(10)		Mann whitny	
		N	%	N	%	Z	*P*-value
Corpus	Absent	3	15.00	4	40.00	1.52	0.130
	Mild	7	35.00	4	40.00		
	Moderate	5	25.00	0	0.00		
	Severe	5	25.00	2	20.00		
Antrum	Absent	0	00.00	0	0.00	4.721	0.025*
	Mild	4	20.00	6	60.00		
	Moderate	8	40.00	2	20.00		
	Severe	8	40.00	2	20.00		

Table (8): Comparison between group I and group II as regard scoring of gastritis (severe gastritis).

	Scoring	Group I (13)		Group II (7)		Mann whitny	
		N	%	N	%	Z	*P*-value
Corpus	Absent	1	7.69	2	28.570	-0.37	0.710
	Mild	6	46.15	1	14.285		
	Moderate	4	30.77	1	14.285		
	Severe	2	15.39	3	42.860		
Antrum	Absent	0	0	0	0	-0.85	0.39
	Mild	2	15.39	2	28.570		
	Moderate	5	38.46	3	42.860		
	Severe	6	46.15	2	28.570		

Table (9): Comparison between total anemic group and control group as regard grading of gastritis.

Grading of gastritis	Total anemic group		Control group		Chi-square	
					X^2	*P*-value
	N	%	N	%		
Grade 0	0	00.00	0	0.00	4.462	0.036*
Grade I	2	10.00	4	40.00		
Grade II	6	30.00	2	20.00		
Grade III	3	15.00	4	40.00		
Grade IV	9	45.00	0	0.00		

Table (10): Comparison between group I and group II as regard grading of gastritis.

Grading of gastritis		Group I		Group II	
		N	%	N	%
Grade 0		0	0.00	0	0.00
Grade I		1	7.69	1	14.29
Grade II		4	30.77	2	28.57
Grade III		3	23.08	0	0.00
Grade IV		5	38.46	4	57.14
Chi-square	X^2	2.173			
	P-value	0.537			

Table (11): Comparison between total anemic group and control group as regards presence of atrophy and its topography.

Atrophy		Total anemic group		Control group		Chi-square	
		N	%	N	%	X^2	*P*-value
Positive		17	85.00	6	60.00	1.141	0.285
Negative		3	15.00	4	40.00		
Site	Antrum	5	25.00	2	20.00	3.357	0.186
	Corpus	1	5.00	2	20.00		
	Antrum & Corpus	11	55.00	2	20.00		

Table (12): Comparison between group I and group II as regards presence of atrophy and its topography.

Atrophy		Group I		Group II		Chi-square	
		N	%	N	%	X^2	*P*-value
Positive		12	92.31	5	71.43	0.349	0.554
Negative		1	7.69	2	28.57		
Site	Antrum	3	23.08	2	28.57	0.711	0.700
	Corpus	1	7.69	0	0.00		
	Antrum & Corpus	8	61.54	3	42.86		

Table (13): Comparison between total anemic group and control group as regards presence of intestinal metaplasia and its topography.

Intestinal metaplasia		Total anemic group		Control group		Chi-square	
		N	%	N	%	X^2	*P*-value
Positive		13	65.00	2	20.00	3.75	0.05*
Negative		7	35.00	8	80.00		
Site	Antrum	3	15.00	2	20.00	4.615	0.099
	Corpus	4	20.00	0	0.00		
	Antrum & Corpus	6	30.00	0	0.00		

Table (14): Comparison between group I and group II as regards presence of intestinal metaplasia and its topography.

Intestinal metaplasia		Group I		Group II		Chi-square	
		N	%	N	%	X^2	*P*-value
Positive		10	76.92	3	42.86	1.065	0.302
Negative		3	23.08	4	57.14		
Site	Antrum	3	23.08	0	0.00	0.264	0.531
	Corpus	3	23.08	1	14.29		
	Antrum & Corpus	4	30.76	2	28.57		

Table (15): Comparison between total patient groups and control group as regards presence of infection by *H.pylori* and its site.

H.pylori infection		Total anemic group		Control group		Chi-square	
		N	%	N	%	X^2	*P*-value
Positive		19	95.00	6	60.00	8.408	0.003*
Negative		1	5.00	4	40.00		
Site	Antrum	6	30.00	4	40.00	1.106	0.2930
	Corpus	0	00.00	0	0.00		
	Antrum & Corpus	13	65.00	2	20.00		

Table (16): Comparison between group I and group II as regards infection by *H.pylori* and its topography.

H.pylori infection		Group I		Group II		Chi-square	
		N	%	N	%	X^2	*P*-value
Positive		13	100.00	6	85.72	0.104	0.747
Negative		0	00.00	1	14.29		
Site	Antrum	3	23.08	3	42,86	0.413	0.520
	Corpus	0	00.00	0	00.00		
	Antrum & Corpus	10	76.92	3	42.86		

Table (17): Relation between Hb level and grading of gastritis, gastric atrophy & *H.pylori* infection in the patient group.

Histological data		Hb		ANOVA	
		Range	Mean ± SD	f	P-value
Grading	GI	11.200 - 11.700	11.450 ± 0.354	11.472	<0.001*
	GII	7.300 - 11.000	9.333 ± 1.329		
	GIII	7.500 - 9.000	8.300 ± 0.755		
	GIV	4.500 - 9.600	6.444 ± 1.419		
Atrophy	Negative	10.500 - 11.700	11.133 ± 0.603	3.953	0.028*
	Antrum	5.200 - 11.000	7.983 ± 2.167		
	Corpus	6.500 - 6.500	6.500 ± .		
	Pan	4.500 - 9.600	7.400 ± 1.606		
H.pylori	negative	11.700 - 11.700	11.700 ± .	t=5.33	<0.001*
	Antrum	9.000 - 11.200	10.117 ± 0.909		
	Pan	4.500 - 9.200	6.877 ± 1.342		

Table (18): Relation between serum ferritin and grading of gastritis, gastric atrophy & *H.pylori* infection in the patient group.

Histological data		S.ferritin(ug/L)		Kruskal-Wallis Test	
		Range	Mean ± SD	X^2	*P*-value
Grading	GI	3.060 - 5.200	4.130 ± 1.513	8.77	0.033*
	GII	3.010 - 25.000	14.590 ± 9.346		
	GIII	2.100 - 19.200	10.433 ± 8.558		
	GIV	0.100 - 6.400	2.622 ± 1.970		
Atrophy	Negative	3.060 - 20.120	9.460 ± 9.294	2.60	0.456
	Antrum	0.100 - 23.100	8.800 ± 9.862		
	Corpus	0.300 - 0.300	0.300 ± 0.000		
	Pan	2.100 - 25.000	6.922 ± 7.031		
H.pylori	Negative	3.060 - 3.060	3.060 ± 0.000	Z=-2.10	0.03*
	Antrum	4.100 - 23.100	12.970 ± 8.704		
	Pan	0.100 - 25.000	5.371 ± 6.732		

4.Discussion

IDA is one of the most common organic disorder in clinical practice [(34)]. The most frequent cause of IDA is GI pathology [(35)].Gastroenterologists who investigate patients with IDA and no GIT symptoms direct most effort at identifying an occult bleeding source. However, upper and lower GI investigations will identify lesions of this type in only around half of the patients [(36)], raising the question whether there are yet unrecognized causes of iron deficiency [(37)].

This association of gastric atrophy with hypochromic anemia was established many years ago by Faber who firstly documented this link in 1913. Subsequently, Wintrobe and Beebe in 1933 reviewed 498 published cases of gastric atrophy with hypochromic anemia. They postulated iron malabsorption due to hypochlorhydria as the major cause of "idiopathic hypochromic anemia" [(38)].

In our study we have enrolled twenty patients already diagnosed as IDA on full clinical and laboratory data and ten subjects without anemia, matching for age and sex, who have had upper endoscopy for any cause rather than anemia as a control group. All patient and control groups have had upper GI endoscopy with fundal and antral biopsies. All biopsy speciements have been examined by the same pathologist and evaluated as regard fixed histopathological parameters. In our study we have found a strong association between IDA and chronic gastritis, *H.pylori* infection and gastric atrophy.

Our anemic patient groups have exhibited significant increase in percentage of severe antral gastritis (40.00%) in comparison to control group (20.00%) with *P*-value=0.025*. Also, there was significant increase in prevalence of grade IV gastritis in patient than control groups (45.00%) Vs (00.00%) respectively, *P*-value =0.036*. Moreover, anemic

patient groups have exhibited increased prevalence of gastric atrophy than control group (85.00%) Vs (60.00%) respectively; the same was as regard intestinal metaplasia with percentage ratio of (65.00%) Vs (20.00%) respectively.

This association between increased prevalence of severe gastritis and gastric atrophy in patients with IDA was supported by many studies. Hershko *et al.* (2005) [39], have found a similar proportion of patients with antral gastritis, in their studies on IDA. Annibale *et al.* (2001) [40], found gastric atrophy in 27% of patients referred for investigation of anemia without another obvious source of blood loss. Kaye *et al.* (2008) [38], have studied 161 patient with IDA, 26% of them had significant atrophy compared with 4% of controls, and this was highly significant.

In this study we have found a strong association between the grade of gastritis and degree of anemia in anemic patient groups with an inverse relationship as follow; grade I gastritis was associated with the highest Hb level (mean11.45±0.35gm/dL), the more the degree of gastritis the less Hb level, grade IV gastritis was associated with the least Hb level (mean 6.44±1.41gm/dL).

This was against Davidson and Markson (1955) [41], who compared gastric histology in 42 patients with IDA and 31 age matched controls and didn't find a relationship between degree of anemia and mucosal changes in patient group. This conflict 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 accurate and to do better relation with degree of anemia.

Our anemic patient groups have exhibited significant increase in the prevalence of *H.pylori* infection than control group (95.00%) Vs (40.00%) respectively (*P* value=0.003*). These results add a view of evidence to the relation between *H.pylori* infection and IDA. Also, an important evidence of this relationship is the normalization of Hb level and iron parameters after *H.pylori* bacterial eradication without iron supplementation. But, unfortunately it was not in our study design.

Annibale *et al.* (1999) [42], have also 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 a noticeable recovery from anemia in 75% of cases with corresponding increase in ferritin level. Twelve months later, 91.7% have also been recovered.

In a study of Korean children 2002, whose age was between 6–12 years (n = 753), *H.pylori* seropositivity correlated with lower serum ferritin levels (24 ng/ml vs 39 ng/ml; $p < 0.001$). The prevalence of iron deficiency (serum ferritin < 15 ng/ml) was also significantly higher in seropositive (13.9%) as compared to seronegative children (2.8%) [43].

However, a few epidemiologic studies didn't support an association between *H.pylori* infection and IDA. Collett *et al.* (1999) [44], found no significant differences in serum ferritin levels between *H.pylori* seropositive or seronegative males or females in a study of 1,060 adult subjects in Christchurch, New Zealand.

In this study we have also noticed that infection in patient group was mainly in both corpus and antrum (pangastritis) (65.00%) in comparison to control that has pangastritis only in (20.00%).This gives the idea that *H.pylori* infection occurs mainly in antrum but, if infection extended to corpus (pangastritis) it is more liable to affect iron absorption and cause IDA. This idea is supported by what proposed by Milman *et al.* (1998) [45], who speculated that if the bacteria in the stomach are diffuse and numerous, it will lead to not only antrum but also corpus affection. So, the affinity of iron binding and uptake by the bacteria may increase. The end of that will be IDA.

5.Conclusion

This study aimed primarily to evaluate the role of gastric biopsy in assessment of gastric mucosa in cases with unexplained iron deficiency anemia and to correlate this with clinical and laboratory data. This study has shown a high incidence of *H.pylori* infection, associated with severe and atrophic gastritis in patients with IDA. It is likely that this is causative or at least contributory in many patients. Based on this and previous work, routine gastric biopsies should be considered in patients undergoing upper gastrointestinal endoscopy as a part of investigations for iron deficiency anemia. In addition, screening for *H.pylori* infection should be a part of the work up done for the patients with iron deficiency anemia of unexplained origin.

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