Celiac disease and *Hp* infection association in Iran

M. Rostami-Nejad¹, V. Villanacci², R. Mashayakhi¹, M. Molaei¹, G. Bassotti³, H. Zojaji¹, D. Mirsattari¹, K. Rostami⁴ and M. R. Zali¹

¹Research Center of Gastroenterology and Liver Disease. Shaheed Beheshti University, M.C. Tehran, Iran.

²2nd Department of Pathology. Spedali Civili of Brescia. Italy. ³Gastroenterology and Hepatology Section. Department of Clinical and Experimental Medicine. University of Perugia. Italy. ⁴School of Medicine. University Hospital Birmingham. United Kingdom

ABSTRACT

Background: we assessed the prevalence, the related symptoms, and the endoscopic and histologic gastric features of celiac disease (CD) in patients with *Helicobacter pylori* (*Hp*).

Methods: 450 dyspeptic patients were studied. Biopsies of gastric antrum and duodenum, CD serology, and total IgA were obtained. Histological findings were scored with the Marsh-Rostami criteria.

Results: 411 (91.3%) patients were *Hp* positive. Duodenal histology was normal in 385 (85.6%) patients, 124 (27.5%) had duodenitis and 28 (6.2%) showed duodenal abnormalities (Marsh I-IIIc). Twenty three/28 (82.1%) patients with malabsorption pattern were also *Hp* positive. Serological analysis: 12 of 31 (38.7%) positive patients had abnormal histology (Marsh I-IIIc). Nine out of 450 patients were IgA deficient; none of them was serologically positive for CD.

Conclusion: although a high prevalence of *Hp* infection was found in this study, the relationship between *Hp* infection and CD was similar to that reported in other geographic areas.

Key words: Celiac disease. *H. pylori*.

INTRODUCTION

Celiac disease (CD) is frequently associated with abnormalities in gastric function and histology (1-8). *Helicobacter pylori* (*Hp*) is the causative agent in more than 90% of cases of chronic gastritis, peptic ulcer disease, primary gastric mucosa-associated lymphoid tissue

Rostami Nejad M, Villanacci V, Mashayakhi R, Molaei M, Bassotti G, Zojaji H, Mirsattari D, Rostami K, Reza Zali M. Celiac disease and *Hp* infection association in Iran. *Rev Esp Enferm Dig* 2009; 101: 850-854.

(MALT) lymphoma, and gastric cancer (9). A high prevalence of this infection would be also expected in patients with celiac disease (1-8). Atrophic gastritis with an increased prevalence of parietal cell antibodies seems to be common in some studies (1), but others have found little evidence to support this in CD (10).

Although the pathogenesis of CD is relatively well understood, the possibility that a chronic gastric infection capable of inducing duodenal ulcers could influence the inflammatory and immune responses in the small intestine and, therefore, the development and evolution of CD, should be considered (11,12).

Hp is now recognized as a major etiological factor in most patients with non-autoimmune chronic gastritis (2). In developing countries, *Hp* infects the majority of the population; for instance, the Iranian population displays a rate of infection of more than 90% (13).

Even though epidemiological studies have failed to reveal a connection between gastritis and CD (11,12), some studies showed that patients with *Hp* gastritis are more likely to have increased numbers of intraepithelial lymphocytes in the duodenal mucosa, and that this can be reversed by the eradication of *H. pylori* (14,15). Other works have focused on *Hp*-related lymphocytic gastritis in CD (16-19) and on the link between anemia and *Hp* infection in celiac patients (20,21).

Purposes of the present study were to assess the prevalence of celiac disease, its related symptoms, and endoscopic features, and to compare the histopathological and clinical features in patients with associated *Hp* infection.

Received: 14-05-09.

Accepted: 01-09-09.

Correspondence: Gabrio Bassotti. Clinica di Gastroenterologia ed Epatologia. Ospedale Santa Maria della Misericordia. Piazzale Menghini, 1. 06156 San Sisto (Perugia), Italy. E-mail: gabassot@tin.it

PATIENTS AND METHODS

In the period January 2007-December 2008, four hundred and fifty patients (211 men, 239 women, mean age 36 years, range 15-83 years) who underwent upper endoscopy for dyspeptic symptoms were recruited in the out-patient clinic of Taleghani hospital. After obtaining a written informed consent from all subjects upper endoscopy with gastric and duodenal biopsies was carried out. Two biopsy specimens were obtained from the gastric antral mucosa and two to four samples from different portions of the duodenum. Biopsies were fixed overnight in buffered formalin, embedded in paraffin, cut to 3- μ m thickness, and stained with hematoxylin-eosin for routine histological evaluation. Giemsa staining was also used for gastric specimens, to identify *Hp*. The slides were blindly evaluated by two expert gastrointestinal pathologists.

In accordance with the Updated Sydney System (22), the degree of gastric mucosal inflammation, polymorphonuclear cell infiltration, glandular atrophy, and intestinal metaplasia was classified into four grades as follows: 0 = none, 1 = mild, 2 = moderate and 3 = severe. *Hp* infection was considered positive if present at least in one of the biopsies examined.

The diagnosis of CD was based on the characteristic histological finding of increased intra-epithelial lymphocytes, villous atrophy, and crypt hyperplasia, classified according to the standard classification proposed by Marsh (23,24) and subsequently modified by Rostami et al. (25) (Table I).

Before endoscopy, a blood sample was drawn for serum anti-tissue transglutaminase (tTGA) and stored at -

70 °C until tested. Patients who had normal biopsies, but tested positive for tTGA in their serum, were asked to undergo retest and an intestinal biopsy 12 months later.

IgA-tTGA levels were measured by a commercially available ELISA method (AESKULISA tTGA, Germany). Serum values of tTGA higher than 15 U/mL were considered positive. Total serum IgA values were measured by an immunoturbidometric assay (Pars Azmoon, Iran), and serum levels below 70 U/mL were considered indicative of IgA deficiency. Immunoglobulin G (IgG) tTG values were further obtained in individuals with IgA deficiency by an ELISA method (AESKULISA tTGG, Germany).

RESULTS

Out of 450 patients 411 (91.3%) were *Hp* positive; associated symptoms/diseases were diabetes 26 (5.8%), anorexia 99 (22%), weight loss 143 (31.5%), nausea and vomiting 145 (32.2%), heartburn 261 (58%), bloating 320 (71.1%), flatulence 140 (31.1%), a concomitant stressful condition 117 (26%), and abdominal discomfort 351 (78%). Nine out of 450 recruited patients were IgA deficient and none of them were positive for IgG tTG.

Duodenal histology was normal in 385 (85.6%) patients, 124 (27.5%) had duodenitis, and 28 (6.2%) showed mucosal abnormalities compatible with CD (Marsh I-IIIc) (Table II).

Twenty three out of 28 patients (82%) with positive histology for CD had gastric biopsies positive for *Hp* (Fig. 1). The prevalence of *Hp* in patients without CD (86.2%) was slightly but not significantly higher. *Hp* in-

Table I. Histological classification for coeliac disease

	Marsh 0	Marsh I	Marsh II	Marsh IIIa	Marsh IIIb	Marsh IIIc
IEL/100 enterocytes	< 25/100EC	> 25/100 EC	> 25/100 EC	> 25/100 EC	> 25/100 EC	> 25/100 EC
Crypt hyperplasia	-	-	Hyper plastic	Hyper plastic	Hyper plastic	Hyper plastic
Villous atrophy	-	-	-	PVA	STVA	TVA
	Microscopic enteritis			Macroscopic enteritis		

EC: enterocytes; PVA: partial villous atrophy; STVA: subtotal villous atrophy; TVA: total villous atrophy.

Table II. Serology and type of mucosal lesions in patients with small bowel mucosal abnormalities

	MCG	ModCG	ModACG	SCG	SACG	Total	tTGA	Hp
Marsh I	5	3	1	1	2	12	4/12	10/12
Marsh II	2	1	1			4	2/4	3/4
Marsh IIIa		1		1	1	3	1/3	2/3
Marsh IIIb	2	2	1	1	1	7	3/7	6/7
Marsh IIIc					2	2	2/2	2/2
Total	9	7	3	3	6	28	12	23

MCG: mild chronic gastritis; ModCG: moderate chronic gastritis; ModACG: moderate chronic active gastritis; SCG: severe chronic gastritis; SACG: severe chronic active gastritis; tTGA: tissue transglutaminase antibody; *Hp*: *H. pylori*.

Fig. 1. Active gastritis with positivity for *Hp* (arrows). H&E x 10.

fection was more prevalent in patients younger than 40; as expected, most (91.4%) *Hp*-positive patients had moderate to severe chronic gastritis, whereas only 12 out of 39 (30.7%) *Hp*-negative patients had moderate or severe chronic gastritis.

Serological analysis showed that 12 out of 31 (38.7%) tTGA-positive patients had abnormal histology (four Marsh I, two Marsh II (Fig. 2) one Marsh IIIa, three Marsh IIIb (Fig. 3) and two Marsh IIIc (Fig. 4). In this group, one had mild chronic gastritis, three moderate chronic gastritis, two moderate active chronic gastritis, three severe chronic gastritis, and the last three had severe active chronic gastritis.

DISCUSSION

In this study we found that, in this geographic area of the Middle East, the prevalence of *Hp* infection in dyspeptic patients was higher than 90%. Patients with a malabsorption pattern had 42.8% positive serologies for CD, with sensitivity values ranging from 33.4% in Marsh IIIa patients to 100% in Marsh IIIc, which is in agreement with previously published results (26-28). According to literature results, in our series also only 33.4% of Marsh I patients showed positive serology. The Marsh degree in 5 patients who were *Hp*-negative included one Marsh I, one Marsh II, two Marsh IIIa and one Marsh IIIc. Moder-

Fig. 2. Marsh Type 1 lesion. A. H&E x 20. B. Immunohistochemistry for CD3 x 20.

ate chronic gastritis was more prevalent in *Hp*-infected patients.

Out of 31 serology positive patients, 19 patients had normal histology. In contrast, in the 28 patients with a malabsorption pattern, 16 had negative serology. Therefore, only 12 patients had both abnormal histology and positive serology related to CD.

Hp has been positively identified as the main cause of active chronic gastritis and its major complications (peptic ulcer disease, gastric adenocarcinoma, and primary gastric MALT lymphoma), with numerous studies carried out to explore the possible etiological role of this bacterium in a variety of both gastrointestinal and extra-intestinal conditions (28,29). Investigations on the relationship between *Hp* infection and CD have yielded conflicting results (11,12), probably because of the different prevalence of *Hp* in the populations studied.

As shown in table II, in our series 23/28 patients with a malabsorption pattern (Marsh I-IIIc) were also *Hp*-positive. Histological findings in these patients included six mild chronic gastritis, eight moderate chronic gastritis, three moderate active chronic gastritis, two severe chronic gastritis, and four severe active chronic gastritis cases.

Fig. 3. Marsh type 3A-3B. A. H&E x 20. B. Immunohistochemistry for CD3 x 20.

Fig. 4. Marsh type 3C. A. H&E x 20. B. Immunohistochemistry for CD3 x 20.

The four patients with Marsh I (two patients), Marsh II and IIIb who were positive for *Hp* and tTG-A had mild chronic gastritis, moderate active chronic gastritis, and severe active chronic gastritis, respectively. Two patients with Marsh II had moderate chronic and moderate active chronic gastritis. For Marsh IIIa, one patient had severe chronic gastritis. Three patients with Marsh IIIb had moderate chronic gastritis, severe chronic gastritis and severe active chronic gastritis. Two patients with Marsh IIIc had severe active chronic gastritis. These results showed that 9 out of 12 with serology positive for CD were infected with *Hp*. On the other hand 24/31 tTG positive were also positive for *Hp*.

It should be noted that, concerning *Hp* positivity, the percentage of *Hp*-positive celiac patients is lower than that of non-celiac patients and, as recently shown (29), the clinical features of CD patients are unrelated to the simultaneous presence of *Hp* gastritis, and there is no relation between gastritis and severity of mucosal damage in CD.

In conclusion, the prevalence of *Hp* infection in Iranian patients complaining of dyspeptic symptoms is high, and the relationships between *Hp* and CD are similar to those described in other geographic areas.

REFERENCES

- Gillberg R, Kastrup W, Mobacken H, Stockbrügger R, Ahren C. Gastric morphology and function in dermatitis herpetiformis and in coeliac disease. *Scand J Gastroenterol* 1985; 20: 133-40.
- Hansky J, Shiner M. Gastric studies in idiopathic steatorrhea. *Gastroenterol* 1963; 45: 49-56.
- O'Donoghue DP, Lancaster-Smith M, Johnson GD, Kumar PJ. Gastric lesion in dermatitis herpetiformis. *Gut* 1976; 17: 185-8.
- Primignani M, Agape D, Ronchi G, Forzenigo L, Bonato C, Meroni P, et al. Gastric histology and function tests in Italian patients with dermatitis herpetiformis. *Scand J Gastroenterol* 1990; 25: 357-62.
- Kastrup W, Mobacken H, Stockbrügger R, Swolin B, Westin J. Malabsorption of vitamin B12 in dermatitis herpetiformis and its association with pernicious anaemia. *Acta Med Scand* 1986; 220: 261-8.
- Stockbrügger R, Kastrup W, Lundquist G, Mobacken H. Development of gastric dysfunction in dermatitis herpetiformis. *Acta Derm Venereol (Stockh)* 1978; 158: 343-8.
- Gawkrödger DJ, McDonald C, O'Mahony S, Ferguson A. Intestinal function and dietary status in dermatitis herpetiformis. *Gut* 1991; 32: 377-82.
- Blaser MJ. Hypotheses on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation. *Gastroenterology* 1992; 102: 720-7.
- Lancaster-Smith MJ, Perrin J, Swarbrick ET, Wright JT. Coeliac disease and autoimmunity. *Postgrad Med J* 1974; 50: 45-8.
- Diamanti A, Maino C, Niveloni S, Pedreira S, Vazquez H, Smecuol E, et al. Characterization of gastric mucosal lesions in patients with celiac disease: a prospective controlled study. *Am J Gastroenterol* 1999; 94: 1313-9.
- Ciacci C, Squillante A, Rendina D, Limauro S, Bencivenga C, Labanca F, et al. *Helicobacter pylori* infection and peptic disease in coeliac disease. *Eur J Gastroenterol Hepatol* 2000; 12: 1283-7.
- Massarrat S, Saberi-Firooz M, Soleimani A, Himmelmalm GW, Hitzges M, Keshavarz H. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. *Eur J Gastroenterol Hepatol* 1995; 7: 427-33.

13. Bonihay YG, Nahon S, Bouzazhah A. Augmentation des lymphocytes intra-épithéliaux sans atrophie villositaire. *Ann Pathol* 2003; 23: S105 (abstract).
14. Memeo L, Jhang J, Hibshoosh H, Green PH, Rotterdam H, Bhagat G. Duodenal intraepithelial lymphocytosis with normal villous architecture: common occurrence in *H. pylori* gastritis. *Mod Pathol* 2005; 18: 1134-44.
15. Crabtree JE, O'Mahony S, Wyatt JI, Heatley RV, Vestey JP, Howdle PD, et al. *Helicobacter pylori* serology in patients with coeliac disease and dermatitis herpetiformis. *J Clin Pathol* 1992; 45: 597-600.
16. Feeley KM, Heneghan MA, Stevens FM, McCarthy CF. Lymphocytic gastritis and coeliac disease: Evidence of a positive association *J Clin Pathol* 1998; 51: 207-10.
17. Wolber R, Owen D, DelBuono L, Appelman H, Freeman H. Lymphocytic gastritis in patients with coeliac sprue or spruelike intestinal disease. *Gastroenterology* 1990; 98: 310-5.
18. Wu TT, Hamilton SR. Lymphocytic gastritis: association with etiology and topology. *Am J Surg Pathol* 1999; 23: 153-8.
19. Cuoco L, Cammarota G, Jorizzo RA, Santarelli L, Cianci R, Montalto M, et al. Link between *Helicobacter pylori* infection and iron-deficiency anemia in patients with coeliac disease. *Scand J Gastroenterol* 2001; 36: 1284-8.
20. Hershko C, Hoffbrand AV, Keret D, Souroujon M, Maschler I, Monselise Y, et al. Role of autoimmune gastritis, *Helicobacter pylori* and coeliac disease in refractory or unexplained iron deficiency anemia. *Haematologica* 2005; 90: 585-95.
21. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis: the Updated Sydney System. *Am J Surg Pathol* 1996; 20: 1161-81.
22. Marsh MN. Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge. *Gut* 1990; 31: 111-4.
23. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiological approach to the spectrum of gluten sensitivity. *Gastroenterol* 1992; 102: 330-54.
24. Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW, Mulder CJ. Sensitivity of antiendomysium and antigliadin antibodies in untreated coeliac disease: disappointing in clinical practice. *Am J Gastroenterol* 1999; 94: 888-94.
25. Rostami K, Villanacci V. Microscopic enteritis: Novel prospect in coeliac disease clinical disease and immunohistogenesis evolution in diagnosis and treatment strategies. *Dig Liver Dis* 2009; 41: 245-52.
26. Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 2002; 347: 1175-86.
27. Ernst PB, Takaishi H, Crowe SE. *Helicobacter pylori* infection as a model for gastrointestinal immunity and chronic inflammatory diseases. *Dig Dis* 2001; 19: 104-11.
28. Villanacci V, Bassotti G, Liserre B, Lanzini A, Lanzarotto F, Genta RM. *Helicobacter pylori* infection in patients with coeliac disease. *Am J Gastroenterol* 2006; 101: 1880-5.
29. Kupcinskas L, Malfeltheiner P. *Helicobacter pylori* and non-malignant diseases. *Helicobacter* 2005; 10 (Supl. 1): 26-33.