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Original research article

DNA variants in *Helicobacter pylori* infected patients with chronic gastritis, dysplasia and gastric cancer

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ABSTRACT

Purpose: The main scope of this study was to evaluate the importance of selected DNA variants for developing inflammation of gastric mucosa and carcinogenesis in gastrointestinal diseases in patients infected with *Helicobacter pylori*.

Patients and methods: Patients subjected to analysis constituted a group of 131 consecutive cases, with control groups consisting of 100 healthy volunteers and 13 dyspeptic patients. Molecular analysis included the following genes: *TP53* (c.743 G > A, c.746 G > A, c.749C > T), *MSH2* (c.942 + 3A > T), *MLH1* (c.2041 G > A), *NOD2/CARD15* (c.3016_3017insC, c.802C > T), *IL1A* (c.-949C > T) and *IL1B* (c.315C > T). DNA variants were detected using PCR-RFLP, pyrosequencing and sequencing.

Results: Mutations of the analyzed genes were observed more frequently in patients with a higher degree of mucosal lesions (50.9%) than in patients with milder mucosal changes (27.6%). Single mutations and polymorphisms did not affect the course of the disease. Our analysis confirms the influence of the *NOD2/CARD15* c.802C > T polymorphism on the development of mucosal changes. A correlation of the frequency of the CT genotype of the *NOD2/CARD15* c.802C > T polymorphism with the *NOD2/CARD15* c.3016_3017insC mutation was observed. The TT genotype frequency in the c.315C > T *IL1B* gene polymorphism was statistically significantly higher in patients with mucosa changes.

Conclusions: Accumulation of molecular abnormalities may increase the susceptibility to inflammatory response of the gastric mucosa in *H. pylori*-infected patients and play an important role in the development of chronic active gastritis, atrophy, intestinal metaplasia, dysplasia and the intestinal type of gastric cancer. The severity of gastric mucosal damage correlates with the presence of mutations in the gastric mucosa and the age of patients.

1. Introduction

Helicobacter pylori (*H. pylori*) bacterium infections of gastric mucosa are one of the causes of chronic gastritis, peptic ulcer disease and gastric cancer [1,2]. Isolation and culture of this bacterium in 1983 by Warren and Marshall were the most important achievements leading to the understanding of this process. Their investigations indicated a correlation between the inflammatory response of the gastric mucosa to

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H. pylori infection and suggested pathogenic association with chronic gastritis, gastric ulcer and gastric cancer [3]. The International Agency for Research on Cancer listed *H. pylori* as a group I carcinogen. Gastric cancer develops through numerous stages beginning from chronic inflammation, atrophic inflammation, intestinal metaplasia and dysplasia and later cancer (intestinal type) [4]. This process is widely known as the Correa cascade [5]. The general prevalence of asymptomatic *H. pylori* infection in US population was estimated to reach approximately 52%. Infections increased with age and were closely correlated with socioeconomic status. The prevalence of *H. pylori* infection increased rapidly with age at 1% per year for the overall population [6]. Meta-analysis of global prevalence of *H. pylori* infection revealed interesting data. The prevalence of *H. pylori* in the general population was 35.6% in the United States, 35.5% in the United Kingdom and 35.3% in Germany; Nigeria (87.7%), Portugal (86.4%) and Estonia (82.5%) had the highest *H. pylori* prevalence, while Switzerland (18.9%), Denmark (22.1%) and New Zealand (24.0%) were in the group of countries with the lowest *H. pylori* prevalence. The prevalence in Poland was estimated in one study at 66.6% [7], but in another study at 58.3% [8].

In the recent years, many studies have been published indicating the key role of *H. pylori* infection in gastric carcinogenesis [9]. These studies have shown an increased risk of severe chronic intestinal metaplasia, dysplasia and subsequent gastric cancer (intestinal type). The involvement of polymorphisms in the interleukin-1 alpha (*IL1A,* NG_008850.1), interleukin-1 beta (*IL1B,* NG_008851.1) and nucleotide binding oligomerization domain (*NOD2/CARD15*, NG_007508.1) genes has not yet been elucidated, and therefore, we performed these analyses in our own well-characterized group of patients. The present study focused on the analysis of the following mutations in four genes: rs11540652 (c.743 G > A, p.R248Q), rs587782329 (c.746 G > A, p.R249 K) and COSM10771 (c.749C > T, p.P250 L) in the tumor protein p53 gene (*TP53*, NG_017013.2), rs193922376 (c.942 + 3A > T) in the mutS homolog 2 gene (*MSH2,* NG_007110.2), rs63750217 (c.2041 G > A, p.A681 T) in the mutL homolog 1 gene (*MLH1,* NG_007109.2) and rs2066847 (c.3016_3017insC, p.L1007Pfs) in the *NOD2/CARD15* gene. Furthermore, we have also analyzed polymorphisms in three other genes: rs2066842 (c.802C > T, p.P268S) in the *NOD2/CARD15* gene, rs1800587 (c.-949C > T) in the *IL1A* gene and rs1143634 (c.315C > T, p.F105 F) in the *IL1B* gene in *H. pylori*-infected patients with chronic gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia and gastric cancer (intestinal type). Both mutations and polymorphisms can be classified as genetic variants and we used the term mutation when there were observable phenotypic characteristics.

NOD2/CARD15 was selected as a potential susceptible gene in Crohn's disease, as an intracellular receptor for bacterial products and transducer of a signal leading to nuclear transcription factor-κB (NF-κB) activation [10]. Recently, reports have been published suggesting the association of the c.3016_3017insC mutation in the *NOD2/CARD15* gene with the predisposition to this disease [11]. Meta-analysis involving 7 studies analyzing the c.3016_3017insC change in the *NOD2/CARD15* gene indicated that there was a significant risk of colorectal cancer in individuals with variant genotypes [12]. Changes in the *NOD2/CARD15* gene were correlated with chronic inflammation of gastric mucosa with a concomitant *H. pylori* infection and chronic gastritis and gastric cancer development [13].

IL1B initiates and enhances the inflammatory response to the infection in the presence of *H. pylori* [14]. It has been found that the c.315C > T variant of the *IL1B* gene plays an important role in this process [15]. The proinflammatory genotype (IL1B-511 T, IL1B-31C and IL1RN*2) of the interleukin-1 gene (*IL1*) occurs together with the increased risk of gastric carcinoma and is a probable precursor of atrophic gastric inflammation and hypochlorhydria [13]. Polymorphisms of host inflammatory response genes may affect the character and extent of the gastric mucosa inflammation associated with *H. pylori* infection. The aim of this study was to evaluate the importance of selected DNA variants for developing inflammation of gastric mucosa and carcinogenesis.

2. Patients and methods

2.1. Patients characteristics

Patients subjected to analysis constituted a cohort of 131 consecutive cases of Caucasian origin collected in western Poland. Patients were 11 to 87 years of age (average age 45.9 years) and all suffered from dyspepsia. A group of 40 patients (average age 35.6 ± 14.6 years) with concomitant *H. pylori* infection and chronic active gastritis, 36 patients (average age 41.0 ± 18.5 years) with gastritis and atrophy, 17 patients (average age 38.4 ± 17.2 years) with gastritis and intestinal metaplasia, 21 patients (average age 52.5 ± 18.7 years) with dysplasia and 17 patients (average age 62.2 ± 12.5 years) with gastric cancer of the intestinal type have been confirmed by microscopic examination of gastric mucosal samples. The study excluded: patients taking, on a long-term basis, non-steroidal anti-inflammatory drugs, drugs reducing gastric secretion, antibiotics and anti-coagulants during the previous two months as well as patients abusing alcohol and smoking tobacco, patients with serious somatic ailments (diseases of the liver, kidneys, cardiovascular and respiratory systems etc.), patients with autoimmune gastritis, patients after operations on bile ducts and gastric resections and patients with active ulcer disease (gastric ulcer and duodenal ulcer) and diffuse gastric cancer. Therefore, the size of the study group was limited to 131, but still provided a high homogeneity of selected groups, representing approx. 10% of cases. The authors selected two control groups. First group (control I) included 100 healthy volunteers of Caucasian origin (50 males and 50 females) aged 21–30 (average age 25.5 ± 2.9 years). Healthy individuals from the first control group were *H. pylori* negative, they passed medical examinations, but they did not undergo endoscopy, because they have not reported any digestive tract symptoms at blood drawing. Second control group (control II) comprised of 13 patients aged 16–57 (average age 37.8 ± 12.9 years) with dyspepsia without macroscopic lesions in endoscopic examination, without histological inflammatory changes and without *H. pylori* infection in gastric mucosal biopsies.

All endoscopic examinations of the stomach were performed by the same endoscopist (A.H). Macroscopic assessment of endoscopic images in terms of inflammatory changes of the gastric mucosa was carried out in accordance with the Sydney System for the classification of chronic gastritis [16]. The following five biopsies were taken during endoscopic examination: from the antrum, angle and from the body of the stomach (from the larger and smaller curvature).

2.2. Pathomorphology

Histological assessment was performed by staining the preparations with hematoxylin and eosin, as described earlier [13,15]. The intensity of gastric mucosal inflammation, chronic atrophic gastritis of glands, intestinal metaplasia were evaluated in each section according to the updated Sydney System for the classification of chronic gastritis [17]. The degree of mucosal inflammation, chronic atrophic gastritis, and intestinal metaplasia was classified using the following four grades: 0 – none; 1 – mild; 2 – moderate; and 3 – severe. Dysplasia was estimated according to WHO classification [18]. Gastric carcinoma was classified into intestinal and diffuse types according to Lauren's criteria [19].

2.3. Detection of the H. pylori infection

The presence of *H. pylori* bacteria was determined using the urease test (Institute of Food and Nutrition, Warsaw), which was read after 2 and 24 h (doubtful cases). Additionally, the presence of *H. pylori* bacteria was confirmed in the gastric mucosa by histological examination employing the Warthin-Starry method modified by Giemsa, according

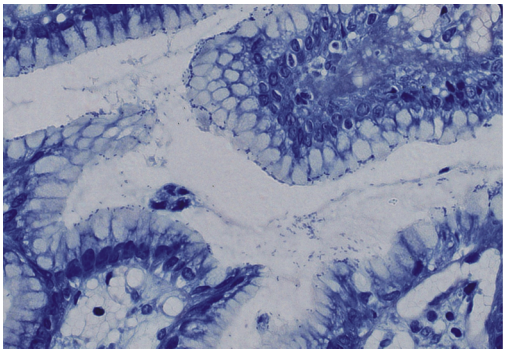

Fig. 1. *Helicobacter pylori* by Giemsa-Romanovsky staining. *Helicobacter pylori* (medium stage of infection) present in a layer of mucus covering the gastric epithelium (magnification 400x).

to the updated Sydney System for the classification of chronic gastritis [16,17]. Both tests were performed for all patients. The most relevant results were obtained by histopathological examination, considering the lower sensitivity and specificity of the rapid urease test. *H. pylori* infection was analyzed in the healthy group of volunteers (control I) using an immunoassay (HELICO Test, Ani Biotech Oy, Finland). This test measures the level of IgG antibodies against *H. pylori* (Fig. 1).

2.4. Molecular analysis

DNA was isolated in the patient group and in the second control group (control II) from paraffin blocks of gastric mucosal biopsies using the High Pure PCR template preparation kit (Roche Diagnostics GmbH, Germany) and from peripheral blood using the guanidine isothiocyanate (GTC) method. DNA in the first control group (control I) was isolated from peripheral blood using the same method. Screening procedures involved analyses of single strand conformational polymorphism (PCR-SSCP) or heteroduplexes (PCR-HD), restriction fragment length polymorphism (PCR-RFLP), followed by direct sequencing using the MegaBACE™ 1000 DNA Analysis System (Amersham Biosciences, United Kingdom) or pyrosequencing using the PSQ™ 96 MA System (Qiagen, Germany), according to the manufacturer's specifications [20]. The presence of germline or somatic mutation was evaluated using paraffin block samples and blood samples from the same patients (patients and control II). Additionally, the presence of germline mutations was evaluated using blood samples from healthy volunteers (control I).

2.5. Ethical issues

The experiments were accepted by the Bioethics Committee of the Poznan University of Medical Sciences (approval numbers: 871/09, 442/13 and 995/17) and the methods were carried out in accordance with the approved guidelines. All patients as well as parents and children had access to full information concerning the experiments and submitted their written consent for their performance. Gastroscopy in a healthy group of volunteers (control I) would have been very helpful, but had not been performed because of the lack of acceptance of an invasive test for people without any symptoms of gastrointestinal disease due to bioethical reasons.

2.6. Statistical analysis

The analyses were carried out using the Statistica 10.0 (StatSoft Inc., 2011) software. The following nonparametric methods were used for hypothesis verification: Fisher's exact test, χ^2 chi-square test (Pearson's test with Yates' correction), Mann-Whitney *U* test and Spearman's rank correlation coefficient. The level of significance was set at $p < 0.05$. Hardy-Weinberg equilibrium for the analyzed DNA variants was tested

Table 1
Overview of mutations and polymorphisms of selected genes in group of *H. pylori* infected patients with chronic active gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia and gastric cancer (intestinal type) in comparison with control groups. Analysis in control I group were performed in blood of volunteers and on tissues and blood of patients in control II group.

Gene	Genotype	Chronic active gastritis n = 40	Chronic atrophic gastritis n = 36	Intestinal metaplasia n = 17	Dysplasia n = 21	Gastric cancer (intestinal type) n = 17	Control group I n = 100	Control group II n = 13	p-value*
TP53	rs11540652 (c.743 G > A)	2	1	2	2	1	0	0	< 0.01
	rs587782329 (c.746 G > A)	2	1	1	3	0	0	0	< 0.02
	COSM10771 (c.749C > T)	0	2	2	1	0	0	0	< 0.1
MSH2	rs193922376 (c.942 + 3A > T)	1	1	1	3	1	0	0	< 0.02
MLH1	rs63750217 (c.2041 G > A)	2	1	1	0	4	0	0	< 0.01
NOD2/ CARD15	rs2066847 (c.3016_3017insC) rs2066842 (c.802C > T)	7	2	2	2	3	0	0	< 0.0001
	CC	19	24	9	10	5	68	10	< 0.0001
	CT	15	7	6	6	8	28	1	
	TT	6	5	2	5	4	4	2	
IL1A	rs1800587 (c.-949C > T)								
	CC	21	18	4	8	9	48	8	< 0.0001
	CT	12	13	9	9	6	41	4	
	TT	7	5	4	4	2	11	1	
IL1B	rs1143634 (c.315C > T)								
	CC	18	22	7	12	9	63	63	< 0.0001
	CT	16	9	5	6	6	28	28	
	TT	6	5	5	3	2	9	1	

* Patients vs control I + control II.

in the patients and population groups.

3. Results

3.1. Evaluation of analyzed groups

The study included a group of 131 patients with chronic active gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia and the intestinal type of gastric cancer with *H. pylori* infection. The latter was confirmed by histological analysis and urease test. Gastric biopsies were classified by the Sydney System for the classification of chronic gastritis.

Two control groups were selected (Table 1): 100 healthy volunteers without *H. pylori* infection in a serological test (control I) and a group of 13 dyspeptic patients with normal gastric mucosa in endoscopic examination without inflammatory changes in histological assessment of gastric mucosal samples (control II). The lack of *H. pylori* infection in this group was confirmed by urease test and histological examination of gastric mucosal samples.

3.2. Influence of genetic background on mucosal changes

Mutations in the analyzed genes (*TP53*, *MSH2*, *MLH1* and *NOD2/CARD15*) were observed more frequently in patients with a higher severity of gastric mucosal damage (intestinal metaplasia, dysplasia or gastric cancer of the intestinal type, 50.9%) versus patients with milder changes (chronic active gastritis, chronic atrophic gastritis, 27.6%) and showed statistical significance ($\chi^2 = 7.384324$, df = 1, $p < 0.01$). Single mutations and polymorphisms did not affect the course of disease and statistically significant changes were not observed between patients with chronic active gastritis and chronic atrophic gastritis versus patients with intestinal metaplasia, dysplasia or gastric cancer of the intestinal type. Our previous analysis [13] confirmed the influence of the *NOD2/CARD15* c.802C > T polymorphism on the development of mucosal changes. The TT genotype of this polymorphism was more frequent in patients with chronic active gastritis and chronic atrophic gastritis (14.5%) as well as patients with intestinal metaplasia, dysplasia or gastric cancer of the intestinal type (20.0%) in comparison to the control group (5.3%) and these differences were statistically significant (Table 2).

Our previous study [15] showed no statistical significance of the c.-949C > T *IL1A* gene polymorphism with respect to mucosal changes in *H. pylori* patients, but a higher frequency of the TT genotype was observed in patients versus the control group (16.8% vs. 10.6%, respectively, $\chi^2 = 5.1$; $p < 0.1$).

The same analysis for the c.315C > T *IL1B* polymorphism showed statistically significant ($\chi^2 = 9.3$; $p < 0.01$) increase in the frequency of CT and TT genotypes in patients (CC 51.9%, CT 32.1% and TT 16.0%) in comparison to the control group (CC 62.0% CT 29.2% and TT 8.85%). A similar situation was observed between patients with intestinal metaplasia, dysplasia or gastric cancer of the intestinal type and the control group ($\chi^2 = 6.1$; $p < 0.05$).

The presence of two changes – mutation and specific polymorphism in the same gene – *NOD2/CARD15* (c.3016_3017insC mutation and the CT genotype of the c.802C > T polymorphism) – was statistically significant in patients in comparison to healthy volunteers ($\chi^2 = 31.87721$; $p < 0.00000$; CC 1.5%; CT 35.7% and TT 0%). A comparison of the presence of all mutations and the *NOD2/CARD15* c.802C > T polymorphism provided similar results, as a higher number of patients with the CT genotype was recorded ($\chi^2 = 13.88140$; $p < 0.001$; CC 29.9%; CT 59.5% and TT 18.2%; respectively).

The severity of changes in the gastric mucosa in *H. pylori*-infected patients was also statistically significantly correlated with the age of patients (mean R = 0.43; $p < 0.0000$). The comparison of patients with chronic active gastritis or chronic atrophic gastritis to patients with intestinal metaplasia, dysplasia or gastric cancer of the intestinal type using Mann-Whitney *U* test confirmed the data obtained using Spearman's rank correlation coefficient (Z=-4.0; $p < 0.0001$), indicating that the group with a higher degree of gastric mucosal damage was older.

4. Discussion

For analysis, we used mucosal biopsies and blood samples from the same patients, which allowed us to focus on germline mutations. For example, patients with diagnosed metaplasia intestinalis in samples obtained from antrum and the body of stomach had the same molecular background. The presence of the control group is an important part of each study. Gastroscopy with a biopsy in the group of healthy volunteers (control I) would be very helpful, but it was not performed because of the lack of acceptance of an invasive test for people without any symptoms of gastrointestinal disease due to bioethical reasons. A group of 100 healthy individuals, not suffering from diseases, in particular without the symptoms associated with the gastrointestinal tract and with a confirmed absence of *H pylori* infection was used as a control. The control group (control II) was extended by including 13 dyspeptic patients without macroscopic mucosal lesion, histological inflammatory changes and *H. pylori* infection.

The risk of gastric cancer is additionally increased by the presence of various genotypes of pro-inflammatory cytokines, such as TNFα, interleukin-1β and receptor antagonist interleukin-1 [21]. Variants found within the *TP53* suppressor gene are of similar significance [22]. TP53, a known tumor suppressor protein, plays a key role in detecting DNA damage and TP53 alterations occur in most cases of gastric cancer. *H. pylori* infection might lead to a deficiency of DNA mismatch repair in gastric epithelial cells, which may increase the risk of mutation accumulation in gastric mucosal cells and the risk of gastric cancer during chronic *H. pylori* infection [23,24]. In our study, the presence of *TP53* gene mutations was not statistically significant, and interestingly, it was not associated with the increased risk of mucosal changes and a consequent gastric cancer development. This could also be due to a low number of patients analyzed. The increased number of *TP53* gene mutations in gastric cancer patients compared to earlier stages of mucosal lesions observed in other studies could be related to different evaluation methods. Our findings showed that mutations in the *TP53*, *NOD2/CARD15, MSH2* and *MLH1* genes were collectively more frequent in the group with severe symptoms of mucosal changes ($p < 0.01$). This could be associated with a reduced DNA mismatch

Table 2
Comparison of *NOD2/CARD15* rs2066842 polymorphism frequency in different patient's groups vs control groups (control I + control II) [13].

NOD2/CARD15 rs2066842	Patients			Control I + Control II
	Chronic active gastritis or chronic atrophic gastritis	Intestinal metaplasia, dysplasia or gastric cancer of intestinal type	Patients total	
CC	43 (56.58%)	24 (43.64%)	67 (51.15%)	78 (69.03%)
CT	22 (28.95%)	20 (36.36%)	42 (32.06%)	29 (25.66%)
TT	11 (14.47%)	11 (20.00%)	22 (16.79%)	6 (5.31%)
Patients vs. control groups	$\chi^2 = 14.0$; $p < 0.001$	$\chi^2 = 29.1$; $p < 0.0001$	$\chi^2 = 39.9$; $p < 0.0001$	

repair, and was at least in part connected to CpG methylation of the *hMLH1* gene promoter. These data support the notion that *H. pylori*-induced mutations and epigenetic alterations in gastric epithelial cells during chronic gastritis may contribute to an increased risk of gastric cancer associated with *H. pylori* infection [25].

Our earlier investigations revealed a significant interrelationship between the c.802C > T polymorphism of the *NOD2/CARD15* gene and the intensity of the chronic inflammation of the gastric mucosa associated with *H. pylori* infection. It was shown that the frequency of the T allele of this polymorphism increased in patient groups with high intensity of morphological changes observed in the mucosa [13]. The immunological response of the gastric mucosa to *H. pylori* infection and the course of the inflammatory reaction are the main factors responsible for the development of chronic inflammation of the gastric mucosa and, consequently, carcinogenesis [1].

Changes within the *NOD2/CARD15* gene are advantageous for the development of Crohn's disease [10], ulcerative inflammation of the large intestine [26] as well as colorectal cancer. The occurrence of the c.802C > T polymorphism of the *NOD2/CARD15* gene can predispose to an altered response to *H. pylori* proteins [13]; it can also be assumed that it predisposes to the enhanced inflammatory response of the gastric mucosa. Our study showed the importance of changes in the *NOD2/CARD15* gene, because of all analyzed mutations (*TP53*, *NOD2/CARD15, MSH2* and *MLH1* genes), only the c.3016_3017insC *NOD2/CARD15* gene variant statistically significantly affected the development of mucosal changes, as observed in patients with the CT genotype of the *NOD2/CARD15* c.802C > T polymorphism. Similar situation was observed between the CT genotype of the *NOD2/CARD15* c.802C > T polymorphism and all other mutations ($p < 0.001$). Other variants, such as c.-949C > T in the *IL1A* gene showed no correlation with the inflammatory response of the gastric mucosa but the TT genotype of the c.315C > T polymorphism in the *IL1B* gene was more frequent in group of *H. pylori* infected patients with chronic active gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia and gastric cancer (intestinal type) in comparison with control groups [15].

The presence of various proinflammatory interleukin-10 genotypes, TNFα, interleukin-1β and interleukin-1 receptor antagonist increases the risk of gastric carcinoma development [9,27]. Such an effect of a proinflammatory interleukin-1 polymorphism (IL1RN*2, IL1B-511 T/-31C) has been demonstrated earlier [13]. However, this relationship was not confirmed by the results of other researchers. The occurrence of *H. pylori* infection in patients with the IL1B-31 T/IL1B-511C polymorphism increased IL1B production by the gastric mucosa, which led to the development of the intestinal type of gastric carcinoma [28]. Meta-analyses suggested that IL-1B-511 T and IL-1RN*2 were associated with an increased risk of gastric cancer (intestinal type) [29]. Our previous study on the c.315C > T polymorphism in the *IL1B* gene demonstrated the association of *H. pylori* infection with inflammatory changes of the gastric mucosa and the intestinal type of gastric carcinoma. The c.-949C > T polymorphism of the *IL1A* gene seemed not to be associated with the risk of gastric carcinoma development in patients with *H. pylori* infection, but we found higher frequency of the TT genotype of this polymorphism close to statistical significance ($p = 0.0769$) [15,28]. Our observations are only a small part of interleukin activity, because various pro-inflammatory factors, such as IL1A, IL1B, IL2, IL4 and TNFα are also involved in the modification of resistance to different treatments of inflammatory bowel disease (IBD) patients [30].

As suggested by some researchers, only 1/3 of histology reports were prepared according to the Sydney System for the classification of chronic gastritis in daily routine practice, indicating that international guidelines are poorly observed in clinical practice [31]. Since histology represents a critical element in gastric cancer surveillance strategies, we suggest that the addition of genetic analysis may be a good supplementation in detecting gastric cancer development.

5. Conclusions

Our findings suggest that the accumulation of molecular abnormalities may increase the inflammatory response of the gastric mucosa of *H. pylori*-infected patients and play an important role in the development of chronic active gastritis, atrophy, intestinal metaplasia, dysplasia and the intestinal type of gastric cancer. Our observation concerning the phenomenon of the intensification of gastric mucosal changes with the age of patients is also typical for this cascade. The number of observed mutations in the gastric mucosa correlated with the progression of gastric mucosal damage. Chronic *H. pylori* infection induces genetic instability in gastric epithelial cells. For this reason, *H. pylori* should always be considered a pro-carcinogenic factor.

Conflict of interests

The authors declare no conflict of interests.

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