Do Gene Expressions Differ Between Gastritis with and without *Helicobacter pylori*?

Helicobacter pylori'nin Eşlik Ettiği ve Etmediği Gastritlerde Gen Ekspresyonları Farklı mıdır?

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ABSTRACT Objective: *Helicobacter pylori* infection is a well-defined risk factor for the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. However, mechanisms of carcinogenesis in Helicobacter gastritis need to be elucidated. We aimed in this study to determine the immunohistochemical expressions of some cancer-associated genes, and to show differences between Helicobacter gastritis and Helicobacter-negative gastritis. **Material and Methods:** Sixty-three endoscopic biopsy samples were selected. The samples comprised of normal gastric mucosa (20 samples), Helicobacter gastritis (22 samples), and Helicobacter-negative gastritis (21 samples). Paraffin sections of samples were processed immunohistochemically with some suppressor genes (Rb, p53), protooncogenes (EGFR, cyclin D1), and a heat shock protein, HSP 105. The gene expressions in both crypt epithelia and lymphoid infiltrate were evaluated separately. **Results:** *In epithelia;* expressions of cyclin D1, p53, HSP 105, and EGFR were higher in Helicobacter-negative gastritis than in Helicobacter gastritis ($p< 0.0001$). Immune expression of HSP 105 in Helicobacter gastritis was lower than in control group ($p= 0.008$) as well as in Helicobacter-negative gastritis. However, expression of Rb was higher in Helicobacter gastritis than in Helicobacter-negative gastritis ($p= 0.034$). *In lymphoid infiltrate;* immune reactions for p53 and cyclin D1 were negative in all samples. Expressions of Rb, EGFR, and HSP 105 in lymphoid infiltrate were similar to expressions in epithelia for all groups. **Conclusion:** We can suggest that development of gastric cancer has a different pathway in Helicobacter gastritis when compared to the one Helicobacter-negative gastritis. In addition, decrease of heat shock proteins in Helicobacter gastritis may lead to sensitivity of crypt epithelia and lymphoid infiltrate for carcinogenic mutations.

Key Words: Cocarcinogenesis; cyclin D1; genes, p53; EGFR protein

ÖZET Amaç: *Helicobacter pylori* enfeksiyonu gastrik adenokarsinom ve mukoza ilişkili lenfoid doku (MALT) lenfoması gelişimi için iyi tanımlanmış bir risk faktörüdür. Ancak Helicobacter gastritinde karsinogenez mekanizmalarının aydınlatılması gerekmektedir. Bu çalışmada bazı kanser ilişkili genlerin immunohistokimyasal ekspresyonunu belirlemeyi ve Helicobacter gastriti ile Helicobacter-negatif gastrit arasındaki farklılıkları göstermeyi amaçlamaktayız. **Gereç ve Yöntemler:** Altmış üç endoskopik biyopsi örneği seçilmiştir. Örnekler normal mide mukozası (20 örnek), Helicobacter gastriti (22 örnek), ve Helicobacter negatif gastritten (21 örnek) oluşuyordu. Örneklerin parafin kesitleri immünohistokimyasal olarak bazı süpresör genler (Rb, p53), protoonkogenler (EGFR, siklin D1) ve bir ısı şok proteini olan HSP 105 ile işlendi. Hem kript epiteli hem de lenfoid infiltrat içindeki gen ekspresyonları ayrı ayrı değerlendirildi. **Bulgular:** *Epitelde;* Helikobakter negatif gastritte siklin D1, p53, HSP 105 ve EGFR ekspresyonu Helicobacter gastritinden daha yüksekti ($p< 0.0001$). Helicobacter gastritinde HSP 105'in immün ekspresyonu kontrol grubu ($p= 0.008$) ve Helicobacter-negatif gastritten daha düşüktü. Ancak, Rb ekspresyonu Helicobacter gastritinde, Helicobacter negatif gastrite göre daha yüksekti ($p= 0.034$). *Lenfoid infiltratta;* tüm örneklerde p53 ve siklin D1 için immün reaksiyonlar negatif bulundu. Tüm gruplar için lenfoid infiltrat içinde Rb, EGFR ve HSP 105 ekspresyonu epiteldekine benzerdi. **Sonuç:** Mide kanserinin, Helicobacter gastritinde Helicobacter negatif gastrite göre daha farklı bir yolla geliştiğini öne sürebiliriz. Ayrıca, Helicobacter gastritinde ısı şok proteinlerinin azalması kript epitelinde ve lenfoid infiltratta karsinojenik mutasyonlara duyarlılığa yol açabilir.

Anahtar Kelimeler: Kokarsinogenez; siklin D1; genler, p53; EGFR proteini

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Helicobacter pylori (H. pylori) infection is one of the most important factor in the development of chronic gastritis, and plays a central role in the etiology of peptic ulcer.[1] It also increases the risk for the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma.[2,3] However, mechanisms of carcinogenesis are not clear, and need to be elucidated.

There are some studies investigating molecular or genetic alterations in epithelia of non-neoplastic gastric mucosa with *H. pylori* infection. For example, Kato et al. studied alterations of Ki67 and CD117 gene expressions, and they found that there were alterations in expressions of these genes associated with *H. pylori* infection.[4] In addition, single-stranded DNA and cleaved caspase-3 were positive in a great number of mucosal epithelial cells in their study. Therefore, they suggested that the direct and indirect effects of *H. pylori* infection on the gastric epithelial proliferation, differentiation, and programmed cell death point to the in situ occurrence of gastric cancer were associated with *H. pylori* infection.[4]

It is known that *H. pylori* produces strong variations in host cell cycle mechanisms determining hyperproliferation and deregulation. At the same time, it evokes host responses such as oxidative stress, which are more dangerous for the host than the bacteria, which have been proposed as alternative mechanisms for gastric carcinogenesis.[5,6] Hundreds of cancer-associated genes have been described. Each of these genes has a specific function in host cell cycle, and their dysregulation contributes to the origin or the progression of the malignancy. We aimed in the present study to show differences in the expressions of various genes such as p53, retinoblastoma (Rb), cyclin D1, epidermal growth factor receptor (EGFR), and heat shock protein 105 (HSP 105) in epithelia and lymphoid infiltrate, in patients with chronic gastritis with and without *H. pylori* infection.

Heat shock proteins are mammalian stress proteins that rescue shock-stressed proteins from misfolding. The mission of these proteins is to prevent genetic mutations and aging. The Rb and p53 are the tumor suppressor genes that play a key role in regulating the cell cycle. Cyclin D1 and EGFR are the protooncogenes that are involved in cell proliferation, and defects in the protooncogenes cause converting them into oncogenes.[7]

MATERIAL AND METHODS

This study has been approved by the Ethic Committee of Abant Izzet Baysal University Faculty of Medicine. Sixty-three endoscopic gastric mucosa biopsies were selected from the pathology archives belonging to the period between 2005 and 2007. Patients' ages ranged between 27 and 79 years, with a mean of 48 years. The biopsies comprised of 20 samples with normal gastric fundic mucosa (control group), 22 samples with moderate to severe chronic gastritis in fundic mucosa with *H. pylori* infection (Helicobacter gastritis), and 21 samples with moderate to severe chronic gastritis in fundic mucosa without *H. pylori* infection (Helicobacter-negative gastritis). None of the patients had previous treatment for *H. pylori* infection. To ensure histological identity for activity between Helicobacter gastritis and Helicobacter-negative gastritis, we excluded the samples with moderate to severe neutrophil infiltration. Samples with Helicobacter-negative gastritis were selected among patients who were not clinically considered to have *H. pylori* -associated gastritis. There was no history of non-steroidal anti-inflammatory drug use or chronic alcohol use in all groups. In order to confirm the diagnoses, the clinical records of patients were re-evaluated, serial sections were taken from paraffin blocks, and hematoxylin-eosin and Giemsa stains were applied on the sections for re-evaluation.

Paraffin sections of 4-μm were obtained, deparaffinized and rehydrated for immunohistochemical staining. Then sections were processed with cyclin D1 (NeoMarkers, Fermont, CA), HSP 105 (Novacastra, Newcastle, UK), EGFR (Santa Cruz Biotechnology, California, U.S.A.), Rb (NeoMarkers, Fremont, CA), and p53 (Clon DO-7, ScyTeek Laboratories, Logan, Utah, U.S.A.) primary antibodies. Before processing with the primary an-

tibodies p53, cyclin D1 and HSP 105, antigen retrieval was applied on the sections, which were boiled in 10 mM citrate buffer pH 6.0 for 20 minutes followed by cooling at room temperature for 20 minutes. Similar antigen retrieval was applied for Rb with EDTA pH 8.0. The primary antibodies were applied accompanying the streptavidin-biotin peroxidase methodology with 3,3' Diaminobenzidin (DAB) chromogen substrate (Lab Vision, Fermont CA).

Negative controls, in which the primary antibodies were replaced by PBS, were carried out for each primary antibody. Colon carcinoma for p53 and Rb, breast carcinoma for cyclin D1 and EGFR, and testis sections for HSP 105 were used as positive controls. The gene expressions in both mucosal crypt epithelia and lymphoid infiltrate of the mucosa were evaluated separately. The evaluation of staining intensity and extensiveness were performed by two pathologists independently, and any discrepancy was resolved by joint review.

The level of staining intensity and extensiveness for Rb, HSP 105, and EGFR were divided into five grades (0: no reaction; 1: minimal; 2: mild; 3: moderate; 4: severe) (Figure 1). Immunohistochemical reactivity for p53 and cyclin D1 was divided into three grades (0: no reaction; 1: sparse nuclei with positive staining; 2: more than 10 % of cells with positive staining) (Figure 2).

The Chi-square and Fishers's exact tests were used for the statistical analysis of the results. For multiple comparisons among the groups (control, Helicobacter gastritis, Helicobacter-negative gastritis) for each immunohistochemical marker, we integrated grade 1 and grade 2 for p53 and cyclin

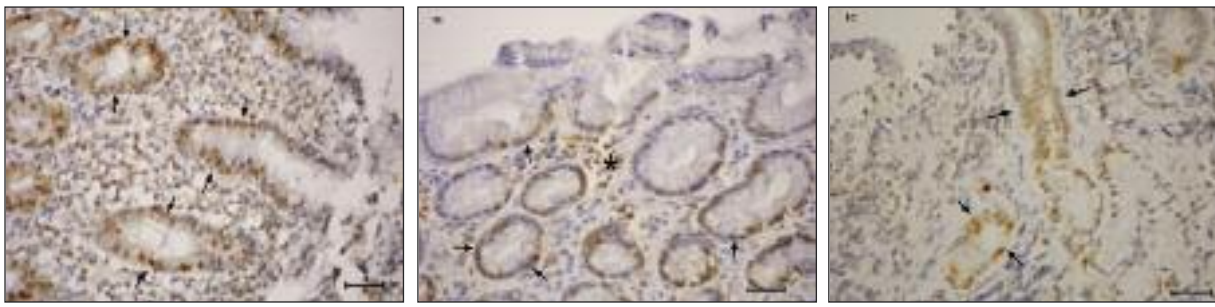

FIGURE 1: Immune expression of Rb, HSP 105, and EGFR in gastric mucosa. Positive expressions are seen in brown. **(1A)** Severe Rb expression is seen in nuclei of crypt epithelia (arrows). **(1B)** Moderate cytoplasmic HSP 105 expression in epithelia (arrows), and lymphoid infiltrate (asterisk) are seen. **(1C)** Moderate cytoplasmic EGFR expression in crypt epithelia is shown (arrows) (immunperoxidase stain, x40, bars show 25 μm).

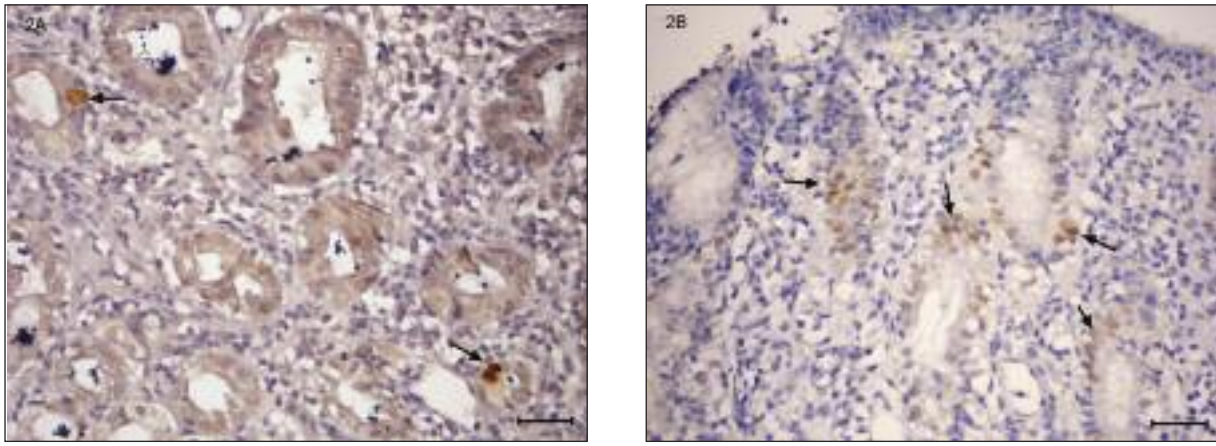

FIGURE 2: Immune expressions of p53 and cyclin D1 in nuclei of crypt epithelia. Positive expressions are seen in brown. **(2A)** p53 immune expression is seen in three nuclei (arrows), consistent with grade 1 immune reaction. **(2B)** Grade 2 immune expression with cyclin D1 is seen in crypt epithelia (arrows) (immunperoxidase stain, x40; bars show 25 μm).

D1. For Rb, EGFR and HSP 105, we integrated minimal and mild; and moderate and severe. Therefore the number of columns in the tables for p53 and cyclin D1 were reduced to 2; and those for the other three markers were reduced to 3. These integrated data were also used for binary comparisons except for Rb, for which the data were integrated in two groups; the first group comprising no reaction, minimal and mild, and the second group comprising moderate and severe.

RESULTS

None of the Helicobacter-negative samples had *H. pylori* in mucous layer of the antral mucosa or the fundic mucosa. Helicobacter-negative gastritis group was determined as a whole group and we did not take etiological subgroups into consideration. Samples of Helicobacter gastritis had mild to moderate density of *H. pylori*. Most of the cases in both gastritis groups had minimal neutrophil infiltration (minimal activity). No samples with chronic gastritis had intestinal metaplasia, dysplasia, or moderate to severe atrophy. Histological criteria such as severity of gastritis, density of *H. pylori*, and severity of activity for sample inclusion were performed in accordance with the Updated Sydney System.[8]

IMMUNE REACTION IN EPITHELIAL CELLS

Table 1 shows the results of p53 and cyclin D1 immunoreaction in gastric epithelial cells in mucosal crypts. The primary antibody of p53 which was used in our study detects mutant and wild types of p53 expressions. Immune reaction with p53 was observed only in Helicobacter-negative gastritis. In this group, p53 expression was detected in seven out of 21 subjects (33.3 %). The difference among groups was statistically significant ($p< 0.0001$). The highest immune expression was similarly observed for cyclin D1 in Helicobacter-negative gastritis. The difference among groups was statistically significant ($p< 0.0001$). There was only one subject that had positive immune expression in Helicobacter gastritis group. Difference between Helicobacter gastritis and control groups was not significant ($p= 0.598$).

Table 2 shows the immune expression of Rb, EGFR and HSP 105. While Helicobacter gastritis

TABLE 1: Grading of p53 and cyclin D1 immunoreaction in crypt epithelia.

		n (%)		
Groups		**None**	**Grade 1**	**Grade 2**
Control n= 20	p53	20 (100)	0 (0)	0 (0)
n= 20	Cyclin D1	18 (90.0)	2 (10.0)	0 (0)
Helicobacter gastritis	p53A	22 (100)	0 (0)	0 (0)
n= 22	Cyclin D1[b]	21 (95.4)	1 (4.60)	0 (0)
Helicobacter-negative gastritis	p53[a]	14 (66.7)	5 (23.8)	2 (9.50)
n= 21	Cyclin D1[b]	9 (42.9)	3 (14.2)	9 (42.9)

Helicobacter, Helicobacter pylori; [a]p= 0.004, Helicobacter gastritis versus Helicobacter-negative gastritis; [b]p< 0.0001, Helicobacter gastritis versus Helicobacter-negative gastritis.

TABLE 2: Grading of Rb, EGFR and HSP 105 immunoreaction in crypt epithelia.

				n (%)		
Groups		**None Reaction**	**Minimal**	**Mild**	**Moderate**	**Severe**
Control group	Rb	0 (0)	6 (30.0)	11 (55.0)	3 (15.0)	0 (0)
n= 20	EGFR	0 (0)	4 (20.0)	11 (55.0)	4 (20.0)	1 (5.00)
	HSP 105	3 (15.0)	9 (45.0)	6 (30.0)	2 (10.0)	0 (0)
Helicobacter gastritis	Rb[a]	0 (0)	2 (9.10)	0 (0)	3 (13.6)	17 (77.3)
n= 22	EGFR[b]	6 (27.3)	6 (27.3)	5 (22.7)	4 (18.2)	1 (4.50)
	HSP 105[c]	13 (59.1)	7 (31.8)	2 (9.10)	0 (0)	0 (0)
Helicobacter-negative gastritis	Rb[a]	1 (4.80)	0 (0)	7 (33.3)	11 (52.4)	2 (9.50)
n= 21	EGFR[b]	0 (0)	1 (4.80)	6 (28.6)	10 (47.6)	4 (19.0)
	HSP 105[c]	0 (0)	1 (4.80)	1 (4.80)	10 (47.6)	9 (42.8)

[a]p= 0.034, Hp gastritis versus Helicobacter-negative gastritis; [b]p= 0.004, Helicobacter gastritis versus Helicobacter-negative gastritis; [c]p< 0.0001, Helicobacter gastritis versus Helicobacter-negative gastritis.

group when had the highest expression, the lowest expression was seen in control group with Rb. Difference among groups was statistically significant (p< 0.0001). Rb positivity was significantly lower in Helicobacter-negative gastritis group compared to Helicobacter gastritis group (p= 0.034). In contrary, Helicobacter-negative group had the highest expression with EGFR and HSP 105. Difference among groups was statistically significant (p< 0.0001). In binary comparisons, the difference for EGFR between Helicobacter-negative gastritis and Helicobacter gastritis groups was statistically significant (p= 0.004). Immune expression of HSP 105 in Helicobacter gastritis was even lower than that in the control group (p= 0.008).

IMMUNE REACTION IN LYMPHOID INFILTRATE

No immune reaction was detected in lymphoid infiltrate for p53 or cyclin D1.

Table 3 shows the immune expressions of Rb, EGFR and HSP 105 in lymphoid infiltrate.

While Helicobacter group had the highest expression, the lowest expression was seen in the control group with Rb. Difference among groups were statistically significant (p< 0.0001). In Helicobacter gastritis group, the Rb positivity was significantly more when compared to Helicobacter-negative gastritis group (p< 0.0001). However, difference between Helicobacter-negative and the control groups was not statistically significant (p= 0.488).

The expressions of EGFR and HSP 105 in Helicobacter-negative gastritis group were statistically higher than those in Helicobacter gastritis group (p= 0.016 and p< 0.0001, respectively). HSP 105 immune expression was even lower in Helicobacter gastritis group than in the control group (p= 0.005).

DISCUSSION

Infection with the Gram-negative bacterium *H. pylori* leads to different clinical and pathological outcomes including malignancies such as gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[9,2] Although *H. pylori* has been classified as a type I carcinogen for gastric cancer by the International Agency for Research on Cancer, the exact nature and strength of the association with gastric malignancies are still being investigated.[10]

Lan et al. have reported that *H. pylori* might cause the severe imbalance between proliferation and apoptosis in the precancerous lesions, first leading to p53-Rb tumor-suppressor system mutation and telomerase reactivation, and finally causing gastric cancer.[10] Some studies showed that *H. pylori*-associated chronic gastritis expressed mutant-type p53, which was significantly associated with more severe atrophic and metaplastic changes.[11,12] However, other studies were not able to detect a significant association between p53 expression and *H. pylori* infection.[10,13]

Zhou et al. suggested that the alterations of Rb protein may play a role in the early stages of gastric

TABLE 3: Grading of Rb, EGFR, and HSP 105 immune expression in lymphoid infiltrate.

		n (%)				
Groups		**None Reaction**	**Minimal**	**Mild**	**Moderate**	**Severe**
Control group	Rb	1 (5.00)	15 (75.0)	4 (20.0)	0 (0)	0 (0)
n= 20	EGFR	0 (0)	0 (0)	6 (30.0)	13 (65.0)	1 (5.00)
	HSP 105	8 (40.0)	9 (45.0)	3 (15.0)	0 (0)	0 (0)
Helicobacter gastritis	Rb[a]	0 (0)	2 (9.10)	1 (4.50)	10 (45.5)	9 (40.9)
n= 22	EGFR[b]	4 (18.2)	7 (31.8)	3 (13.6)	4 (18.2)	4 (18.2)
	HSP 105[c]	18 (81.9)	3 (13.6)	1 (4.50)	0 (0)	0 (0)
Helicobacter-negative gastritis	Rb[a]	1 (4.80)	7 (33.3)	11 (52.4)	2 (9.50)	0 (0)
n= 21	EGFR[b]	0 (0)	0 (0)	5 (23.9)	9 (42.8)	7 (33.3)
	HSP 105[c]	0 (0)	2 (9.50)	3 (14.3)	10 (47.6)	6 (28.6)

[a]p< 0.0001, Helicobacter gastritis versus Helicobacter-negative gastritis; [b]p= 0.016, Helicobacter gastritis versus Helicobacter-negative gastritis; [c]p< 0.0001, Helicobacter gastritis versus Helicobacter-negative gastritis.

cardia carcinogenesis.[14] In that study, *H. pylori* infection was not taken into consideration. In our study, high Rb expression was observed in gastric mucosa with *H. pylori* infection without morphological dysplasia or intestinal metaplasia. However, immunohistochemical reaction of p53 was detected only in Helicobacter-negative gastritis. Therefore, we can suggest that Rb, which is a tumor suppressor gene, may have a role in the earliest phase of carcinogenesis in the gastric epithelia with *H. pylori* infection. In contrast, p53 may have a role in the earliest phase of gastric carcinogenesis in Helicobacter-negative gastritis. Although our results did not reveal p53 expression in the Helicobacter gastritis without intestinal metaplasia and moderate or severe atrophy, p53 may participate in the gastric carcinogenetic pathway in late stage of *H. pylori* gastritis with dysplasia and intestinal metaplasia. Further studies should be performed to clarify this issue.

In a previous study, it was found that EGFR expression was highly related to *H. pylori* infection, and it normalized after treatment of the infection.[15] However, in another study, it was reported that patients with *H. pylori*-associated gastritis and peptic ulcer had reduced levels of gastric luminal EGF which were not restored by *H. pylori* eradication.[16] In another study, the percentage of EGFR-positive cells decreased in *H. pylori*-associated gastritis as compared to the normal epithelium and Helicobacter-negative gastritis.[1] In the present study, EGFR expression did not increase in gastritis with *H. pylori* infection. However, there was high EGFR expression in gastritis without *H. pylori* infection. There are discrepancies among the results of these studies, however according to our results we can say that EGFR expression increases significantly in chronic gastritis without *H. pylori* infection. Therefore, EGFR may play a greater role in early gastric carcinogenesis in chronic gastritis without *H. pylori* infection rather than in early gastric carcinogenesis in Helicobacter gastritis.

High levels of cyclin D1 expression in gastric cancer tissues were reported in the literature.[17,18] In a previous study, while overexpressions of cyclin D2 and D3 were seen in gastric carcinoma, and were significantly correlated with *H. pylori* infection, no overexpression of cyclin D1 was observed in gastric carcinoma, and there was no correlation between cyclin D1 and *H. pylori* infection.[19] In addition, no association was observed between cyclin D1 expression and Helicobacter gastritis in another study.[11] In our study, cyclin D1 was detected only in gastritis without *H. pylori* infection. This result was contrary to the results of the study by Polat et al.[20] In this study, cyclin D1 expression was observed in the late stage of Helicobacter gastritis with intestinal metaplasia and atrophy.[20] Therefore, we can say that cyclin D1 level increases in the late stage of Helicobacter gastritis, however Helicobacter-negative gastritis shows high expression of cyclin D1 in the early stage of chronic gastritis without intestinal metaplasia or atrophy. When we take our results into consideration, we can suggest that cyclin D1 plays a role in gastric carcinogenesis in the early stage of chronic gastritis without *H. pylori* infection, but not in the early stage of Helicobacter gastritis.

HSP 105, a heat shock protein, was found less in both epithelia and lymphoid infiltrate of Helicobacter gastritis in the present study. Due to its protective function, it normally increases in tissues with injury.[21] Deprivation of heat shock proteins in gastric mucosa may lead to sensitization of crypt epithelia and lymphoid infiltrate for carcinogenic mutations, and may prevent healing of mucosal injury.

In mucosal lymphoid infiltrate, no reaction was detected for cyclin D1 and p53 in contrast to epithelial tissue. Therefore, we can say that cyclin D1 and p53 have no role in the early stage of carcinogenesis of lymphoid infiltrate in chronic gastritis with and without *H. pylori*. There are few suppressor gene mutations in gastric mucosa-associated lymphoid tissue lymphoma. In literature, some authors have suggested that additional genetic abnormalities, such as inactivation of the tumor suppressor genes, can lead to high-grade transformation from MALT lymphoma.[22-24] Therefore, absence of p53 abnormality in our results is not surprising.

Rb expression was more in the lymphoid infiltrate of Helicobacter gastritis, whereas HSP 105 and EGFR was more in the lymphoid infiltrate of Helicobacter-negative gastritis in our study. Therefore, hyperproliferation and early carcinogenesis in lymphoid infiltrate for development of MALT lymphoma may be due to the increased expression of Rb gene in Helicobacter gastritis.

We selected only fundic mucosa (corpus and fundus) for this study because the reaction of fundic mucosa against *H. pylori* infection may be somewhat different from that of antral mucosa at molecular level.[5,25] In addition, type A chronic gastritis, a gastritis in the Helicobacter-negative gastritis group, usually settles down in this location.[26] However, we did not classify Helicobacter-negative gastritis group according to etiology. We made an effort to select gastritis samples with similar density of lymphoid infiltrate. Similarly, in gastritis with *H. pylori* group, we noted the similarity of *H. pylori* density among the cases. We preferred Helicobacter gastritis with low activity level to ensure similarity of Helicobacter-negative gastritis samples. In this way, study subjects and data were ensured to be more homogeneous and reliable. Similar studies with higher numbers of subjects and wider spectra of genes may reveal more reliable and interesting results.

The gene expressions in both crypt cells and lymphoid infiltrate of gastric mucosa with *H. pylori* infection were different from those in chronic gastritis without *H. pylori* infection in the present study. These differences between gastritis with and without *H. pylori* infection for gene expressions may have been due to the production of specific virulence factors by *H. pylori* such as urease and vacuolating cytotoxin VacA.[27,28] In addition to the bacterium and its products, some chemical mediators of inflammation in chronic gastritis with *H. pylori* infection may have an effect on immunohistochemical reactivity of some genes or alter the antigenic structure of gene products. Consequently, immunohistochemical expressions of different genes may be decreased or increased in Helicobacter gastritis.

Briefly, our study suggests that alterations of cyclin D1, EGFR and p53 may have a possible role in gastric epithelial carcinogenesis in gastritis without *H. pylori* infection. However, Rb gene and HSP 105 may play a role in gastric carcinogenesis in gastritis with *H. pylori* infection. Alterations of cyclin D1, EGFR and p53 were detected only in chronic gastritis without *H. pylori* infection group. Therefore, we can say that cyclin D1, EGFR and p53 do not play a role in early gastric carcinogenesis in Helicobacter gastritis. Decreased HSP 105 in Helicobacter gastritis may suggest that mucosal protective and healing function is weakened in this type of gastritis, which ensures continuation of inflammation, and can sensitize gastric mucosa to early carcinogenic mutation.

In conclusion, these different expression profiles for some genes may explain the difference in behavior between chronic gastritis with and without *H. pylori* infection. If gastric cancer develops in relation with chronic gastritis, the carcinogenesis may have a different molecular pathway in gastritis with *H. pylori* infection when compared to gastritis without *H. pylori* infection.

REFERENCES

1. Ruzsovics A, Unger Z, Molnar B, Pronai L, Tulassay Z. Effect of Helicobacter pylori infection on epidermal growth factor receptor (EGFR) expression and cell proliferation of gastric epithelial mucosa: correlation to macroscopic and microscopic diagnosis. Int J Exp Pathol 2002;83(5):257-63.
2. Zarrilli R, Ricci V, Romano M. Molecular response of gastric epithelial cells to Helicobacter pylori-induced cell damage. Cell Microbiol 1999;1(2):93-9.
3. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 1993;342(8871):575-7.
4. Kato K, Hasui K, Wang J, Kawano Y, Aikou T, Murata F. Homeostatic mass control in gastric non-neoplastic epithelia under infection of Helicobacter pylori: an immunohistochemical analysis of cell growth, stem cells and programmed cell death. Acta Histochem Cytochem 2008;41(3):23-38.
5. Hofman VJ, Moreilhon C, Brest PD, Lassalle S, Le Brigand K, Sicard D, et al. Gene expression profiling in human gastric mucosa infected with Helicobacter pylori. Mod Pathol 2007;20(9):974-89.
6. De Luca A, Iaquinto G. Helicobacter pylori and gastric diseases: a dangerous association. Cancer Lett 2004;213(1):1-10.

7. Kumar V, Abbas AK, Fausto N. Neoplasia. Robins and Cotran Pathologic Basis of Disease. 7th ed. Philadelphia: Elsevier Inc; 2005. p.269-342.
8. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20(10):1161-81.
9. Blaser MJ, Parsonnet J. Parasitism by the "slow" bacterium Helicobacter pylori leads to altered gastric homeostasis and neoplasia. J Clin Invest 1994;94(1):4-8.
10. Lan J, Xiong YY, Lin YX, Wang BC, Gong LL, Xu HS, et al. Helicobacter pylori infection generated gastric cancer through p53-Rb tumor-suppressor system mutation and telomerase reactivation. World J Gastroenterol 2003;9(1): 54-8.
11. Guarner J, Bartlett J, Seitz R, Whistler T, Herrera-Goepfert R, Mohar A, et al. Cell proliferation and inflammation on biopsy samples with multifocal atrophic gastritis before and 1 year after Helicobacter pylori eradication. Arch Pathol Lab Med 2005;129(11):1451-6.
12. Kodama M, Murakami K, Okimoto T, Sato R, Watanabe K, Fujioka T. Expression of mutant type-p53 products in H pylori-associated chronic gastritis. World J Gastroenterol 2007;13 (10):1541-6.
13. Gobbo César AC, de Freitas Calmon M, Cury PM, Caetano A, Borim AA, Silva AE. Genetic alterations in benign lesions: chronic gastritis and gastric ulcer. World J Gastroenterol 2006;12(4):625-9.
14. Zhou Y, Gao SS, Li YX, Fan ZM, Zhao X, Qi YJ, et al. Tumor suppressor gene p16 and Rb expression in gastric cardia precancerous lesions from subjects at a high incidence area in northern China. World J Gastroenterol 2002;8(3):423-5.
15. Wong BC, Wang WP, So WH, Shin VY, Wong WM, Fung FM, et al. Epidermal growth factor and its receptor in chronic active gastritis and gastroduodenal ulcer before and after Helicobacter pylori eradication. Aliment Pharmacol Ther 2001;15(9):1459-65.
16. Lynch DA, Mapstone NP, Lewis F, Pentith J, Axon AT, Dixon MF, et al. Serum and gastric luminal epidermal growth factor in Helicobacter pylori-associated gastritis and peptic ulcer disease. Helicobacter 1996;1(4):219-26.
17. Gao P, Zhou GY, Liu Y, Li JS, Zhen JH, Yuan YP. Alteration of cyclin D1 in gastric carcinoma and its clinicopathologic significance. World J Gastroenterol 2004;10(20):2936-9.
18. Takasu S, Tsukamoto T, Ushijima T, Yamashita S, Ogasawara N, Ban H, et al. Cyclin D1 overexpression in N-methyl-N'-nitro-N-nitrosoguanidine-induced rat gastric adenocarcinomas. Exp Toxicol Pathol 2007;59(3-4): 171-5.
19. Yu J, Miehlke S, Ebert MP, Szokodi D, Wehvnignh B, Malfertheiner P, et al. Expression of cyclin genes in human gastric cancer and in first degree relatives. Chin Med J (Engl) 2002;115(5):710-5.
20. Polat A, Cinel L, Dusmez D, Aydin O, Egilmez R. Expression of cell-cycle related proteins in Helicobacter pylori gastritis and association with gastric carcinoma. Neoplasma 2002;49 (2):95-100.
21. Feder ME, Hofmann GE. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 1999;61:243-82.
22. Starostik P, Patzner J, Greiner A, Schwarz S, Kalla J, Ott G, et al. Gastric marginal zone B-cell lymphomas of MALT type develop along 2 distinct pathogenetic pathways. Blood 2002;99(1):3-9.
23. Murga Penas EM, Hinz K, Biller L, Zivkovic T, Röser K, Löning T, et al. Frequency of chromosomal aneuploidies and deletions of the RB and TP53 genes in MALT lymphomas harboring the t(14;18)(q32;q21). Cancer Genet Cytogenet 2006;164(1):81-3.
24. Sato H, Sato Y, Ichimura K, Oka T, Kondo E, Tanaka T, et al. p27(Kip1) is detected on most gastric MALT lymphomas, but not large cell lymphomas. J Clin Exp Hematop 2006;46(1) :25-30.
25. Murata H, Tsuji S, Tsujii M, Nakamura T, Fu HY, Eguchi H, et al. Helicobacter pylori infection induces candidate stem cell marker Musashi-1 in the human gastric epithelium. Dig Dis Sci 2008;53(2):363-9.
26. Eidt S, Oberhuber G, Schneider A, Stolte M. The histopathological spectrum of type A gastritis. Pathol Res Pract 1996;192(2):101-6.
27. Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. Clin Microbiol Rev 1997;10(4): 720-41.
28. Sommi P, Savio M, Stivala LA, Scotti C, Mignosi P, Prosperi E, et al. Helicobacter pylori releases a factor(s) inhibiting cell cycle progression of human gastric cell lines by affecting cyclin E/cdk2 kinase activity and Rb protein phosphorylation through enhanced p27(KIP1) protein expression. Exp Cell Res 2002;281(1): 128-39.