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# Expression of cytokeratins in *Helicobacter pylori* –associated chronic gastritis of adult patients infected with *cagA*+ strains: An immunohistochemical study

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## Abstract

**AIM:** To investigate the expression of different cytokeratins (CKs) in gastric epithelium of adult patients with chronic gastritis infected with *Helicobacter pylori* (*H pylori*) *cagA*+ strains.

**METHODS:** The expression of CK 7, 8, 18, 19 and 20 was studied immunohistochemically in antral gastric biopsies of 84 patients. All the CKs were immunostained in *cagA*+*H pylori* gastritis (57 cases), non-*H pylori* gastritis (17 cases) and normal gastric mucosa (10 cases).

**RESULTS:** In *cagA*+ *H pylori* gastritis, CK8 was expressed comparably to the normal antral mucosa from surface epithelium to deep glands. Distribution of CK18 and CK 19 was unchanged, i.e. transmucosal, but intensity of the expression was different in foveolar region in comparison to normal gastric mucosa. Cytokeratin 18 immunoreactivity was significantly higher in the foveolar epithelium of *H pylori*-positive gastritis compared to both *H pylori*-negative gastritis and controls. On the contrary, decrease in CK19 immunoreactivity occurred in foveolar epithelium of *H pylori*-positive gastritis. In both normal and inflamed antral mucosa without *H pylori* infection, CK20 was expressed strongly/moderately and homogenously in surface epithelium and upper foveolar region, but in *H pylori* -induced gastritis significant decrease of expression in foveolar region was noted. Generally, in both normal antral mucosa and *H pylori*-negative gastritis, expression of CK7 was not observed, while in about half *cagA*+ *H pylori*-infected patients, moderate focal CK7 immunoreactivity of the neck and coiled gland areas was registered, especially in areas with more severe inflammatory infiltrate.

**CONCLUSION:** Alterations in expression of CK 7, 18, 19 and 20 together with normal expression of CK8 occur in antral mucosa of *H pylori*-associated chronic gastritis in adult patients infected with *cagA*+ strains. Alterations in different cytokeratins expression might contribute to weakening of epithelial tight junctions observed in *H pylori*-infected gastric mucosa.



**Key words**: cytokeratin 7; cytokeratin 8; cytokeratin18; cytokeratin19; cytokeratin20; *Helicobacter pylori*; Gastritis; *CagA*

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## INTRODUCTION

Cytokeratins (CKs), a family of important cytoskeleton structural proteins, have specific spatial and temporal dynamic locations along the epithelial axis of the gastrointestinal tract (GIT), and their expression is linked to the degree of epithelial differentiation[1-4]. Cytokeratins 7, 8 (intermediate-sized and basic), 18 and 19 (smallest in size and acidic) are exclusively expressed in nearly all simple epithelia, pseudostratified respiratory epithelium and

transitional epithelium. CK8 and CK18 pair together and have a similar distribution, while CK19 can be detected in a broad range of epithelial tissues, including many simple epithelia, diverse stratified epithelia, and cultured keratinocytes. CK20 (intermediate sized and acidic) is expressed in gastric foveolar epithelium, intestinal villi and crypt epithelium, cutaneous and oral Merkel cells[4]. Various changes in CK expression and distribution profile have been noted in inflammatory[5], preneoplastic[6-10] and neoplastic[2,11-13] disorders along GIT, including gastric mucosa. Structural changes in the gastric epithelium of adult and pediatric *H pylori*-infected patients with chronic gastritis have been recently demonstrated using cytokeratin immunohistochemistry[14-19].

Results of previous studies postulated that only a subset of individuals infected with *H pylori* develop severe gastritis and/or metaplasia, peptic ulcer or gastric cancer [14,20]. Bacterial strain, environmental and host factors can converge in the gastroduodenal milieu and control the final outcome of *H pylori* infection. However, to the best of our knowledge, relationship between *cagA*+ *H pylori* and changes in CK expression in the gastric epithelium has not yet been studied in patients with chronic gastritis. Since we have previously demonstrated high seroprevalence of antibodies to *cagA* in *H pylori*-infected patients in Serbia and Montenegro[21], this study aimed to identify and describe immunohistochemical pattern of antral CK expression in *H pylori* –associated chronic gastritis of adult patients infected with *cagA*+ strains.

## MATERIALS AND METHODS

### Subjects

We conducted an outpatient-based prospective study at the Clinic for Gastroenterology and Hepatology (Clinical Center of Serbia, University of Belgrade). All patients gave informed consent for participation in the study and the study protocol was approved by the local Ethics Committee. Adult patients with dyspeptic symptoms and histological signs of gastritis entered the study. Dyspepsia was defined as upper abdominal or retrosternal pain, discomfort, nausea, vomiting or other symptoms referable to the upper abdominal tract lasting for at least one month[22]. Exclusion criteria were in concordance with the recommendations from European *H pylori* Study Group[20].

Ninety-one patients entered the study. After histological examination, we excluded 17 patients (15 with and 2 without *H pylori* infection) due to the presence of intestinal metaplasia (IM) in the antral mucosa. Since specific IM- related changes in CK expression were reported, we aimed to identify changes related exclusively to the presence of *H pylori* infection. Therefore, data from 74 patients (57 *H pylori*-positive and 17 *H pylori*-negative) with chronic gastritis were analyzed. Control group (CG) consisted of 10 asymptomatic healthy volunteers with no histological changes in the gastric mucosa (mean age 32± 11 years, 3 males, 7 females). In the *H pylori*-positive group mean age was 44±13 years (26 males, 31 females), while in the non-infected group mean age was 47±17 years (5 males, 12 females). Esophagogastroduodenoscopy with biopsies from antral and corpus mucosa was performed in all patients and blood was taken for serology and immunoblot.

*H pylori* infection was diagnosed by rapid urease test (RUT), histology, immunohistochemistry and serology. A patient was defined as *H pylori* positive if histology and at least one of the other applied diagnostic methods were positive.

### Routine endoscopy and H pylori status

Each patient underwent upper endoscopy and testing for the presence of *H pylori* by RUT and histology. Six biopsies were taken, 3 from the antrum and 3 from the corpus (2 for histology and 1 for RUT). Biopsies selected for histological examination were stained with hematoxylin-eosin, alcian blue pH 2.5/periodic acid Schiff (AB/PAS) and high iron diamine/alcian blue pH2.5 (HID/PAS). Both Giemsa and immunohistochemical stainings (polyclonal antibody to *H pylori*, dilution 1:10, DAKO A/S, Glostrup, Denmark) were applied for the detection of *H pylori*.

### H pylori serology and immunoblot assay

Blood samples were taken from all patients after endoscopic examination and sera were separated by centrifugation and stored at -20 °C until analyzed. The presence of anti-*cagA* antibodies was detected using the *Helicobacter pylori* Vira blot test kit™ (Viramed Biotech AG, Lich, Germany). The concentration of anti-*H pylori* IgG antibodies was analyzed using Pyloriset EIA-G III™ (Orion Diagnostica, Finland)[23]. Both tests were performed according to the manufacturer's instructions.

### Immunohistochemistry

Immunohistochemical reactions were performed on consecutive sections of one selected antral biopsy of each patient to detect cytokeratins 7, 8, 18, 19 and 20. Only well-oriented biopsies allowing assessment of full mucosal thickness, were selected for immunohistochemical study. The sections were stained with monoclonal antibodies to CK7 (dilution 1:25, DAKO A/S, Glostrup, Denmark), CK8 (dilution 1:20, DAKO Carpinteria, CA, USA), CK18 (dilution 1:25, DAKO A/S, Glostrup, Denmark), CK19 (dilution 1:50, DAKO A/S, Glostrup, Denmark) and CK20 (dilution 1:25, DAKO A/S, Glostrup, Denmark). Immunohistochemical staining was performed according to the manufacturer's instructions using streptavidin-biotin/HRP detection system (DAKO LSAB+/HRP kit, Glostrup, Denmark), followed by counterstaining with hematoxylin. For negative controls, no staining was detectable when the pre-immune serum was used instead of primary antibodies. In addition, human fetal esophagus of the 13$^{th}$ gestation week was used as positive control for evaluation of CK7 immunoreactivity.

### Histological and immunohistochemical evaluation

Mucosal biopsies were evaluated by an experienced pathologist blinded to clinical and endoscopic data. In addition, an experienced gastrointestinal pathologist and a cytologist independently evaluated immunohistochemistry. Differences in immunohistochemical evaluation were resolved by re-examination and consensus. Chronic gastritis was diagnosed and graded according to the

**Table 1 Antral histology according to Sydney classification of gastritis in patients with and without *Helicobacter pylori* infection**

| Antral mucosa (Sydney classification) | *H pylori* + (*n* = 57) | *H pylori*- (*n* = 17) | *P* |
|---|---|---|---|
| Inflammatory infiltrate | | | |
| 0 | 0 | 1 | 0.000 |
| 1 | 19 | 14 | |
| 2 | 31 | 2 | |
| 3 | 7 | 0 | |
| Activity of inflammation | | | |
| 0 | 2 | 9 | 0.000 |
| 1 | 39 | 6 | |
| 2 | 15 | 2 | |
| 3 | 1 | 0 | |
| Atrophy | | | |
| 0 | 45 | 13 | NS |
| 1 | 12 | 4 | |
| *H pylori*-colonization density | | | |
| 0 | 0 | 17 | |
| 1 | 19 | 0 | |
| 2 | 25 | 0 | |
| 3 | 13 | 0 | |

*H pylori*+: *Helicobacter pylori* positive patients; *H pylori* -: *Helicobacter pylori* negative patients; NS: $P > 0.05$.

updated Sydney system[24]. Biopsies showing intestinal metaplasia classified according to previous proposals [13] were not included in this study. Mucosal distribution (surface epithelium, foveolar region, glandular necks and deep glands) and intensity of cytoplasm staining (without staining, weak or moderate/strong staining) were registered together with the expression pattern (focal or diffuse) for each analyzed CK, while an additional semi-quantitative assessment of percentages of immunoreactive cells in each mucosal compartment was performed and graded for CK7 in 3 groups (<10%, 10-20%, and >20% immunoreactive cells).

### Statistical analysis

Results were analyzed using non-parametric tests, Kruskall-Wallis, chi-square or Fisher's exact test for independent samples. $P < 0.05$ was considered statistically significant.

## RESULTS

Results of our study showed that presence of intestinal metaplasia could be detected in 18.6% (17/91) of dyspeptic patients with histological signs of gastritis (15 with and 2 without *H pylori* infection). These patients were excluded from further analysis, since we aimed to investigate the influence of *H pylori* infection on normal gastric epithelium. Out of the remaining 74 patients, 57 (77%) were infected with *H pylori,* while infection was not found in 17. All infected patients harbored *cagA*+ bacterial strain in the gastric mucosa.

### Histology evaluation

Histological examination of antral and corpus mucosa in *H pylori*-infected individuals revealed signs of antral gastritis in 5 (9%), while pangastritis was diagnosed in the remaining 52 (91%) patients. In the *H pylori* negative group antral gastritis was diagnosed in 7 (42%) patients and histological signs of pangastritis were found in 10 (58%). Histological changes in the gastric mucosa were graded using Sydney classification both for antral (Table 1) and corpus mucosa (data not shown).

In the antrum of *H pylori*-infected individuals, inflammatory infiltrate density and activity of inflammation were higher than in the uninfected group ($P < 0.001$ for both histological parameters). Presence of atrophic changes was not different between the two groups, while moderate density of *H pylori*-colonization was most frequent in infected patients.

### Immunohistochemical evaluation of CK expression and distribution

Cytokeratin 8 was identified immunohistochemically in antral mucosa of both *H pylori* positive and negative patients with gastritis and controls (Table 2). Diffuse immunoreactivity to CK8 was the predominant expression pattern in surface epithelium, foveolae and glandular neck (moderate/strong immunoreactivty was observed in 90% of controls, 70.6% of *H pylori* negative and 63.1% of *H pylori*+ gastritis and weak in only 11.8% with and 12.3% without *H pylori* infection). On the other hand, deeper glandular structures did not express CK8 in about 10-30% of cases. No significant difference was found in any of the examined antral mucosa regions between the three groups (Figure 1).

Normal antral mucosa was in general immunostained transmucosal (from the surface to the gland region) when antibodies to CK18 and CK19 were applied (Tables 3 and 4), while in foveolar region inconsistent CK18 immunoreactivity with the expression rate of only 10% was observed. In the foveolar epithelium of *H pylori*+ patients more intense diffuse ($P < 0.05$) and focal ($P < 0.01$) CK18 immunoreactivity was detected when compared to *H pylori*-negative gastritis and controls (Figure 2). As opposed to these results, lower antral CK19 patchy immunoreactivity was noted in foveolar epithelium of *H pylori*+ gastritis compared to *H pylori*-negative gastritis and controls ($P < 0.05$) (Figure 3).

All examined antral biopsies showed positive immunostaining of CK20 (Table 5) restricted to the surface and upper foveolar epithelium. In the surface epithelium strong and homogenous CK20 immunoreactivity was predominant (moderate/strong diffuse immunoreactivity was observed in 90% of controls, 70.6% of *H pylori* negative and 64.9% of *H pylori*+ gastritis, as opposed to the patchy staining observed in 10%, 29.4% and 35.1% of individuals, respectively). No significant differences were noted between controls and patients with gastritis irrespective of the presence of *H pylori* infection. In the antral mucosa of the majority of *H pylori* negative patients with gastritis and controls CK20 was expressed strongly/moderately in all foveolar cells while in *H pylori* positive gastritis patients a significantly higher percentage of patients had focal expression of CK20 (Figure 4). Further analysis revealed different CK20 expressions in upper foveolar region related to the presence of gastritis but not

**Table 2 Expression of CK8 in antral mucosa of patients with gastritis (*H pylori* positive and negative) compared to the control group**

| Cytokeratin 8 expression | Antral mucosa epithelium | | | | | | |
|---|---|---|---|---|---|---|---|
| | *H pylori*+G | | *H pylori*-G | | CG | | *P* |
| | *n* | % | *n* | % | *n* | % | |
| | Surface epithelium, foveolae and glandular necks | | | | | | |
| Diffuse immunoreactivity | 43 | 75.4 | 14 | 82.4 | 9 | 90 | NS |
| Focal immunoreactivity | 14 | 24.6 | 3 | 17.6 | 1 | 10 | NS |
| Without staining | 0 | 0 | 0 | 0 | 0 | 0 | NS |
| *n* (%) | 57 | 100 | 17 | 100 | 10 | 100 | |
| | Deep glands | | | | | | |
| Diffuse immunoreactivity | 24 | 42.1 | 11 | 64.7 | 5 | 50 | NS |
| Focal immunoreactivity | 15 | 26.3 | 2 | 11.8 | 4 | 40 | NS |
| Without staining | 18 | 31.6 | 4 | 23.5 | 1 | 10 | NS |
| *n* (%) | 57 | 100 | 17 | 100 | 10 | 100 | |

CG: control group; *H pylori*+G: *H pylori* positive patients with gastritis; *H pylori*-G -: *H pylori* negative patients with gastritis; NS: $P > 0.05$

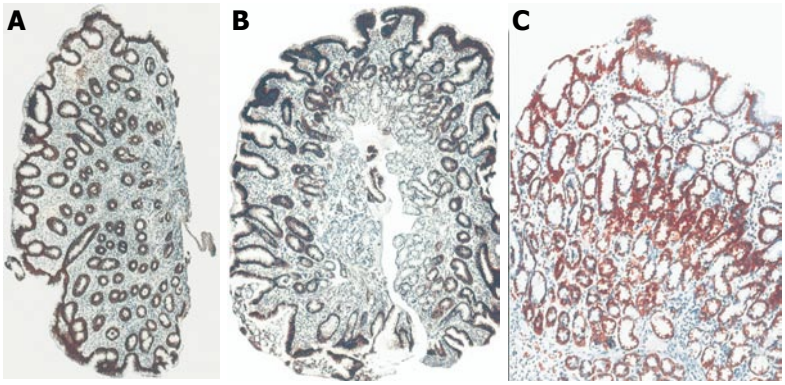


**Figure 1** CK8 immunoreactivity in antral mucosa of patients with cagA+ *H pylori* chronic gastritis (**A**), non-*H pylori* chronic gastritis (**B**) and control subjects with normal gastric mucosa (**C**) with differences of CK8 immunoreactivity in antral mucosa. Original magnification: x10 (**A**,**C**); x20 (**B**).

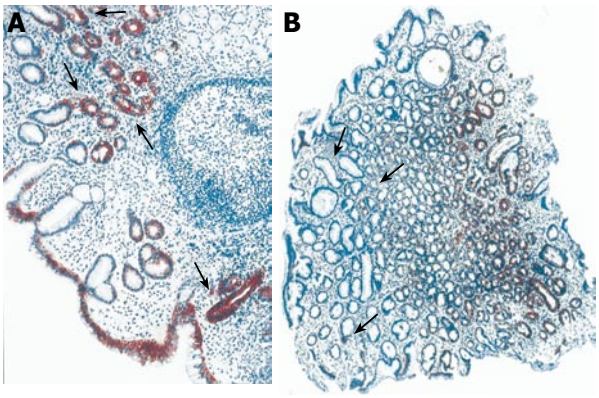


**Figure 2** CK18 immunoreactivity in antral mucosa of patients with cagA+ *H pylori* chronic gastritis (**A**) and control subjects without gastritis (**B**). Original magnification: x10. Cytokeratin 18 immunoreactivity was significantly higher in the foveolar epithelium of *H pylori*-positive gastritis compared with *H pylori*-negative gastritis.

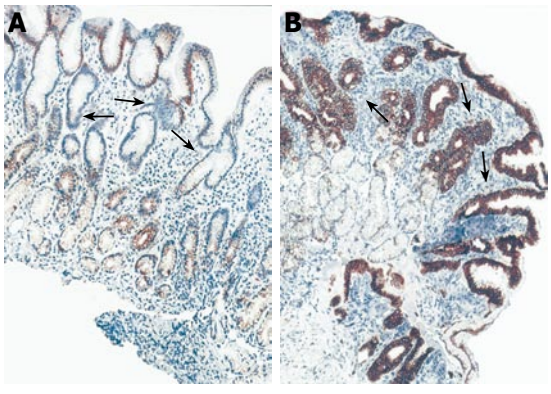


**Figure 3** CK19 immunoreactivity in antral mucosa of patients with cagA+ *H pylori* chronic gastritis (**A**) and non-*H pylori* chronic gastritis (**B**). Decrease in CK19 immunoreactivity was found in foveolar epithelium of antral mucosa of *H pylori*-positive gastritis compared to non-*H pylori* chronic gastritis. Original magnification: x20 (**A**, **B**)

*H pylori* infection. Namely, patchy staining pattern was detected in 44% of *H pylori*+ and 29% of *H pylori* negative gastritis patients and only 10% of controls ($P < 0.05$).

The main findings of CK7 immunoreactivity are listed in Table 6. In both controls and *H pylori*-negative gastritis patients almost no immunoreactivity of CK7 was found in the antral mucosa, with the exception of some inconsistent and faint CK7 immunoreactivity observed in single cells of glandular necks and deep glands in about 2/3 of cases (Figure 5). However, moderate focal CK7 immunoreactivity in the same area was more frequently observed in *H pylori*-positive gastritis compared to both *H pylori*-negative gastritis and control group ($P < 0.01$). Namely, about half in *H pylori*-infected patients (28/55,

**Table 3 Expression of CK18 in antral mucosa of patients with gastritis (*H pylori* positive and negative) compared to the control group**

| Cytokeratin 18 expression | Antral mucosa epithelium | | | | | | |
|---|---|---|---|---|---|---|---|
| | *H pylori*+G | | *H pylori*-G | | CG | | *P* |
| | *n* | % | *n* | % | *n* | % | |
| | | | | Surface epithelium | | | |
| **Diffuse immunoreactivity** | 32 | 56.1 | 14 | 82.3 | 5 | 50.0 | NS |
| **Focal immunoreactivity** | 24 | 42.1 | 2 | 11.8 | 3 | 30.0 | NS |
| **Without staining** | 1 | **1.7** | 1 | **5.9** | 2 | **20.0** | **0.043** |
| *n* (%) | 57 | 100 | 17 | 100 | 10 | 100 | |
| | | | | Foveolar epithelium | | | |
| **Diffuse immunoreactivity** | **31** | **54.4** | **8** | **47.1** | **1** | **10** | **0.035** |
| Moderate/Strong | 30 | 52.7 | 8 | 47.1 | 1 | 10 | **0.045** |
| Weak | 1 | 1.7 | 0 | 0 | 0 | 0 | NS |
| **Focal immunoreactivity** | **20** | **35.1** | **1** | **5.8** | **0** | **0** | **0.008** |
| Moderate/Strong | **5** | 8.8 | 0 | 0 | 0 | 0 | NS |
| Weak | 15 | 26.3 | 1 | 5.8 | 0 | 0 | 0.045 |
| **Without staining** | **6** | **10.5** | **8** | **47.1** | **9** | **90** | **<0.001** |
| ***n* (%)** | **57** | **100** | **17** | **100** | **10** | **100** | |
| | | | | Glandular necks | | | |
| **Diffuse immunoreactivity** | 36 | 63.2 | 13 | 76.5 | 6 | 60 | NS |
| **Focal immunoreactivity** | 18 | **31.6** | 2 | 11.8 | 2 | 20 | NS |
| **Without staining** | 3 | 5.2 | 1 | 5.9 | 2 | 20 | NS |
| *n* (%) | 57 | 100 | 17 | 100 | 10 | 100 | |
| | | | | Deep glands | | | |
| **Diffuse immunoreactivity** | **15** | **26.3** | **8** | **47.0** | **4** | **40** | NS |
| Moderate/Strong | 15 | 26.3 | 5 | 29.4 | 3 | 30 | NS |
| Weak | 0 | **0** | 3 | **17.6** | 1 | **10** | **0.008** |
| **Focal immunoreactivity** | **18** | **31.6** | **3** | **17.7** | **4** | **40** | NS |
| **Without staining** | **24** | **42.1** | **6** | **10.3** | **2** | **20** | NS |
| ***n* (%)** | **57** | **100** | **17** | **100** | **10** | **100** | |

CG:control group; *H pylori*+G -:*Helicobacter pylori* positive patients with gastritis; *H pylori*-G: *Helicobacter pylori* negative patients with gastritis; NS: $P > 0.05$

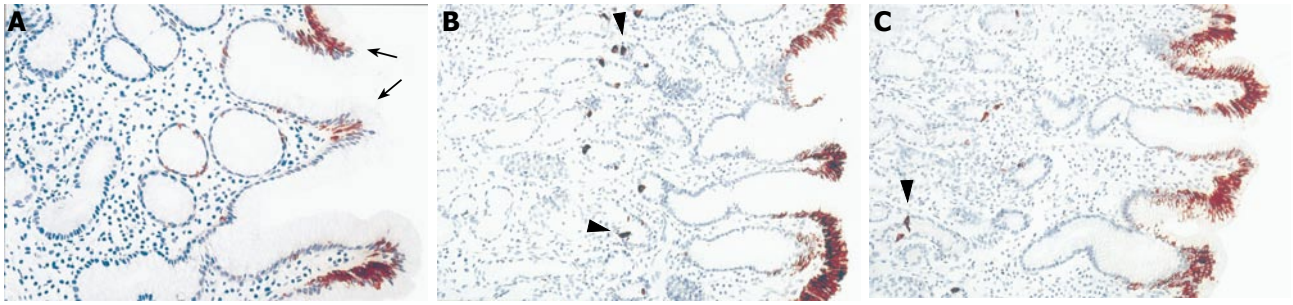


**Figure 4** CK20 immunoreactivity in antral mucosa of patients with cagA+ *H pylori* chronic gastritis (**A**), non-*H pylori* chronic gastritis (**B**) and control subjects with normal gastric mucosa (**C**). In the cagA+ *H pylori* chronic gastritis, CK20 expression was decreased in upper foveolar epithelium in comparison to non-*H pylori* chronic gastritis and control subjects with normal gastric mucosa. Numerous CK20 strong immunoreactive endocrine cells could be observed (►) in the glandular epithelium. Original magnification: x20 (A-C)

50.1%) moderate focal immunoreactivity of CK7 in more than 10% of cells in neck and coiled gland areas was registered, especially in individuals with more severe inflammatory infiltrate (Figure 6).

## DISCUSSION

*H pylori*-induced proinflammatory cytokine expression and inflammation affects various gastric cell populations controlling gastric acid secretion[25], including somatostatin-producing D cells[26], gastrin-producing G cells[27], and parietal cells[28]. In addition, in the course of *H pylori*-induced gastritis, acute foveolitis of the pit proliferative zone with characteristic morphological change of epithelial cells, which is often described as the malgun (clear) cell change[29] occurs. It is postulated that malgun cell change in the course of *H pylori* gastritis reflects epithelial genomic damage and repair processes. In addition, various structural changes of mucosal epithelial cells in *H pylori* gastritis have been reported in both adult[14-16, 19] and pediatric patients[18].

Strain-specific *H pylori* gene, *cagA*, has been recognized as a marker of strains that confer increased risk for peptic

**Table 4 Expression of CK19 in antral mucosa of patients with gastritis (*H pylori* positive and negative) compared to the control group**

| Cytokeratin 19 expression | Antral mucosa epithelium | | | | | | *P* |
|---|---|---|---|---|---|---|---|
| | *H pylori*+G | | *H pylori*-G | | CG | | |
| | *n* | % | *n* | % | *n* | % | |
| **Surface epithelium** | | | | | | | |
| **Expression in all cells** | 34 | 60.6 | 12 | 70.6 | 5 | 55.6 | NS |
| **Patchy staining** | 21 | 37.4 | 5 | 29.4 | 4 | 44.4 | |
| **Without staining** | 1 | 2.0 | 0 | 0 | 0 | 0 | |
| ***n* (%)** | 56 | 100 | 17 | 100 | 9 | 100 | |
| **Foveolar epithelium** | | | | | | | |
| **Expression in all cells** | **39** | **69.7** | **9** | **53.0** | **5** | **55.6** | NS |
| **Patchy staining** | **7** | **12.5** | **5** | **29.4** | **4** | **44.4** | **0.027** |
| Moderate/Strong | 2 | 3.6 | 2 | 11.8 | 2 | 22.2 | |
| Weak | 5 | 8.9 | 3 | 17.6 | 2 | 22.2 | |
| **Without staining** | **10** | **17.8** | **3** | **17.6** | **0** | **0** | NS |
| ***n* (%)** | **56** | **100** | **17** | **100** | **9** | **100** | |
| **Glandular neck** | | | | | | | |
| **Expression in all cells** | 37 | 58.1 | 9 | 53 | 6 | 66.7 | NS |
| **Patchy staining** | 10 | 17.8 | 6 | 25.2 | 3 | 33.3 | |
| **Without staining** | 9 | 16.1 | 2 | 11.8 | 0 | 0 | |
| ***n* (%)** | 56 | 100 | 17 | 100 | 9 | 100 | |
| **Deep gland** | | | | | | | |
| **Expression in all cells** | 22 | 39.2 | 6 | 35.2 | 3 | 33.3 | NS |
| **Patchy staining** | 10 | 17.8 | 5 | 29.5 | 2 | 22.2 | |
| **Without staining** | 24 | 43.0 | 6 | 35.3 | 4 | 44.5 | |
| ***n* (%)** | 56 | 100 | 17 | 100 | 9 | 100 | |

CG: control group; *H pylori*+G: *Helicobacter pylori* positive patients with gastritis; *H pylori*-G: *Helicobacter pylori* negative patients with gastritis; NS: $P > 0.05$.

**Table 5 Expression of CK20 in antral mucosa of patients with gastritis (*H pylori* positive and negative) compared to the control group**

| Cytokeratin 20 expression | Antral mucosa epithelium | | | | | | *P* |
|---|---|---|---|---|---|---|---|
| | *H pylori*+G | | *H pylori*-G | | CG | | |
| | *n* | % | *n* | % | *n* | % | |
| Surface epithelium | | | | | | | |
| Expression in all cells | 37 | 64.9 | 12 | 70.6 | 9 | 90 | |
| Patchy staining | 20 | 35.1 | 5 | 29.4 | 1 | 10 | NS |
| Without staining | 0 | 0 | 0 | 0 | 0 | 0 | |
| *n* (%) | 57 | 100 | 17 | 100 | 10 | 100 | |
| Upper foveolar region | | | | | | | |
| Expression in all cells | 32 | 56.1 | 12 | 70.6 | 9 | 90 | NS |
| Patchy staining | 25 | 43.9 | 5 | 29.4 | 1 | 10 | |
| Moderate/Strong | 20 | 35.1 | 4 | 23.5 | 1 | 10 | |
| Weak | 5 | 8.8 | 1 | 5.9 | 0 | 0 | 0.044 |
| Without staining | 0 | 0 | 0 | 0 | 0 | 0 | |
| *n* (%) | 57 | 100 | 17 | 100 | 10 | 100 | |

CG:control group; *H pylori*+G: *Helicobacter pylori* positive patients with gastritis; *H pylori*-G: *Helicobacter pylori* negative patients with gastritis; NS: $P > 0.05$.

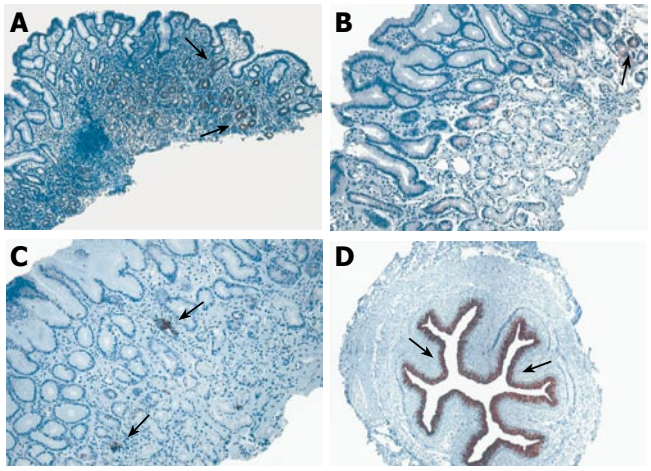


**Figure 5** CK7 immunoreactivity in antral mucosa of patients with cagA+ *H pylori* chronic gastritis (**A**), non-*H pylori* chronic gastritis (**B**), control subjects with normal gastric mucosa (**C**), and control specimen of human fetal esophagus (**D**). Moderate focal CK7 positivity (→) in the pits and the glands of antral mucosa in patients with cagA+ *H pylori* chronic gastritis could be observed. CK7 positive cells covering a single gland (→) of antral mucosa could be found in patients with non-*H pylori* chronic gastritis. Normal antral mucosa with a few single cells or cell clusters (→) were strongly decorated with antibody to CK7. Strong CK7 immunoreactivity was displayed in the stratified columnar epithelium (→) of human fetal esophagus of the 13th gestation week. Original magnification: x10 (**A-D**).

ulcer disease and gastric cancer[25]. Since the seroprevalence of *cagA*+ *H pylori* strains was high in chronic gastritis patients of Serbia and Montenegro[21] and there is no evidence that bacterial strain is related to alterations in cytokeratin expression in gastric epithelium, we aimed in the current study to evaluate the changes in distribution and expression pattern of different CKs in the course of *cagA*+ *H pylori* -associated chronic gastritis in adult patients.

In normal antral gastric mucosa, results of our study revealed broad panmucosal (from the surface to the gland) expressions of CKs8,18 and 19, while CK20 immunoreactivity was strong and homogenous in the tip

**Table 6 Expression of CK7 in antral mucosa of patients with gastritis (*H pylori* positive and negative) compared to the control group**

| Cytokeratin 7 expression | Antral mucosa epithelium | | | | | | *P* |
|---|---|---|---|---|---|---|---|
| | *H pylori*+G | | *H pylori*-G | | CG | | |
| | *n* | % | *n* | % | *n* | % | |
| | Surface and foveolar epithelium | | | | | | |
| Weak focal immunoreactivity | 0 | 0 | 0 | 0 | 0 | 0 | NS |
| Moderate focal immunoreactivity | 5 | 9.1 | 0 | 0 | 0 | 0 | NS |
| <10% cells | 0 | 0 | 0 | 0 | 0 | 0 | |
| 10-20% cells | 3 | 5.5 | 0 | 0 | 0 | 0 | |
| >20% cells | 2 | 3.6 | 0 | 0 | 0 | 0 | |
| Without staining | 50 | 90.9 | 16 | 100 | 10 | 100 | NS |
| *n* (%) | 55 | 100 | 16 | 100 | 10 | 100 | |
| | Glandular necks and deep glands | | | | | | |
| Weak focal immunoreactivity | 6 | 10.9 | 1 | 6.3 | 3 | 30.0 | |
| <10% cells | 3 | 5.5 | 1 | 6.3 | 3 | 30.0 | 0.01 |
| 10-20% cells | 2 | 3.6 | 0 | 0 | 0 | 0 | NS |
| >20% cells | 1 | 1.8 | 0 | 0 | 0 | 0 | NS |
| Moderate focal immunoreactivity | 38 | 69.1 | 10 | 62.5 | 7 | 70.0 | |
| <10% cells | 10 | 18.3 | 10 | 62.5 | 7 | 70.0 | 0.002 |
| 10-20% cells | 14 | 25.4 | 0 | 0 | 0 | 0 | 0.002 |
| >20% cells | 14 | 25.4 | 0 | 0 | 0 | 0 | 0.002 |
| Without staining | 11 | 20.0 | 5 | 31.2 | 0 | 0 | |
| *n* (%) | 55 | 100 | 16 | 100 | 10 | 100 | |

CG: control group; *H pylori*+G: *Helicobacter pylori* positive patients with gastritis; *H pylori*-G: *Helicobacter pylori* negative patients with gastritis; NS: $P > 0.05$.

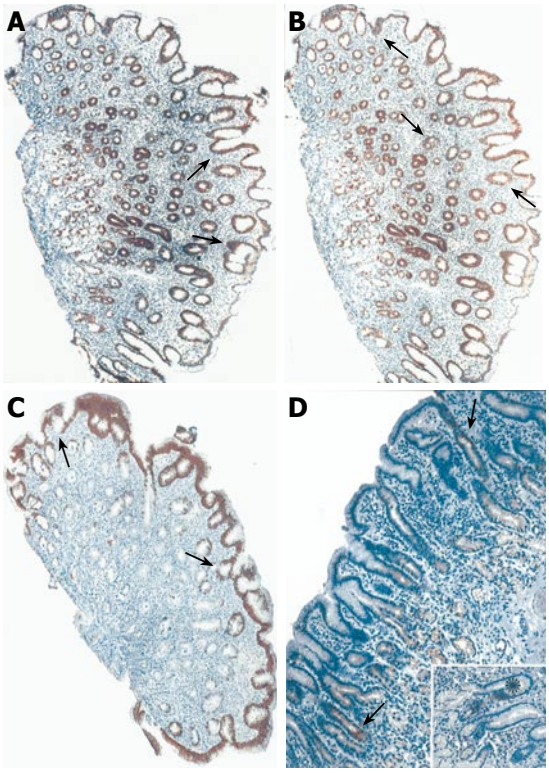


**Figure 6** Typical expression pattern of CK18 (**A**), CK19 (**B**), CK20 (**C**) and CK7 (**D**) in antral mucosa of patients with cagA+ *H pylori* chronic gastritis. Strong diffuse cytokeratin 18, moderate focal CK19 and CK20 (**C**) immunoreactivities were demonstrated in upper foveolar epithelium (→) of the antral mucosa. Moderate focal CK7 positivity (→) was revealed in the pits and the glands of antral mucosa. CK7 immunoreactivity was prominent (*) in some cell clusters of the foveolar epithelium (insert). Original magnification: x10 (**A**-**C**), insert x20.

and upper portion cells of foveolae. As opposed to other examined cytokeratins, CK7 was mostly undetectable. Similar results were obtained for gastritis patients without *H pylori* infection and overall these findings are in line with previous reports[1,2,14,15,17].

Several immunohistochemical studies in the past few years demonstrated that altered gastric cytokeratin expression is closely related with *H pylori* infection in adult patients[1,2,14-17,19]. However only two studies by Schwerer *et al*[15] and Louwers *et al*[17] have investigated multiple CKs simultaneously. To the best of our knowledge, all other studies are focused on particular CK. None of these studies however have provided data concerning bacterial strain.

According to previous observations, CK7 is present in fetal but largely absent in normal adult and is transiently *de novo* expressed in metaplastic and neoplastic epithelial cells[2,11]. Our results suggest that CK7, largely absent in normal adult antral mucosa, is expressed in *H pylori* chronic gastritis patients, which is in line with reports in adults describing slight[17] and markedly increased CK7 expression in *H pylori*-associated chronic gastritis in children[18]. As opposed to these findings, a study by Schwerer and Baczako[14] when investigating CK7 expression in normal foveolar epithelium found that *H pylori* can induce gastritis and intestinal metaplasia with CK7 immunoreactivity detected only in intestinal metaplasia.

Study by Kirchner *et al*[2] using animal experimental model of gastritis-cancer sequence in Mongolian gerbils revealed signs of mild gastritis 2 mo after *H pylori* infection together with CK7 expression in epithelial cells of basal glands followed by loss of specific differentiation and changes to duct-like appearance, while after 6, 12 and 24 mo moderate to severe gastritis with loss of differentiated gastric glands and switch to CK7 positive duct-like structures in large mucosal segments was observed. These results may provide evidence that non-neoplastic stomach with non-atrophic *H pylori* gastritis constantly exhibits low score of CK7 positive cells in antrum and corpus, thus supporting our findings. Furthermore, there is evidence that CK7 expression in metaplastic and neoplastic stomach is related to dedifferentiated epithelial cells that can phenotypically be linked to fetal cells at the beginning of gastric pit development[2]. The dedifferentiated cells exhibit low proliferation and beta-catenin accumulation similar to stem cells. Therefore, observations of Kirchner *et al*[2] imply that metaplasia, gastric intraepithelial neoplasia, early gastric cancer and dedifferentiated epithelial cells defined by CK7 expression are related with each other in *H pylori* induced gastritis. Based on the above stated findings, we speculate that CK7 *de novo* expression in gastric mucosa of patients infected with *cagA*+ *H pylori* strains represents the proliferative/regenerative cells rather than pure dedifferentiated cells because CK7 positive flat duct-like

structures have not been identified.

Our results did not reveal any significant difference in CK8 expression between patients with *cagA*+ *H pylori*-induced gastritis and normal mucosa. These findings are supported by results of other authors[15,17], but not by study of Baek *et al*[19] that described under-expressed CK8 in the gastric mucosa of *H pylori* infected-individuals as a result of oxidative stress-induced cytoskeleton damage.

Higher expression of CK 18 in foveolar epithelium together with a decrease in CK19 expression was noted in patients with *cagA*+ *H pylori*-induced gastritis. These findings differ from previous studies, since two independent studies have revealed unchanged CK18 and CK19 expression in gastric mucosa of *H pylori*-infected patients[15,17]. Normal stomach expresses less CK19 in the upper mucosal compartment in comparison to other mucosal compartments[1]. CK19 expression is thought to be inversely related to cell proliferation, strong CK19 expression implying weak proliferation and vice versa[1]. Intestinal metaplasia of the stomach however exhibits more intense CK19-immunoreactivity than gastric cancer tissue[1]. If CK19 immunoreactivity is negatively correlated with cell proliferation and differentiation in fetal, normal and pathologically transformed adult gastric mucosa, our results may suggest good differentiation and enhanced proliferation of upper foveolar cells in patients with *cagA*+ *H pylori*-induced gastritis.

Botta *et al*[3] studied CK20 immunoreactivity in fetal and neonatal human gut, including stomach and demonstrated that CK20 expression is progressive increased during gestation, suggesting that the degree of CK20 positivity is related to the epithelial maturation stage in gastric mucosa. CK20 expression in adults is restricted to the surface foveolar epithelium and is not detectable in gastric pit or glandular region. Previous investigations revealed that CK20 expression is significantly lower in foveolar epithelium of *H pylori*-induced chronic gastritis[15-17] supporting our results, while available studies suggest that this is a reversible change and CK20 expression is normalized within 6 mo after eradication of *H pylori* infection[15].

Taken together, all these findings imply alterations in epithelial cell maturation in the course of *H pylori*-induced chronic gastritis.

Our results suggest that bacterial strain is of importance in inducing alterations of CK expression. It is well known that *cagA* present in 50-60% of all strains is a part of *H pylori* genome termed *cag* pathogenicity island (*cag*PAI) and that proteins encoded by *cag*PAI are responsible for both NFkB and MAPK activation in gastric epithelial cells. It has also been demonstrated that infection with *cagA*+ strains is more likely to result in peptic ulceration, atrophic gastritis and gastric carcinoma[25]. Therefore, presence of *cag*PAI in *H pylori* genome might play a role in signal transduction leading to *H pylori*-induced host gene expression, thus regulating inflammation, proliferation and carcinogenesis. In addition, bacterial strain-related differences in host gene expression have been reported, implying that protein expression profile including CKs, depends on bacterial strains and is related to the presence of *cagA*+ *H pylori* strains. Amieva *et al*.[31] reported that *cagA* appears to target *H pylori* host cell intercellular junctions and to disrupt junction-mediated functions. Since predominant genotype of *H pylori* in Serbia and Montenegro has been reported to be the *cagA*+ genotype[21], it is very important to further investigate the presence and reversibility of different epithelial alterations induced by different *H pylori* strains.

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