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Please cite this article: **CHARACTERISTICS OF GASTRIC AND DUODENAL MUCOSA IN PATIENTS WITH PRIMARY BILIARY CHOLANGITIS**

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Authors: Dragani Popović*,†, Sanja Zgradic*, Sanja Dragasevic*, Simon Zec†, Marijan Micev*,‡, Tamara Naumovic†‖, Tomica Milosavljevic*,‡, Tamara Alempijevic*,‡; Vojnosanitetski pregled (2017); Online First October, 2017.

**UDC:**

**DOI:** https://doi.org/10.2298/VSP161123141P

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
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KARAKTERISTIKE MUKOZE ŽELUCA I DUODENUMA KOD PACIJENATA SA PRIMARNIM BILIJARNIM HOLANGITISOM

Dragan Popović*†, Sanja Zgradić*, Sanja Dragasević*, Simon Zec†, Marijan Micev†‡, Tamara Naumović†||, Tomica Milosavljević*†, Tamara Alempijević*†

* Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia
† University of Belgrade, Faculty of medicine, Belgrade, Serbia
‡ Department of Pathology, Clinic for Digestive Surgery, Clinical Center of Serbia, Belgrade, Serbia
|| Institute of Public Health of Serbia, „Dr Milan Jovanović Batut“, Belgrade, Serbia

Corresponding author:
Assoc. Prof Tamara Alempijević, MD, PhD, Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia; Dr Koste Todorovica St. 2, 11 000 Belgrade, Serbia; Phone: (381) 11 3663727; Fax: (381) 11 3615432; Email: tamara.alempijevic@med.bg.ac.rs

Krataknaslovrad: Features of gastroduodenal mucosa in primary biliary cholangitis

Tamara Alempijević, Dragan Popović, Tomica Milosavljević have conducted the study and done the esophagogastroduodenoscopy, Marijan Micev performed histopathological examination, Tamara Naumović has done the statistical analysis, Sanja Zgradić, Simon Zec
and Sanja Dragašević wrote the paper.

ABSTRACT
Background and aims: The aim of our study was to determine the correlation between the primary biliary cholangitis (PBC), atrophic gastritis (AG) and gluten-sensitive enteropathy (GSE); to identify the macroscopic and histopathological modifications of gastric and duodenal mucosa which occur in PBC and to analyze the frequency of these changes compared to a control group.

Patients and methods: This study included 50 patients with PBC and 46 controls subjects with dyspeptic symptoms, without liver disease. All of the examined subjects underwent esophagogastroduodenoscopy. Macroscopic and histopathological findings of the gastric and duodenal mucosal samples were recorded and analyzed.

Results: There is no statistically significant association between the PBC and AG or between the PBC and Helicobacter pylori infection. There is a highly significant difference in the frequency of Helicobacter pylori infection and the presence of GSE in patients in the control group compared to those with PBC.

Conclusions: Patients with PBC are at lower risk for Helicobacter pylori infection and atrophic gastritis. Testing for GSE in PBC patients may be beneficial, considering the higher incidence of GSE amongst these patients. GSE represents risk factor for the presence of PBC, and patients with GSE are nearly four times more likely to have PBC.

Key words: primary biliary cholangitis, atrophic gastritis, gluten-sensitive enteropathy, Helicobacter pylori

ABSTRAKT:
Uvod/Cilj: Cilj našeg istraživanja bio je da se utvrdi korelacija između primarnog bilijarnog holangitisa (PBC), atrofičnog gastritisa (AG) i gluten-senzitivne enteropatije (GSE); da se identifikuju makroskopske i histopatološke promene mukoze želuca i duodenuma kod PBC i analizira učestalost ovih promena u poređenju sa kontrolnom grupom.
**Metode:** U studiju je uključeno 50 pacijenata sa primarnim bilijarnim holangitisom i 46 kontrolnih pacijenta sa dispeptičnim tegobama, bez bolesti jetre. Svi ispitanici su bili podvrgnuti ezofagogastroduodenoskopiji. Makroskopski i histopatološki nalaz i uzorak amukoze želuca i duodenuma su snimljeni i analizirani.

**Rezultati:** Nije registrovana statistički iznačajna povezanost između primarnog bilijarnog holangitisa i AG, između primarnog bilijarnog holangitisa i *Helicobacter pylori* infekcije. Uočena je visokostatistički značajna razlika u učestalosti *Helicobacter pylori* infekcije i postojanja GSE kod bolesnika u kontrolnoj grupi u odnosu na one sa primarnim bilijarnim holangitisom.

**Zaključak:** Pacijenti sa primarnim bilijarnim holangitisom imaju manji rizik za *Helicobacter pylori* infekciju i atrofični gastritis. Testiranje za GSE kod pacijenata sa primarnim bilijarnim holangitisom može biti korisno, obzirom na veću učestalost GSE među ovim pacijentima. GSE predstavlja faktor rizika za prisustvo primarnog bilijarnog holangitisa i pacijenti sa GSE imaju skoro četiri puta veću predispoziciju za primarni bilijarniholangitis.

**Ključne riječi:** primarni bilijarniholangitis, atrofični gastritis, glutenskaenteropatija, *Helicobacter pylori*

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**INTRODUCTION**

Primary biliary cholangitis (PBC) is an immune-mediated chronic cholestatic disease of the liver, with a slow progression. Biochemical analysis classical demonstrates persistently higher levels of alkaline phosphatase and gamma-glutamyltransferase (1). Immunological analysis usually shows the presence of anti-mitochondrial antibodies (AMA), while antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) are present in approximately one third of all PBC patients. Diagnosis is confirmed by histopathology acquired through liver biopsy. According to Scheuer's classification, there are four stages
of PBC (Table 1). Although a number of studies have suggested immunological, genetic, infective and ecological factors, the exact mechanisms of the etiopathogenesis of PBC are not fully understood. Autoimmune features of PBC (infiltration of biliary epithelium with Th1 lymphocytes; expression of adhesion molecules (ICAM1, VCAM1, MHC molecules, IL2 receptors, TNF alpha, IFN gamma), including humoral and cellular immunity disorders, indicate potential overlap with other autoimmune diseases including, Sjögren’s syndrome, scleroderma, hypothyroidism and celiac disease (2, 3, 4).

Gluten sensitive enteropathy (GSE), also known as celiac disease, is a chronic disease typically affecting the proximal small intestine of genetically predisposed individuals with an inadequate immune response to gluten and similar proteins found in oat, rye and barley. GSE is diagnosed based on the histopathological findings of biopsy of the duodenal and/or jejunal mucosa, and by determining the serum levels of anti-gliadin, anti-endomysial (AEMA) and anti-transglutaminase antibodies (3). According to the Marsh classification, there are five stages of the disease (Table 2) (5). Celiac disease-associated autoimmune diseases of other organ-systems have already been described in the literature (liver, kidneys, skin; cardiovascular, nervous, endocrine and reproductive system) (6). The best documented are GSE-associated autoimmune diseases of the liver: PBC (with incidence 3 to 7%), autoimmune hepatitis (3 to 6%), and primary sclerosing cholangitis (2 to 3 %) (4,6).

According to the data obtained from available literature, there is significantly less evidence about the overlap of atrophic gastritis and PBC. Two forms of chronic atrophic gastritis have been described: type A autoimmune gastritis, with the presence of anti-parietal autoantibodies (APA) and type B gastritis associated with persistent Helicobacter pylori (H. pylori) infection. These two types have different etiologies, topographic distributions and histopathological features (7,8). The association between type A atrophic gastritis and autoimmune hepatic diseases remains controversial, and small number of data in present literature examining the relationship between H.pylori gastritis and PBC (7).

The group of autoimmune “overlap” syndromes includes syndromes which contain characteristics of at least two diseases.

The aim of this study was to investigate the correlations between PBC and atrophic gastritis and GSE; to determine the difference in the presence of atrophic gastritis and GSE in PBC patients in relation to controls and finally, to identify other macroscopic and...
histopathological changes of gastric and duodenal mucosa present in patients with PBC (*H. pylori* infection)

**METHODS**

This retrospective study included 50 patients with PBC, treated at a tertiary health center in Serbia, from 2009 to 2013. The control group consisted of 46 persons who were examined because of dyspeptic complaints. This study was approved by the Ethics Committee of our hospital. All patients gave informed written consent prior to participation in this study.

Recorded demographic data (age, gender) was analyzed. The diagnosis of PBC was based on laboratory and immunological analysis, as well as histopathological findings of liver biopsy, performed wherever possible. Exclusion criteria were blood coagulation disorders (INR>1.5) and the presence of ascites.

A complete immunological work-up was performed, which included ANA IgG in rodent tissue, ANA HEp2 in human cells, AMA, ASMA, APA, and AEMA, using immunofluorescence technique. Titles more than 1:80 were considered as clinically significant. The titre of anti-transglutaminase antibodies (TGA) was determined using ELISA, and expressed in U/ml. Where possible, percutaneous biopsy of the right lobe of the liver was performed and the liver tissue samples were sent for histopathological analysis. According to Scheuer's classification, histopathological stage of PBC was expressed in four categories(9). In our study, PBC patients were categorized in two groups: patients in the early stage of the disease (mild and moderate fibrosis) and patients with advanced liver disease (severe liver fibrosis and cirrhosis). As a part of the routine diagnostic algorithm, esophagogastroduodenoscopy was performed. Macroscopic findings of endoscopy were recorded and analyzed, while biopsy samples of duodenal and gastric mucosa were taken and sent for processing. A record was taken of the following: the presence and degree of atrophy (graded in three stages, according to the Sydney-Houston classification); *Helicobacter pylori* status (graded in three stages according to the degree of colonization); and the presence and degree of GSE according to the Marsh classification (8,10,5).

In the control group, a record was taken on demographic data and gastrointestinal medical history. Esophagogastroduodenoscopy was performed with biopsies of the duodenal and gastric mucosa, using the same criteria as in the study group.
Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, Version 20.0 (IBM Corp., Armonk, New York, USA). Categorical variables were shown by frequencies and relative numbers (percentages). Basic descriptive statistics included means, standard deviations, ranges and percentages. Chi-squared test was used to verify significant differences in the frequency of *H. pylori* infection, mucosal atrophy and GSE in the PBC and control groups and was followed by a logistic regression analysis – univariantemodel including the factors marked as significant by chi-squared test. Values less than 0.05 for type 1 statistical error (alpha) were considered as statistically significant.

**RESULTS**

The total number of patients diagnosed with PBC was 50, with a mean age of 56±10 years (range 29 – 79 years). The control group consisted of 46 patients, mean age 60±13 years (age range 36 – 84 years). In the study group, 98% of patients were female and 2% were male, while the gender distribution among controls was: 47.8% female and 52.2% male. All patients within the study group were tested for the presence of AMA in the serum, with positive results in 76% of the cases. No statistically significant difference in the presence of AMA was found between the group of patients with the early-stage, and those with the advanced-stage disease (p>0.05). ANAs were positive in 22.5% of PBC patients. In cases where it was possible, the patients were also tested for the presence of APA, which were positive in 7.1% of the patients with PBC, and AEMA, which were positive in 12.5% of the patients with PBC.

A biopsy of the liver was performed in most of the patients in the study group (47 of 50 patients): 60% of the subjects had mild, 2% had moderate and 16% had severe liver fibrosis, while cirrhosis was confirmed in 22% of the patients. Patients were divided into two groups based on stage: 61.7% of the patients with the early-stage, and 38.8% with the advanced disease of the liver.

The correlation between the PBC/PBC stage and gastric mucosal atrophy, *H.pylori* infection, and GSE was analyzed, and no statistical significance was found(Table 3, Table 4).

In our study, we have analyzed frequency of AG, H.pylori gastritis and GSE among
patients in study group and subjects in the control group. We found no statistically significant difference in presence of AG between this two groups. Further, we found highly significant statistical difference in presence of H. pylori infection in the control group compared to the PBC group and the frequency of GSE in patients with PBC (Table 5).

By univariate logistic regression, it was proven that the presence of *H. pylori* infection is a significant protective factor, and that individuals with *H. pylori* infection are less likely to have PBC (OR=0.27; 95%IP=0.11-0.69; p <0.01). The presence of GSE however, was shown to be a risk factor for the presence of PBC, and patients with GSE are almost four times more likely to have PBC (OR=3.79; 95%IP=0.95-15.11; p=0.059) (Table 6).

**DISCUSSION**

This study was conducted in order to find out more about connection of autoimmune disease of liver, duodenum and gast. In our study, study group were patients with PBC. Most of the patients were female, with mean age of 56. Results obtained in our study matched findings from available literature. Presented studies suggested that PBC has a female predominance, and in some studies it is suggested that female-to-male ratio is about 8:1 (11). Also, most of the patients with PBC are age over 40(1).

Regarding to the immunological analysis, most of PBC patients in our study had AMA positive disease. Our findings are in compliance with results from available literature. Sakagauchi et al. conducted a cross-sectional study of PBC in Japan and included 5805 patients. Among them, 86.6% had AMA (11). Joshita et al. reported that a great majority of patients in their study (369 of 395, 93.4%) had positive AMA (12).

Data on the correlation between the GSE and PBC is rather controversial. Results of our study revealed that GSE is present in 20.9% patients with PBC, with statistically significant difference to the control group (6.5% subjects with GSE in control group, p=0.047). Further analysis have shown that GSE is risk factor for presence of PBC, meaning that GSE patients had more than four times risk of having PBC.

Somestudies did not suggest that GSE occurs frequently with liver diseases (Mirzaagha et al., Drastich P et al, Bardella et al., Chatzicostas et al.) (6, 13, 14, 15).

However, studies which have included larger number of patients have indicated positive correlation between the PBC and GSE. Lawson et al. conducted study that includes 4732
patients diagnosed with GSE and 23620 control subjects, within General Medical Services (16). Results from this study suggested that the patients with PBC are three times more likely to develop GSE than the general population. Further, a largestudy was conducted in Sweden and Denmark, including patients diagnosed with GSE from National Registers, indicating a higher risk for developing PBC in patients with GSE (17).

It is suggested within the present literature, that the prevalence of atrophic gastritis is up to 10.9%, annually, while about 50% of the world population is infected with \textit{H. pylori} (18, 19, 20). It was challenging task to try to find out more about correlation between PBC and gastritis, both atrophic and Helicobacter pylori, because there is a small number of information related to this topic within the presented literature and the results are rather controversial. Results from our study did not show statistically significant difference in presence of AG in patients with PBC compared to the control group. Furthermore, we found that \textit{H. pylori} infection occurs less frequently in patients with PBC than in the control group, with statistically significant difference. Results showed in studies from present literature find similar correlation (19, 21).

On the other hand, positive correlation of PBC and pernicious anemia was found mostly as case reports. The relationship between PBC and pernicious anemia was first described by Renoux in 1980 (22). Chen-Shuan Chung presented the case of a 46-year old woman who, three years after being diagnosed with PBC, was also diagnosed with pernicious anemia (23). Abenavoli et al. presented the case of a 36-year old woman diagnosed with GSE, PBC and \textit{H. pylori} infection (24).

In addition to case reports presented in the available literature, there was also a large prospective study including where 289 patients were divided into three groups (control group, patients with cirrhosis and patients with portal hypertension without cirrhosis). Patients were tested for the presence of gastric mucosal atrophy, metaplasia, dysplasia and the presence of \textit{H. pylori} infection. Results obtained in this study indicated that the frequency of gastric mucosal atrophy was higher in the group of patients with cirrhosis than in the control group (25).

\textbf{CONCLUSIONS}
Our study showed no statistically significant difference in the presence of gastric mucosal atrophy between PBC patients and controls. A highly significant statistical difference in the presence of *H. pylori* infection in the control group compared to the PBC group and the frequency of GSE in patients with PBC was also confirmed. Analysis of the results obtained in our study show that GSE represents a risk factor for the presence of PBC, meaning that individuals with GSE have almost four times more chance to develop PBC. This indicates that testing PBC patients for the presence of GSE would be prudent so as to ensure proper treatment. Additionally, for those with GSE, it is necessary to exclude the presence of PBC within the "overlap" syndrome. Based on the results of our study, it may be concluded that patients with PBC, are at lower risk for *H. pylori* infection at the same time. Future studies are expected to identify the complex pathogenetic mechanisms of this phenomenon.

**REFERENCES:**

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17. Sorensen H, Thulstrup A, Blomqvist P etal. Risk of primary biliary liver cirrhosis in


Table 1. Scheuer classification for grading and staging of chronic hepatitis

<table>
<thead>
<tr>
<th>Grade</th>
<th>Portal/perioral activity</th>
<th>Lobular activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Portal inflammation</td>
<td>Inflammation, no necrosis</td>
</tr>
<tr>
<td>2</td>
<td>Mild piecemeal necrosis</td>
<td>Focal necrosis /acidophil bodies</td>
</tr>
<tr>
<td>3</td>
<td>Moderate piecemeal necrosis</td>
<td>Severe focal cell damage</td>
</tr>
<tr>
<td>4</td>
<td>Severe piecemeal necrosis</td>
<td>Damage with bridging necrosis</td>
</tr>
<tr>
<td>Stage</td>
<td>Fibrosis</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Enlarged, fibrotic portal tracts</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Perioral or portal-portal septa, but intact architecture</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Fibrosis with architectural distortion, no obvious cirrhosis</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Probable or definite cirrhosis</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Modified Marsh Classification of GSE

<table>
<thead>
<tr>
<th>Marsh Type</th>
<th>Intraepithelial Lymphocytes per 100 Enterocytes</th>
<th>Crypts</th>
<th>Villi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;40</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>&gt;40</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>&gt;40</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>3a</td>
<td>&gt;40</td>
<td>Increased</td>
<td>Mild atrophy</td>
</tr>
<tr>
<td>3b</td>
<td>&gt;40</td>
<td>Increased</td>
<td>Marked atrophy</td>
</tr>
<tr>
<td>3c</td>
<td>&gt;40</td>
<td>Increased</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Table 3. The association between PBC, AG and H.pylori

<table>
<thead>
<tr>
<th></th>
<th>Present in PBC patients</th>
<th>P value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG†</td>
<td>40%</td>
<td>0.475</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>H.pylori‡</td>
<td>20%</td>
<td>0.916</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

*PBC: Primary Biliary Cholangitis, †AG: Atrophic Gastritis, ‡H.pylori: Helicobacter pylori

Table 4: The association between stage of Primary Biliary Cholangitis, Atrophic Gastritis, Helicobacter pylori and Gluten-Sensitive Enteropathy
<table>
<thead>
<tr>
<th></th>
<th>PBC* gr I and gr II</th>
<th>PBC* gr III and IV</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AG†</strong></td>
<td>50%</td>
<td>50%</td>
<td>p=0.531</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td><strong>H. Pylori‡</strong></td>
<td>55.60%</td>
<td>44.40%</td>
<td>p=1.000</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td><strong>GSE§</strong></td>
<td>42.90%</td>
<td>57.10%</td>
<td>p=0.680</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

*PBC: Primary Biliary Cholangitis, †AG: Atrophic Gastritis, ‡H.pylori: Helicobacter pylori, §GSE: Gluten-Sensitive Enteropathy

Table 5. The difference in presence/absence of the gastric mucosal atrophy, *Helicobacter pylori* infection and Gluten-Sensitive Enteropathy between the study group and the control group.

<table>
<thead>
<tr>
<th></th>
<th>PBC*</th>
<th>Control group</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AG†</strong></td>
<td>40%</td>
<td>50%</td>
<td>p=0.338</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td><strong>H. Pylori‡</strong></td>
<td>20%</td>
<td>47.8%</td>
<td>p=0.005</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>GSE§</strong></td>
<td>20.9%</td>
<td>6.5%</td>
<td>p=0.047</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

*PBC: Primary Biliary Cholangitis, †AG: Atrophic Gastritis, ‡H.pylori: Helicobacter pylori, §GSE: Gluten-Sensitive Enteropathy
Table 6. Logistic regression analysis for *Helicobacter pylori* and Gluten-Sensitive Enteropathy

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
<th>Sig</th>
<th>EXP(B)</th>
<th>95%CI</th>
<th>EXP (B)</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>H.pylori†</td>
<td>1.299</td>
<td>0.475</td>
<td>7.469</td>
<td>1</td>
<td>0.006</td>
<td>0.273</td>
<td>0.107</td>
<td>0.692</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td>0.405</td>
<td>0.264</td>
<td>2.367</td>
<td>1</td>
<td>0.124</td>
<td>0.124</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSE†</td>
<td>1.333</td>
<td>0.705</td>
<td>3.577</td>
<td>1</td>
<td>0.059</td>
<td>3.794</td>
<td>0.953</td>
<td>15.11</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Constant</td>
<td>0.235</td>
<td>0.229</td>
<td>1.047</td>
<td>1</td>
<td>0.306</td>
<td>0.791</td>
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</table>

*H.pylori: Helicobacter pylori; †GSE: Gluten-Sensitive Enteropathy*