ORIGINAL ARTICLE

UDC: 616.361-002:616.33/.34
https://doi.org/10.2298/VSP161123141P

Characteristics of gastric and duodenal mucosa in the patients with primary biliary cholangitis

Karakteristike mukoze želuca i duodenuma kod bolesnika sa primarnim bilijarnim holangitisom

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Abstract

Background/Aim. Primary biliary cholangitis (PBC) is an immune-mediated chronic cholestatic disease of liver, with a slow progression. The aim of our study was to determine the correlation of 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. **Methods.** This study included 50 patients with PBC and 46 control subjects with the 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 was no statistically significant association between the PBC and AG, or between the PBC and *Helicobacter pylori* infection. There was a highly significant difference in the frequency of *Helicobacter pylori* infection and the presence of GSE in the patients in the control group compared to those with PBC. **Conclusions.** The patients with PBC are at a lower risk for *Helicobacter pylori* infectionand atrophic gastritis. Testing for GSE in the PBC patients may be beneficial, considering the higher incidence of GSE amongst these patients. GSE represents a risk factor for the presence of PBC and the patients with GSE are nearly four times more likely to have PBC.

Key words:
liver cirrhosis, biliary; gastritis, atrophic; glutens; celiac disease; comorbidity; histology; helicobacter pylori.

Apstrakt

Uvod/Cilj. Primarni bilijarni holangitis (PBC) je imunski posredovana hronična holestatska bolest jetre sa sporom progresijom. Cilj našeg istraživanja bio je da se utvrdi korelacija između PBC, atrofičnog gastritisa (AG) i glutensenzitivne 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 bilo uključeno 50 bolesnika sa PBC i 46 kontrolnih bolesnika sa dispeptičnim tegobama, bez bolesti jetre. Svi ispitanici su bili podvrgnuti ezofagogastroduodenoskopiji. Makroskopski i histopatološki nalaz i uzorak mukoze želuca i duodenuma su snimljeni i analizirani. **Rezultati.** Nije registrovana statistič iznačajna povezanost između PBC i AG, između PBC 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 PBC. **Zaključak.** Bolesnici sa PBC imaju manji rizik za *Helicobacter pylori* infekciju i AG. Testiranje za GSE kod PBC bolesnika može biti korisno, s obzirom na veću učestalost GSE među ovim bolesnicima. GSE predstavlja faktor rizika od prisustva PBC i bolesnici sa GSE imaju skoro četiri puta veću predispoziciju za PBC.

Ključne reči:
jetra, bilijarna ciroza; gastritis, atrofijski; gluten; celijakija; komorbiditet; histologija; helicobacter pylori.

Introduction

Primary biliary cholangitis (PBC) is an immune-mediated chronic cholestatic disease of the liver, with a slow progression. Biochemical analysis classicaly demonstrates persistently higher levels of alkaline phosphatase and gamma-glutamyltransferase [1]. Immunological analysis usually shows the presence of anti-mitochondrial antibodies (AMA), while anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) are present in approximately one third of all

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PBC patients. Diagnosis is confirmed by histopathology acquired through liver biopsy. 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 epithelial cells 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 the Sjörgen's syndrome, scleroderma, hypothyroidism and celiac disease [2–4].

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 [5]. Celiac disease-associated autoimmune diseases of the 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 the available literature, there is significantly less evidence about the overlap of atrophic gastritis (AG) and PBC. Two forms of chronic AG were 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, AG and autoimmune hepatic diseases remains controversial and, in the literature, small number of data examining the relationship between *H. pylori* gastritis and PBC is presented [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 AG, and GSE, to determine the difference in the presence of atrophic gastritis and GSE in the PBC patients in relation to the controls and finally, to identify other macroscopic and histopathological changes of gastric and duodenal mucosa present in the patients with PBC (*H. pylori* infection).

Methods

This retrospective study included 50 patients with PBC, treated at the 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.

The recorded demographic data (age, gender) were analyzed. The diagnosis of PBC was based on laboratory and immunological analysis as well as the histopathological findings of liver biopsy, performed wherever possible. Exclusion criteria were blood coagulation disorders [international normalized ratio (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 the immunofluorescence technique. Titres 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 the Scheuer's classification, a histopathological stage of PBC was expressed in four categories (Table 1) [9]. In our study, the PBC patients were categorized into two groups: the patients in the early stage of the disease (mild and moderate fibrosis) and the patients with advanced liver disease (severe liver fibrosis and cirrhosis). As a part of the routine diagnostic algorithm, esophagogastroduodenoscopy was performed.

Table 1

Scheuer's classification for grading and staging of chronic hepatitis

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

The 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); *H. pylori* status (graded in three stages according to the degree of colonization); the presence and degree of GSE according to the Marsh classification (Table 2) [8, 10, 5]. In the control group, a record of demographic data and gastrointestinal medical history was taken. Esophagogastroduodenoscopy was performed with biopsies of the duodenal and gastric mucosa, using the same criteria as in the study group.

Table 2

Modified Marsh classification of gluten-sensitive enteropathy (GSE)

Marsh type	Intraepithelial lymphocytes *per* 100 enterocytes	Crypts	Villi
0	< 40	Normal	Normal
1	> 40	Normal	Normal
2	> 40	Increased	Normal
3a	> 40	Increased	Mild atrophy
3b	> 40	Increased	Marked atrophy
3c	> 40	Increased	Absent

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. The χ^2-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 it was followed by the logistic regression analysis – univariante model including the factors marked as significant by the χ^2-test. The values less than 0.05 for the type 1 statistical error (alpha) were considered being statistically significant.

Results

The total number of patients diagnosed with PBC was 50, with the mean age of 56 ± 10 years (age 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 females and 2% were males, while the gender distribution among controls was: 47.8% of females and 52.2% of males.

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 of the disease ($p > 0.05$). ANAs were positive in 22.5% of the 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. The patients were divided into two groups based on a 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 (Tables 3 and 4).

Table 3

The association between PBC, AG and *H. pylori*

Parameters	Present in PBC patients (%)	*p*
AG	40	0.475
H. pylori	20	0.916

PBC – primary biliary cholangitis; AG – atrophic gastritis; *H. pylori – Helicobacter pylori*.

Table 4

The association between stage of PBC, AG, *H. pylori* and GSE

Parameters	PBC gr I and gr II (%)	PBC gr III and IV (%)	*p*
AG	50	50	0.531
H. pylori	55.60	44.40	1.000
GSE	42.90	57.10	0.680

PBC – primary biliary cholangitis; AG – atrophic gastritis; *H. pylori – Helicobacter pylori*; GSE – gluten-sensitive enteropathy.

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

Table 5

The difference in presence/absence of the GA, *H. pylori* infection and GSE between the study group and the control group

Parameters	PBC (%)	Control group (%)	*p* value
AG	40	50	$p = 0.338$
H. pylori	20	47.8	$p = 0.005$
GSE	20.9	6.5	$p = 0.047$

PBC – primary biliary cholangitis; AG – atrophic gastritis; *H. pylori – Helicobacter pylori*; GSE – gluten-sensitive enteropathy.

Table 6

Logistic regression analysis for *H. pylori and* GSE

	Parameter	B	S.E.	Wald	df	Sig	EXP(B)	95% CI EXP (B)	
								lower	upper
Step 1a	*H. pylori*	1.299	0.475	7.469	1	0.006	0.273	0.107	0.692
	Constant	0.405	0.264	2.367	1	0.124	0.124		
	GSE	1.333	0.705	3.577	1	0.059	3.794	0.953	15.11
	Constant	0.235	0.229	1.047	1	0.306	0.791		

***H. pylori* – *Helicobacter pylori*; GSE – gluten sensitive enteropathy; Sig – significance; CI – confidence interval.**

Applying the univariante logistic regression, it was proven that the presence of *H. pylori* infection was a significant protective factor and that individuals with *H. pylori* infection were less likely to have PBC [odds ration (OR) = 0.27; 95% confidence interval (CI) = 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 the patients with GSE were almost four times more likely to have PBC (OR = 3.79; 95% CI = 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 the liver, duodenum and gaster. In our study, the study group consisted of patients with PBC. Most of the patients were females, with the mean age of 56. The results obtained in our study matched findings from available literature. The 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 over 40 years of age [1].

Regarding the immunological analysis, most of the PBC patients in our study had the AMA positive disease. Our findings are in compliance with the results from available literature. Sakauchi et al. [11] conducted a cross-sectional study of PBC in Japan and included 5,805 patients. Among them, 86.6% had AMA. Joshita et al. [12] reported that a great majority of patients in their study (369 of 395, 93.4%) had positive AMA.

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

Some studies did not suggest that GSE occurs frequently with the liver diseases [6, 13–15]. However, studies which included a larger number of patients indicated the positive correlation between the PBC and GSE. Lawson et al. [15] conducted study that included 4,732 patients diagnosed with GSE and 23,620 control subjects within the General Medical Services [16]. The results from this study suggest that the patients with PBC are three times more likely to develop GSE than the general population. Further, a large study conducted in Sweden and Denmark, including the patients diagnosed with GSE from the National Registers, indicates a higher risk for developing PBC in the patients with GSE [16].

It is suggested within the present literature, that the prevalence of AG is up to 10.9%, annually, while about 50% of the world population is infected with *H. pylori* [17–19]. It was challenging task to try to find out more about correlation between PBC and gastritis, both atrophic and *H. pylori*, because there is a small number of information related to this topic within the presented literature and the results are rather controversial. The results from our study did not show a statistically significant difference in the presence of AG in the patients with PBC compared to the control group. Furthermore, we found that *H. pylori* infection occurs less frequently in the patients with PBC than in the control group, with a statistically significant difference. The results showed in the studies from present literature provided similar correlation [18, 20].

On the other hand, a 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 et al. [21] in 1980. Chung et al. [22] presented the case of a 46-year-old woman who, three years after being diagnosed with PBC, was also diagnosed with pernicious anemia. Abenavoli et al. [23] presented the case of a 36-year-old woman diagnosed with GSE, PBC and *H. pylori* infection.

In addition to the case reports presented in the available literature, there was also a large prospective study including 289 patients divided into three groups (control group, patients with cirrhosis and patients with portal hypertension without cirrhosis). The patients were tested for the presence of gastric mucosal atrophy, metaplasia, dysplasia and the presence of *H. pylori* infection. The 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 [24].

Conclusion

Our study showed no statistically significant difference in the presence of gastric mucosal atrophy between the 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 the patients with PBC was also confirmed. The analysis of the results obtained in our study showed that GSE represented a risk factor for the presence of PBC, meaning that the

individuals with GSE have almost four times more chance to develop PBC. This indicates that the testing of the 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 the patients with PBC, are at a lower risk for *H. pylori* infection. Future studies are expected to identify the complex pathogenetic mechanisms of this phenomenon.

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Received on November 23, 2016.
Revised on April 13, 2017.
Accepted on September 11, 2017.
Online First October, 2017.