short communication

The prevalence and characteristics of *Helicobacter pylori*-associated gastritis in dyspeptic patients in Eastern Croatia, determined by immunohistochemistry

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Abstract

Background and purpose: Helicobacter pylori *(Hp), which is associated with many upper gastrointestinal diseases, is found in about half of world's population. Aim od this study is to reveal the prevalence and characteristics of Hp-associated gastritis in dyspeptic patients in the population of Croatia.*

Materials and methods: *Retrospective analysis the data and materials of 871 dyspeptic patients undergoing routine endoscopy. The gastric biopsy specimens were stained by Hp-specific immmunohistocemical stain. Endoscopic and pathology findings were recorded for Hp infection and the presence of active gastritis. Nominal variables in the case of a large number of samples were compared using Pearson χ^2 test, while in the case of a small number of samples was used Fisher's Exact test.*

Results: *In the present study determined prevalence of Hp-associated gastritis in the population of Croatia is 41%. Chronic active gastritis was found in 94% of patients with Hp infection and 5% of patients with gastritis of some other etiology. Gastric mucosal atrophy was observed in 5% of patients older than 40 years. Intestinal metaplasia was detected in 26% of patients. Severe intestinal metaplasia occurs in the age group older than 60 years.*

Conclusion: *This study is the first large study of Hp prevalence by histological and immunohistochemical methods in Croatia, so the results of this study contribute to the understanding of how big a public health problem is Hp infection in a population of Croatia.*

INTRODUCTION

*H*elicobacter pylori (Hp) is a spiral, Gram-negative bacterium which was discovered in 1982 by Marshall and Warren *(1)*. Hp chronically colonizes the human stomach and is currently recognized to play a causative role in the pathogenesis of various gastroduodenal diseases, including gastritis, peptic ulcers, gastric cancer and mucosa-associated lymphoid tissue lymphoma *(2-4)*.

Hp is a highly heterogeneous bacterium and its virulence varies geographically. The explanation for geographic differences in the incidence of gastric cancer and the role of Hp infection in gastric cancer and duodenal, could be the presence of different types of Hp virulence factor, especially CagA (cytotoxin-associated gene A), VacA (vacuolating cytotoxin), OipA (outer inflammatory protein) and DupA (outer inflamma-

tory protein). Some studies have been reported that individuals infected with CagA- positive strains of Hp are at a higher risk of peptic ulcer or gastric cancer than those infected with CagA-negative strains. In some researches DupA is confirmed as a risk factor for duodenal ulcer. *(5)*

Hp shows a remarkable allelic diversity and a large genetic variability, resulting that every individual is infected with Hp's unique strain, but differences between relatives are minimal. Rapid development of high-level resistance to commonly used antibiotics could be explained by a significantly higher mutation rate than found in many other bacteria. *(6)*

Several diagnostic tests are available for the detection of Hp and they could be divided in group of invasive and non-invasive tests. Each of these tests has its usefulness and limitations in different clinical situations. Invasive tests include histology, culture, rapid urease test and molecular methods, and they are performed through endoscopic biopsy specimens. The most widely used non-invasive tests are urea breathing test and stool antigen. In the epidemiological studies the most used non-invasive test is serology. *(7)*

Histological method of detection Hp infecton has been considered to be the gold standard. It is widely available and capable of achieving sensitivity and specificity of more than 95%, also has the ability to evaluate for pathologic changes associated with Hp infection such as inflammation, atrophy, intestinal metaplasia and malignancy. *(7)*

Besides heamtoxylin and eosin (H&E) staining of histological preparations, there are several histochemical staining methods that can improve visualization of microorganisms including Warthin-Starry, Modified Giemsa, Modified Genta, and Hp silver stain. Sensitivity of H&E is comparable to that of Giemsa and Genta, but the specificity is decreasing in low Hp density (90%). Warthin-Starry silver staining allows an excellent visualization, but it is expensive, time consuming and the results are not always reliable. Some researchers showed that immunohistochemical staining (IHC) with specific Hp antibodies has the highest sensitivity and specificity. A significant advantage of IHC is assessing the presence of Hp even if in biopsy specimen are coccoid forms of Hp. *(7)*

According to the Croatian national guidelines 2014, for the diagnosis of Hp infection should be applied a different diagnostic approach in patients according to age. In the group of patients younger than 45 years, without alarming symptoms (weight loss, dysphagia, signs of gastrointestinal bleeding, palpable resistance in the stomach, iron deficiency anemia) diagnostic procedure can be performed through the non-invasive diagnostic methods. After treatment they also should be tested noninvasively to evaluate succes of therapy. For group of patients older than 45 years, and all those present with alarm symptoms, regardless of age, it is recommended to be tested with endoscopy and invasive methods. *(8)*

Guidelines from the last Consensus Conference (Maastricht V) did not change significantly for all populations with a prevalence of Hp infection over 20% (this is the case in Croatia). It was suggested that the age limit between non-invasive and invasive diagnostic methods of Hp infection should be determined by the prevalence of gastric cancer in a given population. *(9)*

METHODS

Population study

This study was conducted at Department of Pathology and Cytology of General Hospital Vinkovci and was approved by Ethics Committee of the General Hospital Vinkovci. The data and biopsy materials of dyspeptic patients undergoing routine endoscopy from two-year period (2011 – 2012) are analyzed. Patients who were in the control gastroscopy after Hp eradication therapy and patients whose findings were insufficient samples were not taken into account.

Endoscopy and biopsy sample

Samples of gastric biopsies were taken during endoscopy, four gastric biopsy were obtained (two from the antrum and two from the corpus). Two pathologists analyzed samples at the Department of Pathology and Cytology of General Hospital Vinkovci.

Histochemistry and immunohistochemistry

Histological features such as gastric mucosal changes for any evidence of gastritis were studied on H&E-stained sections and was evaluated according to the updated Sydney system *(10)*. The degree of bacterial load was classified into four grades: 0 „normal", 1 „mild", 2 „moderate", 3 „marked".

Immunohistochemical staining, also used by Tajalli et al. *(11)*, was performed with the concentrated primary antibody, anti-Hp polyclonal rabbit (DakoCytomation), at a dilution 1:150 with Envision Flex antibody diluent, using the device for automated immunohistochemical staining AUTOSTAINER Link (Dako). Antigen cells in deparaffinized sections were unmasked by treatment with the PT-Link (109 Dako PT) in Target Retrieval Solution, High pH 9. The secondary antibody (streptavidin peroxidase) is used from the set LSAB + System, HRP (Dako). Preparations are contrasted with hematoxylin 2 minutes.

Statistical analysis

Nominal variables in the case of a large number of samples were compared using Pearson χ^2 test, while in the case of a small number of samples was used Fisher's Exact test. In numerical comparisons with nominal variables, with a normal distribution of numerical variables was used parametric Student's t-test. The level of significance was p <0.05.

Table 1. *The prevalence of Hp infection by age groups*

	Hp+ (n)	Hp– (n)	Hp+ (%)	Hp– (%)
≤20	3	14	18	82
21–30	14	34	29	71
31–40	41	55	43	57
41–50	71	75	48	52
51–60	98	137	42	58
>60	133	196	40	60

Hp: *Helicobacter pylori*

RESULTS

In this study, 410 male and 461 female patients were included. Patients were divided into six groups: younger than 20 years (n = 17), 21-30 years (n = 48), 31-40 years (n = 96), 41-50 years (n = 146), 51-60 years (n = 235), older than 60 years (n = 329).

Of the total number of patients, in 41% is determined presence of Hp. There is no statistically remarkable difference between the sexes (p = 0.531). The prevalence of Hp infection by age groups is shown in Table 1.

In the age group 41-50 years occurs the plateau of Hp infection (48%), followed by decreasing to 40% in the age group older than 60.

Hp was found in the antrum and corpus of 77% of patients, in 19 % only in the corpus and in 4% only in the antrum. The intensity of the Hp according to localization is shown in Table 2.

Chronic active gastritis was found in 94% patients in Hp positive group and in 5% patients in Hp negative group. Pangastritis prevalents in both sexes (men 68% and women 62%). After pangastritis in men is more common antral gastritis (20%), while in women corpus gastritis (24%). χ^2 test showed a statistically remarkable difference in the occurrence of chronic active gastritis in relation to the region of the stomach between the sexes (p = 0.005).

Analysis of the components of inflammation in the mucosa of the stomach has shown that in the antrum and corpus predominates moderate acute component of inflammation (2+) and marked chronic inflammation (3+).

Table 2. *The intensity of the Hp according to localization*

	Hp (n)			Hp (%)		
	+	++	+++	+	++	+++
Antrum	77	107	106	26	37	37
Corpus	75	163	107	22	47	31

Hp: *Helicobacter pylori*

Remarkable correlation between the Hp and acute inflammatory components was found in the antrum of both sexes, while in the corpus is confirmed only in men (with correlation coefficient r ranging from ± 0.40 to ± 0.70). In the antrum in both sexes and in the corpus in men exists mild correlation between the Hp and chronic inflammation (r ± 0.20 to ± 0.40).

Atrophy of the gastric mucosa is found in 5% patients in Hp positive group, and in 3.5% patients Hp negative group. In Hp positive group the antrum is atrophic changed in 72% patients, the corpus in 22%, and in 6% of patients atrophy affects antrum and corpus. Severe atrophy occurs in patients older than 60 years.

Intestinal metaplasia of gastric mucosa was found in 26% patients in the Hp positive group and in 25% patients in the Hp negative group. In 88% patients intestinal metaplasia was found in the antrum, in 8% engages the antrum and corpus, and in 4% patients was found only in the corpus. The most of the patients had mild metaplasia, while severe metaplasia occurs only in the group of patients older than 60 years.

In Table 3 are comparatively shown the most remarkable changes (Hp, intestinal metaplasia, atrophy) in relation to the localization.

Table 3. *Comparison of the most remarkable changes in relation to a localization*

	Hp presence (%)	Atrophy (%)	Intestinal metaplasia (%)
Antrum	4	72	88
Corpus	19	22	4
Antrum and corpus	77	6	8

Hp: *Helicobacter pylori*

DISSCUSIONS

It is assumed that about 50% of the world's population is infected with Hp. Seroprevalence of Hp infection in the population differs in developing and developed countries. Serologic indicators of Hp infection in developed countries are rarely positive in children younger than 10 years and prevalence grow to 10% in age group 18-30 years, whereas the maximum (50%) of the infection is reached only after the age of 60 *(12)*. Developing countries are characterized by the majority of children infected before the age of 10, with a plateau of the infection (over 80%) before the age of 50 *(13, 14)*

Epidemiological studies frequently are using serological tests for Hp infection detection, because of their inexpensiveness, rapidity and acceptability to patients. Serological tests are useful for evaluation of Hp infection in children. The other advantage of serological test is that

the accuracy of serological tests is not affected by ulcer bleeding, gastric atrophy as well as the use of PPI or antibiotics, which cause false negative results in other invasive or noninvasive tests. Because the serological tests detect antibodies that can persist in the blood for long periods after infection is cured, they do not distinguish between active infection and past exposure and are not suitable for evaluation in the eradication therapy. (15)

Croatia is located at the crossroads of Mediterranean, Central and Southeast Europe. In Europe, the prevalence of Hp seems to be lower in Northern countries (the Netherlands 32%) than in Southern (Portugal 84%) and Eastern countries (Turkey 83%) (16). Review article, which covers 22 of the 35 European countries, based on 44 studies, cites prevalence which ranges from 17% in Denmark to 88% in Russia (17). In Croatian population in 1997 seroprevalence of Hp infection was between 60% and 68% (18). The latest epidemiological study in Croatia, conducted ELISA, showed seroprevalence of Hp infection 92% in dyspeptic patients and 64% in the control group of voluntary blood donors (19).

This study is the first large study of Hp prevalence by histological and immunohistochemical methods in Croatia, so the results of this study contribute to the understanding of how big a public health problem is Hp infection in a population of Croatia.

The prevalence of Hp infection in this study is determined to be 41% putting Croatia between developed and developing countries, which can be compared with the results of histological studies: 71% in Turkey (20), 69% in Morocco (21), 66% in Vietnam (22), 62% in Bhutan (23), 50% in India (24), 54% of Albanians and 34% of the Greeks (25), 42% in Hungary (17), 38% in Canada (16), 4% to 29% in large sample of patients (78,985) from 35 U.S. states (26), Puerto Rico and Colombia, 15% in Belgium (17), 7% in 251 patients in USA (27).

The plateau of Hp infection occurs in the age group 41-50 years (48%), which coincides with the research Jo-utei et al. in Morocco (21), while Sonnenberg et al. (27) states plateau at the age group 30-39 years.

Vilaichone et al. (23) reported decreasing of the prevalence of Hp infection in the population older than 60 (43%) which correlates with our results, in contrast to Nguyen et al. (22) which quotes the increase in prevalence (75%) in the same age group.

Chronic active gastritis was present in 94% patients in Hp positive group, while in the Hp negative group is present in only 5% patients. Nguyen et al. (22) cites 83% of chronic active gastritis in the Hp positive group, which correlates with our research. In other studies whose results were compared, the percentage of patients with chronic active gastritis is less than 40% (24, 27, 28).

Remarkable correlation is reported between the severity of gastritis and grade of Hp infection which is in accordance with several other authors (29-31).

Gastric mucosal atrophy was observed in 5% of patients which can be compared with study in India (24) and was lower than in other studies: in Vietnam 85% (22), in Sweden 55% (28), in Italy 24% (32), in Iran 45% (33).

Our results show intestinal metaplasia of gastric mucosa in 26% patients in the Hp positive group and in 25% patients in the Hp negative group. Other researches show a lower percentage of patients with intestinal metaplasia of the stomach mucose: in Turkey 18% (20), in Vietnam 15% (22), in USA 2% to 17% (27), in India 2% (24).

The recommended primary therapy for Hp infection includes: a proton pump inhibitor (PPI) and antibiotic (clarithromycin, and amoxicillin or metronidazole). Recent studies have shown a significant decline in the effectiveness of conventional therapy, caused by an increase in primary resistance of Hp to clarithromycin. Although the primary resistance to metronidazole in many European countries is relatively high, this problem can be overcome by increasing the dose and longer treatment or the addition of bismuth preparations.

In addition to conventional therapy, there is sequential therapy based on the hypothesis that during the first stage treatment amoxicillin reduces the number of mutants resistant to clarithromycin, so that in the second stage of the therapy application of clarithromycin is more efficiently. Sequential therapy is conducted such that the first five days administered amoksiclin + PIP, then the next five days clarithromycin + metronidazole + PPI

After the failure of PPI-triple therapy (PPI-metronidazole-amoxicillin) for 10-14 days or sequential therapy for a period of 10 days, in the guidelines recommended therapy is quad therapy with bismuth – 10 days or PPI triple therapy which contains levofloxacin – 10 days. (8)

CONCLUSIONS

The prevalence of Hp infection in this study is determined to be 41% putting Croatia between developed and developing countries.

Chronic active gastritis was present in 94% patients in Hp positive group. Pangastritis prevalents in both sexes (men 68% and women 62%). After pangastritis in men is more common antral gastritis (20%), while in women corpus gastritis (24%).

Atrophy of the mucosa of the stomach was found in 5% of patients, and severe atrophy occurs in age group older than 60 years.

Complete (intestinal) metaplasia was found in 26% and incomplete metaplasia in 1% of patients. In all age groups most of the patients had mild intestinal metaplasia.

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