Smoking habit and gastritis histology

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Abstract

Purpose: Long-term cigarette smoking may increase the risk of digestive tract pathologies, however, what is the influence smoking habit on gastric mucosa histology is still poorly elicited. The aim of the study was to compare histological evaluation of gastritis in smoker and non-smoker groups.

Material and methods: A total of 236 patients of various *H. pylori* status (109 infected, 127 non-infected), clinical diagnosis (107 duodenal ulcer disease, 129 dyspepsia), and smoking habit (92 smokers, 144 non-smokers) were included. Subjects were classified as smokers if they smoked 5 or more cigarettes per day for at least 3 years. A histological examination of endoscopically obtained samples was performed by two experienced pathomorphologists blinded to the diagnoses and smoking habit. Microscopic slices of the gastric mucosa were stained with hematoxylin-eosin and Giemsa. Apart from histological diagnosis, *H. pylori* status was additionally confirmed by an urease test (CLO-test) at least in one of two gastric locations (antrum or corpus).

Results: In the *H. pylori* infected population, *H. pylori* density, neutrophils, and mononuclear cells infiltration in the gastric corpus mucosa were lower in smokers than non-smokers, while in the antrum the differences were not significant. In the non-infected population, no significant differences in neutrophils and mononuclear cells infiltration between smokers and non-smokers were found.

Conclusions: Since the significant differences in studied parameters of chronic gastritis between smokers and non-smokers were found in the corpus mucosa of *H. pylori* infected subjects, smoking should be taken into account when a histological evaluation of the gastric mucosa in the *H. pylori* infected population is performed.

Key words: gastritis, *Helicobacter pylori*, smoking.

Introduction

Clinical and experimental studies have shown that smoking exerts many different effects in living organisms, and most of them are unprofitable. Poisoned components of cigarette smoke reached the stomach either through the circulation or through the gastrointestinal tract, usually within swallowed saliva [1,2]. The mechanism of the action of cigarette smoke constituents on the stomach is not well defined, however, many factors, such as increased synthesis of reactive oxygen species, endothelin 1, disturbences in microcirculation, increased duodeno-gastric reflux, and delayed gastric emptying, should be considered [3-7]. Although chronic exposure to cigarette smoke in *Helicobacter pylori* infected patients enhances the risk of gastric mucosal atrophy and intestinal metaplasia [8], the histological gastritis characterized by *H. pylori* density, neutrophils, and mononuclear cells infiltration (Sydney classification of gastritis [9]) in relation to smoking habit has not been systematically studied. The aim of the present study was to compare histological gastritis severity in smokers and non-smokers with duodenal ulcer disease and dyspepsia.

Material and methods

Patients

The study was performed in 236 patients (both sexes) with various *H. pylori* status (109 infected, 127 non-infected) and clinical diagnosis (107 with duodenal ulcer disease, 129 with

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***Table 1*. Characteristics of the study groups**

	Duodenal ulcer disease		Dyspepsia	
	H. pylori (+)	*H. pylori* (-)	*H. pylori* (+)	*H. pylori* (-)
Total number	45	62	64	65
Age, years (mean ± S.D.)	39.3±9.7	50.4±11.3	52.6±12.3	50.4±14.4
Gender (M/F)	30/15	53/9	27/37	34/31
Cigarette smoking				
None (%)	12 (26.7)	30 (48.4)	44 (68.8)	50 (76.9)
<5/day (%)	1 (2.2)	2 (3.2)	3 (4.7)	2 (3.1)
5-9/day (%)	3 (6.7)	2 (3.2)	1 (1.6)	3 (4.6)
10-19/day (%)	12 (26.7)	13 (21.0)	7 (10.9)	5 (7.7)
20 or more/day (%)	17 (37.8)	15 (24.2)	9 (14.1)	5 (7.7)

dyspepsia). Smokers and non-smokers represent 92 and 144 subjects, respectively (*Tab. 1*). Patients with dyspepsia (pain and/or discomfort in the upper abdomen lasting for at least three months) showed no abnormalities in the gastroscopy. In patients with duodenal ulcer not related to non-steroidal anti-inflammatory drugs (NSAIDs) treatment but infected with *H. pylori*, an ulcer niche 0.5-1.0 cm was present in the duodenal bulb. All patients with duodenal ulcer disease, not currently infected with *H. pylori* and without the ulcer niche, had documented histories of duodenal ulcers not related to NSAIDs treatment. They also underwent eradication therapy at least 36 months before. Subjects with emerge symptoms and abnormal results in basic laboratory tests as well as with the gastric mucosal atrophy and/or intestinal metaplasia in microscopic examination were not included.

Participants that smoked 5 or more cigarettes per day were classified as smokers. Subjects who did not smoke at all were classified as non-smokers. Only those with unchanged smoking habit, according to used criteria for at least 3 years, were included. Patients who were obligated to take any drugs, permanently or sporadically for at least two weeks before undergoing gastroscopy, were excluded. Similarly, subjects using any anti-acid drug, Misopostol, antibiotics, NSAIDs within 4 weeks, or alcoholic beverages within 7 days before the gastroscopy examination were excluded as well.

Endoscopy

Gastroscopy examinations were performed with a gastroscope, GIF V2 or Q145 (Olympus), during which mucosa samples were taken from the prepyloric and corpus region for an urease test (CLO-test) and histological examinations from each side. Separate biopsies were taken from the antrum and corpus to search for possible histological differences in these two locations.

Helicobacter pylori testing

CLO-test was conducted according to Marshall et al. method [10]. Sensitivity and specificity of this test according to a histology examination was 98.1% and 90.2%, respectively. The test was accepted as positive if a 24 hours room temperature incubaction changed its color from orange to pink. Endoscopically taken samples of gastric mucosa were placed in buffered formalin and subjected to standard histological procedures. Subjects classified as infected had positive results in both tests. Subjects classified as non-infected had negative results in both tests. In the case of participants successfully treated for *H. pylori*, the inclusion was possible if eradication had been performed at least 36 month earlier.

Histological study

The examination of endoscopically taken specimens was performed by two experienced pathomorphologists who were blinded to the diagnoses and smoking habit. In the case of inconsistency between pathologists, the final diagnoses were established by joint evaluations of the slide. Endoscopic slices of the gastric mucosa were stained with hematoxylin-eosin and additionally with Giemsa. Gastritis was graded on the basis of a four step scale (0-3), including: 1) *H. pylori* density; 2) activity (neutrophils infiltration); and 3) inflammation (mononuclear cells infiltration) [9]. They were scored as follows:

H. pylori density

(0): no *H. pylori* present

(1): single bacterium or their groups covering up to 1/3 of the gastric mucosa surface

(2): groups of bacteria covering up to 2/3 of the gastric mucosa surface

(3): groups of bacteria covering the whole gastric mucosa surface;

Activity

(0): no neutrophils present

(1): single neutrophil found in the limited number of fields

(2): neutrophils found in the lamina propria

(3): neutrophils found in the lamina propria, gastric glands and epithelium;

Inflammation

(0): no mononuclear cells present

(1): mononuclear cells found in the upper one third of the mucosa or dispersed mildly in the whole mocosa

(2): mononuclear cells found in the upper half of the mucosa or dispersed moderately in the whole mucosa

(3): dense infiltrate of mononuclear cells found within the whole mucosa.

Statistical analysis

The results (means ±S.D or medium and range) were subjected to statistical analysis using Mann-Whitney U-test; significant differences were accepted at $p<0.05$.

***Table 2*. The influence of smoking habit on histology of the gastric mucosa in *Helicobacter pylori* infected subjects**

	Dyspepsia			Duodenal ulcer			Total		
	smoking (+)	smoking (-)		smoking (+)	smoking (-)		smoking (+)	smoking (-)	
	(n=17)	(n=47)	p	(n=32)	(n=13)	p	(n=49)	(n=60)	p
colonisation									
antrum	3 (1-3)	2 (1-3)	NS	3 (1-3)	3 (1-3)	NS	3 (1-3)	3 (1-3)	NS
corpus	1 (0-3)	2 (1-3)	<0.001	1 (0-3)	2 (1-3)	NS	1 (0-3)	2 (1-3)	<0.001
p	<0.001	<0.01		<0.001	NS		<0.001	<0.01	
activity									
antrum	3 (1-3)	3 (1-3)	NS	3 (2-3)	3 (1-3)	NS	3 (1-3)	3 (1-3)	NS
corpus	2 (0-3)	2 (1-3)	<0.005	2 (0-3)	2 (2-3)	NS	2 (0-3)	2 (1-3)	<0.005
p	<0.01	<0.001		<0.001	NS		<0.001	<0.01	
inflammation									
antrum	3 (1-3)	3 (1-3)	NS	3 (2-3)	3 (1-3)	NS	3 (1-3)	3 (1-3)	NS
corpus	1 (0-3)	2 (1-3)	<0.01	1 (0-3)	2 (1-3)	NS	1 (0-3)	2 (1-3)	<0.05
p	p <0.001	<0.001		<0.001	<0.01		<0.001	<0.001	

***Table 3*. The influence of smoking habit on histology of the gastric mucosa of *Helicobacter pylori* non-infected subjects**

	Dyspepsia			Duodenal ulcer			Total		
	smoking (+)	smoking (-)		smoking (+)	smoking (-)		smoking (+)	smoking (-)	
	(n=13)	(n=52)	p	(n=30)	(n=32)	p	(n=43)	(n=84)	p
activity									
antrum	1 (0-2)	0 (0-3)	NS	1 (0-2)	0 (0-1)	NS	1 (0-2)	0 (0-3)	NS
corpus	0 (0-2)	0 (0-2)	NS	0 (0-1)	0 (0-1)	NS	0 (0-2)	0 (0-2)	NS
p	<0.01	<0.05		<0.001	<0.001		<0.001	<0.001	
inflammation									
antrum	1 (0-2)	1 (0-2)	NS	1 (0-2)	0 (0-2)	NS	1 (0-2)	0 (0-2)	NS
corpus	0 (0-1)	0 (0-3)	NS	0 (0-1)	0 (0-1)	NS	0 (0-1)	0 (0-3)	NS
p	<0.001	<0.01		<0.001	<0.001		<0.001	<0.01	

The local Ethical Committee approved the study and all subjects gave informed consent.

Results

H. pylori infected population

In patients with dyspepsia, irrespectively of smoking habit, a significantly higher scores for *H. pylori* density, neutrophils, and mononuclear cells infiltration were found in the antrum compared to the corpus. With regards to smokers and non-smokers, the differences of studied parameters were found only in the corpus mucosa; *H. pylori* density, activity, and inflammation were higher in non-smokers than smokers (*Tab. 2*).

In duodenal ulcer patients, the differences within studied parameters were not significant between smoker and non-smoker groups, both in the antrum and in the corpus (*Tab. 2*). However, when smokers and non-smokers were analyzed separately, only the smokers exhibited higher scores for *H. pylori* density, neutrophils and mononuclear cells infiltration in the antrum versus the corpus. In non-smokers the differences were not significant; however, the number of subjects in this group was small. In joint analysis of subjects with dyspepsia and duodenal ulcer, the differences between smokers and non-smokers were the same as in the group with dyspepsia; although, the differences between the antrum and the corpus were more pronounced in smokers than non-smokers.

H. pylori non-infected population

In patients with dyspepsia and duodenal ulcer disease, the differences in activity and inflammation between smokers and non-smokers were not significant in both separate and joint analysis of these two clinical groups, regardless of the stomach location (antrum, corpus) (*Tab. 3*). Moreover, apart from the clinical diagnosis, there was a significant difference in studied parameters of gastritis between the antrum and the corpus of both smokers and non-smokers.

Discussion

Despite the evidence on the association between smoking habit and atrophic/metaplastic lesions within gastric mucosa [8,11], there was no data on the influence of smoking on histological gastritis characterized by the intensity of *H. pylori* infection, neutrophils, and mononuclear cells infiltration. The results of the current study have shown that smoking influences colonization of *H. pylori* within the stomach and modifies the mucosal distribution of neutrophils and mononuclear cells infiltrates. The mechanism of these changes is not clear. Cigarette

smoke contains about 4000 different compounds of various biological effects. These compounds when act separately or more frequently in combination can cause significant changes in histological image of the mucosa. Also, gender and age may be of relevance. However, since the two clinical groups were not comparable, most ulcer disease patients were men and smokers while patients with dyspepsia women and non-smokers, additionally, duodenal ulcer patients infected with *H. pylori* were younger than remaining, the correlation of histological findings with gender and age has not been made.

The gastric response to a chronic *H. pylori* infection is characterized by the infiltration of polymorpho-cells and mononuclear-cells into the mucosa, and an inflammatory response is a specific reaction to the presence of this bacterium. After the eradication of *H. pylori*, the inflammatory cells population decreases significantly [12]. According to our data in duodenal ulcer patients, the histological changes within the gastric mucosa decrease within a few years after *H. pylori* eradication. Observed severity of gastritis was also lower in *H. pylori* negative than positive subjects with dyspepsia, regardless of smoking habits. We do not know how long-time patients with dyspepsia were *H. pylori* free or how many of them eradicated spontaneously within the last three years. However, as spontaneous eradication from the stomach is rare [13] and currently non-infected subjects with dyspepsia had never been eradicated (patients' report), we can assume that no more than a few *H. pylori* negative subjects with dyspepsia could have the spontaneous eradication within the last few years, and thus the relevant error was small.

Formerly, short time lasting exposition to cigarette smoke did not cause the gastric mucosal accumulation of inflammatory cells, while potentiated ethanol- and indomethacin-induced them [14,15]. We have found that in *H. pylori* positive smokers, histological changes are predominantly located in the antrum; while in non-smokers, they are both in the antrum and corpus. The significant differences in studied parameters of chronic gastritis between smokers and non-smokers were found in the corpus mucosa of *H. pylori* infected subjects (separate analysis of patients with dyspepsia and joint analysis of two clinical groups). This would mean that smoking influences gastritis histology mainly in *H. pylori* infected population and additionally in one stomach location.

Smoking is a known risk factor for duodenal ulcer development in the *H. pylori* infected population [16,17]. The pattern of gastritis correlates strongly with this disease; the antral-predominat gastritis is most frequently recognized. The results of our study provides evidence that not only duodenal ulcer disease is related to the observed pattern of gastritis. The antral-predominat gastritis was also found in subjects with dyspepsia, and this observation is in line with recently published data of Western populations [18]. According to these data, the pattern of gastritis may not be related to the clinical diagnosis but more likely to some other factors, e.g., *H. pylori* infection. It is known that the severity of gastritis is positively associated with the intensity of *H. pylori* infections and is higher in the antrum than in the corpus [19]. Interestingly, the results of our study demonstrates that in non-infected subjects, histological gastrits is also more severe in the antrum than in the corpus, and this is more pronounced in smokers than non-smokers. Therefore, one can speculate that *H. pylori* infection may only cause quantitative shifts in microscopic image of chronic gastritis.

It is unclear why in smokers *H. pylori* colonization is larger in the antrum than in the corpus but this fact may have some clinical implications. We know from earlier reports that in *H. pylori* infected smokers, the risk of gastric carcinoma refers predominantly to prepyloric region [20]. Thus, the combined action of two factors, i.e., cigarette smoke components and *H. pylori* presence over a relatively long period, may predispose to neoplastic transformation, making the association of antral carcinoma with smoking and *H. pylori* infection not incidental.

Although the current study is limited to two clinical groups and the number of analyzed patients is relatively small, we conclude that smoking should be taken into account when histological evaluation of the gastric mucosa in *H. pylori* infected population is performed.

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References

1. Armitage AK, Dollery CT, George CF, Houseman TH, Lewis PJ, Turner DM. Absorption and metabolism of nicotine from cigarettes. Br Med J, 1975; 4: 313-6.
2. Ivey KJ, Triggs EJ. Absorption of nicotine by the human stomach and its effect on gastric ion fluxes and potential difference. Am J Dig Dis, 1978; 23: 809-14.
3. Kalra J, Chaudhary AK, Prasad K. Increased production of oxygen free radicals in cigarette smokers. Int J Exp Pathol, 1991; 72: 1-7.
4. Hayashi N, Kawano S, Tsuji S, Masuda E, Nakama A, Tsujii M, Michida T, Kobayashi I, Nagano K, Fusamoto H, Kamada T. Mechanisms of smoking-induced changes in gastric blood flow and mucosal oxygenation: roles of endothelin and prostacyclin. Gastroenterology, 1993; 104: A97.
5. Fletcher DR, Shulkes A, Hardy KJ. The effect of cigarette smoking on gastric acid secretion and gastric mucosal blood flow in man. Aust N Z J Med, 1985; 15: 417-20.
6. Muller-Lissner SA. Bile reflux is increased in cigarette smokers. Gastroenterology, 1986; 90: 1205-9.
7. Johnson RD, Horowitz M, Maddox AF, Wishart JM, Shearman DJC. Cigarette smoking and rate of gastric emptying: effect of alcohol absorption. Brit Med J, 1991; 302: 20-3.
8. Haruma K, Nakamura M, Imagawa M, Sumioka M, Watanabe C, Hamada H, Mihara M, Kamada T, Yoshihara Y, Sumii K, Kajiyama G. Cigarette smoking promotes gastric atrophy and intestinal metaplasia in *Helicobacter pylori*-positive subjects. Gut, 1999; 45 (Suppl. III): A54.
9. Price AB. The Sydney System: histological division. J Gastroenterol Hepatol, 1991; 6: 209-22.
10. Marshall BJ, Warren JR, Francis GJ, Langton SR, Goodwin CS, Blincow ED. Rapid urease test in the management of *Campylobacter pyloridis*-associated gastritis. Am J Gastroenterol, 1987; 82: 200-10.
11. Fontham ETH, Ruiz B, Perez A, Hunter F, Correa P. Determinants of *Helicobacter pylori* infection and chronic gastritis. Am J Gastroenterol, 1995; 90: 1094-101.
12. Witteman EM, Mravunac M, Becx MJCM, Hopman WPM, Verschoor JSC, Tytgat GNJ, de Koning RW. Improvement of gastric inflammation and resolution of epithelial damage one year after eradication of *Helicobacter pylori*. J Clin Pathol, 1995; 48: 250-6.
13. Valle J, Kekki M, Sipponen P, Ihamaki T, Siurala M. Long-term course and consequences of *Helicobacter pylori* gastritis: results of a 32-year follow-up study. Scand J Gastroenterol, 1996; 31: 546-50.
14. Chow JYC, Ma L, Zhu M, Cho CH. The potentiating actions of cigarette smoke on ethanol-induced gastric mucosal damage in rats. Gastroenterology, 1997; 113: 1188-97.

15. Guo X, Mei QB, Cho CH. Potentiating effect of passive cigarette smoking on gastrointestinal damage induced by indomethacin in rats. Dig Dis Sci, 1999; 44: 896-902.

16. Graham DY, Colon-Pagan J, Morse RS, Johnson TL, Walsh JH, McCullough AJ, Marks JW, Sklar M, Stone RC, Cagliola AJ, Walton-Bowen KL, Humphries TJ and Omeprazole Duodenal Ulcer Study Group. Ulcer recurrence following duodenal ulcer healing wih omeprazole, ranitidine, or placebo: a double-blind, multicenter, 6-month study. Gastroenterology, 1992; 102: 1289-94.

17. Namiot Z, Stasiewicz J, Jaroszewicz W, Namiot DB, Bucki R, Kemona A. Duodenal ulcer recurrences one year after *Helicobacter pylori* eradication. Gastroenterol Pol, 2005; 12: 495-8.

18. Vakil N, Talley NJ, Stolte M, Sundin M, Junghard O, Bolling-Sternevald E. Patterns of gastritis and the effect of eradicating *Helicobacter pylori* on gastro-oesophageal reflux disease in Western patients with non-ulcer dyspepsia. Aliment Pharmacol Ther, 2006; 24: 55-63.

19. Dooley CP, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, Blaser MJ. Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. N Engl J Med, 1989; 321: 1562-6.

20. Koizumi Y, Tsubono Y, Nakaya N, Kuriyama S, Shibuya D, Matsuoka H, Tsuji I. Cigarette smoking and the risk of gastric cancer: a pooled analysis of two prospective studies in Japan. Int J Cancer, 2004; 112: 1049-55.