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ASSESSMENT OF CO-EXISTENCE OF *HELICOBACTER PYLORI* AND *CANDIDA* FUNGI IN DISEASES OF THE UPPER GASTROINTESTINAL TRACT

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Candida spp. were found in the gastric mucosa of 27 (17%) patients, out of whom 18 (11%) showed co-existence of the fungi with *H. pylori*. Analysis of relationship between selected disorders of the upper gastrointestinal tract (non ulcer dyspepsia NUD, gastric ulcer, duodenal ulcer) and infection with *H. pylori* and/or *Candida* revealed a link between co-existence of *H. pylori* with *Candida* and gastric ulcers suggesting synergism of those microorganism in pathogenesis of the disease. On the contrary, according to quantitative studies performed, the fungi alone do not play a significant role in pathogenesis of the above mentioned disorders as they colonize only epithelium to the extent that is not pathologically significant ($<10^3$ CFU/ml). Genetical study was carried out on 57 *Helicobacter pylori* strains isolated from bioptates of the gastric mucosa. The genotypes of the strains (gene cagA and alleles of gene vacA – m1, m2, s1, s2) were determined using the PCR technique. As it was shown, the patients infected with *H. pylori* strains of genotype cagA+, vacA s1 are exposed to higher risk of peptic ulcer disease (PUD) as compared to the patients infected with cagA-, vacA s2 strains. In the case of the NUD patients a correlation with allele m2 was found only ($p<0.001$). This may suggest that in future some of the NUD patients infected with cagA+, vacA s1 strains will fall into the group at higher risk for PUD.

Key words: *Helicobacter pylori, cagA, vacA, Candida spp.*

INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) is one of the most widespread bacterial infections all over the world. An estimated 50% of world human population is infected with this bacterium (1, 2). The incidence of the infection is associated mostly with childhood, socioeconomic and sanitary conditions. In developing countries the incidence accounts for 80-100% while in developed countries 20-40% (3). According to the studies carried out in 1995-1996 by Gosciniak (4), *H. pylori* strains were significantly more frequent in adults than in children (57.4% and 38.1% respectively). The incidence of *H. pylori* infection, as revealed by multicentre studies carried out in Poland in the years 2000-2003, accounted for 58% (adults: 84%, children up to 18 years of age: 32%) (5).

H. pylori plays important role in ethiopathogenesis of the upper gastrointestinal tract disorders. The species is etiological factor of type B gastritis and one of significant risk factors for ulcerous disorder, gastric lymphoma MALT (Mucosa Associated Lymphoid Tissue) and gastric carcinoma (6-11). Chronic gastritis develops in all persons infected with *H. pylori*. However, further development of pathological changes occur in only part of them. Ulcerous disease (gastric ulcer in 70% of cases and duodenum ulcer in 90% of cases) develops in some 15% of infected patients while proliferation of a neoplasm in the stomach occurs in 2-5% of them (12, 13). The role of *H. pylori* in the stomach carcinogenesis was proved in an animal model (Mongolian gerbils) (14, 15).

Intensity of pathological changes in the stomach is influenced by the host characteristics (genetic predispositions, intensity of the immune response to infection, diet, life conditions) as well as the level of strain toxicity (8, 16). In patients infected with low-virulent *H. pylori* strains the development of mild gastritis transforming to the chronic form is more probable while in persons infected with more virulent strains digestive ulcers, atrophy of the gastric mucosa and even neoplasmatic changes can occur (17).

The major markers of cytotoxicity, determining *H. pylori* pathogenicity, include among others: immunogenic protein CagA coded by the gene cagA (cytotoxin associated gene A) which a component of cagPAI, so called pathogenicity island, and vacuolating cytotoxin VacA (18-21). The cagA gene occurs in about 60% of *H. pylori* strains and its presence is associated with higher incidence of ulcerous disease, atrophic gastritis and gastric carcinoma (8, 18, 22, 23). The vacA gene is present in all isolated *H. pylori* strains but only as few as 40-50% of them produce active cytotoxin. This phenomenon is associated with types of the vacA gene alleles (s1, s1a, s1b, s2, m1, m2), whose presence in various combinations determines the activity of VacA cytotoxin. The strains s1/m1 produce cytotoxin VacA of higher activity as compared to s1m2 strains while the presence of the s2/m2 genotype determines expression of cytotoxin VacA which does not show cytotoxic activity. Cytotoxin VacA is responsible, among others, for vacuolization of epithelial cells of the gastric mucosa directly resulting in their destruction and the

onset of chronic inflammation (16, 18, 24). Infection with the vacA s1a *H. pylori* strains induces more intense inflammation of the gastric mucosa and development of duodenal ulcerous disease as compared to infection with the s1b or s2 strains, while the vacA m1 strains are associated with a higher level of gastric epithelium injury (25).

High attention is now paid to the role of *Candida* fungi in chronic gastritis, ulcerous disease and non-specific inflammation of the bowel (26-28). Fungal colonization of the gastric mucosa was shown to be present in 30-50% of patients with active ulcerous disease. Persistency of clinical symptoms is prolonged and the process of ulcer healing is affected. Moreover, the strains isolated from gastric ulcers were demonstrated to show greater proteolytic activity than those isolated from chronic gastritis patients (26).

In most of healthy persons yeast-like fungi of the *Candida* genus, as a natural saprophytic flora, inhabit the oral cavity, pharynx and large intestine areas in an equilibrium with gastrointestinal bacteria. The esophagus, stomach and small intestine stand only for a way of passage for them. In high-risk patients persistent fungal colonization can contribute to the development of pathological changes. Fungal infection of the gastrointestinal tract is usually endogenous. Exogenous infection is also possible from solid food contaminated with fungi (29, 30). Recently, attention has been drawn to the fact that *H. pylori* infection can represent a factor facilitating fungal colonization of gastric mucosa (30, 31).

Low pH in stomach is assumed to be not a barrier to survival and pathogenic action of *Candida* fungi. *In vitro* studies showed the ability of *C. albicans* and *C. tropicalis* to grow in an environment of pH = 2 and *C. lusitaniae* at pH = 3. Which is in line with observations of other authors who reported cases of patients with gastric ulcers localized in the prepyloric area in whom concurrent high fungal colonization at very low pH was found. This phenomenon suggests that there exists not fully investigated efficient mechanism adapting *Candida* fungi to survive at low pH that occurs in the stomach (32, 33).

The aims of the study presented in this paper were:

1. analysis of co-existence of *H. pylori* and *Candida* fungi infections in patients with clinical symptoms from the upper gastrointestinal tract.
2. demonstration of a link between the level of *H. pylori* toxicity and the intensity of inflammation changes within the upper gastrointestinal tract in the examined group of persons infected with *H. pylori* as a result of analysis of the relationship between various cagA and vacA genotypes of *H. pylori* and clinical diagnosis.

MATERIALS AND METHODS

The study was carried out with the permission of the Bioethical Commission of the Jagiellonian University in Cracow.

The study covered a group of 158 persons aged 15-80 who applied to the Center of Medical Care Falck in Cracow and had never been treated for *H. pylori* infection before. In all patients clinical symptoms from the upper gastrointestinal tract occurred. Endoscopic examinations of the patients were carried out to identify the reasons for ailments and clinical symptoms. On gastroscopy, bioptates of changed stomach mucosa were collected from the antrum and the body areas for histopathological and microbiological tests including the rapid urease test for *H. pylori* infection (PRO-MED, Gora Kalwaria, Poland). Material tested for *H. pylori* (2 bioptates, one from the antrum and one from the body) was transferred in a transportation medium Portagerm pylori (PORT-PYL, bioMerieux, Marcy l'Etoile, France) while the bioptates tested for *Candida* fungi (one from the antrum and one from the body) were transferred in 1 ml of 0.85% NaCl. Microbiological examinations were carried out in the Department of Pharmaceutical Microbiology of the Jagiellonian University Collegium Medicum.

Results of endoscopy and histopathological examination represented the diagnostic criteria used to qualify the patients into the groups comprising gastric ulcer, duodenal ulcer, non-ulcerous dyspepsia (NUD). The last mentioned group covered patients without any changes found on endoscopy and patients with erosive and non-erosive diseases including esophagitis, gastritis and duodenitis. The patients with gastric and duodenal ulcers were qualified to the group of peptic ulcer diseases (PUD).

H. pylori culture

Specimens of the gastric mucosa collected during gastroscopy were homogenized in glass sterile mortars and inoculated onto Schaedler medium with 5% sheep blood (bioMerieux, Marcy l'Etoile, France). The culture was incubated microaerophilically at 37°C for 2 to 7 days. Direct Gram-stained preparation from bioptate was made and the test for urease with the use of liquid Urea indole medium (bioMerieux, Marcy l'Etoile, France) was carried out to confirm the presence of *H. pylori*. Cultured strains were identified from macroscopic picture of colonies and positive tests for urease, catalase and oxidase. Additionally, a Gram-stained preparation from the culture was made to check the presence of Gram-negative spiral bacteria.

Candida spp. culture

The test to determine the quantity of *Candida* spp. cells (CFU – colony forming unit) in 1 ml of the material tested as well as the analysis of results was performed according to modified Muller method (34). The analysis of quantitative inoculation of the fungi is presented in *Table 1*. Fungal strains isolated from clinical material were species-identified on the chromogenic medium *Candida* ID 2 (bioMerieux, Marcy l'Etoile, France).

DNA isolation

H. pylori culture incubated for 72 hrs on a solid medium was dispersed in 1 ml 0.85% NaCl and centrifuged (9000 rpm, 10 min). Following that, the genomic DNA was isolated with the use of "Genomic Mini" kit (A&A Biotechnology, Gdynia, Poland), according to manufacturer's recommendations. The obtained DNA was stored at 4°C.

PCR

Genetical examinations comprised testing of 58 *H. pylori* strains isolated from gastric mucosa bioptates for cagA gene and alleles of the gene vacA (s1, s2, m1, m2).

For tests were used reagents of Promega (Promega, Madison, WI, USA). Amplification was performed in the Biometra T personal thermocycler (Biometra, Gottingen, Germany). The sequences of primers (Sigma Genosys, St. Louis, MO, USA) used in PCR are shown in *Table 2*.

Detection of H. pylori cagA gene and alleles of gene vacA

The reaction mixture contained 0.125 µl aTaq DNA Polymerase, 0.5 µl PCR Nucleotide Mix, 1.5 µl 25 mM $MgCl_2$, 2.5 µl 10x aTaqDNA Buffer, 16.375 µl nuclease-free water and primers (cagA or vacA) in a quantity of 0.0025 µmol per sample each. To 23 µl of reaction mixture 30 ng of starting DNA was added.

Table 1. Interpretation of quantitative assessment of *Candida* fungi in gastric mucosa bioptates (34).

Tested material	Pathologically non-significant result	Uncertain result (follow-up test recommended)	Positive result (pathologically significant fungal count)
Bioptates of gastric mucosa	$\leq 10^3$ cells/ml	$10^4 - 10^5$ cells/ml	$\geq 10^5$ cells/ml

Table 2. Primers used in PCR for amplification of gene cagA and vacA alleles.

Gene	Primer	Sequence of primer (5'-3')	PCR product (bp)	Reference
vacA				[35]
m1			290	
	VA3-F	GGTCAAAATGCGGTCATGG		
	VA3-R	CCATTGGTACCTGTAGAAAC		
m2			352	
	VA4-F	GGAGCCCCAGGAAACATTG		
	VA4-R	CATAACTAGCGCCTTGCAC		
s1/s2			259/286	
	VA1-Fc	ATGGAAATACAACAAACACAC		
	VA1-Rc	CTGCTTGAATGCGCCAAAC		
cagA			298	[36]
	D008	ATAATGCTAAATTAGACAACTTGAGCG		
	R008	TTAGAATAATCAACAAACATCACGCCA		

Table 3. Distribution of the vacA genotypes and gene cagA by clinical diagnosis.

Genotype	PUD* (%) n=18	NUD** (%) n=39	p^+
s1	13 (72)	21 (54)	0.183
s2	5 (28)	18 (46)	
m1	6 (33)	11 (28)	0.694
m2	12 (67)	28 (72)	
s1/m1	6 (33)	11 (28)	0.394
s1/m2	7 (39)	10 (26)	
s2/m2	5 (28)	18 (46)	
cagA+	13 (72)	17 (44)	0.045
cagA-	5 (28)	22 (56)	

*PUD – peptic ulcer disease
** NUD – non-ulcerous dyspepsia
+Chi-square test

Thermal profiles of PCR

Amplification of the vacA gene alleles (s1, s2, m1, m2): preliminary denaturation at 94°C for 4 min followed by 35 cycles: 94°C for 1 min, 57°C for 1 min, 72°C for 1 min and a final elongation at 72°C for 5 min, 4°C/pause.

Amplification of the cagA gene: preliminary denaturation at 94°C for 5 min followed by 33 cycles: 94°C for 1 min, 60°C for 1 min, 62.5°C for 1 min, 72°C for 1 min and a final elongation at 72°C for 5 min, 4°C/pause.

Analysis of the PCR products

Products of amplification were fractionated by electrophoresis in a 2% agarose gel with etidine bromide added. The assessment of products size was made with the use of the 100 bp DNA ladder (Fermentas Life Science, Burligton, Canada) as a size marker. Results of fractionation were observed under UV light (Vilber Lourmat, Marne-La Vallee, France) and analyzed with the use of computer.

Statistical analysis

Statistical analysis was performed using chi-square test and difference between two percentages – one-sided test. The value $p \leq 0.05$ was deemed statistically significant.

RESULTS

Gastroscopy was carried out in 158 (100%) persons with symptoms from the upper gastrointestinal tract. Infection with and presence of *H. pylori* in bioptates collected from gastric mucosa was confirmed in 97 (61%) of the persons examined. Genotyping was performed for 58 *H. pylori* strains isolated from the above group of patients.

Candida fungi were found in 27 (17%) patients. Twenty strains were identified as *C. albicans* and 7 as *Candida* spp. Analysis of results of quantitative inoculation showed that all patients presented with pathologically non-significant number of fungi in the stomach ($<10^3$ CFU/ml). One hundred twenty eight (81%) NUD cases, 18 (11%) cases of duodenal ulcer and 12 (8%) gastric ulcer cases were found. Co-existence of *H. pylori* and *Candida* fungi (group *H. pylori*+ *Candida*+) in the stomach was found in 18 (11%) patients.

Presence of *H. pylori* without concurrent *Candida* fungi in the stomach (group *H. pylori*+ *Candida*-) was found in 79 (50%) patients while *Candida* fungi without concurrent presence of *H. pylori* were found in 9 (6%) patients (group *H. pylori*- *Candida*+). In 52 (33%) persons neither *H. pylori* nor *Candida* infections were found (group *H. pylori*- *Candida*-).

We showed that NUD is significantly more frequent (p=0.024) in non-infected group (*H. pylori*- *Candida*-) as compared to the group infected with *H. pylori* alone (*H. pylori*+ *Candida*-). The same correlation can be demonstrated by comparison of non-infected group with the group infected with both microorganisms (p=0.002). The incidence of duodenal ulcer was greater (the level close to statistical significance, p=0.068) in the group infected with *H. pylori* alone as compared to the group infected neither with *H. pylori* nor *Candida*. Gastric ulcers were significantly more frequent in the group with concurrent *H. pylori* and *Candida* infections (*H. pylori*+

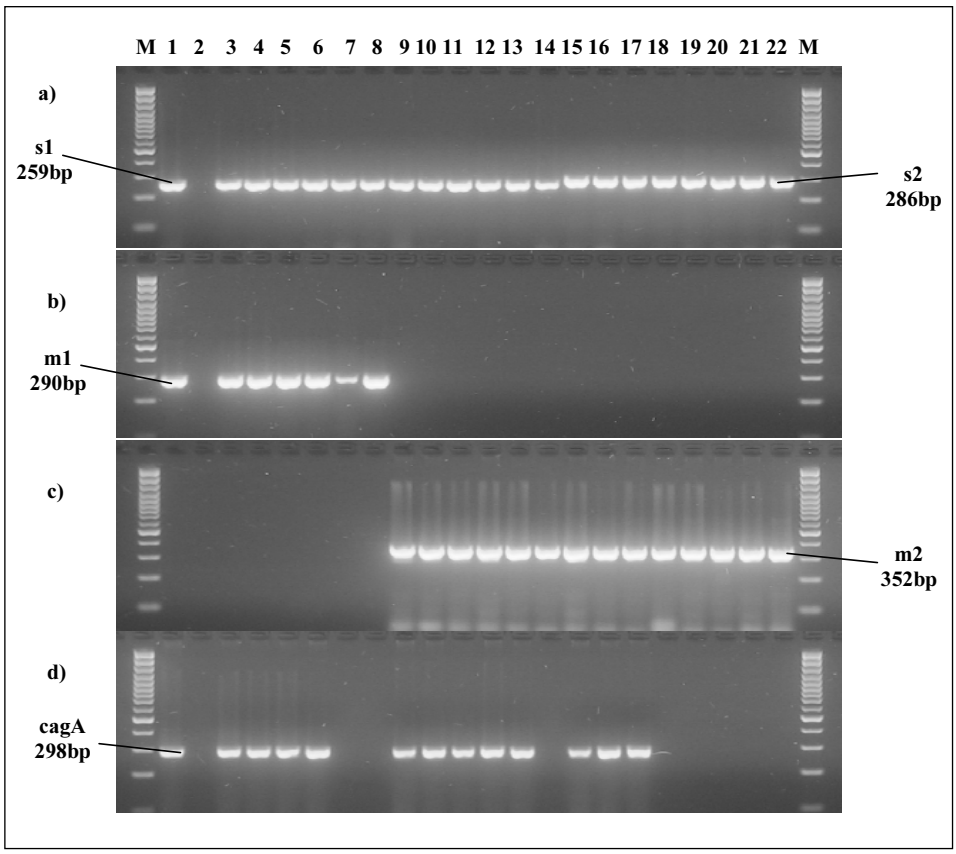

Fig. 1. An example electrophoretic pattern of amplification products of the cagA gene (d) and gene vacA alleles (a-s1/s2, b-m1, c-m2) in 2% agarose gel. M – size marker (DNA ladder 100 bp); 1 – positive control (contains genomic *H. pylori* DNA); 2 – negative control (without DNA); 3-14: s1; 15-22: s2; 3-8: m1; 9-22: m2; 3-6, 9-13, 15-17: cagA.

Candida+) in comparison to the group with single *H. pylori* infection (p=0.007) and to non-infected group (p=0.001). For the remaining cases no statistical differences in the incidence of a specific disease among infection groups studied were found. Fifty eight *H. pylori* strains were PCR tested for the presence of gene cagA and alleles of gene vacA (s1, s2 m1, m2) (*Fig. 1*). In one strain allele s has not been amplified with the use of primers applied, so 57 *H. pylori* were analyzed.

The presence of cagA gene was proved in 30 (53%) out of 57 *H. pylori* strains. The most frequent alleles of vacA gene were: s1 (34 strains – 60%) and m2 (40 strains – 70%). Allele s2 was found in 23 strains (40%) whereas allele m1 in 17 strains (30%). Three out of four possible combinations of gene vacA alleles were detected in the strains studied. They were as follows: s1m1 (17 cases – 30%), s1m2 (17 cases – 30%) and s2m2 (23 cases – 40%). No strains of s2m1 genotype were found.

Twenty seven (48%) *H. pylori* strains carried genotype responsible for higher virulence [cagA+, s1m1 – 15 (27%), cagA+, s1m2 – 12 (21%)]. Allele s1 significantly more frequently occurred together with gene cagA+ while allele s2 with gene cagA- ($p<0.001$). As for alleles m, allele m1 was strongly correlated with cagA+ whereas allele m2 with cagA- ($p<0.001$). Comparison of cagA and vacA genes frequency in the strains isolated from the group of patients with NUD against the group of PUD patients showed that greater frequency of gene cagA in the material collected from the PUD group was the only statistically significant difference (p=0.045) (*Table 3*). A separate scrutiny of those groups showed that within the PUD patients allele s1 was more frequent as compared to allele s2 (p=0.006), allele m2 was more frequent as compared to allele m1 (p=0.025) and gene cagA+ was more frequent as compared to cagA- (p=0.006). In the group of NUD patients only a correlation with allele m2 was found ($p<0.001$).

DISCUSSION

In the study a preliminary attempt to demonstrate importance of colonization of gastric mucosa with *Candida* in pathogenesis of gastric and duodenal disorders was made. It is worth emphasizing that colonization means the presence of fungi in material collected from patients in the quantity not pathologically significant and resulting neither in infection nor in clinical symptoms of fungal infection. Studies carried out by Scott and Katzenstein confirm growing number of such cases (16% and 33% respectively) (37, 38). This phenomenon seems to be alarming as colonization may lead to fungal infection not only in persons of high-risk group in whom favorable factors occur but also in healthy persons (39). The studies carried out by Zwolinska-Wcislo *et al*. showed pathologically significant fungal count in 54.2% of patients with gastric ulcer and in 10.3% of patients with chronic gastritis with *C. albicans* as the most frequent fungi (32). Budak *et al*. demonstrated pathologically significant quantity of fungi in 20.7% of patients with gastric ulcer and in 5% of patients with chronic gastritis. *C. glabrata* was the most frequent (23%) in gastric ulcer cases whereas in chronic gastritis cases the most frequent (15.7%) was *C. albicans* (26).

In our study *Candida* fungi were revealed in the stomach of 27 (17%) patients with ailments from the upper gastrointestinal tract. However, in all patients the fungi counts were not pathologically significant ($<10^3$ CFU/ml). Those patients cannot be deemed as having mycosis, however, the presence alone of fungi in the stomach, even in low quantity, may cause injury in epithelium and changes in mucosa as a result of action of proteolytic enzymes produced by fungal blastoconidia or spores (39).

In present study the importance of fungi presence in gastric mucosa was analyzed in the context of their co-existence with *H. pylori* that may result in intensification of inflammatory changes (40). Co-existence of *Candida* with *H. pylori* was proved in 18

(11%) patients. The diagnoses were as follows: NUD in 11 (61%) persons, gastric ulcer in 5 (28%) persons, duodenal ulcer in 2 (11%) persons. Similarly, the studies carried out by Zwolinska-Wcislo *et al*. revealed co-existence of *Candida* fungi with *H. pylori* in the stomach in 10% cases out of 63 patients (41).

Occurrence of fungi (without concurrent *H. pylori*) was found in 9 patients in whom NUD was diagnosed in 8 (89%) cases and gastric ulcer in 1 (11%) case. Probably, the presence of *Candida* fungi in the stomach of those patients resulted from administered earlier proton pump inhibitors (PPI) or H_2 blockers which, affecting microbiological equilibrium, could contribute to the development of fungal colonization of gastrointestinal tract. Such a situation could also happen during or after therapy with antibiotics and agents neutralizing acidic environment of the stomach (42, 43). The age of patients (the mean: 52 years) is also not without significance as this factor along with other ones represents a risk for candidiasis development (43).

Analysis (Chi-square test) of the correlation between the frequency of selected disorders of the upper gastrointestinal tract and *H. pylori* and/or *Candida* infections showed that fungi had not played an important role in development of those infections what could be a result of their low quantity ($<10^3$ CFU/ml).

It is known that infection with *H. pylori* predispose to duodenal ulcer development. In our patients correlation between infection with *H. pylori* alone and duodenal ulcer frequency was at the level close to statistical significance (p=0.068) as compared to the group not infected with *H. pylori* and *Candida*. On the other hand, in the group with concurrent infection with *H. pylori* and *Candida* gastric ulcer incidence was significantly greater than in the group not infected with those microorganisms at all and the group with *H. pylori* infection alone (p=0.001, p=0.007 respectively).

Thus, justified might be the conclusion that gastric ulcerous disorder can be influenced by the both factors occurring concurrently. However, the number of gastric ulcers in the group *H. pylori*+ *Candida*+ was the same as in the group infected only with *H. pylori*. Maybe, preceding injury of gastric mucosa by *H. pylori* facilitated the presence of fungi. Greenfield substantiates that despaired elimination of fungi from previously injured mucosa can be in favor of fungal colonization (44).

Statistical analysis revealed that NUD was more frequent in the group without both *H. pylori* and *Candida* infection as compared to the group infected with *H. pylori* alone or the group infected with both microorganisms. This finding may be concluded from greater numbers of duodenal ulcers and gastric ulcers in the groups *H. pylori*+ *Candida*- and *H. pylori*+ *Candida*+, respectively. Thus, one can suppose that in part of patients infected with *H. pylori* development of disorders more severe than NUD occurs while non-ulcerous dyspepsia prevails in persons not infected.

The intensity of pathological changes in the stomach is dependent on both the features of host and the level of *H. pylori* strain toxicity (8, 45).

Strains cagA+ are known to raise the risk of ulcerous disease as well as the risk of gastric carcinoma (8, 17, 46). Lage *et al.* reported that 91.7% of patients with ulcerous disease and 73% of patients with gastritis were infected with *H. pylori* cagA+ strains; this finding was confirmed by Weel *et al*. in 93.4% of ulcerous disease patients and in 64.6% of NUD patients (47, 48). In our study gene cagA was found in 72% of patients with peptic ulcer disease. That gene was significantly more frequent in PUD patients than in NUD patients (44%) (p=0.045). In Europe and in USA about 60% of all *H. pylori* strains carries gene cagA although this percentage differs in studied patient populations (6, 18, 47, 49). As it results from the studies carried out in Poland, infection with these strains accounts for 71% while other authors have reported 60% and 72.4% (5, 50, 51). In our study the frequency of infections with the cagA+ *H. pylori* strains was somewhat lower and accounted for 53% (30 cases). It is known that occurrence of the cagA gene is strongly associated with the presence of the signal vacA s1 sequence whereas most cagA- strains carries the s2 allele (18). This was proved by our study where gene cagA was accompanied by allele s1 in 90% of strains while 74% of cagA- strains carried allele s2 (p<0.001).

Analysis of correlation between cagA and vacA genes and clinical diagnosis in our group of patients showed that subjects infected with cagA+, vacA s1 *H. pylori* strain more likely develop ulcerous disease in comparison to patients infected with cagA-, vacA s2 strains. This can mean that part of NUD patients infected with cagA+, vacA s1 strains will fall into the group of higher risk of PUD development in the future. Genotype cagA-, s2m2 was found in 35% of strains tested. Laszewicz *et al*. confirm 27% incidence of strains carrying that genotype in Poland while Dzierzanowska *et al*. report somewhat lower percentage (13.1%) (5, 51).

Multicenter studies carried out in Poland in the years 2000-2003 showed that 54% of *H. pylori* strains carried genotype cagA+, s1m1/s1m2 responsible for higher virulence. Our study revealed high percentage of infections with strains of increased pathogenicity (48% of cagA+, s1m1/s1m2 were found). This may represent a hazard for the Polish population of higher morbidity of some diseases, like gastric ulcer, duodenal ulcer, gastric carcinoma and MALT-type lymphoma (5).

CONCLUSIONS

1. Demonstrated relationship between co-existence of *H. pylori* with *Candida* (in non-significant quantity) and gastric ulcer may confirm their synergistic action and impact on pathogenesis of that disease, but this issue needs further studies.
2. A link between the presence of *Candida* fungi without concurrent *H. pylori* and incidence of gastric or duodenal ulcer was not proved.
3. Peptic ulcer disease of the stomach and duodenum was shown to be related to the cagA+, vacA s1 genotype determining a higher virulence of *H. pylori* strains. The NUD patients infected with cagA+, vacA s1 *H. pylori* strains belong to the group of higher risk of PUD development in the future.
4. This study confirm correlation between toxicity of *H. pylori* strains and intensity of pathological changes in the upper gastrointestinal tract.

Conflict of interests: None declared.

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