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GENOTYPIC AND CLINICAL DIFFERENCES OF SEROPOSITIVE *HELICOBACTER PYLORI* CHILDREN AND ADULTS IN THE POLISH POPULATION

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Helicobacter pylori (*H. pylori*) plays an important role in the pathogenesis of the upper gastrointestinal tract diseases in both children and adults. The aim of this paper was to assess the differences between the clinical course of the disease in children and adults. This paper also presents an analysis of clinical symptoms, endoscopic and histopathological findings, *H. pylori* *cagA* and *vacA* genotypes rates and analysis of the sensitivity of these strains to antibiotics in the Polish population, with possible practical and therapeutic implications. The multicenter study on the frequency of *H. pylori* infections assessed by the presence of antibodies in IgG class against *H. pylori* in serum was conducted in the years 2002 and 2003. The study group included 6565 children and adults, in 3827 of whom antibodies levels were above 24 U/mL. The authors analyzed clinical and endoscopic symptoms and in some patients with *H. pylori* seropositivity also histopathological changes, and *cagA* and *vacA* genes. Sensitivity of *H. pylori* strains to antibiotics were also analyzed. Differences between the frequency of infection between children and adults were determined. Endoscopic examination in adults revealed more frequent cases of gastropathy ($P=0.003$) and erosive gastritis ($P=0.001$), and in children-thick mucosal folds ($P<0.0001$). Histopathological examinations carried out in adults have revealed atrophic gastritis and intestinal metaplasia. In children, *cagA*(+)s1m1 was observed more frequently than in adults (34.0% versus 23.1%; $P=0.02$) contrary to *cagA*(-)s2m2 which occurred more frequently in adults (27.1% versus 14.0%; $P=0.003$). No effect of the infection on nausea, regurgitation, vomiting, heartburn, and abdominal pain in children was detected. However, adults infected with *H. pylori* suffered from more frequent episodes of heartburn and abdominal pain. The *H. pylori* strain exhibited a high resistance to metronidazole (higher in adults: 41.7% versus 27.4%; $P=0.002$), and to clarithromycin (higher in children: 20.2% versus 15.4%; $P>0.05$), and dual resistance to metronidazole and clarithromycin (higher in children: 9.9% versus 8.4%; $P>0.05$). Resistance of the *H. pylori* to amoxicillin and tetracycline was not detected. The conducted study indicated clinical differences in the *H. pylori* infection in children and adults. Among the differences in children, especially the more frequent infections by the *cagA*(+)s1m1/m2 strain could have an influence on further consequences of the infection. The obtained results could be useful in therapeutic decisions.

Key words: *Helicobacter pylori* infection, *Helicobacter pylori* epidemiology, *Helicobacter pylori* histopathology, *cagA* genotype, *vacA* genotype, antibiotic resistance, amoxicillin, clarithromycin, tetracycline, metronidazole

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is widespread all over the world. The greatest number of infected people are in developing countries, whereas in developed countries the rate of infection is the smallest. Among risk factors of infection, the socioeconomic environment is regarded as one of the most important. In developed countries, the incidence of *H. pylori* infection in children is smaller than 12% and shows a tendency to decrease, while in developing countries it may exceed 40% (1). Multicenter studies conducted in Poland in the years 2002–2003

have demonstrated a high rate of *H. pylori* seroprevalence in both children and adults. In children aged 6 month to 18 years *H. pylori* seroprevalence rate was 32% and in adults aged 19 to 89 years 84% and varied depending on the region of the country (2). An influence of poor sanitary and hygiene conditions, economical status and parents education on the infection rate was demonstrated. In the last years a tendency to the decrease of infection rate has been shown, which could be linked to the improvement of social condition (3). *H. pylori* infection is a principal cause of chronic gastritis and peptic ulcer disease in children. In adulthood it leads to many diseases of the gastrointestinal tract including in particular

the development of gastric cancer (4-9). Also other strains of *Helicobacter* (less common) may be involved in pathogenesis of gastrointestinal tract diseases (10-12).

Despite the widespread of *H. pylori* infection in the population only a small fraction of the infected people exhibits clinical symptoms of the disease. The risk of the development of a disease depends on the duration of *H. pylori* infection, on the host factors, environmental factors and virulence of *H. pylori* strain. The diversity of *H. pylori* strains may influence the degree and intensity of the individual inflammatory response, which can result in a variety of clinical manifestation in children and adults (1, 13, 14).

Vacuolizing cytotoxin VacA coded by the vacA gene, CagA protein coded by the cagA gene in Pathogenicity Islands (PAI) and others are listed among the virulence factors of the bacteria (5). Cytotoxin VacA causes inflammation, stimulates apoptosis, inhibits cell reproduction and damages tight junctions between epithelial cells. CagA protein is very immunogenic and leads to the development of metaplasia, dysplasia and gastric cancer (4, 15).

The aim of this study was to analyze the differences between the course of the disease in *H. pylori* seropositive children and adults. The study also assessed the differences in clinical symptoms, endoscopic and histopathological findings, genotypes cagA and vacA of *H. pylori*, as well as the sensitivity of *H. pylori* to antibiotics in the Polish population.

MATERIAL AND METHODS

The study protocol was approved by the local Ethics Committee, affiliated to the Medical University of Wrocław, Wrocław, Poland - approval no. KB-321/2001 and KB-897/2003. Patients gave their informed consent.

Multicenter, prospective, cross-sectional, randomized study on the frequency of *H. pylori* infections among children and adults in Poland was conducted in the years 2002 and 2003 (2). 18,000 people aged from 6 months to 89 years (9000 adults and 9000 children), living in 10 different regions of Poland, were randomly chosen and invited for the participation in the study. From these, 7127 people volunteered to participate, 6565 of whom were examined for *H. pylori* infection (Table 1). *H. pylori* seropositivity was diagnosed on the basis of the presence of IgG antibodies for *H. pylori* using ELISA (Enzyme Linked Immunosorbent Assay) Microgen-recom Well Helicobacter IgG commercial kit (Germany). Studies were carried out using Mini Bos produced by DiaSorin. Concentration of antibodies above 24 U/mL was treated as a positive result that confirmed infection. The presence of antibodies was reported in 3827 (58.29%) subjects (Table 1). The presence of the following symptoms was assessed in children and adults with and without

infection: heartburn, nausea, vomiting, epigastric discomfort, flatulence, stomach aches.

Endoscopic examination

Among the total number of 3827 *H. pylori* seropositive patients, 801 (20.9%) gave consent to the endoscopic examination of the upper gastrointestinal tract. Endoscopy was carried out in 441 children and 360 adults. An endoscopic examination was done in the morning, on fasting, with videoendoscopy. During examination, endoscopic changes in the stomach and duodenum were assessed and four specimens were taken from the stomach mucosa (two from the gastric body and two from the pylorus) for histopathological examination and culture for *H. pylori* infection.

Microbiological examination

A bacteriological examination for *H. pylori* infection was carried out in stomach mucosa specimens taken from 441 children and 360 adults.

Primary isolation was performed on Wilkins Chalgren agar with 7% horse blood and Dent's selective supplement SR 147 (Oxoid, UK). The plates were incubated under microaerophilic conditions (CampyPak Plus, BBL generators) at 37°C for up to 7 days. *H. pylori* was identified by colony morphology, Gram staining and urease, catalase and oxidase activities.

Determination of cagA and vacA genotype

The cagA gene and alleles of vacA gene were detected in 400 patients by PCR as described previously (16). Briefly, genomic DNA was extracted from *H. pylori* isolates (A&A Biotechnology, Poland) and subjected to PCR with specific primers listed in Table 2. The same amplification conditions were used for all the reactions, i.e.: 94°C for 5 min for predenaturation, followed by 35 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 1 min and a final extension at 72°C for 7 min.

Estimation of sensitivity for antibiotics

In 423 isolated *H. pylori* strains, the authors assessed the sensitivity to amoxicillin, clarithromycin, tetracycline and metronidazole. This assessment was carried out on the basis of the standardized E-test method, which permits the determination of the minimal inhibitory concentration (MIC) that inhibited the increase of examined *H. pylori* strain. 222 *H. pylori* strains isolated from children and 201 strains isolated from adults were subjected to sensitivity assessment. In the categorization of strains into sensitive/resistant the following border values were taken into consideration: for metronidazole resistant strains MIC

Table 1. Number of examined children and adults and the number of examinations performed.

Group	Age of patients (years)	Number of studied patients	Number of patients tested for <i>H. pylori</i> antibodies	Number of patients with positive anti- <i>H. pylori</i> IgG antibodies (above 24 U/ml)		Esophagogastro-duodenoscopy and gastric biopsies in seropositive patients		<i>H. pylori</i> strains isolated from in gastric biopsies in seropositive patients		The genotypes <i>H. pylori</i> determined by PCR		Study on antibiotic sensitivity of <i>H. pylori</i> strains	
				n	%	n	%	n	%	n	%	n	%
I	Children (0.5-18)	3546	3258	1043	32.01	441	42.3	256	58.0	209	81.6	222	86.7
II	Adults (19-89)	3581	3307	2784	84.19	360	12.9	211	58.6	191	90.5	201	95.2
Total		7127	6565	3827	58.29	801	20.9	467	58.3	400	85.6	423	90.6

Table 2. Primers used for detection of the *cagA* gene and s1, s2, m2 alleles of the *vacA* gene.

Amplified region	Sequences (5' → 3')	Product (bp)
<i>cagA</i>	ACA TTT YGG CTA AAT AAA CGA TG TCT CCA TGT TGC CAT TAT GCT	360
s1/s2	ATG GAA ATA CAA CAA ACA CAC CTG CTT GAA TGC GCC AAA A	s1:259 s2:286
m1	5'GGT CAA AAT GCG GTC ATG G CAA TTG GTA CCT GTA GAA AC	290
m2	GGA GCC CAG GAA ACA TTG CAT AAC TAG CGC CTT GCA C	

Table 3. Seroprevalence of *H. pylori* infection in children and adults depending on age in the years 2002–2003.

Age to patients (years)	Number of children and adults tested for <i>H. pylori</i> antibodies	Number of patients with positive anti <i>H. pylori</i> IgG antibodies (above 24 IU/ml)	
	n	n	%
0.5 – 1	19	7	36.8
2 – 3	231	61	26.4
4 – 6	433	102	23.5
7 – 9	534	156	29.2
10 – 12	636	201	31.6
13 – 15	739	272	36.8
16 – 18	676	247	36.5
19 – 29	505	332	65.7
30 – 39	478	384	80.3
40 – 49	785	660	84.1
50 – 59	737	677	91.9
60 – 69	491	453	92.3
70 – 79	238	214	89.9
80 – 89	46	43	93.5

>8 mg/L, for clarithromycin resistant strains MIC >1 mg/L, for tetracycline resistant strains MIC >2 mg/L and for amoxicillin resistant strains MIC >0.50 mg/L.

Statistical analysis

Differences in genotypes, resistance of *H. pylori*, symptoms and findings frequency between children and adults were evaluated by t-student and chi-square tests. Statistical analysis was performed using MedCalc for Windows, version 12.6.1. (MedCalc Software, Ostend, Belgium). The level of $P \leq 0.05$ was considered as statistically significant.

RESULTS

Table 3 presents the rate of occurrences of *H. pylori* seropositivity in particular age groups. Worth to note is the large percentage of *H. pylori* seropositivity during the first years of life, which amounted to 36.8% in the first year of life and 26.4% between the second and third year of life. Between the fourth and sixth year of life, this percentage was smaller and amounted to 23.5%. In the subsequent age groups the percentage of seropositive *H. pylori* both in children and adults incrementally increased to 93.5% between the age of 80 and 89.

In the examined patients, the analysis of symptoms from the upper gastrointestinal tract such as heartburn, nausea, regurgitation of food, vomiting, epigastric discomfort, flatulence,

and abdominal pain was not conclusive in determining a connection of the listed symptoms and *H. pylori* seropositivity in children. However, it was found that there was a statistically significantly higher amount of cases of *H. pylori* seropositivity in adults suffering from heartburn and abdominal pain. From 1119 adults with heartburn, seropositive *H. pylori* strains were found in 968 (86.5%), when from 1665 patients without such symptoms, seropositive *H. pylori* strains were found in 1377 (82.7%) ($P=0.004$); and stomach aches respectively in: 86.5% versus 83.0% ($P=0.025$). The assessment of the frequency of seropositive *H. pylori* infections in relation to gastric or duodenal ulcer indicated a statistically significant correlation in children and adults between seropositive *H. pylori* and duodenal ulcer. No association between seropositive *H. pylori* and gastric ulcers was reported neither in children nor adults.

Endoscopic examinations revealed that comparing to children, in adults with seropositive *H. pylori* there was more cases with changes suggesting gastropathy ($P=0.003$), or erosive gastritis ($P=0.001$). In children, thick mucosal folds were observed more frequently than in adults ($P<0.0001$). In nine (5.1%) adults and six (1.7%) children gastric or duodenal ulcers were diagnosed. Deformation or scars of the duodenal bulb was found in 5.6% of adults and in 2.3% of children. In one adult patient the MALT stomach lymphoma was found.

The histopathological analysis of the stomach mucosa carried out in patients with *H. pylori* seropositive patients showed that the bacterium was more often detected in the gastric mucosa in adults (83.3%) than in children (65.6%; $P<0.01$). Moreover,

Table 4. The genotypes *cagA* of *H. pylori* strains isolated from 400 patients.

Group	Age (Years)	n	Genotypes of <i>H. pylori</i> strains					
			<i>cagA</i> (+)			<i>cagA</i> (-)		
			n	%	P I/II	n	%	P I/II
I	Children (0.5–18)	209	161	77.0	P<0.001	48	23.0	P<0.001
II	Adults (19–89)	191	122	63.8		69	36.2	
Total		400	283	70.4 40.6–91.6%*		117	29.6 8.4–59.4%*	

n - number of patients; * - range of frequency in particular regions.

Table 5. Genotypes of *H. pylori* strains isolated from 185 children and 179 adults.

vacA alleles	<i>cagA</i> gene status									
	<i>cagA</i> positive					<i>cagA</i> negative				
	Children		Adults		p	Children		Adults		p
	n	%	n	%		n	%	n	%	
s1m1	63	34.0	41	23.1	0.0279	6	3.2	3	1.7	ns
s1m2	59	31.9	55	31.0	ns	9	4.8	8	4.5	ns
s1m1/m2	11	5.9	8	4.5	ns	1	0.5	0		ns
s1/s2m1	0		0			0		0		
s1s2m2	1	0.5	2	1.1	ns	1	0.5	0		ns
s1/s2m1/m2	2	1.0	1	0.5	ns	1		0		ns
s2m1	0		0			0		0		
s2m2	4	2.1	5	2.8	ns	26	14.0	48	27.1	0.003
s2m1/m2	0		2	1.1	ns	1	0.5	6	3.4	ns

ns - statistically non-significant.

Table 6. Prevalence of *H. pylori* strains resistant to metronidazole (MZ) and clarithromycin (Cl) in children and adults.

Studied group	Number of E-tests	Number of resistant <i>H. pylori</i> strains					
		MZ		Cl		MZ + Cl	
		n	%	n	%	n	%
Children	222	61	27.4 (16-43)*	45	20.2 (9-26)*	22	9.9 (0-16)*
Adults	201	84	41.7 (27-52)*	31	15.4 (3-27)*	17	8.4 (3-11)*
Statistical significance (P)		0.0028		ns		ns	

ns- statistically non-significant

*- range of frequency in particular regions

histopathological examinations have also indicated that there were statistically more cases of atrophic gastritis (35.0% versus 16.5%), $P < 0.0001$ and intestinal metaplasia (35.8% versus 6.3%, $P < 0.0001$) in adult patients as compared to children. However, children were more often diagnosed with chronic active inflammation in the gastric mucosa (70.6% versus 53.7%, $P = 0.0002$). In 2 adults (1.5%) and 1 child (0.4%) dysplasia in the antrum was observed. In 256 children (58.0%) and in 211 adults (58.6%), the culture of *H. pylori* in specimens from gastric mucosa was positive.

In the isolated *H. pylori* strains, the *cagA* gene was found in 283 out of 400 examined strains, which constitutes 70.4% (Table 4). The *cagA* strain was more often isolated in children (77.0%) than in adults (63.8). Regional differentiations of *H. pylori* *cagA*(+) strain were found in both children and in adults, 40.6% and 91.6% respectively, *cagA*(-) in 8.4% and 59.4%.

The distribution of *Helicobacter pylori* genotypes is reported in Table 5. The most frequent genotype in children was genotype

cagA(+)s1m1 (34.0%) and *cagA*(+)s1m2 (31.9%), whereas in adults genotype *cagA*(+)s1m2 (31.0%) and *cagA*(+)s1m1 (23.1%). The presence of *cagA*(+)s1m1 in children was statistically more frequent than in adults ($P = 0.0279$). *H. pylori* strains *cagA*(-)s2m2 were found more frequently in adults than children (27.1% versus 14.0%, $P = 0.003$). In about 10% of the examined patients mixed genotypes, characterized by the presence of both alleles s and/or m, were detected, a similar percentage in children and adults.

Analysis of the sensitivity of *H. pylori* strains to metronidazole indicated a higher number of resistant strains in adults (41.7%) than in children (27.4%) ($P = 0.0028$). However, more strains resistant to clarithromycin were isolated in children (an average of 20.2%) than in adults (15.4%); however, the results were not statistically significant (Table 6). There was also a large regional variation in the resistance of *H. pylori* strains: resistance to metronidazole in children was from 16% to 43%, in

adults from 27% to 52%; resistance to clarithromycin in children was between 9% and 26%, in adults between 3% and 27%. Moreover, instances of high double resistance to metronidazole and clarithromycin, which was found in 9.9% (between 0% and 16%) of children and in 8.4% of adults (from 3% to 11%) were observed.

All the examined *H. pylori* strains isolated from children and adults were sensitive to tetracycline and amoxicillin. It is important to point out that the activity of amoxicillin towards the examined strains was very high. Ninety percent of the strains in both children and adults were inhibited at value MIC₉₀ = 0.016 mg/l. Tetracycline was also very active against the examined *H. pylori* strains (MIC₉₀ = 0.094 mg/l).

DISCUSSION

Our study demonstrated the difference in *H. pylori* seropositivity in relation to the age of the patients. A high percentage of seropositivity in children in their first three years of life, lower in the case of children aged from 4 to 6 years of age and increasingly more cases in older age groups were observed. As it has already been determined in our previous work, the high percentage of infected patients is connected with poor socioeconomic conditions (2). This has been confirmed by other studies (17-20). However, the decreased amount of *H. pylori* seropositivity in the age from 4 to 6 years could be associated with the possibility of spontaneous elimination of the bacteria. Baldassarre *et al.* (21) have conducted research on the *H. pylori* antigen in stool samples taken from 172 children aged 1, 6, 12 and 18 months. The authors have determined infections in 5 children in the first year of life, however, they have not determined any *H. pylori* infection in the 18th month of life. According to the authors this points to the possibility of spontaneous elimination of the bacteria. According to Duque *et al.* (22) in 4.7% per year of children infected with *H. pylori* the disease is spontaneously eliminated.

The analysis of symptoms from the upper gastrointestinal tract in seropositive patients revealed differences in their occurrence in children and adults. In seropositive children compared to children without the seropositivity, no difference in the frequency of nausea, vomiting, epigastric discomfort, heartburn or stomach aches was reported. Heartburn, regurgitation and stomach aches were statistically more frequent in *H. pylori* seropositive adults. The assessment of the dependence of gastric or duodenal ulcer on the *H. pylori* infection demonstrated a statistically significant dependence only in the case of duodenal ulcers both in children and in adults. This relation was not observed in the case of gastric ulcer. Dore *et al.* (23) in their analysis of the occurrence of symptoms in *H. pylori* seropositive schoolchildren have reported the connection between the infection and nausea and vomiting but they have not reported any association between the infection and abdominal pain or heartburn. Kalach *et al.* (24), in a multicenter European research, have reported the low frequency of *H. pylori* infection in children with ulcer and/or gastric and/or duodenum. From 56 of the examined children with endoscopic changes in the form of erosions and/or ulcers, *H. pylori* infection was reported only in 15 children (27%). *H. pylori* infection appeared more frequently in children with duodenal erosions or duodenal ulcer than with gastric ulcer. In our endoscopic research, the gastric ulcer or duodenal ulcer was reported only in 5.1% of adults and 1.7% of children with *H. pylori* seropositivity.

The histopathological examination of specimens of the mucous membrane revealed that in the majority of patients a chronic active inflammatory process was present. Atrophic gastritis and intestinal metaplasia were seen more often in adults

than in children. In individual cases, dysplasia in the pylorus was observed. It should be mentioned that in stomach's inflamed gastric mucosa the virulence factors of *H. pylori* and the stomach's acidity disrupt the balance between the processes of apoptosis and proliferation, leading to erosions, ulcers and also to intestinal metaplasia, dysplasia and finally to the development of cancer (4-9, 25).

In isolated *H. pylori* strains the frequency of *cagA* gene was assessed, which is the marker of the presence of the so-called pathogenicity island (PAI) and allele *vacA* gene responsible for synthesis of vacuolating toxin. *CagA(+)*s1m1 strain is considered to be the most pathogenic, whereas the *cagA(-)*s2m2 strain is the least pathogenic. In our studies *cagA* gene was reported in 70.7% of all examined strains. The frequency of the *cagA* gene was higher in children (77.0%) compared to adults (63.8%). The analysis of *H. pylori* genotype has demonstrated that *H. pylori* *cagA(+)*s1m2 and *cagA(+)*s1m1 strains occurred most frequently. It was determined that the occurrence of *cagA(+)*s1m1 was statistically higher in children than in adults; it was also found that there were more frequent occurrences of *cagA(-)*s2m2 in adults. A significant difference in the percentage of *cagA(+)* strains in particular studied regions of Poland has also been observed and ranged from 40.6% to 90.9%. This difference may be associated with the clonal expansion of *H. pylori* in the environment. In 10% of the examined patients mixed genotypes were reported, which most probably reflected the infection with more than one *H. pylori* strain. It should be noted that *cagA(+)*s1m1 genotypes give rise to an increased risk of pre-cancer changes and DNA damage in stomach epithelial cells, and the increase is even higher if the infection with these strains takes place in the childhood (26). Gonzales *et al.* (26) analyzed the progression of pre-cancer changes in stomach in 312 patients. The average observation time amounted to 12.8 years of age. These authors have reported the progression of pre-cancer changes in the stomach in patients infected with *H. pylori* *cagA(+)*s1m1 strains compared to patients infected with *cagA(-)*s2m2 strains. According to the authors, genotyping of *H. pylori* strains may be useful in identifying patients with a high risk of developing gastric cancer that would require intensive monitoring. De Sablet *et al.* (27) examined the phylogenetic origin of *H. pylori* strains, which are the cause of the increased risk of gastric cancer. The authors have examined 64 patients infected with *cagA(+)* *vacA* s1m1 strains from the Pacific coast and the Andes in Colombia. All strains in the populations with high risk of gastric cancer were confirmed to be in 34% of European or in 66% of African origin. The strains of European origin have strongly forecasted an increased risk of malignant histological pre-cancer changes and DNA damage, even in the low-risk populations. The strains of African origin have been associated with a reduced risk of these changes.

Krzysiek-Maczka *et al.* (8) in their very interesting experimental work on rats studied the role of fibroblasts in pathogenesis of inflammation and carcinogenesis caused by *H. pylori*. Cultures of isolated fibroblasts were infected by *cagA(+)* and *vacA(+)* *H. pylori* strains and the process of their differentiation into myofibroblasts was studied. The authors observed two processes occurring after *H. pylori* infection: increased hypoxia inducible factor-1 α , early carcinogenic marker, and decrease in apoptotic marker Bax which could indicate an inhibition of apoptosis, amplified inflammatory process and carcinogenesis from one side, but also an increased expression of heat shock protein HSP70 mRNA which could represent the protective mechanism from other side.

Wiese *et al.* (28) studied the influence of *H. pylori* *cagA(+)* and *Lactobacillus plantarum* on leukocytes in whole blood culture and the possible role of *Lactobacillus plantarum* restoring immune function of mucosa during infection with *H.*

pylori. The authors observed that only *Lactobacillus plantarum* but not *H. pylori* increased the level of antibacterial cytokine INF- γ in culture. In contrast, *H. pylori* both alone and in combination with *Lactobacillus plantarum* influenced strongly the production of anti-inflammatory interleukin-10, which could be the possible mechanism of the pathogen escaping the immune system of the host.

The study demonstrated a high rate of resistance of *H. pylori* strain to metronidazole, especially in the adult group of patients as well as an increased resistance to clarithromycin in children in comparison to adults. It is noteworthy, that there are large regional variations regarding the resistance of *H. pylori* strains to the examined antibiotics, which certainly is dependent on how frequently they are administered in the treatment of other diseases.

Moreover, we have determined a high dual resistance of the strains to metronidazole and clarithromycin. This high resistance of *H. pylori* is significant in eradicating *H. pylori*. As indicated by Megraud (29) and Koletzko *et al.* (30), the resistance to metronidazole in Europe and the USA varies from 20% to 40% and from 5% to 28% to clarithromycin, with a rising tendency. The most recent studies conducted by Megraud *et al.* (31) indicated an increase in the resistance to clarithromycin as dependent on the administration of macrolides. Studies on resistance have shown significant differences in the frequency of resistance of *H. pylori* in various regions of the world and European countries. Koletzko *et al.* (30) researched the resistance to clarithromycin in 14 European countries. They have been able to determine that resistance to clarithromycin amounted to an average of 24%, in Western European countries it was from 8% to 15%, depending on the region. Gosciniak *et al.* (32, 33) showed that in the Lower Silesian region there is an increasing resistance to clarithromycin in children during the last decade. Between 1997–2001 the primary resistance to clarithromycin was 8.6%, and to metronidazole 35%. However, between 2007–2008 the resistance to clarithromycin was 24%, and to metronidazole 32%. The research conducted by Dierzanowska-Fangrat *et al.* (34) has also indicated that between 2001–2004 the resistance to clarithromycin in children was 28% and in adults 15%. Simultaneous resistance to clarithromycin and metronidazole was high and amounted to 20%. Our results confirmed the earlier reports on significant *H. pylori* resistance to clarithromycin and metronidazole. In our research we did not find *H. pylori* resistance to amoxicillin and tetracycline. That is why the appropriate choice of drugs to eradicate is important depending on the *H. pylori* resistance in respective regions. It is necessary to determinate the resistance of *H. pylori* before the following eradication treatment after the failure of the first therapy, especially in the group of high risk patients. In regions where clarithromycin resistance exceeds 15–20%, this antibiotic should not be used to eradicate the first-line *H. pylori*, without having checked sensitivity to this antibiotic (35, 36).

In our study we concluded that it is necessary to emphasize the existing differences between the course of *H. pylori* infection in children and adults. The authors have reported a significant percentage of seropositive *H. pylori* in the first three years of life and beginning from the 6th year of life with a gradual increase of infection with age. In infected children no statistically more frequent presence of symptoms such as nausea, vomiting, epigastric discomfort, heartburn, stomach aches, gastric ulcers was reported. Dependence of duodenal ulcers on *H. pylori* seropositivity was also noted. However in adults, there was a statistically significant correlation between *H. pylori* seropositivity and the occurrence of heartburn, stomach aches, and recent duodenal ulcer. In the histopathological examination of stomach specimens in children a chronic inflammation prevailed, whereas atrophic gastritis and intestinal metaplasia

was common in adults. The authors reported differences in genotype distribution and the resistance to antibiotics. CagA(+)*s1m1* strains were statistically more frequently reported in children, whereas cagA(-)*s2m2* strains in adults. The authors reported significant differentiation of cagA(+) rate in particular regions of Poland. Resistance to metronidazole and clarithromycin was reported, as well as the resistance to both aforementioned drugs. Metronidazole resistance was statistically significantly lower in children than in adults. Amoxicillin and tetracycline resistance were not proven. Due to the high resistance of *H. pylori* to drugs used in its eradication, it is advised to determine the sensitivity of *H. pylori* after the failure of the first medication/therapy. Proven differences in the course of *H. pylori* infections in children and adults should be considered in individual therapy and in planning actions to eliminate infection in a given population. It is also noteworthy that early elimination of infection in children may prevent carcinogenesis in adults.

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