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Assessment of Sequential and Standard Triple Therapy in Treatment of *Helicobacter pylori* Infection in Children Dependent on Bacteria Sensitivity to Antibiotics*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. In the last decade a 10-day schema of sequential therapy of *Helicobacter pylori* infection based on proton pomp inhibitor (PPI), amoxicillin (AMO), clarithromycin (CLA) and metronidazole (MET) has been introduced. Many studies have emphasized greater efficacy of this therapy in comparison to the efficacy of the standard 7-day triple therapy (PPI + AMO + CLA or MET).
Objectives. The aim of the study was to assess the sequential and standard triple therapy.
Material and Methods. Sixty-nine children, aged 5 to 17 years, with symptoms of dyspepsia and gastric or duodenal ulcer were included in the study. The children were randomly divided into three groups. Group I – 23 children treated with PPI + AMO + CLA, group II – 23 children treated with PPI + AMO + MET, and group III – 23 children treated with sequential therapy. The diagnosis of *Helicobacter pylori* infection was based on histopathological evaluation of gastric mucosa sample and on culture. The sensitivity of bacterial strains to antibiotics was assessed based on E-tests. The efficacy of *Helicobacter pylori* eradication was assessed 6–8 weeks after the completion of the treatment.
Results. In children infected with *Helicobacter pylori* strains, which were sensitive to clarithromycin, the highest rate of eradication was obtained in the group treated with PPI + AMO + CLA (100%) and in the group treated with sequential therapy (90.48%), the lowest was in the group treated with PPI + AMO + MET.
Conclusions. Efficiency of treatment of *Helicobacter pylori* infection in children depended on sensitivity of the strains to clarithromycin. Sensitivity to metronidazole did not influence significantly the eradication rate (**Adv Clin Exp Med 2016, 25, 4, 701–708**).

Key words: *Helicobacter pylori,* resistance, sequential therapy, standard triple therapy.

Helicobacter pylori (H. pylori) is a major cause of gastritis, peptic ulcer disease and a risk factor of gastric cancer and mucosa-associated lymphoid tissue lymphoma [1]. The objective of treatment regimens recommended by scientific associations is to reach the highest eradication rate by means of introducing new drugs, prolonging treatment periods and using antibiotics fitted to the sensitivity of species [2–5]. Graham et al. assumed rates of intention-to-treat of the therapy for *H. pylori* infection would be acceptable greater than 85%, good at 90-95%, and excellent above 95% [6]. Numerous reports have demonstrated that the most frequently used 7-day triple therapy, including proton pump inhibitor (PPI), amoxicillin (AMO) and clarithromycin (CLA) or metronidazole (MET),

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reaches 70–80% eradication rate both in adults and children [7]. Higher eradication rates were achieved after a 10-day sequential therapy or concomitant quadruple therapy containing PPI, AMO, CLA and MET [7–12]. Sequential therapy consists of initial 5-day therapy with PPI and AMO, followed by 5 days of PPI, CLA and MET. According to some authors, initial therapy decreases the density of bacteria, damages bacterial wall and, in consequence, facilitates the influx of CLA into bacterial cell and more effective eradication with smaller side effects [10, 13]. In many reports attention is drawn to the fact that efficacy of *H. pylori* infection eradication depends mainly on the sensitivity of the species to clarithromycin and to a smaller degree on the sensitivity to metronidazole and patient compliance [7, 14–18].

In the last decade, an increase in primary and secondary resistance of *H. pylori* to clarithromycin and metronidazole and a decrease in the eradication rate have been observed. Resistance of *H. pylori* to the used drugs is different in various regions of the country and worldwide [17–19]. In Poland, primary resistance to clarithromycin in some regions surpassed 20% and to metronidazole – 40% [17]. By contrast, resistance to amoxicillin has been not observed.

The objective of the present work was to assess the efficacy of 10-day sequential therapy as a first line therapy and 7-day standard therapy consisting of either PPI, AMO and CLA or PPI, AMO and MET. In this assessment eradication efficacy of sensitivity of *H. pylori* to clarithromycin and metronidazole was taken into consideration.

Material and Methods

The study was designed as a prospective and was conducted during the years 2009–2011. All children with *H. pylori* infection were randomly assigned to one of three groups with various schemes of treatment. Group I was treated for 7 days with omeprazole, amoxicillin and clarithromycin, group II with omeprazole, amoxicillin and metronidazole also for 7 days, and group III with omeprazole and amoxicillin for 5 days followed by 5 days the treatment with omeprazole, clarithromycin and metronidazole. The physician responsible for the treatment did not know in which group a child was included. Doses and scheme of the sequential treatment is presented in Table 1. Only those children who had not been eradicated earlier were included into the study. The children who had been reciving antibiotics or PPI for 4 weeks before the beginning of the study, and those in whom hypersensitivity to antibiotics was observed were excluded from the study. Sixty-nine children, aged 5 to 17 years, admitted to and treated in our clinic due to the symptoms of dyspepsia or due to gastric and/or duodenal ulcer were included into the study. In all the children the upper gastrointestinal endoscopy was performed. During the endoscopy, biopsies were taken both from the gastric antrum and corpus for histopatological analysis according to the updated Sydney classification [20]. Biopsy samples from the stomach were used for bacteriological culture and antimicrobial susceptibility test using epsilometer (E test) [19]. The diagnosis was based on histology and culture of the biopsy specimens. The specimens for direct microscopic examinations were stained using Gram staining method. Bacterial culture was conducted on medium containing Columbia agar with 7% hemolyzed horse blood. The plates were incubated at 37°C in microaerophylic atmosphere (5% O_2, 10% CO_2, 85% N_2) for 6 days. The susceptibility of *H. pylori* to metronidazole (MET), clarithromycin (CLA) and amoxycilin (AMO) was determined by the E-test (AB-Biodisk, Sweden) on Mueller-Hinton agar (Oxoid Ltd, UK) supplemented with 10% horse blood. The plates were incubated at 35°C under microaerophilic and were read after 72h. Minimal inhibitory concentration break points for resistance were defined as follows: MET $\geq$ 8 mg/L, CLA $\geq$ 1 mg/L, AMO $\geq$ 0.5 mg/L. *H. pylori* eradication was assessed with respect to the culture of biopsy specimens sampled from the

Table 1. Sequential therapy and dosage of the medicament used in children

Days of the treatment	Medicament	Dosage
1–5	PPI-omeprazole	1 mg/kg of body weight per 24 h, max 20 mg/24 h twice daily
	amoxicillin	50 mg/kg of body weight per 24 h, max 1000 mg/24 h twice daily
6–10	PPI-omeprazole	1 mg/kg of body weight per 24 h, max 20 mg/24 h twice daily
	clarithromycin	15 mg/kg of body weight per 24 h, max 500 mg/24 h twice daily
	metronidazole	20 mg/kg of body weight per 24 h, max 500 mg/24 h twice daily

PPI – proton pomp inhibitor.

stomach 6–8 weeks after completion of the treatment. Adverse events and compliance with the treatment were assessed basing on interview and a specific questionnaire.

The differences between eradication rates were on analyzed by the χ^2 test. The eradication rates with their 95% confidence intervals (CI) and odds ratios (OR) were calculated, p-value < 0.05 was considered to be significant (STATISTICA PL10).

Approval of Regional Bioethics Committee for the study was obtained.

Results

Table 2 presents the data of the patients and endoscopic diagnosis. No statistically significant differences in sex, age and chronic diseases between groups were demonstrated. Significantly more frequent erosive esophagitis in children from group I ($p < 0.036$) and more frequent granulosis of the stomach mucosa in group III ($p < 0.036$) in comparison to group II were observed. There was no statistical difference between the studied groups in erosions or ulceration of the stomach or duodenum.

In Table 3 susceptibility of *H. pylori* to the used drugs: clarithromycin (CLA), amoxicillin (AMO) and metronidazole (MET) and the number of resistant strains too particular antibiotics in all groups are presented. Significant differences in *H. pylori* susceptibility between groups were demonstrated. The number of *H. pylori* strains was too small to make comparisons.

In Table 4 the results of eradication with regard to *H. pylori* strains susceptibility are presented. The rate of *H. pylori* eradication was the highest in sequential therapy and amounted to 91.3% and was higher than in triple therapy (78.2%), however the difference was not statistically significant. In the assessment of eradication efficacy dependent on susceptibility, the highest rate of eradication was observed in groups of strains susceptible to clarithromycin and treated with this antibiotic (group I – 100%, group III – 90.5%) and also susceptible to clarithromycin and metronidazole (group I – 100%, group III – 94.1%). In the case of strains susceptible to metronidazole the best result was obtained in group III, where eradication rate of *H. pylori* was 94.7% while in group II – only 78.9%.

The strains resistant to clarithromycine treated with O + AMO + CLA (group I) were not eradicated contrary to the *H. pylori* strains resistant to clarithromycin from group III where out of two resistant strains two were eradicated (100%). One may speculate that eradication activity should be ascribed to amoxicillin and to metronidazole. In the case of strains resistant to metronidazole in scheme with metronidazole, efficacy of eradication was only 75%.

In Tables 5, 6, 7 the comparisons of *H. pylori* eradication results depending on susceptibility of particular strains between all groups are present-

Table 2. Clinical and endoscopic patients' characteristics

Parameter	Therapy			p-value		
	O + AMO + CLA (7 days)	O + AMO + MET (7 days)	Sequential therapy (10 days)			
Group	I	II	III	I/II	I/III	II/III
No. patients	23	23	23	–	–	–
Median age Range	12.1 5–17	10.2 5–15	12.4 5–17	ns.	ns.	ns.
Gender: female male	 9 14	 11 12	 15 8	ns.	ns.	ns.
No. chronic disease, n(%)	10 (43.4)	8 (34.7)	4 (17.4)	ns.	ns.	ns.
Erosive esophagitis, n (%)	4 (17.4)	0	2 (8.7)	0.036	ns.	ns.
Nodular gastritis, n (%)	21 (91.3)	19 (82.6)	23 (100)	ns.	ns.	0.036
Gastritis erosions or ulcer, n (%)	9 (39.1)	7 (30.4)	4 (17.4)	ns.	ns.	ns.
Duodenal erosions or ulcer, n (%)	6 (26.1)	5 (21.7)	5 (21.7)	ns.	ns.	ns.

n – number; O – omeprazole; AMO – amoxicilline; CLA – clarithromycin; MET – metronidazole; ns. – not significant.

Table 3. Microbiological patients characteristics

***H. pylori* strains**	**O + AMO + CLA (23) group I**	**O + AMO + MET (23) group II**	**Sequential terapy (23) group III**	**Statistic significance (p)**		
				I/II	**I/III**	**II/III**
H. pylori strains susceptible to CLA, n (%)	18 (78.3)	20 (86.9)	21(91.3)	ns.	ns.	ns.
H. pylori strains susceptible to MET, n (%)	18 (78.3)	19 (82.6)	19 (82.6)	ns.	ns.	ns.
H. pylori strains susceptible to AMO, n (%)	23 (100)	23 (100)	23 (100)	ns.	ns.	ns.
H. pylori strains susceptible to both CLA and MET, n (%)	14 (60.8)	16 (69.5)	17 (73.9)	ns.	ns.	ns.
CLA resistant strains, n (%)	4 (17.39)	3 (13.04)	2 (8.70)	ND	ND	ND
MET resistant strains, n (%)	4 (17.39)	4 (17.39)	4 (17.39)	ND	ND	ND
AMO resistant strains, n (%)	0	0	0	–	–	–
CLA and MET resistant strains, n (%)	1 (4.35)	0	0	–	–	–

ns. – not significant; ND – not done.

Table 4. Treatment eradication rates according to triple and sequential therapy to depending on antimicrobial susceptibility

H. pylori	**O + AMO + CLA (23)**		**O + AMO + MET (23)**		**Sequential therapy (23)**		**Statistic significance (p)**		
	n	**%**	**n**	**%**	**n**	**%**	**I/II**	**I/III**	**II/III**
Successful rates of eradication	18/23	78.2	18/23	78.2	21/23	91.3	ns.	ns.	ns.
H. pylori strains susceptible to CLA	18/18	100.0	15/20	75.0	19/21	90.5	0.023	ns.	ns.
H. pylori strains susceptible to MET	14/18	77.7	15/19	78.9	18/19	94.7	ns.	ns.	ns.
H. pylori strains susceptible to both CLA and MET	14/14	100.0	12/16	75.0	16/17	94.1	0.044	ns.	ns.
CLA resistant strains	0/4	0	3/3	100.0	2/2	100.0	ND	ND	ND
MET resistant strains	4/4	100.0	3/4	75.0	3/4	75.0	ND	ND	ND
CLA and MET resistant strains	0/1	0	0	0	0	0	–	–	–

n – number; ns. – not significant; ND – not done.

ed, as well the calculation of odds ration (OR), 95%-confidence intervals (CI) and significance of the difference (p).

In group I (Table 5), in the case of clarithromycin-susceptible strains a 100% eradication rate was obtained in the O + AMO + CLA treatment scheme. In group II, *H. pylori* eradication rate of metronidazole-susceptible strains in the treatment with O + AMO + MET was 78.9%. Similar rate (75.0%) was obtained in the case of susceptibility of strains to CLA and MET. Strains resistant to metronidazole were eradicated in 75% in O + AMO + MET scheme, in 100% in O + AMO + CLA scheme.

In Table 6 the efficacy of eradication of strains treated with O + AMO + CLA and sequential therapy are compared. The obtained eradication rates were high and amounted to 100.0% vs. 90.4% and in the case of strains sensitive to clarithromycin and metronidazole – 100.0% and 94.1%, respectively.

In Table 7 the results of eradication of *H. pylori* strains with O + AMO + MET scheme and sequential therapy are compared. In children infected with strains susceptible to clarithromycin or metronidazole the best results were obtained in sequential therapy. Eradication of *H. pylori* strains resistant to metronidazole was 75%.

Table 5. Comparison the treatment eradication rates of triple (O + AMO + CLA and O + AMO + MET) to depending on antimicrobiol susceptibility

H. pylori	**Triple therapy**				**OR**	**95% CI**	**p**
	O + AMO + CLA (7 days)		**O + AMO + MET (7 days)**				
	n	**%**	**n**	**%**			
H. pylori strains susceptible to CLA	18/18	100.0	15/20	75.0	0.07	0.003–1.49	0.02
H. pylori strains susceptible to MET	14/18	77.7	15/19	78.9	1.07	0.22–5.13	ns.
H. pylori strains susceptible to both CLA and MET	14/14	100.0	12/16	75.0	0.13	0.006–2.55	0.04
CLA resistant strains	0/4	0	2/2	100.0	–	–	–
MET resistant strains	4/4	100.0	3/4	75.0	–	–	–
CLA and MET resistant strains	0/1	0	0	0	–	–	–

Table 6. Comparison of the treatment eradication rates of triple therapy (O+AMO+CLA) and of sequential therapy

H. pylori	**Therapy**				**OR**	**95% CI**	**p**
	O + AMO + CLA (7 days)		**sequential therapy (10 days)**				
	n	**%**	**n**	**%**			
H. pylori strains susceptible to CLA	18/18	100.0	19/21	90.4	0.21	0.009–4.69	ns.
H. pylori strains susceptible to MET	14/18	77.7	18/19	94.7	5.14	0.52–51.3	ns.
H. pylori strains susceptible to both CLA and MET	14/14	100.0	16/17	94.1	0.38	0.014–10.06	ns.
CLA resistant strains	0/4	0	2/2	100.0	–	–	–
MET resistant strains	4/4	100.0	3/4	75.0	–	–	–
CLA and MET resistant strains	0/1	0	0	0	–	–	–

Table 7. Comparison of the treatment eradication rates of triple therapy (O + AMO + MET) and of sequential therapy

H. pylori	**Therapy**				**OR**	**95% CI**	**p**
	O + AMO + MET (7 days)		**sequential therapy (10 days)**				
	n	**%**	**n**	**%**			
H. pylori strains susceptible to CLA	15/20	75.00	19/21	90.48	3.17	0.54–18.7	ns.
H. pylori strains susceptible to MET	15/19	78.95	18/19	94.74	4.8	0.48–47.7	ns.
H. pylori strains susceptible to both CLA and MET	12/16	75.00	16/17	94.12	5.33	0.53–54.0	ns.
CLA resistant strains	3/3	100.0	2/2	100.0	–	–	–
MET resistant strains	3/4	75.0	3/4	75.0	–	–	–
CLA and MET resistant strains	0	0	0	0	–	–	–

Frequency of adverse events was similar in treated children in particular groups. Incompliance was not reported.

Discussion

The aim of *H. pylori* infection treatment is to obtain the eradication rate as high as possible. As demonstrated in meta-analysis of Graham and Shiotaui the standard therapy with PPI plus amoxicillin and clarithromycin is unsatisfactory [7]. The analysis has shown that only in Hong-Kong and Taiwan the eradication rate was higher than 80%. In the USA, Europe, Korea, China and Japan it was below 80%. Low eradication rates prompted the search of more efficient schemes of the treatment. New antibiotics and bismuth salts were introduced to the eradication of *H. pylori* and the period of treatment was prolonged to 10 and even to 14 days; a quadruple therapy was introduced [2–4, 21–24]. Among new therapies was a quadruple sequential therapy. Tong et al., in meta-analysis showed superiority of sequential therapy over the standard triple therapy conducted for 7 or 10 days [10]. Fischbach and Evans, in their meta-analysis including more than 10 000 patients demonstrated that in obtaining a high rate of *H. pylori* eradication the most important role is played by *H. pylori* resistance to clarithromycin and next to metronidazole [14]. Similar results were obtained by other authors [11, 16, 25, 26]. Wu et al. evaluated efficacy of sequential therapy and concomitant treatment with four drugs for 10 days [11]. They demonstrated that sequential therapy and concomitant therapy with PPI, amoxicillin, clarithromycin and imidazole agent are equally effective and safe for eradication of *H. pylori* infection. According to these authors, resistance to clarithromycin, compliance and adverse events reduced the level of eradication [11]. Graham and Shotaui conducted a meta-analysis of efficacy of sequential therapy and concomitant administration of the same drugs for 3 to 7 days [7]. Intention-to-treat analysis was similar: 93.4% vs. 91.7%. Schwarzer et al. assessing sequential therapy in 160 children demonstrated that eradication rate depended on susceptibility of *H. pylori* strains to clarithromycin and metronidazole and was greater than 91% if the strains were susceptible [16]. Also Bontems et al., comparing sequential therapy and triple therapy, demonstrated that eradication efficiency depends on the susceptibility of the strains to clarithromycin. According to many authors, sequential treatment can be used as a first-line therapy, but only in areas with a low CLA resistance [26].

Our results are parallel to those of other authors. In our analysis we demonstrated that the result of eradication depended on strains susceptibility to clarithromycine. If the strains were susceptible, the scheme consisting of O + AMO + CLA yielded a 100% efficacy of eradication. Such a dependence was not observed in the case of metronidazole. In the cases of *H. pylori* strains resistant to clarithromycin the scheme O + AMO + CLA was ineffective, which is very important in a case of increasing resistance to clarithromycin. Multicenter studies conducted in Poland demonstrated high regional variation of antibiotic resistance of *H. pylori* strains [17]. In children the rate of clarithromycin-resistant *H. pylori* strains was 9 to 26% and in adults from 3 to 27%. As for of metronidazole, treatment resistance in children was 16–43% and in adults 27–52%. High resistance to both antibiotics, in children 10% (0–16%) and in adults 3 to 11% is disturbing [17]. In the studied patients no resistance to amoxicillin was observed. Similar resistance of *H. pylori* strains was demonstrated by Koletzko et al. in other European countries [20]. Gościniak et al. demonstrated an increasing primary *H. pylori* resistance in children in Lower Silesia [28, 29]. In the years 1997–2000 resistance of *H. pylori* to clarithromycin was 5.7% and to metronidazole 30.4%. During the years 2007–2008 primary resistance to clarithromycin increased to 24% and to metronidazole to 32%. Also a secondary resistance increase was observed. For example, in the years 1997–2000 resistance to clarithromycin increased from 5.7% to 15.7% and to metronidazole from 30.4% to 51%. Recent studies conducted in the years 2009–2011 demonstrated the resistance to clarithromycin – 24%, to metronidazole – 68% and to both drugs – 20% in adults [30]. Hunt et al., analyzing resistance of *H. pylori* strains worldwide, demonstrated a strong variation of clarithromycin-resistance from 6.6% in Taiwan to 45.3% in Greece. In Taiwan and Korea resistance to amoxicillin was noted [27]. According to the recommendation of European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), in a population with a high resistance of *H. pylori* to clarithromycin, i.e., higher than 20%, the use of clarithromycin should be limited in first-line therapy or the resistance of the strains should be determined and antibiotics should be applied according to the susceptibility of *H. pylori* [3]. Also, quadruple therapy consisting of PPI, clarithromycin, amoxicillin and metronidazole should be considered, limiting the duration of the treatment to 5–7 days [11, 13]. In the case of double resistance of *H. pylori* strains to clarithromycin and metroni-

dazole, Schwarzer et al. applied a 14 days course of high-dose of amoxycillin, metronidazole and PPI, which resulted in the eradication rate of 73% [21]. In those cases other authors recommend the use of other antibiotics and bismuth salts [22–24, 32, 33].

In the summary it should be stated that, based on our results of the treatment of children with *H. pylori* infection, the selection of antibiotics for eradication should take into consideration various aspects, including the susceptibility to the used antibiotics. In regions of high resistance of *H. pylori* to clarithromycin, the efficacy of the standard triple therapy is low. If the resistance of the *H. pylori* is unknown, any prior use of antibiotics in patients within proceeding 4-6 weeks should be analyzed. Based on the analysis, the resistance of the strains should be suspected. In regions with a high resistance of *H. pylori* to clarithromycin, i.e., greater than 20%, quadruple therapy should be recommended or a therapy dependent on the susceptibility of *H. pylori* strains to antibiotics. However, in regions with low resistance to clarithromycin, the O + AMO + CLA treatment may be applied as a first-line therapy of *H. pylori* infection.

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