Research Article

Risk factors for the preservation of morphological changes in the gastric mucosa after eradication therapy for *Helicobacter pylori* infection in children with chronic gastritis

Margarita M. Gurova[1,2]*, Tatyana A. Romanova[1], Valeria P. Novikova[2], Valentina S. Popova[1]

ABSTRACT

We conducted a clinical, morphofunctional examination and standard treatment of 155 children with chronic gastroduodenitis associated with *Helicobacter pylori* (HP) infection. Subsequently, 100 children were examined after 3 and 6 months of proven HP eradication. It was revealed that after 6 months lymphoplasmacytic infiltration of the gastric mucosa (GM) preserved in 37% of cases, and unspecified atrophy - in 9%, increased C_3 and C_4 components of the compliment - in 19% of children, antroduodenal discoordination on the background of postprandial secretin and cholecystokinin level - in 25%, as well as disorders of the intestinal microbiota. Factors that have a prognostic effect on the preservation of inflammatory changes in the GM according to discriminant analysis include the presence of the pathogenicity factor HP (CagA+), changes in the intestinal microbiota, impaired motor function of the stomach, changes in the immune system (increased C_3 and C_4 components of the compliment), a vegetative dysregulation, and the presence of comorbid pathology (more than three associated diseases).

KEY WORDS: Children, Chronic gastroduodenitis, *Helicobacter pylori* infection, Morphological changes, Gastric motility, Microbiota

INTRODUCTION

Pathology of the digestive system is one of the main causes of the deterioration of health in children and adolescents. Along with the steady increase in the incidence of chronic gastroduodenitis (CGD), exacerbation of the course of the pathological process is noted - the proportion of erosive, subatrophic, and atrophic forms increases by 2.5 times,[1] Lazarev and Gordeeva.[2] The above changes are due to the high incidence of helicobacteriosis (HP) in childhood, reaching, according to data of various authors, 50–80%,[3] Baingana *et al.*[4] and Lee *et al.*,[5] and the increased resistance of HP to the therapy,[6] Ivashkin *et al.*[7], Megraud *et al.*[8], and Dekhnich *et al.*[9] Given that in 60–80% of adult patients with CGD, the formation of the inflammatory process begins in childhood, the progression of the disease with subsequent atrophy increases the risk of dysplasia and metaplasia underlying carcinogenesis,[10] Koshimbetova.[11] One of the unresolved and insufficiently studied questions remains the management of patients with CGD in remission after successful HP eradication. The continuing changes in the regulatory systems of the body, underlying impairments of the motor function of the upper gastrointestinal tract (GIT), immune system, and changes in the intestinal microbiota can contribute not only to the recurrence of the disease but also to the development of comorbid conditions and metabolic disorders.[12]

The relevance of this study is determined by the need to study the clinical and morphofunctional characteristics of the state of the upper digestive tract in children with CGD in remission after eradication of *Helicobacter pylori* (HP) infection.

Objective

The objective of this study was to identify the risk factors for the development of persistent morphological changes in the gastric mucosa (GM) on the basis of studying the characteristics of the

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[1]Department of Pediatrics With a Course of Children's Surgical Diseases, Belgorod State University, 308015, Belgorod, Russia, [2]St. Petersburg State Pediatric Medical University, 194100, St. Petersburg, Russia

***Corresponding author:** Margarita M. Gurova, Department of Pediatrics with a Course of Children's Surgical Diseases, Belgorod State University, 308015, Belgorod, Pobeda Street, 85, Russia. E-mail: itely@mail.ru

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morphofunctional state of the upper GIT, the state of the intestinal microbiota, and the immune system in the acute phase, 3 and 6 months after eradication therapy.

MATERIALS AND METHODS

The study included 155 children aged 12–16 years (mean age 14.9 ± 2.1 years) with CGD associated with HP (38% of children were infected with toxigenic strains containing the CagA+ pathogenicity islet) being observed in the phase of exacerbation and remission (100 children): 3 and 6 months after a successful eradication. The control group consisted of 30 children of health Group 1.

Inclusion Criteria for the Main Group

1. Morphologically confirmed diagnosis of CGD associated with HP
2. Voluntary informed consent of the parents (and/or their legal representatives) of patients for inclusion in the study, the invasive examinations and processing the data obtained during the subsequent observation of the child 3 and 6 months after the conducted treatment (in accordance with the ethical standards of the Helsinki Declaration [2000] and the Order of the MH RF No. 266 of June 19, 2003).

Exclusion Criteria

The following criteria were excluded from the study:
1. Gastric and/or duodenal ulcer, the presence of other serious organic diseases of the GIT
2. Severe concomitant somatic diseases
3. Acute infectious diseases at the time of the study
4. Celiac disease and/or other diseases manifested by malabsorption syndrome (based on the negative results of the determination of AB immunoglobulins A (IgA) to transglutaminase)
5. Activation of latent viruses (cytomegalovirus, Epstein–Barr virus, type 1 and 2 herpes viruses).

In the acute phase of CGD, children underwent three-component eradication therapy for 10 days, which included a proton-pump inhibitor (Omeprazole), amoxicillin (Flemoxin Solutab), clarithromycin (Klacid), and a probiotic (live acidophilic lactobacilli 10^7 CFU and kefir fungal polysaccharide 400 μg) in standard age-specific does.

In the remission phase (after 6 months), depending on the morphological features of the GM, the children (n = 100) were divided into two subgroups: Subgroup 1 (n = 37) - with persisting inflammatory changes in the GM, subgroup 2 (n = 63) - with the absence of inflammatory changes, followed by a discriminant analysis to identify risk factors that contribute to the maintenance of the inflammatory process in the GM.

All children underwent the evaluation of their complaints, physical examination according to standard methods. In addition, routine laboratory methods were carried out: Immunological examination - determination of C_3 and C_4 complement components, interleukin (IL-1β), IL-8, IL-4, and IL-10, IG of classes M, G, and A, using the ProCon reagent kit (LLC Protein Contour, St. Petersburg) by enzyme-linked immunosorbent assay, determination of the level of gastrointestinal neuropeptides (cholecystokinin [CCK] and secretin by ELISA using a reagent kit from Peninsula Laboratories, Inc. [USA]), and diagnosis of HP with the definition of facto pathogenicity of CagA. The method of gas chromatography–mass spectrometry determining the species-specific fatty acids of the structural components of bacterial cells was used to assess the state of the parietal microbiota of the small intestine.

Instrumental Research Methods Included

Esophagogastroduodenoscopy with histological examination of the biopsy specimens obtained, intragastric topographic pH-metry, ultrasound examination of the abdominal organs, and ultrasound examination of the motor function of the stomach. Since the cross-section of the antrum of the stomach in the sagittal plane has the shape of an ellipse, its area was calculated based on the formula of the Greek ellipse - LDxAP/4, where LD is the longitudinal diameter and AP is the anteroposterior diameter. Measurements were performed on an empty stomach, immediately after taking a standard breakfast and then every 20 min for 1 h.

Statistical analysis was performed using the statistical package SSPS 13.0 for Windows. The resulting data were analyzed using descriptive statistics with the calculation of arithmetic mean (M) and standard deviation. The normal distribution was estimated using the Shapiro–Wilk criterion. The evaluation of the statistical significance of differences for data with a normal distribution was carried out using Student's t-test for dependent samples. To compare the frequency of occurrence of qualitative data in two groups, the criterion of compliance χ^2 was used, the confidence interval for the odds ratio was calculated. The results were evaluated as statistically significant at a probability level of $P < 0.05$.

RESULTS

The study of the characteristics of the endoscopic picture showed that in the acute phase of CGD, common gastritis was unreliable more often diagnosed in 58.1% of children against 41.9% with antral gastritis ($P > 0.05$), signs of impaired motility such as gastroesophageal reflux were revealed in 12.2% and 26.4%, respectively. 3 months and 6 months later, visual changes of the GM in the form of moderate hyperemia persisted in 21% and 24% of

children, $P > 0.05$ (signs of pangastritis were observed in 19% and 11% of children, respectively).

The morphological picture in the exacerbation phase was characterized by the presence of lymphoplasmacytic infiltration in the body of the stomach (74.8%), mostly slightly (43.%), and moderately (23.9%) expressed in the antrum of the stomach (100%). At the same time, morphological changes in the body of the stomach were more frequent than endoscopic manifestations (74.8% vs. 58.3%, $P = 0.0037$). Signs of vague atrophy were found in 23.2% of children (16.1% - in the antrum and fundus). 3 and 6 months later, the number of children with signs of inflammatory changes significantly decreased to 37%, while the number of patients with signs of unspecified atrophy decreased significantly only due to patients with changes in the antrum. The obtained results demonstrated that, despite the conducted eradication therapy and clinical improvement, the morphological changes in the GM in the form of moderate lymphoplasmacytic infiltration and atrophy persisted after 6 months in 37% and 9%, respectively [Table 1].

The pH metrics showed that in the acute phase in children, the normacid state prevailed (67.1%/104 children), the hyperacid state was detected in 30.3%/47 cases. 6 months after treatment, the hyperacid state persisted in 14% of children. At the same time, in the exacerbation phase, a predominantly acid-neutralizing dysfunction of the stomach was noted, which positively correlated with the acceleration of emptying the antrum of the stomach ($r_1 = 0.38$ and $r_1 = 0.44$, respectively, $P < 0.05$). In the remission phase, normalization of the acid-producing function was noted with the maintained acid-neutralizing dysfunction.

Changes in the motor-evacuation function of the stomach were characterized in the exacerbation phase by disturbances in the accommodation processes in response to food intake and accelerated emptying of the stomach in 98% of children. A positive correlation between the acceleration of gastric motility and the severity of pain after eating ($r = 0.68$, $P < 0.05$) is shown. 3 and 6 months after eradication therapy, an improvement in the accommodation of the stomach was noted (after 6 months, it was approaching the indicators of healthy children) and the rate of gastric emptying (Δ area of the antrum) comparable to that of healthy children. However, no complete restoration of consistency in the motility of the stomach and the duodenum (dimensions of the antrum after 20 and 60 min) was noted [Table 2]. When assessing the dispersion of indicators of the size of the antrum in the acute phase and in remission, we found that in 25% of children with CGD, changes in gastric motility persisted after 3 and 6 months with Δ antral area of 57.7% ± 10.5 compared with the same indicator in healthy children - 34.4% ± 6.8 ($P = 0.04$).

The revealed motor disorders were accompanied by a decreased postprandial level of gastrointestinal hormones - secretin and CCK in children with acute CGD, compared with healthy children, which positively correlated with an increase in the secretion of hydrochloric acid, acceleration of the evacuation activity of the antrum of the stomach, and the detection of hypomotor dyskinesia of the gallbladder of the antrum (GB) ($r = 0.43$, $r = 0.54$ and $r = 0.62$, respectively, $P < 0.05$). In the remission phase, the indicators of these hormones increased but did not reach the level of healthy children [Table 3]. The resulting changes on the part of secretin and CCK, which are involved in regulating the functioning of the gastroduodeno-pancreatic hepatobiliary complex,

Table 1: Characteristics of the morphological picture of the GM in children with CGD before treatment and 3 and 6 months after therapy

Morphological signs	Fundal part of the stomach			Antral part of the stomach		
	1 group *n*=155 abs. (%)	2 group *n*=100 abs. (%)	3 group *n*=100 abs. (%)	1 group *n*=155 abs. (%)	2 group *n*=100 abs. (%)	3 group *n*=100 abs. (%)
Lymphoplasmocytic infiltration						
Mild	67 (43.2)	25 (25)*	20 (20)**	31 (31)	20 (20)	21 (21)
Moderate	37 (23.9)	11 (11)*	7 (7)**	69 (44.5)	13 (13)*	9 (9)**
Severe	12 (7.7)	0	0	39 (25.2)	10 (10)*	7 (7)**
Total	116 (74.8)	36 (36)*	27 (27)**	155 (100)	43 (43)*	37 (37)**
Atrophy						
Mild	19 (12.2)	10 (10)	7 (7)	22 (14.2)	9 (9)	5 (5)**
Moderate	6 (3.9)	1 (1)	2 (2)	14 (9)	2 (2)*	2 (2)**
Severe	0	0	0	0	0	0
Total	25 (16.1)	11 (11)	9 (9)	36 (23.2)	11 (11)*	7 (7)**
Small intestine metaplasia						
Mild	0	0	0	17 (11)	3 (3)*	4 (4)**
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Total	0	0	0	17 (11)	3 (3)*	4 (4)**

*Differences between children with CGD in the exacerbation phase and 3 months after eradication therapy are significant, $P<0.05$. **Differences between children with CGD in the exacerbation phase and 6 months after eradication therapy are significant, $P<0.05$

emphasize the complex nature of changes in the paracrine regulation, affecting not only motility and secretion of the stomach but also GB and pancreas.

Evaluation of the status of indicators of the innate and adaptive immunity in children with acute CGD revealed changes characteristic of the infectious process with an increase in the blood plasma level of the components of the complement system (2 times higher C_3, 3 times higher C_4), and increased IgM, IgG, and lower IgA. Changes in the level of cytokines were characterized by a 2-fold increase in the level of IL-1β, a moderate increase in IL-10 values, tumor necrosis factor α, and a decrease in IL-8 [Table 4].

After 3 and 6 months of eradication, a tendency toward normalization of altered indices of innate and adaptive immunity was revealed. Despite the positive dynamics, in 19% of children, an elevated content of C_3 and C_4 components of the complement remained, positively correlated with the presence of the pathogenicity factor CagA HP ($r = 0.64$, $P < 0.01$) and an increase in the number of conditionally pathogenic flora (CPF), especially *Bacteroides* ($r = 0.58$ and $r = 0.49$, $P < 0.01$).

The study of microbial metabolites of the parietal microflora in children with CGD allowed us to document the reduction in the number of representatives of the symbiotic microbiota - bifidobacteria, lactobacilli, propionibacteria, eubacteria (*Eubacterium moniliforme*, *Eubacterium nodatum*, and *Eubacterium sabureum*), *Faecalibacterium prausnitzii* in excessive concentrations of representatives of CPF - clostridia (*Clostridium hystolyticum, Clostridium propionicum*,

Table 2: Dynamics of changes in motor-evacuation indicators in children with CGD 3 and 6 months after eradication therapy

US findings (M±SD)	Children with CGD, exacerbation, *n*=60	3 months after treatment, *n*=60	6 months after treatment, *n*=60	Healthy children, *n*=30
Antrum size on an empty stomach (cm^2)	8.6±2.1	8.9±1.8	9.0±1.7	9.5±1.8
Antrum size immediately after eating (cm^2)	10.0±1.3#	9.7±1.4*	10.3±1.7	10.8±2.05
Antrum size 20 min after eating (cm^2) (M±SD)	8.5±1.45#	9.11±1.3*/**	8.1±0.9**/***	9.8±1.8
Antrum size 40 min after eating (cm^2) (M±SD)	9.4±0.98#	9.67±1.3**/***	8.01±0.8*/***	8.4±1.2
Antrum size 60 min after eating (cm^2) (M±SD)	8.9±1.2#	9.3±1.4**/***	8.0±0.9*/**/***	7.0±1.4
Dynamics of changes in the antrum area (gastric emptying rate), %	48.03±6.4#	41.4±5.3	42.5±6.4	34.4±7.1

#Differences between children with acute CGD and the control group are significant, $P<0.05$. *Differences between children with acute CGD and 3 months after treatment are significant, $P<0.05$. **Differences between children with CGD 3 and 6 months after treatment and the control group are significant, $P<0.05$. ***Differences between children with CGD 3 and 6 months after treatment are significant, $P<0.05$

Table 3: The content of secretin and CCK in children with CGD in different phases of the disease

Data	Secretin, ng/ml (M±SD)		Cholecystokinin (CCK), ng/ml (M±SD)	
	Basal level	Postprandial level	Basal level	Postprandial level
CGD exacerbation p-d (*n*=30)	0.81±0.04	0.86±0.02#	0.51±0.03	0.6±0,04#
3 months after treatment (*n*=30)	0.8±0.02	0.9±0.04*	0.52±0.02	0.67±0.03*
3 months after treatment (*n*=30)	0.78±0.02**/***	0.87±0.03**/***	0.55±0.03**/***	0.63±0.02**/***
Healthy children (*n*=30)	0.79±0.03	0.91±0.04	0.54±0.02	0.68±0.03

#Differences between children with acute CGD and the control group are significant, $P<0.05$. *Differences between children with CGD in the exacerbation phase and 3 months after eradication therapy are significant, $P<0.05$. **Differences between children with CGD in the exacerbation phase and 6 months after eradication therapy are significant, $P<0.05$. ***Differences between children with CGD 3 and 6 months after the eradication therapy are significant, $P<0.05$

Table 4: The content of components of the complement system, cytokines, and immunoglobulins (IG) in the blood plasma of children with CGD during exacerbation and remission

Indicators	Exacerbation p-d of CGD, *n*=155, (M±SD)	3 months after treatment, *n*=100, (M±SD)	6 months after treatment, *n*=100, (M±SD)	Healthy children, *n*=30, (M±SD)
C_3 (g/l)	3.37±0.8#	2.8±0.7*	2.07±0.8**/***	1.46±0.5
C_4 (g/l)	0.84±0.13#	0.72±0.1	0.68±0.08**	0.25±0.1
IgM (g/l)	2.37±0.5#	1.86±0.7*	1.38±0.5**/***	1.14±0.6
IgG (g/l)	16.87±3.8#	16.62±5.3	15.1±4.6**/***	14.4±2.3
IgA (g/l)	1.36±0.2#	1.58±0.5*	1.74±0.6**/***	2.39±0.4
IgE (U/ml)	67.8±13.6#	67.06±10.3	61.3±9.7**/***	57.2±10.8
IL-1β (pg/ml)	5.20±1.2#	4.82±1.1*	4.40±1.3**	3.22±0.6
IL-8 (pg/ml)	26.23±6.5#	29.7±7.4*	36.5±6.6**/***	46.5±8.4
IL-10 (pg/ml)	8.42±1.8	8.35±2.1	8.34±1.5	8.24±2.3
TNF-α (pg/ml)	3.51±0.7#	3.45±0.6	2.99±0.8**	2.66±0.4
CIC (U/ml)	18.7±4.1#	17.68±3.8*	14.5±2.6**/***	9.77±2.4

#Differences between children with acute CGD and the control group are significant, $P<0.001$. *Differences between children with CGD in the exacerbation phase and 3 months after eradication therapy are significant, $P<0.05$. **Differences between children with CGD in the exacerbation phase and 6 months after eradication therapy are significant, $P<0.05$. ***Differences between children with CGD 3 and 6 months after the successful eradication therapy are significant, $P<0.05$

and *Clostridium ramosum*), actinomycetes, streptococci, and *Bacteroides fragilis*.

We evaluated the risk factors for the preservation of GM morphofunctional changes in children with CGD after 6 months. To this end, all the children studied were divided into two subgroups depending on the presence (subgroup 1, $n = 37$) or absence (subgroup 2, $n = 63$) of inflammatory changes in GM - the canonical correlation coefficient is 0.34, $\chi^2 = 55.34$, $P < 0.01$. Based on our discriminant analysis, we identified the following risk factors: The presence of the pathogenicity factor HP (CagA+), changes in the intestinal microbiota (deficiency of obligate microorganisms, excessive proliferation of CPF), impaired motor function of the stomach and duodenum (presence of antroduodenal dysregulation), immune changes (increased levels of C_3 and C_4 components of the compliment), impaired vegetative regulation (reduced vegetative resistance), and the presence of comorbid pathology (more than three concomitant diseases).

DISCUSSION

Thus, it was shown that after 3 and 6 months of successful eradication of HP infection and clinical improvement in CGD, the following changes remained in children: Morphological changes in GM were detected in the form of moderate lymphoplasmacytic infiltration and atrophy in 37% and 9%, respectively, incomplete normalization of the secretory function of the stomach - hyperacid state preserved in 14% of children after 6 months, signs of impaired gastric motility remained in 25% of children (decrease in accommodation, accelerated motility) comparable with the data of the acute phase, and changes in the level of gastrointestinal hormones (decrease in stimulated secretin and CCK) involved in the regulation of the functioning of the gastroduodeno-pancreatic hepatobiliary complex with impaired motility and secretion of the stomach, gallbladder, and pancreas, persisted; there were changes in the immune system - a reduced level of IgA with increasing levels of C_3 and C_4; on the part of the parietal microflora, a decrease in the number of symbiotic representatives of normal flora (bifidobacteria, lactobacteria, propionobacteria, and eubacteria) with excessive proliferation of conditionally pathogenic microflora (clostridia, streptococci, and *Bacteroides*) has been revealed.

Summary

During remission of CGD in 37% of cases, lymphoplasmacytic infiltration of GM and uncertain atrophy persisted in 9% of children. The risk factors that support GM pathological changes include the presence of the pathogenicity factor HP (CagA+), changes in the intestinal microbiota (deficiency of obligate microorganisms, excessive proliferation of CPF), impaired motor function of the stomach and duodenum (presence of antroduodenal dysregulation), immune changes (increased levels of C_3 and C_4 components of the compliment), impaired vegetative regulation (reduced vegetative resistance), and the presence of comorbid pathology (more than three concomitant diseases). The obtained data dictate the need to isolate a group of patients with CGD with risk factors for the maintaining inflammatory changes and manifestations of unspecified atrophy to further explore the possibilities of the individualized prevention programs.

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