

Case Control Study

Innate immunity – the hallmark of *Helicobacter pylori* infection in pediatric chronic gastritis

Lorena Elena Meliț, Cristina Oana Mărginean, Maria Oana Săsăran, Simona Mocan, Dana Valentina Ghiga, Alina Bogliș, Carmen Duicu

ORCID number: Lorena Elena Meliț 0000-0001-8857-2289; Cristina Oana Mărginean 0000-0003-2119-1726; Maria Oana Săsăran 0000-0001-8156-7460; Simona Mocan 0000-0001-8067-5072; Dana Valentina Ghiga 0000-0003-4185-606X; Alina Bogliș 0000-0001-9025-0959; Carmen Duicu 0000-0002-8056-1339.

Author contributions: Meliț LE, Duicu C, Mărginean CO, and Săsăran MO conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript; Bogliș A performed the genetic analyses; Mocanu S performed the histopathological exam; Ghiga DV performed the statistical analyses; All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Lorena Elena Meliț, Cristina Oana Mărginean, Carmen Duicu, Department of Pediatrics I, “George Emil Palade” University of Medicine, Pharmacy, Sciences and Technology Târgu Mureș, Târgu Mureș 540136, Romania

Maria Oana Săsăran, Department of Pediatrics III, “George Emil Palade” University of Medicine, Pharmacy, Sciences and Technology Târgu Mureș, Târgu Mureș 540136, Romania

Simona Mocan, Department of Pathology, Emergency County Hospital Târgu Mureș, Târgu Mureș 540139, Romania

Dana Valentina Ghiga, Scientific Medical Research Methodology, “George Emil Palade” University of Medicine, Pharmacy, Sciences and Technology Târgu Mureș, Târgu Mureș 540136, Romania

Alina Bogliș, Department of Genetics, Center for Advanced Medical and Pharmaceutical Research, “George Emil Palade” University of Medicine, Pharmacy, Sciences and Technology Târgu Mureș, Târgu Mureș 540136, Romania

Corresponding author: Cristina Oana Mărginean, MD, PhD, Professor, Department of Pediatrics I, “George Emil Palade” University of Medicine, Pharmacy, Sciences and Technology Târgu Mureș, Gheorghe Marinescu Street No. 38, Târgu Mureș 540136, Romania.

marginean.oana@gmail.com

Abstract

BACKGROUND

Innate immunity was found to be associated with both persistence of *Helicobacter pylori* (*H. pylori*) infection and increased risk of gastric cancer.

AIM

To identify the risk factors associated with *H. pylori* infection and to establish the role of TLR9 rs352140 in suppressing or promoting inflammation related to this infection in children.

METHODS

We performed a study of 155 children with digestive symptoms, who were divided into two groups according to the histopathological exam: Group 1 – 48 children with *H. pylori*-induced chronic gastritis, and Group 2 – control group.

17th 2016, and No. 792/March 11th 2020), being performed according to the Helsinki Declaration.

Informed consent statement: All the parents/caregivers signed the informed consent prior to their children inclusion in the study.

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RESULTS

Rural area and poor living conditions were significantly associated with *H. pylori* chronic gastritis ($P = 0.0042/P < 0.0001$). Both positive immunoglobulin A anti *H. pylori* and the rapid urease test were significantly associated with *H. pylori* infection ($P < 0.0001$). Significantly higher values of leukocytes and neutrophils within the peripheral blood were found in children with *H. pylori* chronic gastritis ($P = 0.111/P = 0.284$). We found a significant positive correlation between the variant TT genotype of TLR9 rs352140 polymorphism and both leucocytes and neutrophils ($P = 0.0225/P = 0.0292$).

CONCLUSION

Variant TT genotype carriers of the TLR9 rs352140 gene polymorphism might have a more severe degree of inflammation.

Key Words: Innate immunity; *Helicobacter pylori* infection; Children; Chronic gastritis; TLR9; Systemic inflammation

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Core Tip: Environmental factors such as poor living conditions and rural area might result in an increased risk of *Helicobacter pylori* (*H. pylori*) chronic gastritis in children. Serological and rapid urease tests could be used as reliable tests for the detection of *H. pylori* infection in children. Moreover, leukocytes and neutrophils are important non-invasive biomarkers for detecting the low-grade inflammatory status associated with this infection in pediatric patients. In terms of innate immunity, our findings emphasized that variant TT genotype carriers of the TLR9 rs352140 gene polymorphism might have a more severe degree of inflammation.

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INTRODUCTION

It is a well-documented fact that *Helicobacter pylori* (*H. pylori*) infection is most commonly acquired during early childhood and that age-related peculiarities are important for delineating the specific features of this infection in children, which are completely different than those encountered in adults. Recent epidemiological studies have proved that the trends are rapidly changing in terms of *H. pylori* prevalence, indicating a decreasing pattern among children as reported in a study performed in Chinese symptomatic children, which identified a decrease from 25.6% in 2005 to 12.8% in 2017[1]. Although abdominal pain, nausea, vomiting, anorexia, weight loss, or heartburn are commonly encountered in children with *H. pylori* infection, the clinical picture remains unspecific[2]. Recent studies have also noted several extra-digestive manifestations such as thrombocytopenia, anemia, eosinophilic esophagitis, or otitis media that might be related to the presence of *H. pylori* in children[3-5]. Nevertheless, taking into account the difficulties related to performing endoscopic studies in children and therefore the scarcity of information in this age group, a precise cause-effect relationship between these manifestations and *H. pylori* has been difficult to establish. Multiple invasive and non-invasive diagnostic tests are currently available for detecting *H. pylori* infection in children, but the gold standard remains tissue culture or concordant-positive histopathology test results and the rapid urease test[6]. Although new and improved non-invasive tests such as stool antigen have relatively good sensitivity and specificity in detecting this infection in children, current recommendations do not sustain the use of these tests for the diagnosis of *H. pylori* infection in pediatric patients[7]. European Society for Pediatric Gastroenterology Hepatology and Nutrition/North American Society For Pediatric Gastroenterology,

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Hepatology & Nutrition guidelines recommend a 2 wk regimen consisting of an association between a proton pump inhibitor and two antibiotics at high doses for the proper eradication of *H. pylori* infection in children[7]. Antimicrobial resistance is the main factor that influences the treatment success, which varies depending on the geographic area, but inadequate dose/duration, poor compliance, bacterial virulence factors, improper penetration of antibiotics at the level of gastric mucosa, antibiotic inactivation due to low gastric pH or fast metabolism of certain proton pump inhibitors might also be responsible for therapeutic failure[8].

The persistence of *H. pylori* infection into adulthood might carry a life-threatening risk since the World Health Organization considered this bacterium as a class I carcinogen[9]. *H. pylori* was proven to bind gastric epithelial cells activating the host innate immune system *via* toll-like receptors (TLRs), which subsequently trigger the secretion of pro- and anti-inflammatory cytokines, resulting in gastric atrophy, hypochlorhydria, and a high risk of carcinogenesis[10]. Gastric cancer is the fourth most common type of neoplasia and the second leading cause of death due to cancer among adults[11]. Because *H. pylori* infection occurs most frequently during early childhood, identifying the risk factors for this infection in pediatric populations might be the cornerstone for the development of further effective prevention strategies that will result in a decrease of *H. pylori*-related gastric cancer during adulthood. As already mentioned, innate immunity plays an essential role in the eradication of multiple microorganisms by stimulating the adaptive immunity *via* TLR activation and subsequent induction of inflammatory cytokines, chemokines, costimulatory and antigen-presenting molecules[12]. Multiple TLRs have been identified to date; they express specific receptors for the recognition of different bacterial components such as lipids, lipoproteins, proteins, or microbial nucleic acids[13].

TLR9 recognizes unmethylated CpG oligonucleotides, an important component of bacterial DNA[14]. These oligonucleotides are believed to be transferred to the intracellular domain *via* non-specific endocytosis since TLR9 itself is located in this compartment[15]. The complex pathway of TLR9 activation is associated with both infectious diseases and a wide spectrum of cancers[16].

The objectives of this study were to identify the risk factors associated with *H. pylori* infection and to establish the role of TLR9 rs352140 in suppressing or promoting the inflammation related to this infection in children.

MATERIALS AND METHODS

Study sample

We performed a cross-sectional prospective study on 183 children admitted to the Pediatrics Clinic 1 Târgu Mureş, Romania between March 2016 and July 2020. The children included in the study had dyspeptic symptoms (*e.g.*, abdominal and/or epigastric pain, nausea, vomiting, heartburn) suggesting gastritis, were between 1 and 18 years of age, and did not have a history of chronic disorders or recent infectious disease. All children who complained of dyspeptic symptoms were consecutively included in the study, but only those whose parents/caregivers agreed to provide signed informed consent underwent upper digestive endoscopy. The exclusion criteria were: Age below 1 year due to the characteristics of the video endoscope; clinical or laboratory signs suggestive of an infectious disorder (*e.g.*, fever, leukocytosis, positive acute phase reactants); children with *H. pylori* acute gastritis, *H. pylori*-negative gastritis, *H. pylori* infection without histopathological changes, and *H. pylori*-negative pediatric patients with pathological findings at histopathology exam; the parents'/caregivers' refusal to sign the informed consent for their children inclusion in the study, and those with incomplete information. Taking into account these criteria, we excluded 26 children from the study: 22 with *H. pylori*-negative gastritis, 2 with *H. pylori*-induced acute gastritis, and 2 due to their parents' refusal to sign the informed consent form. All the children included in this study were clinically assessed and a thorough anamnesis was provided by their parents/caregivers. Children without any history of bed sharing or living in crowded homes, with a relatively good economical level and proper hygiene conditions were considered to have good living conditions. A blood sample was taken from each child, and we assessed the following laboratory parameters: Complete cellular blood count (hemoglobin, leukocytes, lymphocytes, neutrophils, eosinophils), erythrocyte sedimentation rate, serum iron, liver enzymes (alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transpeptidase), and neutrophil/lymphocyte ratio (NLR). A single trained experienced gastroenterologist performed the upper digestive endoscopies and three biopsy series

of the gastric mucosa were obtained: The first consisted of two biopsies, one from the antrum and one from the corpus, that were used for rapid urease test; the second consisted of two biopsy specimens from the same locations, which served as a samples for the histological exam; and the third biopsy from the gastric antrum was collected in an Eppendorf tube filled with a stabilization solution for proper transportation to the Genetics Laboratory in order to rule out the need for immediate DNA isolation and purification. The histological exam was based on Giemsa staining and provided information such as the presence or absence of *H. pylori* infection, the acute or chronic inflammatory pattern, or the presence of metaplasia. All histological interpretations were also performed by a single experienced pediatric pathologist who provided the final diagnosis of chronic gastritis and *H. pylori* infection. Chronic gastritis was defined according to the Sydney classification[17] based on the inflammatory infiltrate thoroughly assessed by the pediatric pathologist (*i.e.* the presence of lymphocytes and/or plasma cells indicating the chronic pattern of inflammation). The diagnoses of gastroesophageal and biliary reflux were established based on history and endoscopic findings. *H. pylori* infection was diagnosed based on the histopathology exam with special Giemsa staining, and only *H. pylori*-positive children were included in the study group. Thus, taking into account only the result of the histopathological exam, the children were divided into two groups: Group 1 - 48 children with *H. pylori*-induced chronic gastritis, and group 2 - control group, 107 *H. pylori*-negative children without any histopathological changes.

Ethics

Our study was approved by the Ethics Committee of the University of Medicine, Pharmacy, Sciences and Technology 'George Emil Palade' Târgu Mureş (No. 27/March 17th 2016, and No. 792/March 11th 2020), and was conducted according to the Helsinki Declaration. All parents/caregivers provided signed informed consent prior to inclusion of their children in the study.

Genotyping analysis

We used the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, United States) for extraction of genomic DNA (gDNA) from fresh gastric tissue samples obtained by upper digestive endoscopies. gDNA obtained according to the manufacturer's protocol was quantified by using an Eppendorf BioSpectrometer (Eppendorf Austria GmbH, Wien, Austria). For genotyping of TLR9 rs352140, we used TaqMan technology on the Applied Biosystems™ 7500Fast Dx Real-Time PCR System (Applied Biosystems, Waltham, MA, United States) and a predesigned assay, namely C_2301954_20.

Statistical analyses

The statistical analyses comprised elements of descriptive statistics (frequency, percentage, mean, median, standard deviation) and elements of inferential statistics. The Shapiro-Wilk test was applied in order to assess the distribution of the analyzed series. The Student's *t*-test, analysis of variance, Mann-Whitney, Kruskal-Wallis, and Dunn's multiple comparison tests were used for comparing means and medians. The chosen significance threshold for *P* value was 0.05. Statistical analyses were performed using the trial variant of the GraphPad Prism program.

RESULTS

Demographic analyses of the sample

Our final sample consisted of 155 children who were divided into two groups according to the histopathological exam: Group 1 - 48 children with *H. pylori*-induced chronic gastritis, and group 2 - control group, 107 children without any histopathological changes. We found a similar mean age between the two groups ($P = 0.1987$), and the girls predominated in both groups without significant differences ($P = 0.7725$). In terms of the originating area, there were significant differences between the study groups, namely, 31.25% of the children came from the urban area, 68.75% from the rural area in comparison to 56.07% from the urban area, and 43.93% from the rural area in the control group ($P = 0.0042$). There were significant differences in terms of living conditions between children with *H. pylori*-induced chronic gastritis, 64.59% of cases stating to have good living conditions, and those from the control group, where 92.52% of cases declared living in good conditions ($P < 0.0001$). The following findings

were noticed in terms of TLR9 rs352140 genotypes distribution: In the study group, 43.75% were heterozygotes CT, 33.33% were homozygotes TT and 21.92% were homozygotes CC, whereas in the control group 58.88% were heterozygotes CT, 18.69% were homozygotes TT and 22.43% were homozygotes CC, without any significant differences ($P = 0.1075$). No significant differences were noticed in terms of gastroesophageal reflux and biliary reflux between the two groups ($P = 0.6657/P = 0.6095$). Both positive immunoglobulin A anti *H. pylori* and rapid urease test were significantly associated with *H. pylori* infection ($P < 0.0001$). The demographic analysis of the two groups is presented in Table 1.

In terms of the assessed hematological parameters, we noticed higher mean values in children with *H. pylori* chronic gastritis, but significant differences were only encountered for leukocytes and neutrophils ($P = 0.011/P = 0.028$). Similar trends were observed for biochemical parameters except the alanine amino transferase value, which was significantly higher in the control group (i.e. 12.65 ± 3.960 U/L vs 15.48 ± 11.55 U/L in the study group [$P = 0.044$]). Assessing the erythrocyte sedimentation rate as an acute phase marker, we found higher values in children with *H. pylori* chronic gastritis compared to those in the control group, but without statistical significance ($P = 0.172$). All of the assessed parameters are described in Table 2.

We compared the mean and median values of the laboratory parameters between the two groups and encountered a significant difference in terms of both leucocytes and neutrophils ($P = 0.0225/P = 0.0292$) for the variant TT genotype of TLR9 rs352140. The results of these comparisons are noted in Table 3.

DISCUSSION

H. pylori infection occurs most commonly during early childhood and can result in life-threatening conditions during adulthood if left untreated. Thus, the persistence of acute gastric inflammation associated with this bacterium and its imminent transformation into a chronic process will definitely result in an increased risk for gastric neoplasia[18]. Based on this fact, we assessed children with *H. pylori* chronic gastritis to delineate the features associated with this disorder in terms of environmental factors, laboratory parameters, and innate immunity since it is of major importance to define and characterize this disorder at young ages. Several environmental factors are associated with an increased risk of *H. pylori* infection such as low socio-economic status, bed sharing between children and adults, and improper sanitary conditions within the household[19,20]. Moreover, a study performed in children showed that those originating from the rural area were more predisposed to developing gastritis independently of the presence or absence of *H. pylori* infection[3]. Similarly, our study revealed that both poor living conditions and rural area might be risk factors for *H. pylori* chronic gastritis in children. Serology and rapid urease tests might be used as additional tests for diagnosing *H. pylori* infection, but their sole use is not recommended[7]. Similarly, the false-positive results of the rapid urease test and serology were also observed in our study. In terms of the rapid urease test, a possible explanation might be related to the presence of other urease-secreting bacteria within the stomach, such as *Proteus* spp[21], whereas regarding serology, this fact might be due to a previous infection with this bacteria[7].

Low-grade systemic inflammation associated with *H. pylori* infection has been the main focus of multiple recent studies. It has been proved that once *H. pylori* colonizes the gastric mucosa, it exerts chemotactic effects on neutrophils and lymphocytes, which together with other cells present at this level such as mononuclear cells and macrophages as well as several signaling cytokines, result in a low-grade systemic inflammatory status[22]. Other studies have identified a strong association between this inflammatory status associated to *H. pylori* infection and a wide spectrum of disorders such as cardiovascular diseases, stroke, anemia, idiopathic thrombocytopenic purpura, diabetes, glaucoma, thyroid disease, Alzheimer's disease, rosacea, eczema, and chronic hives[23]. Moreover, some studies have noted that elevated levels of *H. pylori* antibodies are significantly correlated with increased systolic blood pressure and arterial stiffness in patients with diabetes[24] and coronary artery disease [25]. Most of the studies that have aimed to assess the markers of this systemic inflammatory status have been performed in adult populations and proved that acute phase reactants are significantly higher in patients with *H. pylori* infection[26]. Thus, a study that included 50 adult patients with *H. pylori*-induced gastritis and 50 with *H. pylori*-negative gastritis showed higher values of leukocytes, lymphocytes, and neutrophils in patients with gastritis induced by this infection compared to those with other types

Table 1 Demographic analysis of the two groups, n (%)

Parameters	Study group, n = 48	Control group, n = 107	P value
Age in yr	12.96 ± 3.83 (13.50)	12.09 ± 3.80 (12.00)	0.1987
Gender			
Girls	29 (60.42)	62 (57.94)	0.7725
Boys	19 (39.58)	45 (42.06)	
Originating area			
Urban	15 (31.25)	60 (56.07)	0.0042
Rural	33 (68.75)	47 (43.93)	
TLR9 rs352140			
CT genotype	21 (43.75)	63 (58.88)	0.1075
TT genotype	16 (33.33)	20 (18.69)	
CC genotype	11 (21.92)	24 (22.43)	
Family history			
Negative	47 (97.92)	106 (99.07)	
Positive	1 (2.08)	0 (0.00)	
Living conditions			
Good	31 (64.59)	99 (92.52)	< 0.0001
Poor	17 (36.41)	8 (7.48)	
IgA anti- <i>Helicobacter pylori</i>			
Positive	30 (62.50)	3 (2.80)	< 0.0001
Negative	17 (36, 41)	104 (97.20)	
Gastro-esophageal reflux			
Present	9 (18.75)	25 (23.36)	0.6657
Absent	39 (81.25)	82 (76.64)	
Biliary reflux			
Present	12 (25)	31 (28.97)	0.6096
Absent	36 (75)	76 (71.03)	
Rapid urease test			
Positive	31 (64.58)	15 (15.01)	< 0.0001
Negative	17 (35.42)	91 (85.05)	

CC: Homozygous wild-type genotype; TT: Homozygous variant genotype.

of gastritis[27]. White blood cell subtype along with NLR and platelet/lymphocyte ratio (PLR) also proved their utility in assessing systemic inflammation associated with pediatric obesity[28]. In terms of pediatric gastritis, the scarcity of available information hinders establishment of the precise role of these biomarkers in assessing the systemic inflammatory status related to this infection. Thus, our team has focused on clarifying this issue, and we found that leukocyte and neutrophil counts are important indicators for *H. pylori*-positive gastritis in children, but we did not identify any association between this infection and NLR, nor PLR[3,29]. Moreover, lymphocyte count might be a better marker for non-*H. pylori* gastritis[3]. Neutrophils might better reflect the inflammation, while lymphocytes could be a better indicator of the body's nutritional status and general stress[30]. Similar to the aforementioned findings, our study also showed significantly higher values of both leukocytes and neutrophils in children with *H. pylori* chronic gastritis compared to the healthy controls, but failed to establish an association with NLR.

Table 2 Laboratory parameters in the two groups

Parameters	Study group, n = 48, mean ± SD (median)	Control group, n = 107, mean ± SD (median)	P value
Hemoglobin (g/dL)	13.70 ± 1.848 (13.35)	13.45 ± 1.394 (13.50)	0.930
Leucocytes (10 ³ /μL)	7.950 ± 2.312 (7.81)	7.133 ± 2.394 (6.69)	0.011
Lymphocytes (10 ³ /μL)	2.486 ± 0.6978 (2.51)	2.461 ± 0.7779 (2.43)	0.593
Neutrophils (10 ³ /μL)	4.455 ± 2.130 (3.925)	3.813 ± 2.222 (3.290)	0.028
Eosinophils (10 ³ /μL)	0.2894 ± 0.4060 (0.13)	0.2188 ± 0.2932 (0.11)	0.368
ESR (mm/h)	11.19 ± 9.546 (9.00)	9.327 ± 7.504 (7.00)	0.172
Iron μmol/L)	15.60 ± 6.941 (14.61)	15.59 ± 7.060 (14.94)	0.893
AST (U/L)	20.82 ± 6.756 (19.55)	22.95 ± 11.07 (20.40)	0.291
ALT (U/L)	12.65 ± 3.960 (12.00)	15.48 ± 11.55 (13.10)	0.044
GGT (U/L)	11.40 ± 3.331 (11.00)	12.63 ± 4.308 (12.00)	0.053
NLR	2.031 ± 1.478 (1.485)	1.756 ± 1.349 (1.320)	0.154

AST: Alanine aminotransferase; ALT: Aspartate aminotransferase; ESR: Erythrocyte sedimentation rate; GGT: Gamma-glutamyl transpeptidase; NLR: Neutrophil/lymphocyte ratio, estimated significance value obtained from the non-parametric Mann-Whitney test.

TLR9 is an endosome-transmembrane receptor with an essential role in the DNA recognition of both pathogens and damaged host cells *via* saccharide backbone, a structural component, in a sequence-independent manner[15]. A study that compared *H. pylori*-infected individuals with healthy controls showed a distinct distribution in these individuals, with predominant expression of this TLR in the apical compartment of the gastric epithelium in the control group compared to *H. pylori*-positive ones, where this receptor was mainly encountered in the basolateral compartment[31]. Controversial findings have been reported regarding the role of TLR9 in the development of *H. pylori* infection. Therefore, a study performed in murine models proved that the recognition of *H. pylori* DNA by TLR9 triggers proinflammatory responses[32], while the study by Otani *et al*[33] performed in mice indicated that this receptor might act as a suppressor for *H. pylori* infection during the acute phase of this infection. Based on these findings, Varga *et al*[34] recently stated that TLR9 might have a dichotomous role, and its role in suppressing or promoting might be influenced by the gastric microenvironment. Thus, the microenvironment that contains inflammatory cells without polarity acts as a trigger for the promotion of proinflammatory cascades *via* TLR9 resulting in the development of gastric cancer[31,35]. Moreover, cancer tissue expresses upregulates TLR9[15]. Multiple TLR9 single nucleotide polymorphisms (SNPs) were assessed in patients with gastric malignancies. Thus, a study that included 314 Chinese patients with gastric cancer and 314 healthy controls showed that TLR9 -1486C carriers express both an increased risk of gastric cancer and a poorer prognosis[36]. Moreover, a more recent study proved that the 2848A allele of TLR9 is associated with an increased risk for duodenal ulcer, as well as alteration of inflammatory cytokine expression within the gastric mucosa[37]. By contrast, no association was encountered between TLR9 rs5743836 (or -1237T/C) promoter polymorphisms and the risk of gastric cancer[38]. The role of TLR9 rs352140 polymorphism was assessed in patients with different conditions such as renal transplantation[39], systemic lupus erythematosus[40], bacterial meningitis[41], primary immune thrombocytopenia[42], cervicitis[43], cervical cancer[44], or placental inflammation[45]. In terms of gastropathy and TLR9 rs352140 SNP, the data are scarce. A recent study performed in adults from India underlined that the heterozygous variant CT genotype of this polymorphism is significantly associated with the persistence of *H. pylori* infection[46]. Nevertheless, our study found no correlation between TLR9 rs352140 genotypes and *H. pylori*-induced chronic gastritis, suggesting that this TLR might not influence the persistence of this infection in children. Another study that included patients with chronic gastritis, peptic ulcer disease, and gastric carcinoma showed that the TT genotype was more commonly expressed by patients from the chronic gastritis group[47]. Similarly, the meta-analysis of Zhang *et al*[48] pointed out a significant association between the TT variant genotype of TLR9 rs352140 SNP and the overall risk of neoplasia, emphasizing that this polymorphism

Table 3 Correlations between TLR9 rs352140 polymorphism and laboratory parameters in the two groups

CC genotype of TLR9 rs 352140, n = 35	Study group, n = 11, mean ± SD (median)	Control group, n = 24, mean ± SD (median)	P value
Hemoglobin (g/dL)	14.17 ± 3.300 (13.10)	13.57 ± 1.600 (13.55)	0.900 ¹
Leucocytes (10 ³ /μL)	9.076 ± 3.074 (9.76)	8.522 ± 2.787 (8.01)	0.557 ¹
Lymphocytes (10 ³ /μL)	2.525 ± 0.6571 (2.500)	2.414 ± 0.844 (2.385)	0.704
Neutrophils (10 ³ /μL)	5.600 ± 2.727 (5.72)	5.151 ± 2.967 (4.49)	0.709 ¹
Eosinophils (10 ³ /μL)	0.1831 ± 0.1207 (0.18)	0.2246 ± 0.2376 (0.11)	0.844 ¹
ESR (mm/h)	15.64 ± 16.14 (11.00)	10.04 ± 11.13 (6.00)	0.225 ¹
Iron μmol/L)	17.35 ± 11.07 (12.52)	16.72 ± 8.777 (13.91)	0.872 ¹
AST (U/L)	22.43 ± 6.421 (20.80)	21.84 ± 6.481 (21.30)	0.803
ALT (U/L)	13.64 ± 4.125 (13.20)	14.16 ± 6.511 (12.60)	0.901 ¹
GGT (U/L)	11.64 ± 3.443 (11.00)	12.38 ± 2.667 (12.00)	0.169 ¹
NLR	2.374 ± 1.426 (1.660)	2.620 ± 2.150 (1.845)	0.859 ¹
CT genotype of TLR9 rs 352140, n = 84	Study group, n = 21, mean ± SD (median)	Control group, n = 63, mean ± SD (median)	P value
Hemoglobin (g/dL)	13.37 ± 1.001 (13.00)	13.49 ± 1.435 (13.70)	0.435 ¹
Leucocytes (10 ³ /μL)	7.580 ± 2.331 (7.55)	6.826 ± 2.262 (6.64)	0.075 ¹
Lymphocytes (10 ³ /μL)	2.545 ± 0.6196 (2.56)	2.546 ± 0.8016 (2.45)	0.713 ¹
Neutrophils (10 ³ /μL)	4.032 ± 2.055 (3.85)	3.488 ± 1.898 (3.11)	0.253 ¹
Eosinophils (10 ³ /μL)	0.272 ± 0.3047 (0.175)	0.218 ± 0.3075 (0.110)	0.332 ¹
ESR (mm/h)	10.48 ± 6.668 (9.00)	8.841 ± 5.995 (7.00)	0.288 ¹
Iron μmol/L)	14.84 ± 5.340 (15.00)	14.87 ± 5.832 (15.13)	0.985
AST (U/L)	19.22 ± 4.518 (19.50)	23.54 ± 13.10 (20.10)	0.174 ¹
ALT (U/L)	12.73 ± 3.659 (11.80)	15.93 ± 13.55 (13.10)	0.128 ¹
GGT (U/L)	11.10 ± 3.646 (11.00)	12.76 ± 4.950 (11.00)	0.094 ¹
NLR	1.664 ± 1.028 (1.330)	1.489 ± 0.8915 (1.300)	0.492 ¹
TT genotype of TLR9 rs 352140, n = 36	Study group, n = 16, mean ± SD (median)	Control group, n = 20, mean ± SD (median)	P value
Hemoglobin (g/dL)	13.80 ± 1.332 (13.50)	13.16 ± 0.9517 (13.20)	0.101
Leucocytes (10 ³ /μL)	7.660 ± 1.410 (7.510)	6.434 ± 1.618 (6.045)	0.022
Lymphocytes (10 ³ /μL)	2.383 ± 0.8418 (2.305)	2.254 ± 0.5870 (2.210)	0.590
Neutrophils (10 ³ /μL)	4.221 ± 1.537 (3.865)	3.232 ± 1.472 (2.745)	0.029¹
Eosinophils (10 ³ /μL)	0.3844 ± 0.5999 (0.110)	0.2145 ± 0.3203 (0.175)	0.786 ¹
ESR (mm/h)	9.063 ± 5.709 (8.50)	10.00 ± 6.720 (7.00)	0.747 ¹
Iron μmol/L)	15.39 ± 5.304 (14.45)	16.50 ± 8.347 (13.36)	0.646
AST (U/L)	21.82 ± 9.044 (20.25)	22.44 ± 8.341 (21.50)	0.831
ALT (U/L)	11.88 ± 4.311 (10.20)	15.65 ± 9.539 (13.35)	0.107 ¹
GGT (U/L)	11.63 ± 2.986 (11.00)	12.50 ± 3.846 (12.50)	0.642 ¹
NLR	2.276 ± 1.933 (1.555)	1.559 ± 0.8576 (1.130)	0.232 ¹

¹Mann-Whitney test.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CC: Homozygous CC genotype of TLR9 rs352140 polymorphism; CT: Heterozygous CT genotype of TLR9 rs352140 polymorphism; ESR: Erythrocyte sedimentation rate; GGT: Gamma-glutamyl transpeptidase; NLR: Neutrophil/lymphocyte ratio; TT: Homozygous TT genotype of TLR9 rs352140 polymorphism, estimated significance value obtained from non-parametric.

might alter innate immune responses promoting chronic inflammation and subsequent

Our study had several limitations that must be acknowledged: The relatively small number of patients originating from a single area of our country; the diagnosis of *H. pylori* infection established only based on the histopathological exam without being confirmed by an additional method; we did not assess the gastric microenvironment, the influence of gastric microbiota or the degrees of gastritis severity; we did not perform esophageal pH monitoring in order to confirm the diagnosis of gastroesophageal reflux; the lack of longitudinal follow-up of the patients endoscopically after the eradication therapy, lack of TLR9 gene expression; and we did not take into account the *H. pylori* pathogenicity features in the development of chronic gastritis and subsequent increased risk for a more severe degree of systemic inflammation.

The main two strengths of this study were the small age of the assessed population and the fact that the role of TLR9 rs352140 polymorphism was assessed for the first time in children with *H. pylori* chronic gastritis. Therefore, this study might be considered a pilot study providing valuable results of great multidisciplinary importance that might serve as a solid basis for the development of effective preventive strategies for gastric cancer during adulthood. Other strengths of this study consist of the fact that we established the diagnosis of *H. pylori* gastritis based on the histological exam of the gastric biopsy, as well as the fact that all upper digestive endoscopies and histological exams were performed by two experienced pediatric gastroenterology and pathology specialists.

CONCLUSION

Environmental factors, such as poor living conditions and rural area might result in an increased risk for *H. pylori* chronic gastritis in children. Serological and rapid urease tests could be used as reliable tests for the detection of *H. pylori* infection in children. Moreover, leukocytes and neutrophils are important non-invasive biomarkers for detecting the low-grade inflammatory status associated with this infection in pediatric patients. In terms of innate immunity, our findings emphasized that variant TT genotype carriers of the TLR9 rs352140 gene polymorphism might express a more severe degree of inflammation. Nevertheless, further studies are required on bigger samples of pediatric patients to identify the precise role of innate immunity and its TLR9 polymorphisms in the development of *H. pylori* gastritis.

ARTICLE HIGHLIGHTS

Research background

Innate immunity was found to be associated to both persistence of *Helicobacter pylori* (*H. pylori*) infection and increased risk for gastric cancer.

Research motivation

To identify the risk factors associated with *H. pylori* infection and to establish the role of TLR9 rs352140 in suppressing or promoting the inflammation related to this infection in children.

Research objectives

Environmental factors represent a major risk factor for *H. pylori* chronic gastritis in children. Peripheral blood parameters might be reliable indicators of systemic inflammation triggered by *H. pylori* infection. TLR9 polymorphisms seem to be involved in promoting or suppressing systemic inflammation in the setting of pediatric *H. pylori* chronic gastritis.

Research methods

A cross-sectional prospective study on 183 children.

Research results

Rural area and poor living conditions were significantly associated to *H. pylori* chronic gastritis ($P = 0.0042/P < 0.0001$). Both positive immunoglobulin A anti *H. pylori* and rapid urease test were significantly associated to *H. pylori* infection ($P < 0.0001$). Significantly higher values of leukocytes and neutrophils within the peripheral blood were found in children with *H. pylori* chronic gastritis ($P = 0.111/P = 0.284$). We found

a significant positive correlation for variant TT genotype of TLR9 rs352140 polymorphism and both leucocytes and neutrophils ($P = 0.0225/P = 0.0292$).

Research conclusions

Environmental factors such as poor living conditions and rural area might result in an increased risk for *H. pylori* chronic gastritis in children. Serological and rapid urease tests could be used as reliable tests for the detection of *H. pylori* infection in children. Moreover, leukocytes and neutrophils are important non-invasive biomarkers for detecting the low-grade inflammatory status associated with this infection in pediatric patients. In terms of innate immunity, our findings emphasized that variant TT genotype carriers of the TLR9 rs352140 gene polymorphism might express a more severe degree of inflammation.

Research perspectives

Nevertheless, further studies are required on bigger samples of pediatric patients to identify the precise role of innate immunity and its TLR9 polymorphisms in the development of *H. pylori* gastritis.

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