The Neutrophil/Lymphocyte Ratio in *Helicobacter Pylori* Infected Patients: A Comparative Therapeutic Study to Reduce Risk of Gastric Cancer

Dhifaf R. Reesn [1], Majid S. Jabir [2]

Abstract

Background: Helicobacter pylori (HP) infection is known to induce localized inflammation in the gastric region and trigger a systemic humoral immune response. Previous research in adults has shown a significant correlation between HP infection and changes in the neutrophil/lymphocyte ratio (NLR) as well as the mean levels of neutrophils and lymphocytes. Determining the optimal course of therapy for HP infection relies on results from antibiotic susceptibility testing. Methods: This study aimed to investigate the association between HP infection and immunological responses before and after two distinct treatment modalities. The study included individuals across different age groups and health conditions: children aged 12–16 years, pregnant women aged 19–35 years, individuals with chronic diseases (diabetes and hypertension) aged 45–75 years, youth patients aged 18–45 years, and healthy controls. A total of 240 patients scheduled to undergo gastroduodenoscopy were included, with 120 testing positive for HP and 120 testing negative. The average age of both HP-positive and HP-negative patients was 45.56±12.16 years. Results: The study found that the eradication rate for H. pylori infection was satisfactory using second-line therapies. Specifically, standard 5-week sequential therapy showed a higher effectiveness rate (100%) compared to standard triple therapy (4%), especially when the strains were susceptible to clarithromycin. There were no significant differences observed in the levels of NLR, neutrophils, and lymphocytes values between the two treatment modalities ($p > 0.05$). Conclusion: These findings suggest that standard 5-week sequential therapy may be more effective in eradicating HP infection compared to standard triple therapy, particularly in cases where the strains are susceptible to clarithromycin. However, the study emphasizes the importance of generating regional data on the success rates of various therapeutic approaches to establish effective treatment strategies for HP infection.

Keywords: *Helicobacter pylori*, Gastritis, Neutrophil-to-Lymphocyte Ratio (NLR), Antibiotic Resistance, Treatment Strategies

Significance | The study investigated Helicobacter pylori infection, its impact on inflammatory markers, and treatment efficacy, offering insights into diagnosis and management.

*Correspondence. Majid S. Jabir, Department of Applies Science, University of Technology, Iraq.
E-mail: 100131@uotechnology.edu.iq

Editor Md Shamsuddin Sultan Khan, And accepted by the Editorial Board Apr 04, 2024 (received for review Feb 28, 2024)

Introduction

Helicobacter pylori (HP) is a gram-negative, spiral, flagellated, and microphillic bacterium (Sung et al. 2021). It's colonizing the inimical microenvironment of the human stomach. More than half of Iraqi people are stomachic by HP. *H. pylori will* continue to survive for the host's full life if not treated effectively. In this case, some patients may develop a series of diseases, including gastritis, gastroduodenal ulcer, dysplasia, intestinal metaplasia, and gastric cancer (Jim et al. 2017). The main characteristic of HP-induced

Author Affiliation.
[1] Department of Applies Science, University of Technology, Iraq.

Please cite this article.
Dhifaf R. Reesn, Majid S. Jabir. (2024). The Neutrophil/Lymphocyte Ratio in Helicobacter Pylori Infected Patients: A Comparative Therapeutic Study to Reduce Risk of Gastric Cancer, Journal of Angiotherapy, 8(4), 1-13, 9638

gastritis is the increased drain of inflammatory cells, which is usually the first detectable change among these diseases. Inflammation plays a critical role in restricting HP along with gastric mucosa damage and causing symptoms such as dull or burning pain in the stomach (more often after eating and at night), unplanned weight loss, bloating, loss of appetite, nausea and vomiting, sometimes bloody vomit, dark stool, indigestion (dyspepsia), and burping (Diaconu et al. 2017). HP induces local inflammation in the stomach and a systemic humoral immune response. Most patients have asymptomatic but inveterate inflammation (Savoldi et al. 2018). Previous studies have investigated several parameters, such as compounds of the complete blood count (CBC), a simple and inexpensive test, such as leukocytes, neutrophils, and lymphocytes, and the neutrophil-to-lymphocyte ratio (NLR), which can help recognize the causes of hematological diseases and prognosticate some inflammatory events. NLR is the most important parameters (Polk and Peek 2010).

The World Health Organization (WHO) has identified HP as a universal priority pathogen due to its increasing diffusion and the emergence of antibiotic resistance (Fox and Wang 2007). The history of HP treatment has improved over the past few decades. In the 1980s, *H. pylori was* detected as a causative agent of gastritis and peptic ulcer disease, and treatment firstly consisted of acid suppression therapy alone (Peek and Blaser 2002). Later, it was found that antibiotics could be used to eradicate HP, and a combination of antibiotics and acid suppression drugs became the norm treatment for HP infection. However, antibiotic resistance has become an important problem, and the efficacy of treatment had slump in recent years. The bacterium could be exterminate with a combination of antibiotics and acid suppressant treatments, but antibiotic resistance has become a significant problem that can minimize the efficacy of treatment (Soutto et al. 2015). The WHO has released a list of 12 bacterial families that are the most risky to human health, and one of them is H. *pylori* as a priority pathogen, classified into three priority statuses: critical, high, and medium (Fox and Wang 2007). Clarithromycin and metronidazole-resistant HP are of the same high vantage (Zhu et al. 2017; Correa 1988). In recently years, the WHO has agreed to triple treatment in one kit that consists of PPIs, clarithromycin, and metronidazole (Adachi et al. 2017). This study was to investigated the presence and severity of HP infection linked with these parameters of CBC in patients, the comparative effect of two ways of treating them, and which one of them was best for patients through these parameters. The aim of this study was to determine the effect of HP on the immune system before and after two treatments and which one of them gave us the best results and safety for patients.

2. Materials and methods

2.1 Study Design

Retrospective file scanning was done for this study on the 240 patients ages 12-75 years old who had acute gastritis, gastric ulcers, and dyspepsia. The study protocol was authorize by the Al_technologia University of Clinical Studies Ethics Committee authorized (Decision numberASD-UOT-020921). The Declaration of Helsinki's tenets served as a framework for this investigation. The patients were classified as having *H pylori* positive or not based on pathology reports and laboratory data. The research eliminated patients whose data could not be obtained. The patients' demographic information was documented. According to laboratory results and pathology reports, patient's samples were divided into five groups according to their ages, as follows:

Group 1: 40 children ages (12–16) years were diagnosed with HP gastritis.

Group 2: 46 pregnant women ages (19-35) years were diagnosed with HP gastritis

Group 3: 52 patients with chronic disease (diabetics and blood pressure) ages 45-75 years.

Group 4: 138 youth patients ages between 18-45 years.

Group 5: healthy (controls) for each group above.

After two patients' treatment modalities, the aforementioned categories remain unchanged. In our study, patients aged between 12-75 years presented for dyspeptic symptoms (e.g., nausea, vomiting, abdominal pain, bloating, dark stool, weight loss, and loss of appetite).

Patients having clinical or Para clinical evidence of acute infectious illness, both before and after treatment in two different methods, as well as certain patients with incomplete clinical and Para clinical data and treatment refusal were excluded from the research. Participants in our research had anamnesis based on their acceptance, as well as a full clinical evaluation. The following laboratory parameters were examined such as a full blood count, as well as inflammatory indicators such as neutrophils, lymphocytes, and the neutrophil-to-lymphocyte ratio (NLR), are all available. These measures were examined prior to and after treatment with a triple therapy combikit (**Brawn medicine**) (lanzoprazole capsules 30mg, tinidazole tablets 500 mg, and clarithromycin tablets 250 mg).

2.2 Sample collection

Blood samples, totaling 2mL per patient, were aseptically collected using a sterile syringe and transferred into EDTA-K2 tube (manufactured by malak company) for the purpose of assessing the complete blood count (CBC). The CBC includes the counting of neutrophils and lymphocytes as well as the calculation of the neutrophil-lymphocyte ratio using the BC-30s machine (Hematology analyzer, mindray).

2.3 Diagnosis of HP and current treatment with tested neutrophil, lymphocyte, and neutrophil lymphocyte ratio

Diagnosis of HP infection is based on serological test, urea breath test, and stool antigens test (Fox and Wang 2007). A pre-treatment evaluation of the complete blood count (CBC) was carried out after the diagnosis of *Helicobacter pylori* (HP) infection in order to examine neutrophil levels, lymphocyte counts, and the neutrophil-lymphocyte ratio. The patient was then started on the current treatment plan, which includes the commercially available triple therapy (combikit). This treatment plan combines two antibiotics (a 500 mg tablet of tinidazole and a 250 mg tablet of clarithromycin) with a proton pump inhibitor (PPI) (a 30 mg capsule of lanzoprazole).

A follow-up complete blood count (CBC) analysis was performed to monitor changes in neutrophil and lymphocyte counts, as well as the neutrophil-lymphocyte ratio (NLR), following a week-long pharmacological intervention. But not every patient could follow this specific medication schedule; patients who were between the ages of 12 and 16 were precluded from participating in accordance with the drug protocol. Furthermore, several individuals who were above 18 years old had side effects that included nausea, vomiting, dizziness, and stomach discomfort. Even though the medication was stopped due to these adverse effects, a reanalysis was carried out to look for any changes in the test results as a result.

The remaining group of patients who completed the prescribed course of treatment successfully had a reevaluation for *H. pylori* infection, with findings that continued to be positive. Simultaneously, the effect of the first therapy was evaluated by measuring the neutrophil and lymphocyte counts as well as the neutrophil-lymphocyte ratio. During the inquiry, a new treatment strategy was presented. This treatment plan was carried out for five weeks using the same pharmacological agents, but at different doses, and adding another medicine with the supervision of a physician. The initiation of this protocol started as follows:

Patients received PIs for the first two weeks of therapy, which included 20 mg of omeprazole capsules and 8 mg of ondansetron tablets. The recommended use of this mixture was twice a day, before meals. Furthermore, 500 mg metronidazole pills, marketed in pharmacies under the brand name Negazole, were used to carry out antibiotic therapy. Metronidazole was used twice a day, just after meals.

During the subsequent two weeks, patients were given PPIs, consisting of omeprazole capsules at a dosage of 20 mg and ondansetron tablets at 8 mg. This treatment was given twice a day, right before meals. Clarithromycin 500 mg pills were recommended twice a day after meals to continue the antibiotic medication concurrently. The third week of therapy involved a change to the regimen: omeprazole pills, 20 mg twice a day, before meals for a week.

2.4 Statistical Analysis:

The statistical analysis involved both descriptive statistics elements (frequency, percentage, mean, median, and standard deviation). Correlation was applied in order to identify possible correlations between inflammatory markers and immune parameters for different ages before and after treatment. Choosing a significance threshold for the *P* value of 0.05. The statistical results were obtained using the GraphPad Prism programme version 8 (Ali etal., 2018).

3. Results:

The 240 individuals in our research had gastritis that was linked to *Helicobacter pylori* (HP) positive. Of them, 40 were children, between the ages of 12 and 16; 46 were pregnant women; 52 had pre-existing chronic illnesses, such diabetes and hypertension; and 138 belonged to the 18 to 45 age group. Out of the entire sample, 120 patients (or 50%) tested positive for *H. pylori*, and the other 50% showed negative findings. These patients (PH-negative) made up the control group, which was then further separated for analysis.

The HP-positive subgroup showed varying degrees of positivity: 33 patients had mild HP-positivity, 46 had moderate positivity, and 41 had severe HP-positivity (Table 1).

The research participants' average age was determined to be 45.56±12.16 years. Initial laboratory assessments by patient groups showed the following neutrophil levels: 9.250±1.462 for children between the ages of 12 and 16 as shown in (**Figure1 A**); 8.133±2.060 for pregnant women between the ages of 21 and 28 as shown in (**Figure 1 B**); 7.875±2.866 for patients with chronic diseases (diabetes and hypertension) between the ages of 50 and 75 as shown in (**Figure 1 C**); and 6.429±2.488 for young patients between the ages of 18 and 45 as shown in (**Figure 1 D**) (Table 2).

Values for lymphocytes were established in this manner: shows 1.450±0.5196 for children as shown in (figure 2 A), 1.167±0.2517 for pregnant women as shown in (figure 2 B), 2.000±1.086 for patients with chronic conditions as shown in (figure 2 C), and 4.778±4.610 for young patients as shown in figure 2 D), respectively (Table 2).

Table 2 displays the Neutrophil-to-Lymphocyte Ratio (NLR) values for the same groups. The values for children are 7.200±3.573, as shown in (Figure 3 panel A); for pregnant women, it is 6.850±2.193, as shown in (Figure 3 panel B); for patients with chronic diseases, it is 4.273±1.495, as shown in (Figure 3 panel c); and for young patients, it is 2.947±2.598, as shown in (Figure 3 panel D) (Table 2).

After administering the Brawn kit of the HP combination kit to the patient for a week, the results showed that only four patients in the younger age group had negative HP results. In accordance with the results, the neutrophil count was 4.65±0.7141, as shown in (Figure 1 D), the lymphocyte count was 2.380±0.4764, as shown in (Figure

2 D), and the NLR (neutrophil-to-lymphocyte ratio) was 2.100±0.7176, as shown in (Figure 3 D) table 3.

The subjects who remained tested consistently positive for HP. The results showed that their neutrophil counts were 9.250±1.462, their lymphocyte counts were 1.450±0.5196, and their neutrophil-to-lymphocyte ratio (NLR) was 7.200±3.573 (Table 3).

Because of intolerance, the other groups declined to administer the Brawn kit. As a result, these groups received different treatment plans that included giving them the recommended medications for a period of five weeks.

In order to mitigate the risk of vomiting and abdominal pain, patients were given metronidazole (500 mg) after meals together with ondansetron (8 mg) and omeprazole (20 mg) twice a day before meals. In the subsequent two-week period, the prescribed dosage was 500 mg of clarithromycin twice a day after meals, combined with 8 mg of ondansetron and 20 mg of omeprazole twice a day before meals. However, patients were only given omeprazole (20 mg) twice a day, before meals, during the third week.

For this treatment regimen, the following results were obtained from laboratory assessments across different groups: the neutrophil count for the children's group was 4.050±1.857, as shown in (Figure 1 A); the neutrophil count for pregnant women was 3.467±1.002, as shown in (Figure 1 B); the neutrophil count for patients with chronic diseases was 3.638±1.042, as shown in (Figure 1 C); and the neutrophil count for young patients whose Helicobacter pylori (HP) remained positive after the combo kit (Brawn treatment) was 4.047±1.159, as shown in (Figure 5 A).

Moreover, the lymphocyte results showed that: the lymphocyte count for the children's group was 2.375±0.8382, as shown in (Figure 2 A); the lymphocyte count for pregnant women was 2.633±0.1155,as shown in figure (Figure 2 B); the lymphocyte count for patients with chronic illnesses was 2.388±0.4565, as shown in (Figure 2 C); and the lymphocyte count for young patients who continued to test positive for HP after using the combo kit was 2.282±0.7282, as shown (Figure 5 B).

The results for these groups' Neutrophil-to-Lymphocyte Ratios (NLR) were as follows: the NLR for the children's group was 1.650±0.2646, as shown (Figure 3 A); the NLR for pregnant women was 2.225±0.6551, as shown in (Figure 3 B); the NLR for patients with chronic illnesses was 1.575±0.4773, as shown in (Figure 3 C); and the NLR value for young patients was 2.947±2.598, (Figure 5 C) (Table 4).

Prior to therapy, baseline values for neutrophils, lymphocytes, and the Neutrophil-to-Lymphocyte Ratio (NLR) were as follows in the presence of HP infection: In children, the neutrophil level was <0.0001, the lymphocyte level was 0.0798, and the NLR was 0.0123. Among pregnant women, the neutrophil level was 0.0009, the lymphocyte level was 0.0241, and the NLR was <0.0001. For patients with chronic diseases, the neutrophil level was 0.0021, the lymphocyte level was 0.7991, and the NLR was 0.0003. In young patients, the neutrophil level was 0.0068, the lymphocyte level was 0.1142, and the NLR was 0.3741 (Table 5).

After the initial combination kit (Brawn medicine) treatment regimen, special observations were made for the young patients who still tested positive for HP even after taking the medication. The neutrophil level was 0.0157, the lymphocyte level was 0.1101, and the neutrophil-to-lymphocyte ratio (NLR) was 0.3243 for this group. On the other hand, the following results were recorded for the young patients who were HP negative following the administration of the Brawn drug: neutrophil level of 0.1871, lymphocyte level of 0.4012, and NLR level of 0.2406 (Table 6).

On the other hand, after using this unique 5-week treatment strategy, the observed values for various groups were as follows:

In the children's group, the neutrophil level was 0.7933, the lymphocyte level was 0.5316, and the Neutrophil-to-Lymphocyte Ratio (NLR) was 0.2109. Among pregnant women, the values were recorded as follows: neutrophil level of 0.0807, lymphocyte level of 0.1620, and NLR of 0.1881. For patients with chronic diseases, the observed values were: neutrophil level of 0.6438, lymphocyte level of 0.3979, and NLR of 0.2175. In the young patient group, the values were documented as follows: neutrophil level of 0.6734, lymphocyte level of 0.5680, and NLR of 0.9793 (Table 7).

There were statistically significant variations seen in the neutrophil, lymphocyte, and neutrophil-to-lymphocyte ratio (NLR) levels between the control and patient groups ($p>0.05$).

Prior to treatment, the HP values for the following groups showed statistically significance, with p-values documented as follows: < 0.0001 in children; 0.299 in pregnant women as shown in Table 8 ; 0.0001 in patients with chronic disease ; and 0.0002 in young patients ($p > 0.05$).

Just four patients (value = 0.1995) had negative outcomes following the combination kit therapy (Brawn medicines), which was only given to the younger patient group. The other patients (value = 0.0036; Figure 5) had positive results ($p > 0.05$).

After a novel treatment approach was used, the HP values for the various groups were found to be statistically significant, with the following p-values: 0.2271 for young patients; 0.1994 for pregnant women; 0.4551 for patients with chronic disease; and 0.4371 for children.

The mean±SD values for several groups were as follows, based on the classification of Helicobacter pylori (HP) positive into mild, moderate, and severe categories: The values in the children's group were 2.225±0.9356 for mild positive, 6.143±1.241 for moderate positivity, and 11.21±1.713 for sever positivity. The values among pregnant women were 2.578±0.8694 for mild positive, 5.582±0.8445 for moderate positivity, and 10.37±1.526 for severe positivity. The values for mild, moderate, and severe positive in individuals with chronic illnesses were 2.260±0.6037, 5.489±0.8145,

and 15.31±5.093, respectively. The results recorded for mild positive in the young patient group were 2.320±0.8464, moderate positivity was 5.413±0.9588, and severe positivity was 16.95±4.979 (Table 9).

Discussion

Over half of all adults worldwide have an infection with the stomach bacteria *H. pylori* (Correa and Piazuelo 2008). Certain cytokine components, patient genetic predisposition, receptor gene polymorphisms, gene editing factors, and environmental effects all seem to have a role in the implications of H. pylori infection (Bagheri et al. 2013; Salimzadeh et al. 2015). The majority of children who get the illness will not have any difficulties; nonetheless, the course of the infection is largely determined by immunological processes that arise in the child's stomach mucosa. This pathogenic bacterium colonises the stomach, resulting in an inflammatory response that includes damage to the epithelium and the accumulation of neutrophils, T and B lymphocytes, plasma cells, and macrophages. In addition, almost everyone infected with *H. pylori* has a chronic stomach irritation (Suzuki et al. 2002; Bagheri et al. 2015; Razavi et al. 2015). Despite the early onset of the infection, the related disorders primarily manifest in patients. The type of immune response and the variety of inflammatory mediators that are present in the patents gastric mucosa might likely influence how an illness turns out as an adult. Consequently, it's critical to thoroughly assess every instance of infection in kids to stop the emergence of serious stomach disorders linked to *H. pylori* infection in adults (Ortiz-Princz et al. 2016). In our study, the inflammatory markers (neutrophils, lymphocytes and Neutrophil-to-Lymphocyte Ratio) were assessed in several groups such as children, adults, pregnant women and chronic illness patients. In general, neutrophil, lymphocytes and NLR results revealed a substantial (p<0.05) increase in the *H. pylori* positive group. As a result, we concluded that neutrophil, lymphocytes and NLR counts are useful indicators of the inflammatory stages of an *H. pylori* infection. They could turn into helpful guidelines for the investigation. The average age of the patients in our study who tested positive for *H. pylori* was found to be equal to the patients who tested negative, therefore no statistically significant difference was found. Age and *H. pylori* positive may be correlated, although no meaningful relationship between the two has been found in the many previous research that have investigated this possibility (Koc and Gedikli 2022; Dorji et al. 2014). In addition we found no statistically significant difference was seen (p>0.05), even though the *H. pylori*-positive patient group had a higher mean age. We propose that the previously proposed relationship between age and *H. pylori* positive is explained by differences in sample selection, financial status, and environmental variables in prior investigations (Benberin et al. 2013; Hanafi and Mohamed 2013). A study conducted the systemic inflammatory markers linked to *H. pylori* infection were assessed in order to differentiate between carriers who did not exhibit any symptoms and those who did. It was established through a study done specifically for this purpose that patients with *H. pylori* positivity had considerably higher levels of acute phase reactants (C-reactive protein) (Jackson et al. 2009). With age comes a rise in the percentage of children who are infected. In underdeveloped nations, the majority of children have *H. pylori* infection by the time they are ten (Eusebi, Zagari, and Bazzoli 2014). The mean age of *H. pylori* positive patients in our investigation was much greater than that of negative patients (p<0.05). In both groups, the female to male ratio was favourable to the female patients (p>0.05). 240 *H. pylori* negative patients had an average age of 10.0±5.8 years. These findings indicate that *H. pylori* infection is more prevalent in the elderly and females. Furthermore, a new analysis reveals that within the H. pylori positive group, women predominated. On the other hand, there was no discernible difference between the two groups (H. pylori both positive and negative) in terms of age or gender (Kovacheva-Slavova et al. 2021). The impact of *H. pylori* positive and negative paediatric gastritis on parameters such as erythrocytes, thrombocytes, MPV, NLR, and PLR was assessed in the study carried out by Melit et al. This study found no significant correlation between childhood and teenage gastritis in regards to count MPV, PLR, or NLR. It has been determined that severe gastritis may result in a considerable drop in Hb and Htc levels, and that a significant rise in lymphocyte count may indicate non-*H. pylori* paediatric gastritis (Melit et al. 2019).

A study conducted to measure the total leukocyte count and NLR in adult patients with peptic ulcers infected with *H. pylori* and asymptomatic people, and to assess if these parameters are related. Leukocyte, neutrophil count, and NLR were found to be considerably greater in patients with *H. pylori*-infected peptic ulcers and asymptomatic individuals than in the control group in this investigation. Additionally, there were notable changes in these characteristics between patients with peptic ulcers and those without any symptoms (Jafarzadeh et al. 2013). Other study found NLR and MPV levels did not correlate with childhood *H. pylori* infection, severity categorization, or treatment status before and after (Sahin, Gubur, and Tekingunduz 2020).

In our research, we observed that there was statistically significant disparity in the groups count between patients who tested positive for *H. pylori* and those who tested negative. However, we did find a statistically significant rise in neutrophil values (p<0.05) and a non-significant drop in lymphocytes in some groups values (p>0.05) among the *H. pylori* positive group. The NLR value in the *H. pylori* positive group was found to be considerably lower (p<0.05) compared to the *H. pylori* negative group. In contrast, the positive group for *H. pylori* exhibited a substantially greater in chronic disease groups value compared to the negative group for *H. pylori*

Table 1. Demographic features of patients at presentation

Patients Groups	**(n)**
Children	40
Pregnant women	46
Chronic diseases	52
Young patients	138
HP infection	**(n)**
Negative	120
Positive	120
HP positivity degree	**(n)**
Mild	33
Moderate	46
Sever	41
Participants' average age	45.56±12.16

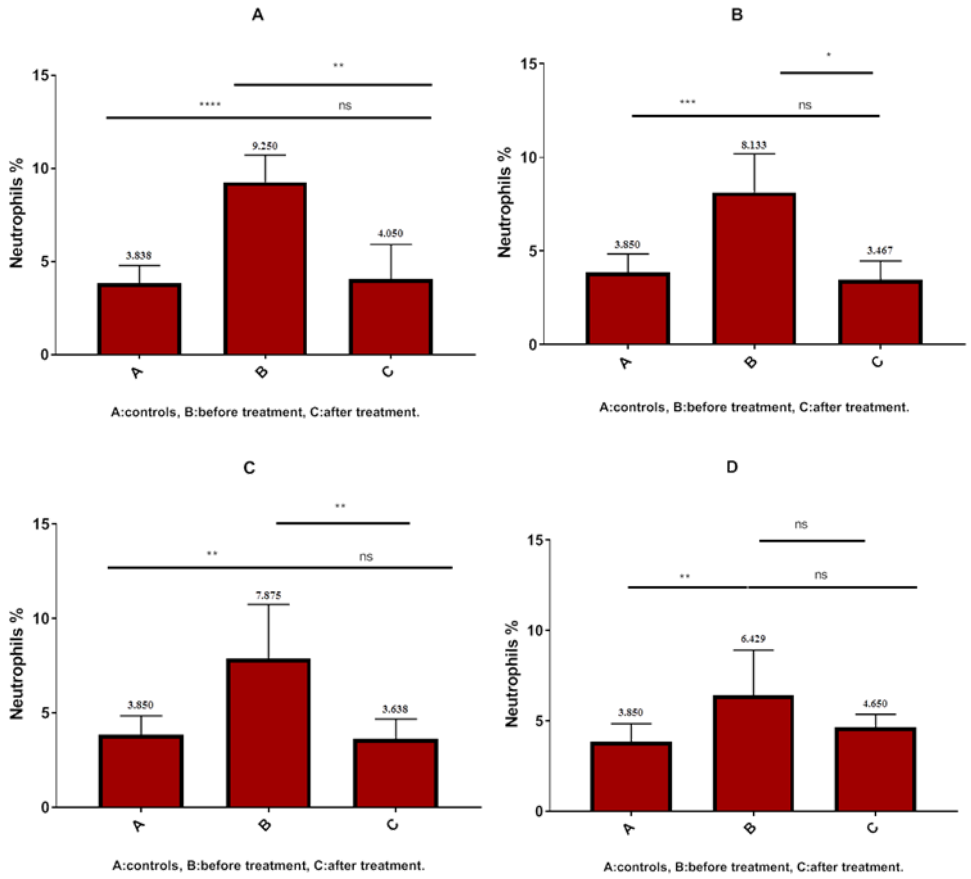

Figure 1. Neutrophils count for all groups, A: children groups, B: Pregnant women groups, C: Chronic disease groups, D: Young patient groups.

Table 2. Comparison of laboratory assessment (neutrophil, lymphocytes and NLR levels) for the patient group before treatment

Patients Groups	**Age (years)**	**Neutrophils values**	**Lymphocytes values**		**NLR* values**
Children	12-16	9.250±1.462	1.450±0.5196		7.200±3.573
Pregnant women	21-28	8.133±2.060	1.167±0.2517		6.850±2.193
Chronic diseases	50-75	7.875±2.866	2.000±1.086		4.273±1.495
Young patients	18-45	6.429±2.488	4.778±4.610		2.947±2.598

** NLR: Neutrophil-to-Lymphocyte Ratio*

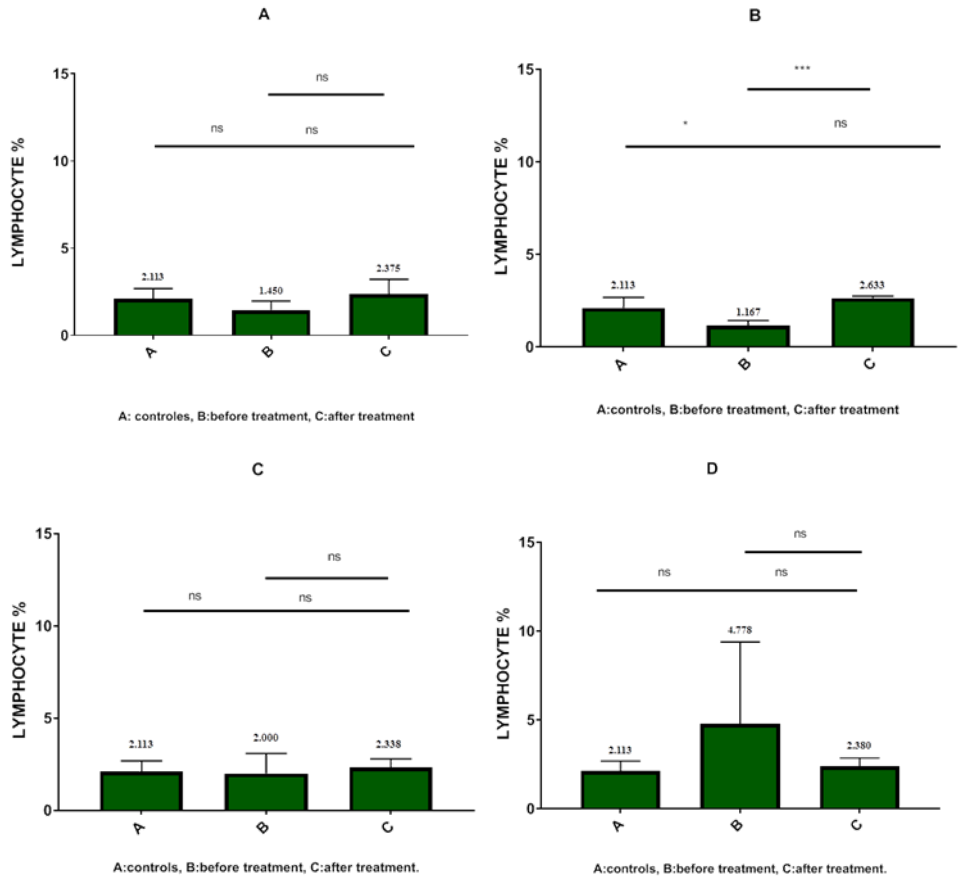

Figure 2. lymphocyte values for all groups, panel A childes group, panel B pregnant women group, panel C chronic diseases group, and C young patients' group.

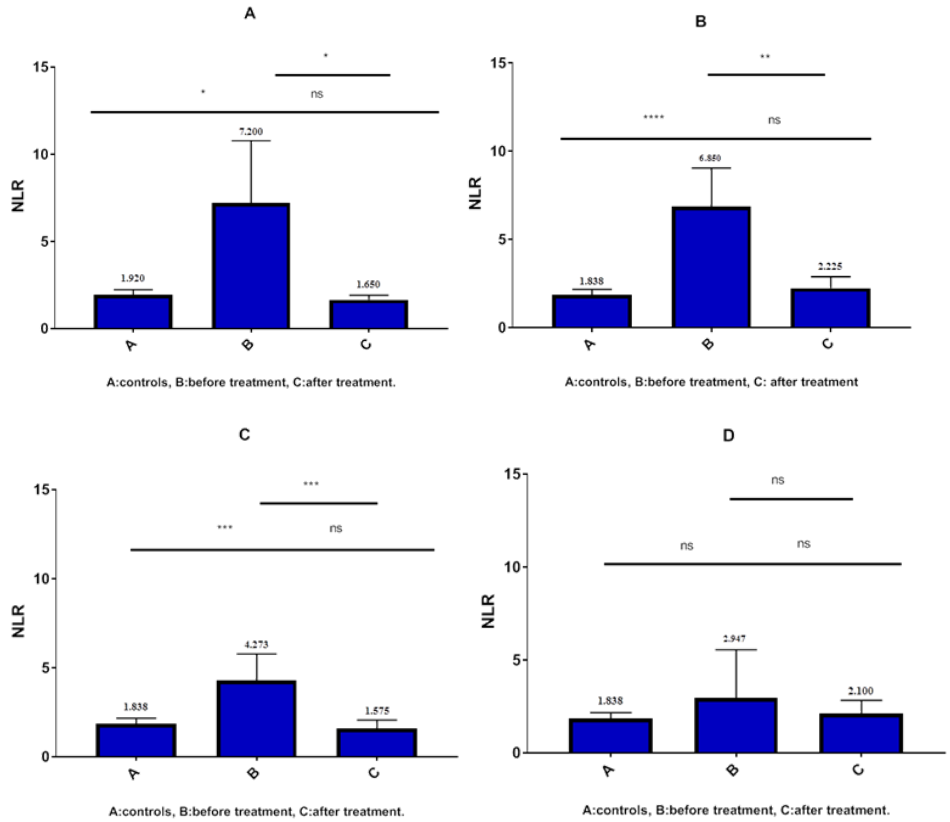

Figure 3. NLR values for all groups: A Childs group, B: pregnant women group, C: chronic disease patients, and D: young patients

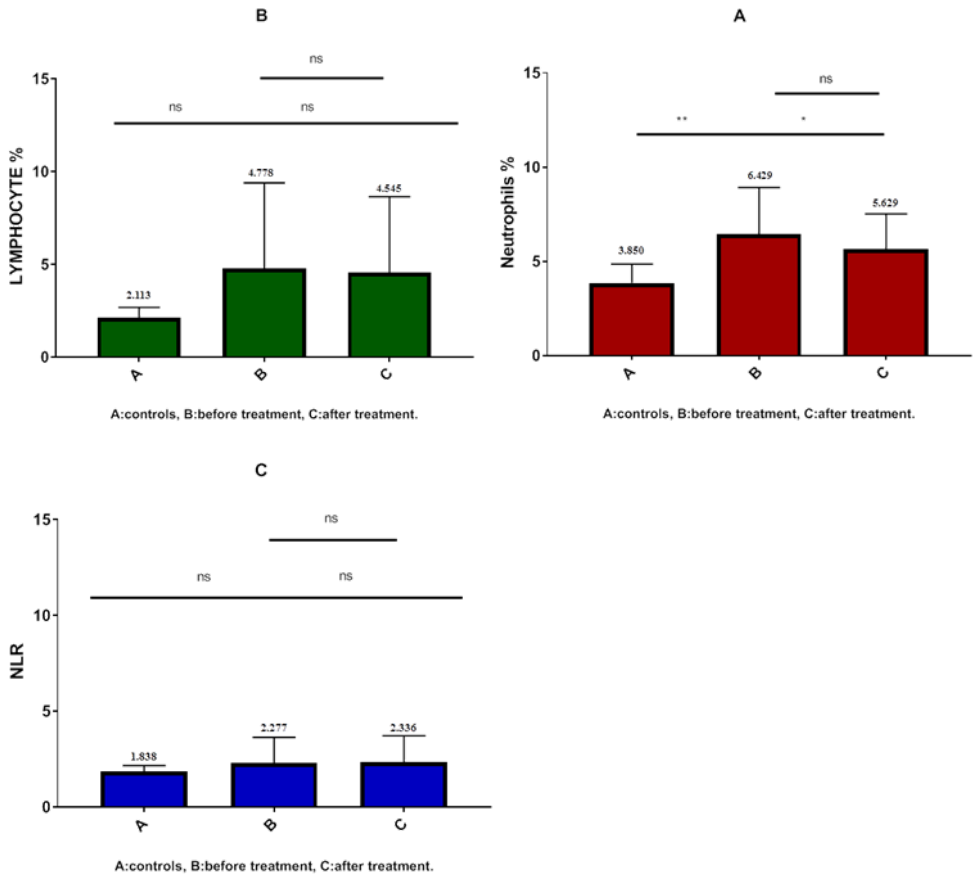

Figure 4. values after Brawn kit for young patients that still HP positive, A: neutrophils, B: lymphocytes, and C: NLR*

Table 3. Laboratory evaluation following a week of providing young age group with the HP combo kit (Brawn kit).

Young age	(n)	Neutrophils values	Lymphocytes values	NLR* values
HP positive	134	9.250±1.462	1.450±0.5196	7.200±3.573
HP negative	4	4.65±0.7141	2.380±0.4764	2.100±0.7176

* *NLR: Neutrophil-to-Lymphocyte Ratio*

Table 4. Comparison of laboratory assessment (neutrophil, lymphocytes and NLR levels) for the patient group following five weeks treatment.

Patients Groups	Neutrophils values	Lymphocytes values	NLR* values
Children	4.050±1.857	2.375±0.8382	1.650±0.2646
Pregnant women	3.467±1.002	2.633±0.1155	2.225±0.6551
Chronic diseases	3.638±1.042	2.388±0.4565	1.575±0.4773
Young patients*	4.047±1.159	2.282±0.7282	2.947±2.598

* *NLR: Neutrophil-to-Lymphocyte Ratio*

Table 5. Comparison of laboratory assessment (neutrophil, lymphocytes and NLR levels) for the patient group prior therapy.

Patients Groups	Neutrophils values	Lymphocytes values	NLR* values
Children	<0.0001	0.0798	0.0123
Pregnant women	0.0009	0.0241	<0.0001
Chronic diseases	0.0021	0.7991	0.0003
Young patients	0.0068	0.1142	0.3741

* *NLR: Neutrophil-to-Lymphocyte Ratio*

Table 6.

Young patients	Neutrophils values	Lymphocytes values	NLR* values
HP positive	0.0157	0.1101	0.3243
HP negative	0.1871	0.4012	0.2406

* *NLR: Neutrophil-to-Lymphocyte Ratio*

Table 8: P values for HP before and after treatment for the various patient groups

Patients Groups	HP before treatment	HP after treatment
Children	< 0.0001	0.4371
Pregnant women	0.299	0.1994
Chronic diseases	0.0001	0.4551
Young patients	0.0002	0.2271

Table 9: The mean±SD values for patient groups based on the classification of HP positive into mild, moderate, and severe categories

Patients Groups	Mild	Moderate	Severe
Children	2.225±0.9356	6.143±1.241	11.21±1.713
Pregnant women	2.578±0.8694	5.582±0.8445	10.37±1.526
Chronic diseases	2.260±0.6037	5.489±0.8145	15.31±5.093
Young patients	2.320±0.8464	5.413±0.9588	16.95±4.979

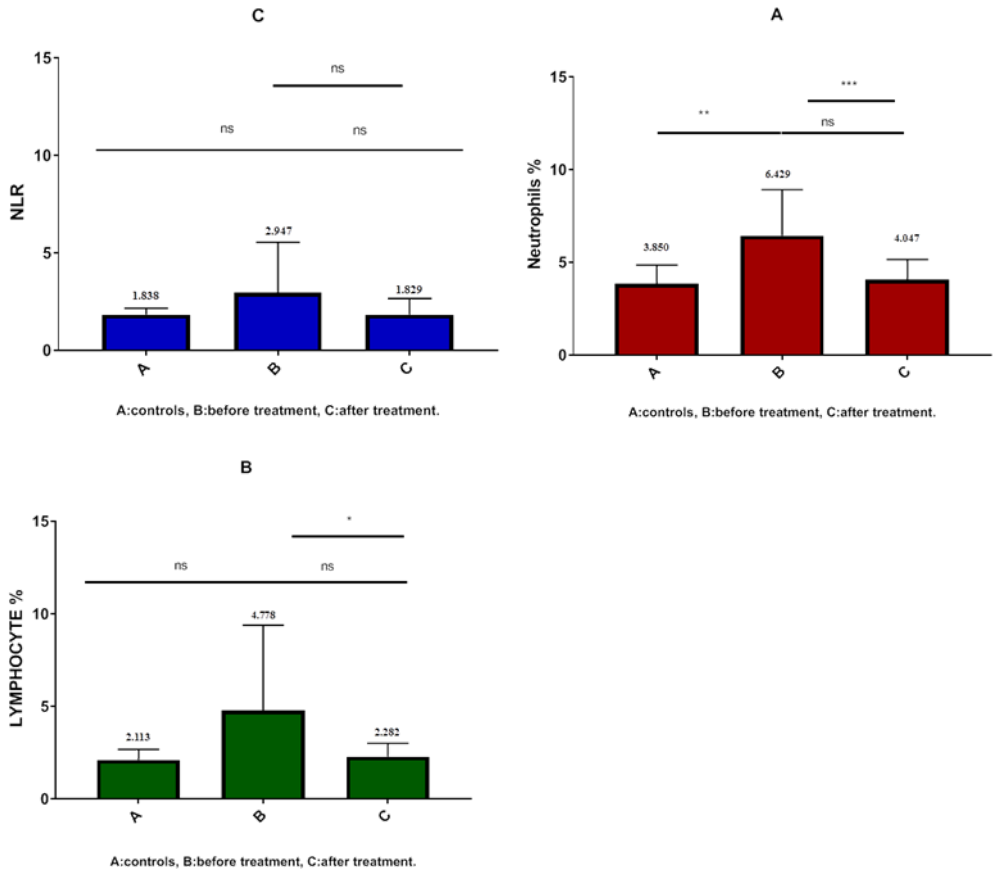

Figure 5. Different values after a unique way treatment for young patients that still positive HP after Brawn drug, A: neutrophils, B: lymphocytes, C: NLR

($p < 0.05$). Based on the obtained findings, it was hypothesised that the neutrophils a count and NLR may serve as reliable indicators of inflammation in cases of chronic gastritis and *H. pylori* infection. A notable rise in leukocyte and neutrophil-to-lymphocyte ratio (NLR) measurements was seen in a research conducted on adult individuals (Jafarzadeh et al. 2013). Additionally, another study revealed a considerable elevation in lymphocyte levels in patients diagnosed with severe chronic gastritis (Melit et al. 2019). Furthermore, the lack of variation in these parameters shown in our investigation including children might be attributed to the progressive nature of this illness, which tends to intensify with age, resulting in chronic inflammation and thus increasing the susceptibility to carcinogenesis. In the future, it may be beneficial to use these indicators when doing follow-up assessments on children with *H. pylori*.

The effective elimination of *H. pylori* infection is contingent upon a comprehensive understanding of the sensitivity of *H. pylori* to antibiotics and strict adherence to the prescribed treatment regimen (Schwarzer et al. 2016). The primary objective of first treatment is to achieve a minimum eradication rate of 90%. A high initial rate of eradication will effectively mitigate the establishment of antibiotic-resistant pathogens (Aguilera Matos et al. 2020). Nevertheless, inadequate rates of eradication were seen in children patients who had an *H. pylori* infection by using the first line treatment with Brawn kit components. Paediatric and pregnant women patients on *H. pylori* eradication treatment (Brawn kit) showed symptoms such as stomach discomfort, diarrhoea, nausea, and vomiting. The selection of an alternate first treatment for paediatric *H. pylori* infection should be based on the geographical rates of resistance to clarithromycin and metronidazole. The resistance of *H. pylori* to drugs exhibits regional and temporal variations. A research conducted in Zagreb revealed the presence of antibiotic resistance in treatment-naïve pediatrics patients infected with *H. pylori*. Specifically, the resistance rates were found to be 11.9% for clarithromycin, 10.1% for metronidazole, and 0.6% for amoxicillin (Hojsak et al. 2012). In 2015, a research used a registry encompassing nine European centers to document the rates of antibiotic resistance. The findings revealed that clarithromycin exhibited a resistance rate of 17.7%, while metronidazole shown a resistance rate of 18.6% (Schwarzer et al. 2016). According to a study conducted from 2005 to 2016, it was shown that 21% (46 out of 222) of *H. pylori* strains in Swedish children exhibited resistance to clarithromycin (Jansson and Agardh 2019). The present study conducted a meta-analysis on six studies including Iranian children, aiming to determine the prevalence rates of clarithromycin, metronidazole, and ciprofloxacin resistance. The findings revealed that the prevalence rates of resistance to clarithromycin, metronidazole, and ciprofloxacin were 12%, 71%, and 16%, respectively (Yousefi-Avarvand et al. 2018). The Vietnamese research revealed a significantly elevated resistance rate of 50.9% for clarithromycin and 65.3% for metronidazole among children aged 3-15 years (Nguyen et al. 2012). However, the resistance rate for amoxicillin remained low at 0.5%. In general, the prevalence of resistance to clarithromycin varied between 11.9% and 50.9%, while resistance to metronidazole ranged from 10.1% to 71%. The resistance rate to amoxicillin was found to be between 0.5% and 0.6%. Ciprofloxacin exhibited a resistance rate of 16% across different areas and time periods. In 2018, Savoldi et al. conducted a comprehensive systematic review with meta-analysis on the topic of antibiotic resistance in *Helicobacter pylori* across various World Health Organization (WHO) regions. The study encompassed a total of 178 studies conducted in 65 countries. The findings of this review indicated that the rates of resistance to clarithromycin, metronidazole, and levofloxacin were observed to be 15% across all WHO regions (Savoldi et al. 2018).

The occurrence of adverse effects was reported in many trials, with the majority of these occurrences being resolved with discontinuation of the medication. In their study, Bontems et al. (2011) observed that paediatric patients undergoing H. pylori eradication therapy experienced various adverse effects. Abdominal pain was reported in 20% of the patients, with a higher incidence observed in those receiving sequential therapy (24%) compared to triple therapy (17%). Diarrhoea was observed in 14% of the patients, with a slightly lower occurrence in the sequential therapy group (12%) compared to the triple therapy group (16%). Nausea was reported in 6% of the patients, with a higher prevalence in the sequential therapy group (8%) compared to the triple therapy group (5%). Vomiting occurred in 2% of the patients, with a higher incidence observed in the sequential therapy group (4%) compared to the triple therapy group (0%) (Bontems et al. 2011; Nyssen et al. 2016). Concomitant treatment is a viable option in the absence of susceptibility testing. In their study, Anania et al. used a concurrent treatment regimen consisting of proton pump inhibitor (PPI), amoxicillin, clarithromycin, and tinidazole for a duration of 5 days. The researchers reported a notable eradication rate of 93.3%. In their study, Zhou et al. conducted a comparison of four distinct treatment regimens. The results indicated that only bismuth-based therapy and concurrent medication exhibited significantly higher rates of eradication.

For the previous reasons, a new treatment strategy was presented in the current study. The treatment protocol was implemented for duration of five weeks, using consistent pharmacological agents but at varying dosages. Additionally, under the guidance and oversight of a medical professional, an additional medication was introduced (omeprazole, ondansetron, metronidazole and Clarithromycin). The therapy intervention was shown to result in improved patient well-being and increased medication adherence due to enhanced comfort levels. The subsequent testing results for *Helicobacter*

pylori (HP) infection yielded negative outcomes, and a reduction in the levels of the CBC values suggesting the effective eradication of the illness. The transmission of H. *pylori* by intrafamilial means is a significant pathway. The study conducted by Zhao et al. (year) found that implementing a comprehensive family-based treatment approach for *H. pylori* infection resulted in a partial improvement in eradication rates among children, as well as a reduction in recurrence rates compared to a treatment strategy focused only on individual patients (Osaki et al. 2015; Zhao et al. 2021). Nevertheless, more investigation is required to assess the advantages and disadvantages of family-based eradication treatment in terms of antibiotic exposure to both the host and the environment, as well as its impact on the gut microbiota.

Conclusions

The rates of eradicating children and pregnant women *H. pylori* infection, in comparison to adult populations, are deemed unsatisfactory regardless of the therapeutic approaches used, including triple therapy combikit (Brawn medicine) (lanzoprazole capsules 30mg, tinidazole tablets 500 mg, and clarithromycin tablets 250 mg) treatments. However, there is a desired eradication rate of 100% or above for second-line treatment. In accordance with current recommendations adapted from children and adult guidelines, it is advised to consider retreatment with the new treatment strategy concurrent medication for a duration of 5 weeks in cases where there is a presence of double resistance strains. Nevertheless, the treatment procedure continues to differ across different regions and is implemented based on clinical expertise without reaching a consensus. The efficacy of the regimen may demonstrate a higher level of effectiveness when compared to other regimens in some research; however, conflicting findings may be shown in other investigations. Further research is necessary to enhance the efficacy of eradication treatment for *H. pylori* infection in all patients groups. The optimal approach remains to be individualized therapy informed by cultural considerations, including antibiotic sensitivity testing. Furthermore, it is essential to generate regional statistics on the success rates of various therapeutic interventions in order to establish an effective policy.

Author contributions

D.R.R. 1, M.S.J. performed the tests, analyzed the data, and wrote the original draft.

Acknowledgment

The authors thanked the Department. The Authors equally contributed in each part of the study.

Competing financial interests

The authors have no conflict of interest.

References

Adachi, Teppei, Shigenaga Matsui, Tomohiro Watanabe, Kazuki Okamoto, Ayana Okamoto, Masashi Kono, Mitsunari Yamada, et al. 2017. "Comparative Study of Clarithromycin- versus Metronidazole-Based Triple Therapy as First-Line Eradication for Helicobacter Pylori." Oncology 93 (Suppl. 1): 15–19. https://doi.org/10.1159/000481224.

Aguilera Matos, Idalmis, Sarah Esther Diaz Oliva, Angel A Escobedo, Oscar Manuel Villa Jiménez, and Yamila del Carmen Velazco Villaurrutia. 2020. "Helicobacter Pylori Infection in Children." BMJ Paediatrics Open 4 (1): e000679. https://doi.org/10.1136/bmjpo-2020-000679.

Ali, I. H., Jabir, M. S., Al-Shmgani, H. S., Sulaiman, G. M., & Sadoon, A. H. (2018, May). Pathological And immunological study on infection with escherichia coli in ale balb/c mice. In Journal of Physics: Conference Series (Vol. 1003, No. 1, p. 012009). IOP Publishing).

Bagheri, Nader, Fatemeh Azadegan-Dehkordi, Hedayatollah Shirzad, Mahmoud Rafieian-Kopaei, Ghorbanali Rahimian, and Alireza Razavi. 2015. "The Biological Functions of IL-17 in Different Clinical Expressions of Helicobacter Pylori-Infection." Microbial Pathogenesis 81 (April): 33–38. https://doi.org/10.1016/j.micpath.2015.03.010.

Bagheri, Nader, Afshin Taghikhani, Ghorbanali Rahimian, Loghman Salimzadeh, Fatemeh Azadegan Dehkordi, Farid Zandi, Morteza Hashemzadeh Chaleshtori, Mahmoud Rafieian-Kopaei, and Hedayatollah Shirzad. 2013. "Association between Virulence Factors of Helicobacter Pylori and Gastric Mucosal Interleukin-18 MRNA Expression in Dyspeptic Patients." Microbial Pathogenesis 65 (December): 7–13. https://doi.org/10.1016/j.micpath.2013.08.005.

Benberin, Valery, Roza Bektayeva, Raushan Karabayeva, Aleksandr Lebedev, Kenzhekhan Akemeyeva, Lea Paloheimo, and Kari Syrjänen. 2013. "Prevalence of H. Pylori Infection and Atrophic Gastritis among Symptomatic and Dyspeptic Adults in Kazakhstan. A Hospital-Based Screening Study Using a Panel of Serum Biomarkers." Anticancer Research 33 (10): 4595–4602. http://www.ncbi.nlm.nih.gov/pubmed/24123036.

Bontems, Patrick, Nicolas Kalach, Giuseppina Oderda, Assad Salame, Laurence Muyshont, D. Yvette Miendje, Josette Raymond, Samy Cadranel, and Michèle Scaillon. 2011. "Sequential Therapy Versus Tailored Triple Therapies for Helicobacter Pylori Infection in Children." Journal of Pediatric Gastroenterology & Nutrition 53 (6): 646–50. https://doi.org/10.1097/MPG.0b013e318229c769.

Correa, P., and M.B. Piazuelo. 2008. "Natural History of Helicobacter Pylori Infection." Digestive and Liver Disease 40 (7): 490–96. https://doi.org/10.1016/j.dld.2008.02.035.

Correa, P. 1988. "A Human Model of Gastric Carcinogenesis." Cancer Research 48 (13): 3554–60. http://www.ncbi.nlm.nih.gov/pubmed/3288329.

Diaconu, S, A Predescu, A Moldoveanu, C S Pop, and C Fierbinţeanu-Braticevici. 2017. "Helicobacter Pylori Infection: Old and New." Journal of Medicine and Life 10 (2): 112–17. http://www.ncbi.nlm.nih.gov/pubmed/28616085.

Dorji, Dorji, Tashi Dendup, Hoda M. Malaty, Kinley Wangchuk, Deki Yangzom, and James M. Richter. 2014. "Epidemiology of H Elicobacter Pylori in B Hutan: The Role

of Environment and Geographic Location." Helicobacter 19 (1): 69–73. https://doi.org/10.1111/hel.12088.

Eusebi, Leonardo H., Rocco M. Zagari, and Franco Bazzoli. 2014. "Epidemiology of Helicobacter Pylori Infection." Helicobacter 19 (s1): 1–5. https://doi.org/10.1111/hel.12165.

Fox, James G., and Timothy C. Wang. 2007. "Inflammation, Atrophy, and Gastric Cancer." Journal of Clinical Investigation 117 (1): 60–69. https://doi.org/10.1172/JCI30111.

Hanafi, Manal I., and Aida M. Mohamed. 2013. "Helicobacter Pylori Infection." Journal of the Egyptian Public Health Association 88 (1): 40–45. https://doi.org/10.1097/01.EPX.0000427043.99834.a4.

Hojsak, Iva, Tea Kos, Jelena Dumančić, Zrinjka Mišak, Oleg Jadrešin, Alemka Jaklin Kekez, Amarela Lukić Grlić, and Sanja Kolaček. 2012. "Antibiotic Resistance of Helicobacter Pylori in Pediatric Patients — 10 Years' Experience." European Journal of Pediatrics 171 (9): 1325–30. https://doi.org/10.1007/s00431-012-1722-8.

Jackson, Louisa, John Britton, Sarah A. Lewis, Tricia M. McKeever, John Atherton, Donna Fullerton, and Andrew W. Fogarty. 2009. "A Population-Based Epidemiologic Study of Helicobacter Pylori Infection and Its Association with Systemic Inflammation." Helicobacter 14 (5): 460–65. https://doi.org/10.1111/j.1523-5378.2009.00711.x.

Jafarzadeh, A, V Akbarpoor, M Nabizadeh, M Nemati, and M T Rezayati. 2013. "Total Leukocyte Counts and Neutrophil-Lymphocyte Count Ratios among Helicobacter Pylori-Infected Patients with Peptic Ulcers: Independent of Bacterial CagA Status." The Southeast Asian Journal of Tropical Medicine and Public Health 44 (1): 82–88. http://www.ncbi.nlm.nih.gov/pubmed/23682441.

Jansson, Love, and Daniel Agardh. 2019. "Prevalence of Clarithromycin-Resistant Helicobacter Pylori in Children Living in South of Sweden: A 12-Year Follow-Up." Scandinavian Journal of Gastroenterology 54 (7): 838–42. https://doi.org/10.1080/00365521.2019.1637452.

Jim, Melissa A., Paulo S. Pinheiro, Helena Carreira, David K. Espey, Charles L. Wiggins, and Hannah K. Weir. 2017. "Stomach Cancer Survival in the United States by Race and Stage (2001-2009): Findings from the CONCORD-2 Study." Cancer 123 (S24): 4994–5013. https://doi.org/10.1002/cncr.30881.

Koc, Suleyman, and Mustafa Asım Gedikli. 2022. "The Role of Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratios in Predicting H Pylori Positivity And Severity in Patients with Chronic Gastritis." Cumhuriyet Medical Journal, March. https://doi.org/10.7197/cmj.1084756.

Kovacheva-Slavova, M, H Valkov, T Angelov, R Tropcheva, and B Vladimirov. 2021. "Screening for Helicobacter Pylori Infection and Clarithromycin Resistance Using Real-Time Polymerase Chain Reaction." European Review for Medical and Pharmacological Sciences 25 (15): 5042–46. https://doi.org/10.26355/eurrev_202108_26461.

Melit, Lorena Elena, Maria Oana Mărginean, Simona Mocan, and Cristina Oana Mărginean. 2019. "The Usefulness of Inflammatory Biomarkers in Diagnosing Child and Adolescent's Gastritis." Medicine 98 (26): e16188. https://doi.org/10.1097/MD.0000000000016188.

Nguyen, Thi Viet Ha, Carina Bengtsson, Li Yin, Gia Khanh Nguyen, Thi Thu Ha Hoang, Dac Cam Phung, Mikael Sörberg, and Marta Granström. 2012. "Eradication of Helicobacter Pylori in Children in Vietnam in Relation to Antibiotic Resistance." Helicobacter 17 (4): 319–25. https://doi.org/10.1111/j.1523-5378.2012.00950.x.

Nyssen, Olga P, Adrian G McNicholl, Francis Megraud, Vincenzo Savarino, Giuseppina Oderda, Carlo A Fallone, Lori Fischbach, Franco Bazzoli, and Javier P Gisbert. 2016. "Sequential versus Standard Triple First-Line Therapy for Helicobacter Pylori Eradication." Cochrane Database of Systematic Reviews 2016 (6). https://doi.org/10.1002/14651858.CD009034.pub2.

Ortiz-Princz, D, G Daoud, A Salgado-Sabel, and M E Cavazza. 2016. "Helicobacter Pylori Infection in Children: Should It Be Carefully Assessed?" European Review for Medical and Pharmacological Sciences 20 (9): 1798–1813. http://www.ncbi.nlm.nih.gov/pubmed/27212173.

Osaki, Takako, Mutsuko Konno, Hideo Yonezawa, Fuhito Hojo, Cynthia Zaman, Michiko Takahashi, Shinichi Fujiwara, and Shigeru Kamiya. 2015. "Analysis of Intra-Familial Transmission of Helicobacter Pylori in Japanese Families." Journal of Medical Microbiology 64 (1): 67–73. https://doi.org/10.1099/jmm.0.080507-0.

Peek, Richard M., and Martin J. Blaser. 2002. "Helicobacter Pylori and Gastrointestinal Tract Adenocarcinomas." Nature Reviews Cancer 2 (1): 28–37. https://doi.org/10.1038/nrc703.

Polk, D. Brent, and Richard M. Peek. 2010. "Helicobacter Pylori: Gastric Cancer and Beyond." Nature Reviews Cancer 10 (6): 403–14. https://doi.org/10.1038/nrc2857.

Razavi, Alireza, Nader Bagheri, Fatemeh Azadegan-Dehkordi, Mahsa Shirzad, Ghorbanali Rahimian, Mahmoud Rafieian-Kopaei, and Hedaytollah Shirzad. 2015. "Comparative Immune Response in Children and Adults with H. Pylori Infection." Journal of Immunology Research 2015: 1–6. https://doi.org/10.1155/2015/315957.

Sahin, Yasin, Ozlem Gubur, and Emine Tekingunduz. 2020. "Relationship between the Severity of Helicobacter Pylori Infection and Neutrophil and Lymphocyte Ratio and Mean Platelet Volume in Children." Archivos Argentinos de Pediatria 118 (3): e241–45. https://doi.org/10.5546/aap.2020.eng.e241.

Salimzadeh, Loghman, Nader Bagheri, Behnam Zamanzad, Fatemeh Azadegan-Dehkordi, Ghorbanali Rahimian, Morteza Hashemzadeh-Chaleshtori, Mahmoud Rafieian-Kopaei, Mohammad Hossein Sanei, and Hedayatollah Shirzad. 2015. "Frequency of Virulence Factors in Helicobacter Pylori-Infected Patients with Gastritis." Microbial Pathogenesis 80 (March): 67–72. https://doi.org/10.1016/j.micpath.2015.01.008.

Savoldi, Alessia, Elena Carrara, David Y. Graham, Michela Conti, and Evelina Tacconelli. 2018. "Prevalence of Antibiotic Resistance in Helicobacter Pylori: A Systematic Review and Meta-Analysis in World Health Organization Regions." Gastroenterology 155 (5): 1372-1382.e17. https://doi.org/10.1053/j.gastro.2018.07.007.

Schwarzer, Andrea, Patrick Bontems, Pedro Urruzuno, Nicolas Kalach, Barbara Iwanczak, Elefteria Roma-Giannikou, Josef Sykora, et al. 2016. "Sequential Therapy for Helicobacter Pylori Infection in Treatment-naïve Children." Helicobacter 21 (2): 106–13. https://doi.org/10.1111/hel.12240.

Soutto, Mohammed, Judith Romero-Gallo, Uma Krishna, M. Blanca Piazuelo, M. Kay Washington, Abbes Belkhiri, Richard M. Peek, and Wael El-Rifai. 2015. "Loss of TFF1 Promotes Helicobacter Pylori -Induced β-Catenin Activation and Gastric Tumorigenesis." Oncotarget 6 (20): 17911–22. https://doi.org/10.18632/oncotarget.3772.

Sung, Hyuna, Jacques Ferlay, Rebecca L. Siegel, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, and Freddie Bray. 2021. "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries." CA: A Cancer Journal for Clinicians 71 (3): 209–49. https://doi.org/10.3322/caac.21660.

Suzuki, Tatsuhiko, Katsuaki Kato, Shuichi Ohara, Kenji Noguchi, Hitoshi Sekine, Hiroshi Nagura, and Tooru Shimosegawa. 2002. "Localization of Antigen-presenting Cells in Helicobacter Pylori -infected Gastric Mucosa." Pathology International 52 (4): 265–71. https://doi.org/10.1046/j.1440-1827.2002.01347.x.

Yousefi-Avarvand, Arshid, Hamid Vaez, Mohsen Tafaghodi, Amir Hossein Sahebkar, Mohsen Arzanlou, and Farzad Khademi. 2018. "Antibiotic Resistance of Helicobacter Pylori in Iranian Children: A Systematic Review and Meta-Analysis." Microbial Drug Resistance 24 (7): 980–86. https://doi.org/10.1089/mdr.2017.0292.

Zhao, Jun-Bo, Lin Yuan, Xue-Chun Yu, Qiao-Qiao Shao, Jing Ma, Miao Yu, Yue Wu, et al. 2021. "Whole Family—Based Helicobacter Pylori Eradication Is a Superior Strategy to Single-infected Patient Treatment Approach: A Systematic Review and Meta-analysis." Helicobacter 26 (3). https://doi.org/10.1111/hel.12793.

Zhu, Shoumin, Mohammed Soutto, Zheng Chen, DunFa Peng, Judith Romero-Gallo, Uma S Krishna, Abbes Belkhiri, M Kay Washington, Richard Peek, and Wael El-Rifai. 2017. "Helicobacter Pylori- Induced Cell Death Is Counteracted by NF-κB-Mediated Transcription of DARPP-32." Gut 66 (5): 761.1-762. https://doi.org/10.1136/gutjnl-2016-312141.