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Original Article

Influence of Helicobacter pylori Treatment Protocol on Vitamin B12 Bioavailability in Pediatric Libyan Patients

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A B S T R A C T

Helicobacter pylori (H. pylori) infection is a common gastrointestinal condition associated with impaired micronutrient absorption, particularly cobalamin (vitamin B12). Vitamin B12 deficiency in children may contribute to hematological and neurological complications. This study aims to evaluate the influence of various H. pylori eradication protocols on the bioavailability of vitamin B12 in pediatric Libyan patients. A prospective cohort study was conducted at Souq Al Khamis Hospital, Al Khoms City, from October 2023 to March 2024. Thirty children with biopsy-confirmed H. pylori gastritis were enrolled. Patients received standard eradication therapy based on the current treatment protocol. Serum vitamin B12 levels, hematologic indices (hemoglobin, MCV, RDW), and symptom duration were assessed before and after treatment. Statistical analysis evaluated changes in vitamin B12 levels and their correlation with clinical and laboratory parameters. Post-treatment assessment demonstrated a significant increase in serum vitamin B12 levels following H. pylori eradication. A positive correlation was observed between post-treatment vitamin B12 levels and hemoglobin concentration. Conversely, an inverse correlation was found between vitamin B12 levels and both symptom duration and indices of red cell distribution (MCV, RDW). Children with a longer history of symptoms showed a less pronounced response in B12 recovery, suggesting early intervention improves outcomes. Eradication of H. pylori infection leads to significant improvement in vitamin B12 bioavailability in pediatric patients. The findings support incorporating H. pylori screening and treatment in the diagnostic workup of pediatric patients presenting with unexplained anemia or suspected micronutrient deficiencies.

Introduction

Helicobacter pylori (H. pylori) is a gram-negative, spiral-shaped bacterium first identified by Warren and Marshall in the late 1970s. Since then, it has been recognized as a highly prevalent pathogen worldwide, particularly in developing countries, where infection commonly begins in childhood and persists throughout life. H. pylori colonizes the gastric mucosa and is strongly associated with chronic gastritis, peptic ulcer disease, and long-term complications, including gastric mucosa–associated lymphoid tissue (MALT) lymphoma and gastric carcinoma [1,2].

Vitamin B12 (cobalamin) is an essential, water-soluble micronutrient obtained primarily from animal-derived foods. Its absorption depends on the integrity of the gastric mucosa and the availability of intrinsic factor (IF), which binds dietary B12 and facilitates uptake in the terminal ileum. Any disruption of gastric acid secretion, parietal cell function, or intrinsic factor production can impair B12 absorption. Pernicious anemia and chronic atrophic gastritis are among the most common pathological causes of impaired cobalamin bioavailability [3]. Vitamin B12 plays a critical role in cellular metabolism, DNA synthesis, hematopoiesis, and maintenance of neuronal myelination. Deficiency may lead to macrocytic anemia, glossitis, neurocognitive impairment, peripheral neuropathy, and, in severe or prolonged cases, irreversible neurological damage [4].

In pediatric populations, H. pylori infection has been identified as a contributor to impaired B12 absorption due to chronic gastritis, reduced acid secretion, and compromised intrinsic factor availability. Consequently, eradication of H. pylori may offer significant improvement in cobalamin bioavailability, preventing hematological and neurodevelopmental complications. Understanding the impact of different H. pylori treatment protocols on B12 levels is particularly relevant in Libya, where infection rates remain high, and treatment regimens vary [5]. This study was conducted to evaluate the effect of different H. pylori eradication treatment protocols on vitamin B12 bioavailability in pediatric Libyan patients.

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Methods

Study design and patients

This prospective cohort study was conducted at Souq Al Khamis Hospital, Al-Khoms City, from October 2023 to March 2024. A total of 30 pediatric patients presenting with upper gastrointestinal symptoms and confirmed H. pylori infection were enrolled. Diagnosis of H. pylori infection was established using endoscopic biopsy and histopathological examination, ensuring high diagnostic accuracy.

Ethical approval

Ethical approval was obtained from the Research Ethics Committee of Al-Marqab University (Institutional Review Board). Written informed consent was secured from patients' parents or legal guardians. All procedures adhered to the ethical principles outlined in the Declaration of Helsinki for research involving human subjects.

Sample Size

Based on the expected number of eligible cases attending the hospital (approximately 5 cases/month), a comprehensive sample of 30 patients fulfilling the inclusion criteria was selected.

Inclusion Criteria

The inclusion criteria required that participants have a confirmed diagnosis of *Helicobacter pylori* infection established through biopsy and histological examination. Eligible individuals were between 5 and 18 years of age, and both males and females were included. Only patients who had not received any previous treatment for *H. pylori* infection were considered for enrollment.

Exclusion criteria

Individuals were excluded if they had inflammatory bowel disease, liver disease, celiac disease, diabetes mellitus, or any other systemic condition requiring continuous medical therapy. Patients were also excluded if they had used steroids, proton pump inhibitors, antibiotics, antacids, nonsteroidal anti-inflammatory drugs, or H2-receptor antagonists within the preceding month. Additional exclusion criteria included a history of eating disorders, cachexia, malnutrition, or being underweight, as well as any prior intake of vitamin B12 supplements. All enrolled patients underwent comprehensive history taking. This included personal history, the nature and duration of presenting symptoms, and an evaluation of potential risk factors such as parental educational level, household overcrowding, exposure to unsanitary living conditions, and bed-sharing practices. Dietary habits were assessed, with particular attention to excessive consumption of tea, coffee, spicy foods, and fats. The history also covered extra-gastrointestinal manifestations potentially associated with *H. pylori* infection, detailed medication use, and any family history of gastrointestinal disorders.

Clinical examination including

Anthropometric measurements, assessment of vital signs, a general physical examination, and a comprehensive systemic evaluation were performed for all participants. Each patient subsequently underwent upper gastrointestinal endoscopy, during which gastric mucosal biopsies were obtained for histopathological examination. Patients diagnosed with Helicobacter pylori infection received a two-week course of triple therapy consisting of clarithromycin at a dose of 15 mg/kg, metronidazole at 20 mg/kg, and a proton pump inhibitor at 1 mg/kg, administered according to standard therapeutic protocols.

Follow up

Patients were reassessed by serum B12 and CBC one month following the end of treatment for the H. Pylori infection; they were not given PPI or vitamins during this time.
An endoscopic examination was performed using a Pentax upper gastroduodenoscopy (IPEX-5000). The following were the reasons it was done: 1. The identification of significant endoscopic anomalies. 2. Gastric antrum and corpus biopsy specimens were obtained for histological analysis.

Histological examination

Antral and corpus biopsies are used for histological evaluation and to verify that H. pylori bacilli are present. assessment of gastritis by B-histopathology using the most recent Sydney system. After being immediately fixed in 105 formalin solution, the biopsies underwent automated tissue processing until paraffin embedding, after which they were serially sliced into two slides with 5-µm-thick sections. One slide was stained with hematoxylin and eosin for microscopic analysis in order to identify the degree of glandular atrophy, intestinal metaplasia, the presence of lymphoplasmocytic inflammatory infiltrate and density, the

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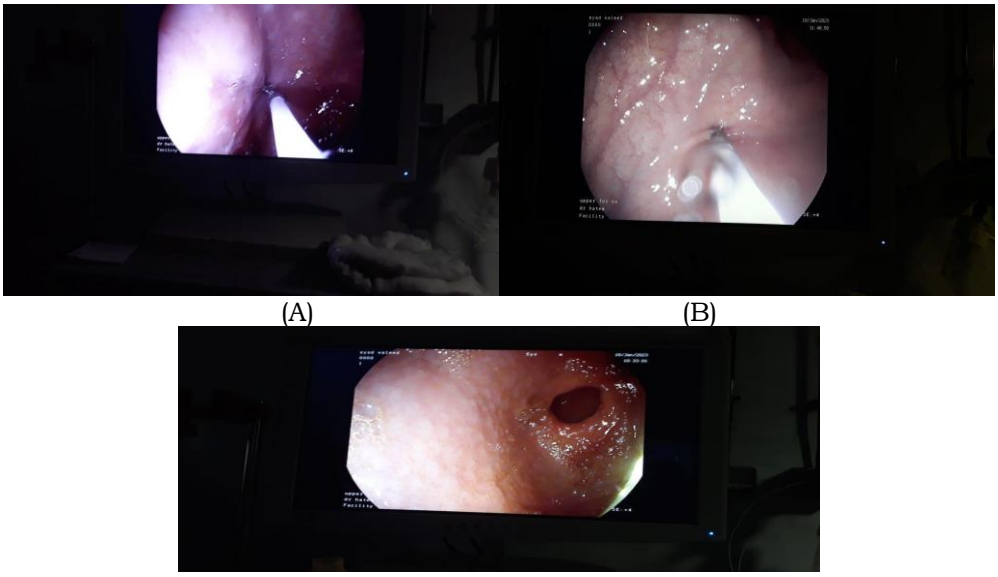
presence of lymphoid follicles, and the presence of neutrophils as a marker of inflammatory activity. Giemsa dye was applied to the other slide in order to detect H. Pylori bacilli under a microscope.

Histopathological assessment was conducted in accordance with the Updated Sydney classification. Neutrophilic activity was evaluated by examining polymorph infiltration within the lamina propria, gastric pits, and surface epithelium. In cases where polymorphs were scarcely detectable, activity was considered absent. Mild activity was defined as infiltration involving less than one-third of the pit and surface epithelium, whereas moderate activity corresponded to infiltration of approximately one-half to two-thirds. Severe activity was characterized by infiltration exceeding two-thirds of the pit and surface epithelium. Chronic inflammation was determined by the presence of increased plasma cells and lymphocytes in the lamina propria. When the density of these cells remained within normal limits, inflammation was graded as absent (grade 0). A slight increase in cellular density was classified as mild (grade 1), a moderate increase as moderate (grade 2), and a marked increase as severe (grade 3). Glandular atrophy, defined as the loss of specific glands from the gastric body or antrum, was also assessed. The absence of glandular loss was considered normal. A minimal reduction in glandular structures was classified as mild atrophy, a more pronounced but modest reduction as moderate atrophy, and extensive glandular loss as severe atrophy.



(A) (B)

(C)

Fig. (1). Endoscopic pictures of some of the studied patients; (A): biopsy taking, (B): Erythema, (C): fine nodularity

Laboratory investigations, including
Sampling

Following an overnight fast, ten milliliters of venous blood were drawn via sterile vein puncture from a peripheral vein. Five milliliters were collected on a plain tube, allowed to clot for twenty minutes, centrifuged for ten minutes at 3000 rpm, and then frozen at -20 °C until the vitamin B12 analysis. For the total blood count, 2 milliliters of blood were drawn using a K2 EDTA-containing tube. For PT, INR, and PTT, two milliliters of blood were drawn in a citrate-containing tube.

Routine laboratory investigations

The evaluation included a complete blood count assessing leucocytic count, hemoglobin concentration, and platelet count, performed both before and after treatment for *Helicobacter pylori*. In addition, a coagulation profile comprising prothrombin time, international normalized ratio, and partial thromboplastin time was obtained. Serum vitamin B12 levels were measured at baseline and again one month after completion of therapy to determine any treatment-related changes.

Specific laboratory investigations
Assessment of serum level of vitamin B12

Before and after therapy, the serum level of vitamin B12 was measured using an ELISA device that was acquired from SunRed with catalog number 201-12-1545.

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Statistical Analysis

Every piece of information was gathered, tabulated, and statistically examined using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., 2015; Armonk, NY). While quantitative data were displayed as the mean ± SD and median (range), qualitative data were displayed as numbers and percentages. The paired t-test was used to compare the paired continuous normally distributed variables. When comparing more than two sets of normally distributed variables, the ANOVA test was employed. The quantitative characteristics were correlated using Pearson's correlation coefficient (r). When two variables have an r value greater than 0.7, the link is usually regarded as strong. A number between -1 and 1 is usually the correlation coefficient, or r. When there is a positive correlation between the variables, the r-value is positive; when it is negative, the r-value is negative. Every test had two sides. P-values less than 0.05 were considered statistically significant, whereas those more than 0.05 were regarded as statistically non-significant (NS).

Results

The current Study included 30 children infected with H.pylori, 18 of them. There were 12 (40%) females and 60 (60%) males. Their mean age was 9.3±2.5 years, with a range of 6 to 15 years. The mean weight was 35.6±11.5 kg, the mean Height (cm) was 137.2 ±15.6, the mean Body mass index (kg/m^2) was 18.2 ± 4.5.

Table (1). Demographic characteristics of the studied group (n=30)

Sex	**No(%)**
Males	18(60%)
Males	12(40%)
Characteristic	Study group (n=24)
Age (years) Mean ±SD Median (Range)	 9.3 ±2.5 9 (6-15)
Weight (kg) Mean ±SD Median (Range)	 35.6±11.5 30.2 (18-65)
Height(cm) Mean ±SD Median (Range)	 137.2 ±15.6 136.5(108-158)
Body mass index(kg/m^2) Mean ±SD Median (Range)	 18.2 ± 4.5 17.3(12-32)

Duration of complaint from symptoms ranged from 5 to 35 months, with a mean of 12.5±8.4 years. The main complaint of H pylori infection was loss of appetite (63.3%). Then abdominal pain (46.7%), then vomiting (40%), in 10 patients reported epigastric pain (33.3%), 6 children (16.7%) had hematemesis, lastly 5 children (16.7%) had melena.

Table (2). Symptoms of the studied group (n=24)

Symptoms		**Study group (n=30)**	
Duration of symptoms (months=)	Mean ±SD Median (Range)	12.5 ±8.4 6 (5-35)	
		No.	%
Symptoms	loss appetite	19	63.3
	abdominal pain	14	46.7
	vomiting	12	40
	Epigastric pain	10	33.3
	hematemesis	6	20
	Melena	5	20.8

Treatment of H pylori infection improves the Hemoglobin level significantly by 7. 41%, P<0.05. MCV significantly decreased by 5.08%, and RDW decreased significantly by 10.24%, P<0.05. There is no difference in both WBC, PLT post-treatment, P>0.05.

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Table (3). Pre and post-CBC findings in the studied group

Investigations	Study time		% of improvement	t	p-value
	Pre treatment	Post treatment			
Hemoglobin Mean ± SD Median (range)	11.4± 1.1 11.4(9.7-13)	12.4±0.51 12.3(11.1-13.1)	7.41	4.73	.0001*
WBC Mean ± SD Median (range)	8.3±2.09 8.2(5.9-12.9)	8±1.7 7.8(5.4-12.8)	3.38	0.524	.605
MCV Mean ± SD Median (range)	83.7±4.6 83.7(75-90.5)	79.4±4.8 78(72-90.3)	-5.08	5	0.0001*
RDW Mean ± SD Median (range)	14.2±1.3 13.9(12.6-16.5)	12.7±0.91 12.35(11.1-15)	-10.24	4.7	0.0001*
PLT Mean ± SD Median (range)	289±61.3 293(173-385)	271±56.4 259(203-426)	6.11	1.265	0.219

*t: paired t-test, *P<0.05 significant*

Treatment of H. Pylori infection improves serum vitamin B12 level by 19.75%, P<0.05.

Table (4). Vitamin B12 level before and after treatment in the studied group

Vitamin B12(pg /L) Mean ± SD Median (range)	Study time		% of improvement		Paired t	p-value
	Pre treatment	Post treatment				
	1027.4±223.4 1051.4(415-1450)	1228±194.5 1250.7 (786-1500)	19.75	7.3	0.0001*	

*t: paired t-test, *P<0.05 significant*

The table shows a statistically significant positive association between the pretreatment vitamin B12 value and the hemoglobin value, indicating that when vitamin B12 levels rise, so do hemoglobin levels. While the pretreatment vitamin B12 value and the length of symptoms, MCV, and RDW have an inverse relationship, this indicates that vitamin B12 decreases with a prolonged H. pylori infection, and vitamin B12 decreases with an increase in MC, RDW, P<0.05.

Table (5). Correlation between (Vitamin B12 value pretreatment) and age, weight, height, BMI, head circumference, mid arm circumference, duration of symptom, HB, WBC, PLT, MCV, RDW(N=24)

Variables		Vitamin B12 pre-treatment	
		r	p
Age in years		0.029	0.892
Weight kg		-0.027	0.81
Height (cm)		-0.032	0.883
BMI (kg/ml)		-0.031	0.887
Duration of the symptom		-0.467*	0.021
Pre treatment	Hemoglobin	0.483*	0.017
	WBC	0.065	0.763
	PLT	-0.202	0.343
	MCV	-0.520**	0.009
	RDW	-0.446*	0.029

*r:Correlation coefficient >0.05 no significant ,*p<0.05 significant*

The table shows a statistically significant positive association between hemoglobin value and vitamin B12 value after therapy, indicating that when vitamin B12 levels rise, so do hemoglobin levels. While the duration of symptoms, MCV, and RDW are inversely correlated with the vitamin B12 value after treatment, this indicates that vitamin B12 does not react well to a prolonged H. pylori infection. A drop in MCV and RDW P<0.05 is linked to an increase in vitamin B12.

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Table (6). correlation between (Vitamin B12 post treatment) and age, weight, height, BMI, head circumference, mid arm circumference, duration of symptom, HB, WBC, PLT, MCV, RDW (N=24)

Variables		Vitamin B12 post-treatment	
		r	P
Age in years		0.082	0.702
Weight kg		-0.157	0.464
Height (cm)		-0.013	0.951
BMI (kg/ml)		-0.196	0.359
Duration of the symptom		-0.498*	0.013
Post treatment	Hemoglobin	0.600**	0.002
	WBC	0.324	0.39
	PLT	0.082	0.702
	MCV	-0.472*	0.02
	RDW	-0.611**	0.002

*R : Correlation coefficient >0.05 no significant ,*p<0.05 significant*

Discussion

Helicobacter pylori infections affect half of the world's population, a helical, flagellated, Gram-negative bacterium that has evolved specifically to live in the stomach lumen. It is regarded as the most effective human pathogen. It is a frequent and possibly treatable cause of peptic ulcer disease and dyspepsia. One of the causes of iron deficiency anemia (IDA) is an H. pylori infection, and it is very common in developing nations. However, this can be fixed with an H. pylori eradication program [7]. Children frequently suffer from vitamin B12 deficiency, which can lead to neurological disorders and megaloblastic anemia. A cluster of neurological symptoms in youngsters and immature erythrocytes in peripheral smears are its defining features, and vitamin B12 administration quickly improves them. Among children, its prevalence ranges from 7% to 50% [5]. Most H. pylori-infected patients do not have peptic ulcers or cancers, despite the fact that H. pylori is linked to both conditions, which can cause bleeding and iron-deficient anemia (IDA). Chronic gastritis, which is not linked to gastrointestinal bleeding, is typically present in infected individuals [8].

A H. pylori infection may result in reduced absorption of vitamin B12, which could result in a deficit [9].

Of the thirty infants infected with H. pylori in the current study, twelve (40%) were female and eighteen (60%) were male. They were between the ages of 6 and 15, with an average age of 9.3±2.5 years. Average height (cm) was 137.2 ±15.6, average weight was 35.6±11.5 kg, and average body mass index (kg/m2) was 18.2 ± 4.5. In agreement with our study, Akcam et al. [10] showed that the mean age was 10.1 ± 3 years, and Mwafy and Afana [9] showed that 50.7% of cases were males and 49.3% were females. The mean age was 10.1 ± 3 years, according to Akcam et al. [10], and Mwafy and Afana [9] revealed that 49.3% of cases were female and 50.7% of cases were male

The current study showed a mean of 12.5±8.4 years, and the duration of complaints from symptoms varied from 5 to 35 months. The primary symptom of a H pylori infection was appetite loss (63.3%). Five children (16.7%) experienced hematemesis and melena, followed by abdominal pain (46.7%) and vomiting (40%). Ten kids also complained of epigastric discomfort (33.3%). Makhmonov et al. [11] discovered that morning headaches, fainting, dizziness, increased susceptibility to infections, changes in skin and mucous membranes, weakness, increased fatigue, impaired attention, anxiety, forgetfulness, irritability, and dyspeptic disorders are some of the symptoms that patients with H. pylori infections experience and that negatively impact their quality of life.

The current study showed that when H pylori infection is treated, hemoglobin levels rise by 7.41%, P<0.05. Both MCV and RDW significantly decreased by 5.08% and 10.24%, respectively, with a P-value of less than 0.05. Following treatment, neither WBC nor PLT changed. P < 0.05. In agreement with our study, Kurekci et al. (12) demonstrated that children's anemia resolved when the H. pylori infection was eradicated without iron therapy. Elsaghier et al. [4] revealed that the mean hemoglobin level, as determined by CBC, was 11.38 ± 1.31, which was below the normal threshold. Mwafy and Afana [9] discovered that at the start of the trial, the Hb, RBC, Hct, and MCHC levels were noticeably below normal.

Ciacci et al. [13] proposed a potential pathogenic explanation for anemia and explained it by citing poor iron absorption from chronic gastritis and hypochlorhydria, as well as blood loss from chronic erosive gastritis.

The current study demonstrated that serum vitamin B12 levels are considerably improved by 19.75%, P<0.05, when H. pylori infection is treated. In agreement with our study, Mwafy and Afana [9] demonstrated that OAC treatment resulted in a considerable rise in mean vitamin B12 levels (pretreatment: 137.5 ± 19.5 pg/mL; posttreatment: 317.28 ± 65.26 pg/mL), with a 230.8% increase (p-value <0.001).

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Also, Serin et al. [14] revealed that following H. pylori eradication treatment, there was a significant improvement in the serum level of vitamin B12, a drop in antrum and corpus inflammation, and a decrease in the neutrophil activation score. Furthermore, Cai et al. [15] discovered that following effective H. pylori eradication medication, the serum vitamin B12 levels of individuals with H. pylori improved. However, a pediatric study that looked into the relationship between H. pylori infection and vitamin B12 insufficiency found no evidence of a strong correlation between vitamin B12 deficiency and H. pylori infection [16]. This discrepancy could result from various laboratory test normal values and methods.

The current study showed that Hemoglobin levels and vitamin B12 levels before therapy showed a statistically significant positive connection, indicating that when vitamin B12 levels rise, so do hemoglobin levels. Although the pretreatment vitamin B12 value and the duration of symptoms have an inverse relationship, vitamin B12 decreases with a prolonged H. pylori infection, and vitamin B12 decreases with an increase in MC, RDW $P<0.05$. Also, Hemoglobin and vitamin B12 levels after therapy showed a statistically significant positive connection, indicating that when vitamin B12 levels rise, hemoglobin levels. While the duration of symptoms, MCV, and RDW are inversely correlated with the vitamin B12 value after treatment, this indicates that vitamin B12 does not react well to a prolonged H. pylori infection. A drop in MCV and RDW $P<0.05$ is linked to an increase in vitamin B12. In agreement with our study, Mwafy et al. [9] demonstrated that significant relationships between vitamin B12 and other hematological and biochemical indicators were found by analysis employing the Pearson correlation coefficient. A negative connection with RDW and positive correlations with Hb, RBC, and Hct ($r = 0.5, 0.7, 0.7$, and 0.6, respectively; p-value <0.001) are among these noteworthy associations.

Hemoglobin was much below normal prior to beginning the Omperazol-Amoxicillin-Clarithromycin therapy regimen. The findings also showed statistically significant correlations between H. pylori and anemia, with those who test positive for the bacteria having a 3.5-fold increased risk of anemia in comparison to healthy persons [17]. This mechanism could explain the occurrence of anemia in H. pylori-infected patients.

A persistent H. pylori infection in the stomach mucosa may hinder vitamin B12 absorption. [18]. Salgueiro et al. [19] discovered that the presence of an H. pylori infection was associated with reduced levels of vitamin B12, folate, vitamin A, and vitamin C. Ravi et al. [20] showed that there was a significant relation between H. pylori status and serum B12 level. Ulasoglu et al. [21] showed that serum vitamin B12 levels were negatively impacted by H. pylori, in contrast to prior observational studies. Kakehasi et al. [22] and Soyocak et al. [23] found that there was no connection between H. pylori and vitamin B12. Different methods for measuring H pylori, vitamin B12, and infection duration could be the cause of this discrepancy.

Conclusion

The findings of our study imply that H. pylori infection has a reversible detrimental impact on children's vitamin B12 status and seems to reduce vitamin B12 levels and certain hematological markers. However, following treatment protocol with omeprazole, clarithromycin, and metronidazole, they nearly returned to normal. Since H. pylori is linked to iron and vitamin B12 deficiencies, it could be a helpful indicator and potential treatment for anemic youngsters with gastritis. Further studies on a large sample of patients are required for more comprehensive statistical analysis and better conclusions.

Conflict of interest. Nil

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