Original Article

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Vitamin B12 Status in Children with *Helicobacter pylori* Gastritis

Helicobacter pylori Gastriti Tanılı Çocuklarda Vitamin B12 Düzeyi

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ABSTRACT

Objective: *Helicobacter pylori is rdescribed as the major etiological factor for gastritis and also associated with gastroesophageal reflux disease, and vitamin B12 deficiencies. Therefore, we aimed to evaluate the relationship between Helicobacter pylori gastritis and vitamin B12 status as well as to determine prevalence of esophagitis in children with H.pylori infections.*
Method: *A total number of 556 children who underwent eso-gastro-duodenoscopy were evaluated retrospectively. Diagnosis of H pylori infection, esophagitis, and gastritis was performed with histopathologic examination. Patients were divided into H.pylori (+) and (-) groups. Patients' demographic characteristics, physical examination, imaging and laboratory findings were recorded and evaluated.*
Results: *Patients included in the study consisted of 310 (55.8%) females, and 246 (44.2%) males. The mean age was significantly lower in males (9.43±5.69) than females (11.10±5.32) (p<0.001). The most common symptom was abdominal pain (41,5%). According to the histopathological examination H.pylori was positive in 24.5% (n=136) of our patients. Of the patients 28.6% (n=159) were diagnosed with esophagitis and 55.4% (n=308) with chronic gastritis. Esophagitis was detected in 30.1% of patients diagnosed with H.pylori and all chronic gastritis patients were found to be positive for H.pylori (p<0.001). There were no statistically significant differences found in mean levels of vitamin B12 between H.pylori negative and positive groups of patients with chronic gastritis. But the mean serum levels of vitamin B12 measured in the H.pylori positive group (382.93±245.50 pg/mL) was statistically significantly lower than H.pylori-negative group (467.90±305.36 pg/mL) (p=0.028). There were also no significant differences found in mean levels of iron between H.pylori-negative and positive groups.*
Conclusion: *Although all children with chronic gastritis were positive for H.pylori, our findings provide no evidence for a link between esophagitis, iron deficiency and H.pylori infection. In addition, H.pylori infection has been demonstrated to be a risk factor for vitamin B12 deficiency.*

Keywords: *bacterial meningitis, aseptic meningitis, mortality, pediatric intensive care unit*

ÖZ

Amaç: *Helicobacter pylori gastritin ana etiyolojik faktörü olarak tanımlanmakta, ayrıca gastroözofageal reflü hastalığı ve B12 vitamini eksiklikleri ile de ilişkilendirilmektedir. Bu nedenle, bizde H.pylori gastriti ve B12 vitamin statüsü arasındaki ilişki yanında H.pylori enfeksiyonu olan çocuklarda özofajit prevalansının değerlendirmeyi amaçladık.*
Yöntem: *Özogastroduodenoskopi yapılan toplam 556 çocuk retrospektif olarak değerlendirildi. H pylori enfeksiyonu, özofajit ve gastrit tanıları histopatolojik inceleme ile konuldu. Hastalar H.pylori pozitif ve negatif gruplara ayrıldı. Hastaların demografik özellikleri, fizik muayene, görüntüleme ve laboratuvar bulguları kaydedildi ve değerlendirildi.*
Bulgular: *Çalışmaya dahil edilen hastalar 310'u (%55,8) kız ve 246'sı (%44,2) erkek idi. Erkek hastalarda ortalama yaş (9,43±5,69) kızlardan (11,10±5,32) anlamlı şekilde düşüktü (p <0,001). Hastalarımızda en sık bildirilen semptom karın ağrısıydı (%41,5). Histopatolojik incelemeye göre H.pylori hastalarımızın % 24.5'inde (n=136) pozitifti. Hastaların % 28.6'sı (n=159) özofajit ve % 55.4'ü (n=308) kronik gastrit tanısı aldı. H.pylori tanısı alan hastaların % 30.1'inde özofajit saptandı ve tüm kronik gastrit hastalarının H.pylori için pozitif olduğu belirlendi (p<0,001). H.pylori negatif ve pozitif kronik gastritli hasta grupları arasında ortalama B12 vitamini düzeyleri açısından istatistiksel olarak anlamlı bir fark bulunmadı. Ancak H.pylori pozitif grupta (382,93±245,50 pg/mL) ölçülen ortalama B12 vitamin düzeyi istatistiksel olarak anlamlı şekilde H.pylori negatif gruptan (467,90±305,36 pg/mL) düşük olduğu belirlendi (p=0,028). Bununla beraber H.pylori negatif ve pozitif gruplar arasında ortalama demir düzeyleri açısıdan anlamlı bir fark bulunmadı.*
Sonuç: *Her ne kadar kronik gastritli tüm çocuklar H.pylori için pozitif olsa da, çalışmamızda H.pylori enfeksiyonu ile özofajit ve demir eksikliği arasında ilişki kurulamamıştır. Bunlara ek olarak, H.pylori enfeksiyonunun B12 vitamini eksikliği için bir risk faktörü olduğu gösterilmiştir.*

Anahtar kelimeler: *Helicobacter pylori, gastrit, Vitamin B12, özofajit*

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INTRODUCTION

H.pylori is a spiral-shaped, microaerophilic, noninvasive and gram-negative bacteria that colonize gastric epithelial cells with facilitation of flagellum-associated proteins, adhesins, and chemotactic activities [1]. It is the most common chronic bacterial infection worldwide particularly in developing countries (75-90%) [2]. *H.pylori* infection is generally acquired in early infancy particularly before the age of 10 years and persists throughout entire life without treatment [3]. The colonization of bacteria in stomach triggers gastric inflammation. Production of heat shock protein, urease and antigen presented by bacteria lead to activation of T-cells and increase in the levels of certain proinflammatory cytokines which results in gastric mucosal damage [4]. Thus, local and systemic immune responses of the host against *H.pylori* is related with chronic gastritis, peptic ulcer, mucosa-associated tissue lymphoid lymphoma and gastric cancer [5].

H.pylori is widely accepted as the major etiological factor for gastritis and peptic ulcer [6]. Although *H.pylori* infections are mostly asymptomatic, majority of patients develop acute gastritis which alters into chronic gastritis. In addition, childhood *H.pylori* infections are frequently associated with antral gastritis [7].

Recently, childhood *H.pylori* infections have been also associated with other digestive disorders such as gastroesophageal reflux disease. Moreover limited number of published data with controversial results, have indicated the presence of an inversely relation of *H.pylori* with esophagitis severity [8,9]. Besides, *Helicobacter pylori* gastritis has been linked to malabsorption and has been demonstrated as the potential causative agent of vitamin B12 deficiency in numerous studies [10,11]. Therefore, we aimed to evaluate the relationship between *Helicobacter pylori* gastritis and vitamin B12 status as well as to determine the prevalence of esophagitis in children with *H.pylori* infections.

MATERIALS and METHODS

This study was performed with the Institutional Review Board protocol approval date 18/02/2019 and number 2019/13 in Istanbul Dr. Sadi Konuk Training and Research Hospital, Department of Pediatric Gastroenterology, Hepatology and Nutrition, between January 2017 and June 2018. A total number of 556 children, aged between 0-18 years, who underwent eso-gastro-duodenoscopy were evaluated retrospectively. The diagnosis of *H.pylori inf*ection, esophagitis, and gastritis was made based on histopathologic examination. Patients were divided into *H.pylori* (+) and *H.pylori* (-) groups. Patients' demographic characteristics, physical examination, imaging and laboratory findings were recorded.

Blood cell count analysis was performed using patients' venous blood samples. Haematological parameters were analysed using a hematology analyser (Cell-Dyne 3700, Abbott, Abbott Park, IL, USA). Biochemical analysis was performed with serum samples using electro-chemiluminescence immunoassay on Beckman Coulter Unicel DXI 800 analyzer. Serum vitamin B12 analysis was performed using an immunodiagnostic system (Siemens, Advia Centaur xp, Germany) at a normality level of 220 pg/ml.

Statistical analysis

All the data were analysed with SPSS (Statistical Package for the Social Sciences) software for Windows (v21.0; IBM, Armonk, NY, USA). Individual and aggregate data were summarized using descriptive statistics including mean, standard deviations, medians (min-max), frequency distributions and percentages. Normality of data distribution was verified by Kolmogorov-Smirnov test. Comparison of the variables with normal distribution was made using Student t test. For the intergroup comparisons of variables which were not normally distributed, the Mann Whitney and Kruskal Wallis tests were performed. Evaluation of categorical variables was performed using chi-square test. P-Values of <0.05 were considered statistically significant.

RESULTS

Patients included in this study consisted of 310 (55.8%) females, and 246 (44.2%) males. Mean age of all patients (n=556) was 10.22±4.87 months (range: 0-18 years). In addition, the mean age was significantly lower in male patients (9.43±5.69) than

Table 1. Comparison of hronic gastritis and esophagitis rates in patients with *H.pylori*.

	Clinical Variables	*H.pylori* Negative n (%)	*H.pylori* Positive n (%)	p-value
Esophagitis	Absent	302 (71.9%)	95 (69.9%)	0.645
	Present	118 (28.1%)	41 (30.1%)	
Chronic gastritis	Absent	248 (59.6 %)	0 (0.0%)	0.000*
	Present	172 (41.0%)	136 (100.0)	

** = p<0.05 statistically significant.*

Table 2. Comparison of laboratory findings between *H.pylori* negative and positive groups in patients diagnosed with chronic gastritis.

Laboratory results	*H.pylori* Negative (Mean±SD)	*H.pylori* Positive (Mean±SD)	P-value
Iron (ug/dL)	64.83±48.75	68.62±35.75	0.230
Hemoglobin (g/dL)	12.46±1.32	12.74±1.54	0.050*
MCV (fL)	85.46±53.38	80.81±5.41	0.860
RDW (%)	13.64±1.98	13.52±1.60	0.848
PLT ($x10^9$/L)	303.8±897.5	332.4±985.0	0.058
Vitamin B12 (pg/mL)	431.7±263.4	382.9±245.5	0.171

** = p<0.05 statistically significant.*

Table 3. The comparison of laboratory findings between esophagitis- negative and positive groups in *H.pylori* positive cases.

Laboratory results	Esophagitis Negative (Mean±SD)	Esophagitis Positive (Mean±SD)	P-value
Iron (ug/dL)	66.44±33.96	73.71±40.19	0.606
Hemoglobin (g/dL)	12.54±1.56	13.19±1.40	0.121
MCV (fL)	80.41±5.39	81.70±5.45	0.496
RDW (%)	13.58±1.70	13.39±1.39	0.734
PLT ($x10^9$/L)	332.0±973.0	333.1±102.8	0.697
Vitamin B12 (pg/mL)	389.38±261.74	361.27±187.36	0.877

female patients (11.10±5.32) (p<0.001).

The most common symptom reported in our patients was abdominal pain seen in 41.5 % (n=220) of the cases, followed by abdominal pain + nausea (n=118, 22.3%), vomiting (n=57, 10.8%), and weight loss + sour liquid rushing into the mouth + puffiness + dysphagia (n=45, 8.5%) respectively. According to the histopathological examination *H.pylori* was positive in 24.5% (n=136) of our patients. In addition, 28.6% (n=159) of the patients were histopathologically diagnosed with esophagitis and 55.4% (n=308) of them with chronic gastritis. In our study, esophagitis was detected in 30.1% of patients diagnosed with *H.pylori*-positive and 28.1% of *H.pylori* -negative patients (p=0,645). Moreover, all of our patients with chronic gastritis were found to be positive for *H.pylori* (p<0,001) (Table 1).

According to the evaluation of laboratory findings; the mean values of: iron, hemoglobin, MCV, RDW, vitamin B12 and PLT in the study population were 70.67±42.40 ug/dl, 12.62±1.39 g/dL, 82.48±30.85 fL, 13.50±1.75 %, 448.11±294.28 pg/mL, and 315.8±103,8 $x10^9$/L, respectively. The comparison of laboratory findings between *H.pylori* -negative and positive groups in patients diagnosed with chronic gastritis is presented in Table 2. Mean level of hemoglobin was found to be statistically higher in *H.pylori* -positive group (12,74±1,54) than *H.pylori* -negative group (12,46±1,32) (p=0.05). Additionally, there were no statistically significant differences in mean serum levels of iron, MCV, RDW, vitamin B12 and POLİTİKA between *H.pylori* -negative and positive groups (p>0,05) (Table 2).

Furthermore, the comparison of laboratory findings between esophagitis- negative and positive groups in *H.pylori* -positive cases is presented in Table 3. Any statistically significant differences were found in mean serum levels of iron, hemoglobin, MCV, RDW, vitamin B12 and PLT between esophagitis -negative and positive groups (p>0,05) (Table 3).

Additionally, only *H.pylori*- positive and negative cases were compared according to the laboratory findings; the mean serum levels of vitamin B12 measured in the *H.pylori* -positive group (382,93±245,50 pg/mL) was statistically lower than the *H.pylori* -negative group (467,90±305,36 pg/mL) (p=0,028) (Table 4).

Table 4. Comparison of laboratory findings according to H.pylori diagnosis.

Laboratory results	*H.pylori* Negative (Mean±SD)	*H.pylori* Positive (Mean±SD)	P-value
Iron (ug/dL)	71.28±44.24	68.62±35.75	0.996
Hemoglobin (g/dL)	12.58±1.34	12.74±1.54	0.120
MCV (fL)	82.94±34.75	80.81±5.41	0.929
RDW (%)	13.50±1.79	13.52±1.60	0.515
PLT ($x10^9$/L)	311.1±104.0	332.4±985.0	0.064
Vitamin B12 (pg/mL)	467.90±305.36	382.93±245.50	0.028*

** = p<0.05 statistically significant.*

DISCUSSION

Helicobacter pylori infection is the most common infection worldwide and it is estimated that approximately half of the world's population is infected with Helicobacter pylori. Since majority of the infected patients are asymptomatic, it seems difficult to eradicate *H.pylori* infection. Moreover, *H.pylori* is commonly responsible for etiology of gastritis [7]. Development of chronic gastritis depends on bacterial virulence factors, host and environmental factors [3]. In a meta analysis, Weck et al. concluded that a very strong association existed between *H.pylori* infection and chronic gastritis by evaluating 66 relevant articles [12]. Similarly, Langner et al. reported chronic active gastritis in all children with *H.pylori* infection in their study investigating 132 gastric biopsies [13]. In accordance with these published data, in our study all of our patients with chronic gastritis were found to be positive for *H.pylori.*

Although a link has been identified between decreased serum iron status and childhood *H.pylori* infection, it is debated in published data whether *H.pylori* infection causes iron deficiency or iron deficiency anemia [14]. In a study, Vendt et al. found no relationship between *H.pylori* infection and iron deficiency in 363 children with *H.pylori* infection [15]. In addition, older age is documented to be more responsible for iron deficiency [15,16]. Supportively in our study, no statistically significant differences were found between *H.pylori* -negative and positive groups according to the mean serum iron levels.

It is appears to be controversy in the limited number of published data whether *Helicobacter pylori* infection is protective or triggering factor for gastroesophageal reflux disease. Prevalence of gastroesophageal reflux disease reported to be increased as a result of decrease in the *Helicobacter pylori* incidence [9]. Daugule et al. reported a higher prevalence of *H.pylori* in patients with reflux oesophagitis among 130 children [17]. Similarly, Moon et al. concluded that *Helicobacter pylori* infection is a risk factor for reflux oesophagitis development [18]. On the contrary, Emiroglu et al. found no significant association between the prevalence of *H.pylori* infection and reflux oesophagitis or the oesophagitis severity in 206 children [19]. Supportively, Zagorski et al. compared 308 children with reflux esophagitis and 418 patients with chronic gastritis without reflux esophagitis. *Helicobacter pylori* infection was detected in 44.5% of children with reflux esophagitis and it was not significantly differed in patients without reflux esophagitis. Researchers concluded that the development of reflux esophagitis was not associated with *Helicobacter pylori* infection [9]. In accordance with these data, 28.6% (n=159) of our patients were histopathologically diagnosed with esophagitis. Esophagitis was detected in 30.1% of patients diagnosed with *H.pylori* and 28.1% in *H.pylori* negative patients. Thus, *Helicobacter pylori* infection was not significantly effected prevalence of esophagitis in our study.

Although various conditions may lead to vitamin B12 deficiency, it is frequently caused by chronic gastritis. *H.pylori*-induced gastritis damages the parietal cells which are essential for vitamin B12 absorption. Kaptan et al. reported *Helicobacter pylori* infection in 56% of patients with pernicious anemia. Researchers demonstrated a post-treatment improvement of vitamin B12 in 40% of the patients [20]. In a study by Sarari et al. which, compared *H.pylori* -infected and non-infected patients consisting of 60 children, mean levels of vitamin B12 were 207.7+21.9 and 419.7+39.8, respectively (p=0,000). Vitamin B12 was found to be lower than 200 pg/ml in 67.4% of patients with *H.pylori* infection [21]. Similarly, Akcam et al. reported mean levels of vitamin B12 as 303±135 pg/mL in *Helicobacter pylori* positive and 393±166 pg/mL *Helicobacter pylori* -negative groups in a study included 50 children aged 5-18 years [22]. In accordance with these data, the mean serum level of vitamin B12 measured in the *H.pylori* -positive group

(382,93±245,50 pg/mL) was statistically lower than the *H.pylori* -negative group (467,90±305,36 pg/mL) in present study.

In conclusion, although all children with chronic gastritis were positive for *H.pylori*, our findings have not provided any evidence for a link between esophagitis, iron deficiency and *H.pylori* infection. In addition, *H.pylori* infection has been demonstrated to be a risk factor for vitamin B12 deficiency. In this respect a rapid, accurate diagnosis and an effective treatment approach are crucial to achieve appropriate management of *H.pylori* infection in children.

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Ethics Committee Approval: This study was performed with the Institutional Review Board protocol approval date 18/02/2019 and number 2019/13 in Istanbul Dr. Sadi Konuk Training and Research Hospital, Department of Pediatric Gastroenterology, Hepatology and Nutrition, between January 2017 and June 2018.
Conflict of Interest: None
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Informed Consent: Informed consent was obtained from all individual participants included in the study.

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