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Evaluation of the diagnosis of helicobacter pylori from stomach biopsy samples by staining methods

Ulku Karaman[1], Sahin Direkel[2], Havva Erdem[3], Yasemin Kaya[4], Yeliz Kasko Arici[5], Tuba Gul[6]

[1]Ordu University Medical Faculty, Department of Parasitology, Ordu Türkiye
[2]Giresun University Medical Faculty, Department of Microbiyology, Giresun, Türkiye
[3]Ordu University of Medical Faculty, Department of Pathology, Ordu, Türkiye
[4]Ordu University Medical Faculty, Department of Internal Medicine, Ordu, Türkiye
[5]Ordu University Medical Faculty, Department of Biostatistics, Ordu, Türkiye
[6]Ordu University of Medical Faculty,Department of Norology, Ordu, Türkiye

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Abstract

In this study, it was aimed to retrospectively investigate the presence of *H. pylori* with Modified Giemsa and Hematoxylin & Eosin in gastric antrum biopsy samples of patients who applied to Ordu University hospital with dyspeptic complaints. Also, cross-sections were stained with Giemsa, Wright's eosin methylene blue and modified Giemsa dyes to compare their effectiveness in diagnosis. The population of the study consisted of 2679 gastric biopsy samples sent to the pathology laboratory between 2014 and 2018. Gastric biopsy samples were screened, and samples stained with Hematoxylin & Eosin, Giemsa and Periodic Acid Schift (PAS) were re-examined for *H. pylori* In the microscopic examination, the samples were also evaluated in terms of intestinal metaplasia, activation and atrophy. In the study, 37 negative, 31 mildly positive, 31 moderately positive and 31 severely positive samples were randomly selected, in addition to routine staining methods. Selected samples were re-sectioned, stained with Giemsa and Wright's eosin dye, and Mayg Grunwald-Giemsa (MGG) dye, Giemsa and Wright's eosin methods were compared. A total of 2679 patients, 49.15% male and 50.85% female, were included in the study. The mean age of patients aged 17-93 was 50.42±15.32. *H. pylori* positivity was found to be 46.8% in the study. It was determined in the study that there was a significant association between *H. pylori* severity and inflammation ($p<0.01$). The increase in *H. pylori* severity also increased the incidence of activation positivity. The ratio of atrophic patients (56.4%) was higher in patients with severe *H. pylori* positivity (43.6%). Conclusion: It was observed that the incidence of the bacterium was high in Ordu province, and it was suggested that the public should be informed about the transmission and prevention ways of *H. pylori* . Moreover, since similar results were obtained with the staining methods applied in the study, it was concluded that Giemsa and Wright's eosin dyes can be used because they are easy and cheap to diagnose.

Keywords: *H. pylori* stomach, antrum biopsy, Ordu

Introduction

H. pylori can cause acute gastritis and chronic gastritis in the gastric mucosa. Bacteria, which can sometimes be asymptomatic, colonize the apical membrane of the surface epithelial cells. The local humoral immune response occurring is not sufficient for the elimination of this microorganism, therefore, the bacteria has been accepted as a pathogen in the stomach [1,2].

The National Institute of Health of United States of America has also specified H. pylori among the causes of peptic ulcer and emphasized the necessity of both its treatment and control.

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Corresponding Author: Ulku Karaman, Ordu University Medical Faculty, Department of Parasitology, Ordu Türkiye, Email: ulkukaraman44@hotmail.com

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Again, the International Agency for Research on Cancer (IARC) has reported that *H. pylori* is a carcinogen in humans [3].

H. pylori exhibits host and tissue tropism and is primarily localized in the antrum region of the stomach in humans. It can subsequently colonize the corpus region and all regions with gastric cell metaplasia [1].

It was reported that the bacterium infected 50% of the world's population, and the incidence of infection in developing countries reached 70-90%, while this rate was between 25-50% in developed countries. It was indicated that the prevalence of *H. pylori* in Turkey was between 45% and 100%, with an average of 85% [4,5].

C13 urea breath tests, stool culture, serological method, stool antigen test can be listed as noninvasive tests in the diagnosis of *H. pylori*, and also polymerase chain reaction (PCR) from stool can be used. Invasive methods are histopathological evaluation, gastric biopsy sample culture, rapid urease test and molecular methods [6,7].

In the literature, no research on the epidemiology of *H. pylori* performed in Ordu province was found. In this study, it was aimed to retrospectively investigate the presence of *H. pylori* with Modified Giemsa and Hematoxylin & Eosin in gastric antrum biopsy samples of patients who applied to Ordu University with dyspeptic complaints. Moreover, cross-sections were stained with Giemsa, Wright's Eosin Methylene Blue and Modified Giemsa dyes in parallel in order to compare the effectiveness in diagnosis.

Materials and Methods

Study population

Approval was obtained from Ordu University Clinical Research Ethics Committee before starting the study (2019/60). The population of the study consisted of 2679 gastric biopsy samples sent to the pathology laboratory between 2014 and 2018.

In the study, 37 negative, 31 mildly positive, 31 moderately positive and 31 severely positive samples were randomly selected, except for routine staining methods. Selected samples were re-sectioned, stained with Giemsa and Wright's eosin dye, and Mayg Grunwald-Giemsa (MGG) dye, Giemsa and Wright's eosin methods were compared.

Examination of the samples

In the microscopic examination, the samples were also evaluated in terms of intestinal metaplasia, activation and atrophy. After negative and positive evaluations were made, the positives were grouped as mild, moderate, and severe according to the Sidney classification (8). In addition to routine staining methods, 37 negative, 31 mildly positive, 31 moderately positive and 31 intensely positive samples were examined under the light microscope with 100x objective lens, and the results were compared.

Data analysis

Categorical variables were presented as frequency values and continuous variables were presented as mean ± standard deviation. Normal distribution control of the data was performed with Kolmogorov-Smirnov test and homogeneity control of group variances was realized with Levene test. Afterwards, comparisons of two independent groups in continuous variables were conducted with the t-test. Two-way chi-square test was used to determine the association between categorical variables. Comparison of the frequencies in *H. pylori* positive patients was performed with one-way chi-square test. In chi-square tests, if the expected frequencies were ≥5, Pearson's chi-square value was calculated, and when <5, the likelihood ratio was calculated with chi-square value, and the significance level (α) was considered as 5%. Statistical calculations were realized with SPSS v26 (IBM Inc., Chicago, IL, USA) package program.

Results

A total of 2679 patients, 49.15% male and 50.85% female, were included in the study, and *H. pylori* positivity was detected in 1255 (46.84%) patients. *H. pylori,* atrophy, intestinal metaplasia, inflammation and activation (increased neutrophil count) were given in Figures 1 and 2.

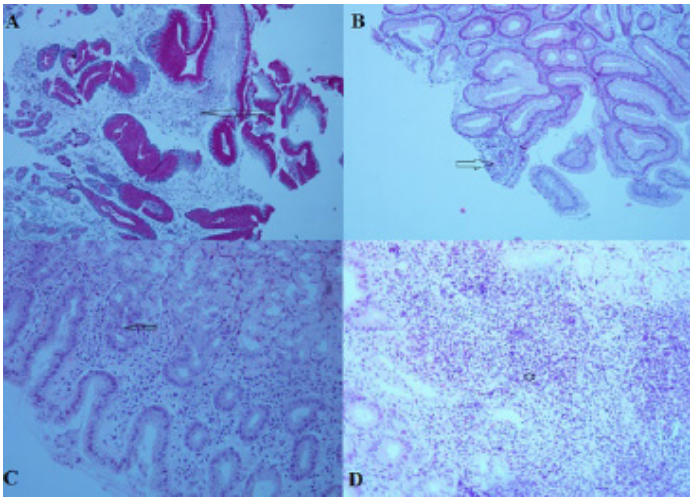

Figure 1. A: Atrophy in gastric biopsy sample stained with PAS-Alcian Blue (100X), B: Intestinal metaplasia in gastric biopsy sample stained with PAS-Alcian Blue (100X), C: Activation in gastric biopsy sample stained with H&E (200X), D: Inflammation in gastric biopsy specimen stained with H&E (200X)

The mean age of patients aged 17-93 was 50.42±15.32. In the study, which was performed by retrospective analysis of patient records of biopsy samples admitted to Department of Medical Pathology laboratory between 2014 and 2018, the descriptive statistical values of the ages of *H. pylori* positive and negative patients were given in Table 1.

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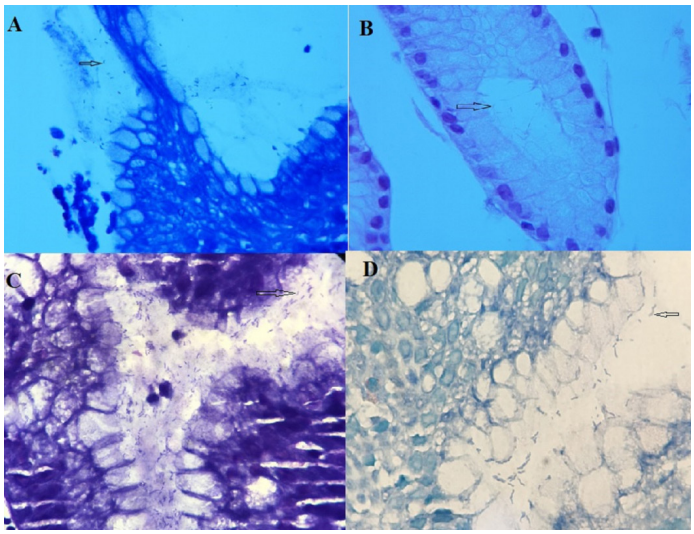

Figure 2: A: *H. pylori* in gastric biopsy sample stained with MGG dye (1000X), B: *H. pylori* in gastric biopsy sample stained with H&E stain (1000X), C: H. pylori in gastric biopsy sample stained with Giemsa stain (1000X), D: *H. pylori* in gastric biopsy specimen stained with Wright's eosin dye (1000X)

Table 1. Descriptive statistical values and comparison results of patients' ages

H. pylori	n	Mean±Std. Deviation	Minimum	Maximum	p
Negative	1424	51.90±15.41	17	93	<0.001
Positive	1255	48.75±15.04	17	86	
Total	2679	50.42±15.32	17	93	

As a result of comparison with Student's t-test, the mean age of *H. pylori* positive patients (48.75±15.04) was found to be statistically significantly lower than the mean age of *H. pylori* negative patients (51.90±15.41) (p<0.001).

The association between *H. pylori* positivity and gender by years was examined with a two-way chi-square test and the results were given in Table 2.

Table 2. Distribution of patients according to years and gender in terms of *H. pylori* positivity

Year		*H. pylori*				p
		Negative		Positive		
		n	%	n	%	
2014	Female	97	56.7	194	64.2	0.107
	Male	74	43.3	108	35.8	
2015	Female	300	58.1	198	55.9	0.518
	Male	216	41.9	156	44.1	
2016	Female	135	57.9	145	61.2	0.474
	Male	98	42.1	92	38.8	
2017	Female	130	67.7	74	61.2	0.236
	Male	62	32.3	47	38.8	
2018	Female	201	64.4	136	56.4	0.056
	Male	111	35.6	105	43.6	
p				0.568		

According to the table, *H. pylori* positivity was found in 508 (18.9%) male and 747 (27.8%) female patients. Chi-square test performed separately in all the years examined showed that *H. pylori* positive or negative status did not differ according to gender (p>0.05). The chi-square test performed considering all patients between 2014 and 2018, without any difference in years, also confirmed that *H. pylori* positivity did not differ according to gender (p>0.05).

The distribution of patients according to months and years in terms of *H. pylori* positivity was given in Table 3.

As a result of the two-way chi-square tests performed in the study, it was observed that *H. pylori* positivity in 2014 (p<0.001) and 2015 (p<0.05) showed significant changes according to months. In 2016, 2017 and 2018, *H. pylori* positivity did not differ significantly according to months (p>0.05). As a result of the chi-square test performed considering all patients between 2014 and 2018, without any difference in years, it was detected that there was a significant association between *H. pylori* positivity and months (p<0.001). In March, April and May, the ratio of positive patients was higher than the ratio of negative patients. In the light of these findings, it can be said that *H. pylori* positivity increases in spring.

The distribution of disease severity according to the presence of inflammation, intestinal metaplasia, atrophy and activation in *H. pylori* positive patients was given in Table 4.

In the study, a significant correlation was detected between *H. pylori* severity and inflammation as a result of the two-way chi-square test (p<0.01). In patients with severe *H. pylori*, the ratio of patients without inflammation increased as the disease severity decreased while inflammation was observed in all patients (100%). The association between the positivity of intestinal metaplasia and the severity of *H. pylori* was found to be statistically significant (p<0.01). The increase in the severity of *H. pylori* caused a mild decrease in the incidence of intestinal metaplasia. Activation positivity also showed a significant correlation with *H. pylori* severity (p<0.001). The increase in *H. pylori* severity increased the incidence of activation positivity. While the rate of patients with mild and moderate *H. pylori* positivity was higher than those without atrophy, the rate of patients with severe *H. pylori* positivity (56.4%) was higher than the rate of patients without atrophy (43.6%). However, the two-way chi-square test showed that there was no significant association between atrophy positivity and *H. pylori* severity (p>0.05).

The comparison of intestinal metaplasia types with *H. pylori* positivity was given in Table 5.

According to the table, a significant correlation was found between inflammation, atrophy, activation severity and intestinal metaplasia types and the positivity of the bacteria (p<0.001). *H. pylori* positivity was detected more in type 1 patients. Bacterial positivity was observed to be higher in moderate inflammation. Bacterial positivity was found to be higher in mild atrophy and activation.

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Table 3. The distribution of patients according to months and years in terms of *H. pylori* positivity

		2014		2015		2016		2017		2018		Total		p
		n	%	n	%	n	%	n	%	n	%	n	%	
January	Negative	8	30.8	58	62.4	22	56.4	17	53.1	31	81.6	136	9.6	
	Positive	18	69.2	35	37.6	17	43.6	15	46.9	7	18.4	92	7.3	
February	Negative	2	25.0	61	64.2	74	47.7	13	76.5	26	61.9	176	12.4	
	Positive	6	75.0	34	35.8	81	52.3	4	23.5	16	38.1	141	11.2	
March	Negative	4	15.4	53	52.5	71	55.5	0	0.0	14	48.3	142	10.0	
	Positive	22	84.6	48	47.5	57	44.5	0	0.0	15	51.7	142	11.3	
April	Negative	11	16.7	56	66.7	1	16.7	8	40.0	23	57.5	99	7.0	
	Positive	55	83.3	28	33.3	5	83.3	12	60.0	17	42.5	117	9.3	
May	Negative	6	8.7	36	52.2	21	44.7	23	56.1	23	59.0	109	7.7	
	Positive	63	91.3	33	47.8	26	55.3	18	43.9	16	41.0	156	12.4	
June	Negative	0	0.0	51	63.7	11	50.0	13	65.0	56	54.9	131	9.2	
	Positive	0	0.0	29	36.3	11	50.0	7	35.0	46	45.1	93	7.4	
July	Negative	0	0.0	22	53.7	10	45.5	22	71.0	46	52.3	100	7.0	<0.001
	Positive	0	0.0	19	46.3	12	54.5	9	29.0	42	47.7	82	6.5	
August	Negative	12	30.8	26	52.0	7	63.6	11	61.1	1	100.0	57	4.0	
	Positive	27	69.2	24	48.0	4	36.4	7	38.9	0	0.0	62	4.9	
September	Negative	19	39.6	51	76.1	0	0.0	10	41.7	21	67.7	101	7.1	
	Positive	29	60.4	16	23.9	0	0.0	14	58.3	10	32.3	69	5.5	
October	Negative	23	59.0	28	56.0	5	29.4	30	71.4	13	50.0	99	7.0	
	Positive	16	41.0	22	44.0	12	70.6	12	28.6	13	50.0	75	6.0	
November	Negative	39	58.2	40	50.6	2	50.0	31	67.4	16	45.7	128	9.0	
	Positive	28	41.8	39	49.4	2	50.0	15	32.6	19	54.3	103	8.2	
December	Negative	47	55.3	34	55.7	9	47.4	14	63.6	42	51.2	146	10.3	
	Positive	38	44.7	27	44.3	10	52.6	8	36.4	40	48.8	123	9.8	

Table 4. The distribution of disease severity according to the presence of inflammation, intestinal metaplasia, atrophy and activation in H. pylori positive patients

		H. pylori severity						p
		Mild		Moderate		Severe		
		n	%	n	%	n	%	
Inflammation	Negative	19	3.5	6	1.3	0	0.0	**0.002****
	Positive	527	96.5	439	98.7	264	100.0	
Intestinal metaplasia	Negative	332	60.8	319	71.7	170	64.4	0.002**
	Positive	214	39.2	126	28.3	94	35.6	
Atrophy	Negative	278	50.9	226	50.8	115	43.6	0.108
	Positive	268	49.1	219	49.2	149	56.4	
Activation	Negative	143	26.2	58	13.0	14	5.3	<0.001
	Positive	403	73.8	387	87.0	250	94.7	

** p<0.01

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Table 5. The distribution of *H. pylori* positivity according to intestinal metaplasia types

		H. pylori severity						p
		Negative		Positive		Total		
		n	%	n	%	n	%	
Metaplasia	Negative	1280	90.0	821	65.4	2101	78.5	**<0.001**
	Type 1	112	7.9	294	23.4	406	15.2	
	Type 2	22	1.5	112	8.9	134	5.0	
	Type 3	9	0.6	28	2.2	37	1.4	
Inflammation	Negative	798	56.0	25	2.0	823	30.7	
	Mild	426	29.9	396	31.6	822	30.7	
	Moderate	140	9.8	449	35.8	589	22.0	
	Severe	60	4.2	385	30.7	445	16.6	
Atrophy	Negative	1341	94.2	619	49.3	1960	73.2	
	Mild	78	5.5	501	39.9	579	21.6	
	Moderate	5	0.4	115	9.2	120	4.5	
	Severe	0	0.0	20	1.6	20	0.7	
Activation	Negative	1249	87.7	215	17.1	1464	54.6	
	Mild	132	9.3	460	36.7	592	22.1	
	Moderate	32	2.2	417	33.2	449	16.8	
	Severe	11	0.8	163	13.0	174	6.5	

n: number, %: percentage

Discussion

It has been reported that there may be a risk of developing functional changes and gastritis in the stomach of almost all patients, peptic ulcer in 15-20%, ulcer complications in 2-12%, gastric cancer in 1-3%, primary gastric lymphoma in 0.1%, and less frequently functional dyspepsia in *H. pylori* infection (9). Again, it has been stated that *H. pylori* may localize in the antrum first and cause chronic active gastritis, followed by atrophic gastritis, intestinal metaplasia, dysplasia, and gastric cancer (10-12). The patients were evaluated in terms of intestinal metaplasia, atrophy, inflammation and activation for their status and follow-up. In addition, Giemsa and Wright's eosin dyes, which were used in the study, were found to be as effective as the routinely used MGG method in the diagnosis of bacteria. These data can be interpreted as Giemsa and Wright's eosin dyes can also be used in routine diagnosis. Moreover, it was thought that the use of a single dye in both dyeing methods was cheaper and more applicable in practice.

In the present study, the mean age of *H. pylori* positive patients (48.75±15.04) was statistically significantly lower than the mean age of *H. pylori* negative patients (51.90±15.41) ($p<0.001$). Again, *H. pylori* positivity was found to be 46.5% in 35 and younger age group, 48.9% in the 36-45 age range, 53.4 in the 46-54 age range, and 58.2% in the 55 and older age group (Table 4.9). Accordingly, it was determined that *H. pylori* positivity increased with age ($p<0.001$). Similarly, Chalise et al. (13) and Erdoğan et al. (14) reported that it increased with age. Again, Korkmaz et al. (15) reported that *H. pylori* positivity may increase with increasing age. Çıkman et al. (16) noted in their study that the prevalence of *H. pylori* increased in parallel with age. Tarhane et al. (17) also found *H. pylori* positivity to be higher in individuals aged 25-44 ($p<0.05$) and attributed this to the fact that these age groups were active working age and had a high causative exposure. However, Uyanıkoğlu et al. (18) expressed that they could not find a significant association between age and bacterial positivity. This difference may be due to the population of the study, the study area and the method. Moreover, as age progresses, the localization and colonization of bacteria may be easier. Again, it was found in the study that inflammation, intestinal metaplasia and activation of gastric mucosa caused by *H. pylori* positivity increased significantly with age ($p<0.001$). Although there was an increase in percentage in the presence of atrophy, no significant association was found ($p>0.05$). This can be explained by the fact that age facilitates the localization of bacteria and can cause more serious damage to the stomach.

In the study, *H. pylori* positivity was observed in 508 (18.9%) male and 747 (27.8%) female patients. When the association between gender and *H. pylori* was evaluated, the chi-square test performed separately in all the years examined showed that *H. pylori* positive or negative status did not differ according to

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gender (p>0.05). The chi-square test performed considering all patients between 2014 and 2018, without any difference in years, also confirmed that *H. pylori* positivity did not differ according to gender (p>0.05). In similar studies, Uyanıkoğlu et al. (18) reported that the incidence of bacteria did not differ between genders. Again, Erdogan et al. (14) did not find a significant assocaition between gender and bacterial positivity. However, Çıkman et al. (16) observed positivity to be significantly higher in women in the experimental group consisting of children and adults. This difference may be due to diet, study group, study area and method.

According to epidemiological studies conducted in Turkey and in the world, Gisbert and Calvet (19) found bacteria positivity in 81.2% of 16080 patients with duodenal ulcers in 1999-2008. There are many studies on the prevalence of *H. pylori* in Turkey. In a retrospective study conducted by Salih et al. (20) in Istanbul, they applied rapid urease test on 4471 patients who had upper GIS endoscopy between 1999-2003 and reported the frequency of *H. pylori* as 62.7%. Umit et al. (21) included 4714 patients who underwent upper GIS endoscopy in the Thrace region between 2003 and 2007 and investigated the presence of *H. pylori* with the rapid urease test. In different studies conducted with children, the prevalence was found to be 80% in Bangladeshi children and 16% in 695 school children in Sweden (22,23). In Turkey, Gökşen et al. found *H. pylori* antigen positivity to be 17.8% in stool samples of children with chronic abdominal pain (24).

According to the literature, it was reported that bacteria positivity was found 48.8% of celiac patients, 85.4% of control patients in the study of Kahramanoğlu Aksoy et al. (25), 53.8% of 52 patients in the study of Topal et al. (11), in 66.7% of the patients in Adıyaman in the study of Aygün et al. (26) and 43% in Siverek region in the study of Tosun and Çakır (27). Korkmaz et al. (15) observed 49.5% of *H. pylori* positivity in 2189 patients in Ankara with the urea breath test. Again, Uyanıkoğlu et al. (28) reported positivity in 71% of 1298 patients who had antrum biopsy. Cikman et al. (16) searched for antigen in 8402 stool samples in Van between January 2006 and December 2010. Of the samples, 4304 were children and 4098 were adults, and the positivity rate was 20.7% in children and 25.5% in adults. Researchers found 23% positivity in total. Again, Tarhane et al. (17) collected samples from 195 patients in Kars by endoscopy and evaluated them for helicobacteria *(H. pylori, H. felis and H. heilmannii)*. Researchers observed that 163 (83.58%) of 195 samples were positive with May-Grunwald-Giemsa. Erdogan et al. (14) performed C14 urea breath test on 252 patients and reported positivity in 62.7% women and 66.3% men. Konakçı et al (9) detected the positivity of bacteria in 50.5% of 218 patients in Bursa. Mete et al (29) stated a positivity rate of 73.7% in Tekirdag region. Kurtulus et al. (30) found *H. pylori* in 69.5% of 262 patients in Antalya. In the presented study, 2679 gastric biopsy samples were examined between 2014 and 2018 and bacterial positivity was detected in 46.84%. The result obtained was found to be similar to the results of some studies (10,31). The difference in the rates obtained in the studies may be due to the study method, the patient, the region where the biopsy was taken, and the socio-economic level of the region where the study was conducted.

"Triple therapy" is recommended in the treatment of *H. pylori* infection, and treatment with a proton pump inhibitor, clarithromycin and amoxicillin or metronidazole are generally preferred. Researchers have reported that there is an association between acute neuropsychiatric symptoms and antibiotics, as a result of examining patients with chronic *H. pylori* infection and receiving antibiotic treatment (32). Again, chronic *H. pylori* infection or proton pump inhibitor drugs taken continuously for this reason cause vitamin B12 absorption defect, resulting in an increase in blood homocysteine levels and consequently an increase in clinical findings of dementia, pseudodementia, a tendency to ischemic stroke, and neurological diseases such as subacute combined degeneration. It has a clinical aggravating effect in Parkinson's disease by reducing the absorption of L.dopa taken orally with both food and drugs. 1-methyl-4-phenyl-1,2,36-tetrahydropyridine, a by-product of *H. pylori* infection (MPTP), has a degenerative effect on the substantia nigra and may contribute to Parkinson's disease by causing a decrease in dopamine levels (33). In this context, it was thought that patients should be treated after diagnosis and controlled in terms of neuropsychiatric symptoms in this process.

It was determined that there was a significant assocaition between the colonization status of the bacteria and inflammation in patients with positive *H. pylori* (p<0.01). In patients with severe *H. pylori* infection, inflammation was observed in all patients (100%), while the rates of patients without inflammation increased as the disease severity decreased. The association between intestinal metaplasia positivity and *H. pylori* density was also statistically significant (p<0.01). An increase of 50.7% in intestinal metaplasia was detected in bacterial positivity. A significant correlation was also found with activation positivity and *H. pylori* severity (p<0.001). The increase in the severity of *H. pylori* infection increased the incidence of activation positivity. While the rate of patients with mild and moderate *H. pylori* positivity was higher than those without atrophy, the rate of patients with severe *H. pylori* positivity (56.4%) was higher than the rate of patients without atrophy (43.6%). As a result of the chi-square test performed considering all patients, without any difference in years, a significant association was found between *H. pylori* positivity and months (p<0.001). In March, April and May, the rate of positive patients was higher than the rate of negative patients. In the light of these findings, it can be said that *H. pylori* positivity increases in spring.

Conclusion

In the study Giemsa and Wright's eosin dyes were found to be as effective as MGG. Accordingly, it has been presented that Giemsa and Wright's eosin dyes can also be used in routine

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diagnosis. Moreover, a significant association was found between atrophy, inflammation, intestinal metaplasia and activation and bacterial positivity in presented study. In this context, it has been concluded that these parameters are important in the follow-up of the patients, and the old samples should be evaluated in parallel during the follow-up periods. It has been considered that information studies should be carried out about the transmission and protection ways of *H. pylori*, infection may be easier due to the increased incidence of bacteria in the spring season and the region receives a lot of rains in those seasons, and it has been suggested that measures for protection should be increased in these months.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

The written permission of the Dokuz Eylul University Non-Interventional Research Ethics Committee was obtained for this study (September 27, 2018, 2018/23-02).

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