Available online at www.medicinescience.org

ORIGINAL RESEARCH

Medicine Science International Medical Journal

Medicine Science 2019;8(4):786-91

Does anti-Helicobacter pylori immunohistochemical staining has a confirmative role in the definitive diagnosis of negative gastric biopsy samples stained with routine histological stains?

Cengiz Kocak[1], Sirin Kucuk[1], Ersoy Ercihan[2] , Mehmet Gundogan[2], Canan Sakar[2] , Asli Ucar Uncu[2] , Bulent Mizrak[1]

[1]Usak University, Faculty of Medicine, Department of Pathology, Usak, Turkey
[2]Usak Training and Research Hospital, Department of Pathology, Usak, Turkey

Received 20 August 2019; Accepted 16 September2019
Available online 21.10.2019 with doi:10.5455/medscience.2019.08.9087

Abstract

Helicobacter pylori (H. pylori) causes chronic gastritis, and is associated with gastric lymphoma and carcinoma. Although severe infections with many bacteria can be identified easily on hematoxylin-eosin (H&E)–stained tissue samples; the identification ratio is only 66% with many false-positive and false-negative results. Furthermore, identification of the low-density bacteria, primarily following the therapy, requires special staining methods. The purpose of this study was to investigate whether the use of anti-helicobacter immunohistochemical (IHC) methods in the histopathological diagnosis of H. pylori is necessary to obtain accurate results. We investigated whether the use of specific immunohistochemical methods can detect H. pylori, which previously undetected by classical histological staining methods, in endoscopic gastric biopsy specimens. The study involved 210 endoscopic gastric biopsies considered as H. pylori negative with routine histological stainings and subsequently applied IHC staining methods to confirmative diagnosis. Inflammation was seen in all cases. One hundred thirty-eight patients had chronic inactive, 72 patients had chronic active gastritis. While H. pylori was found as negative in all cases with H&E and Giemsa stainings, in 60 of all these cases, H. pylori was found as positive with anti-H. pylori IHC staining. We suggest that in cases of chronic gastritis suggestive of H. pylori infection, when H. pylori cannot be seen by routine histological stainings, the IHC staining method is a definitive diagnostic tool for identification of H. pylori to avoid from false negative results.

Keywords: Gastritis, helicobacter pylori, immunohistochemistry

Introduction

Gastric infection by Helicobacter pylori (H. pylori) causes chronic active gastritis, and is strongly associated with gastric lymphoma and carcinoma [1]. Epidemiological studies have revealed that H. pylori gastritis is associated with the risk of developing gastric cancer [2,3]. Subsequently, H. pylori was classified as a group I carcinogen by the International Agency for Research on Cancer, World Health Organization (IARC/WHO) in 1994 [4]. Therefore, the identification of H. pylori in the gastric biopsy samples is extremely important because of it is associated with both chronic gastritis and also gastric carcinoma [5].

Since H. pylori was demonstrated in patients with gastritis by Robin Warren, pathologists have been trying to find the optimal staining method for identification of bacteria in gastric biopsy samples [6]. Although severe infections with many organisms can be identified easily on hematoxylin-eosin (H&E)–stained tissue samples; the identification ratio is only 66% with many false-positive and false-negative results [7]. Furthermore, detection of the low-density bacteria, especially following the therapy, requires special staining techniques. Treatment of H. pylori gastritis can change the shape of bacteria and decrease the density of the bacteria. These alterations cause difficulties in identification of bacteria and differentiation from extracellular debris or mucin globules. In such cases, immunohistochemical (IHC) methods would be recommended. Immunohistochemistry can improve the exact and accurate identification of the bacteria even though histopathological examination and culture are false-negative [7,8].

The purpose of this retrospective study was to investigate whether the use of anti-helicobacter IHC methods in the histopathological diagnosis of H. pylori is necessary to obtain accurate results. So that we investigated whether the use of specific immunohistochemical methods can detect H. pylori, which previously undetected by classical histological staining methods, in endoscopic gastric biopsy specimens.

***Coresponding Author:** Cengiz Kocak, Usak University, Faculty of Medicine, Department of Pathology, Usak, Turkey
E-mail: drcengizkocak@hotmail

Material and Methods

Study design and data collection

The study involved 210 endoscopic gastric biopsies considered as H. pylori negative with routine histological stainings and subsequently applied IHC staining methods to confirmative diagnosis. Endoscopic gastric biopsy reports were gathered from the archive of the Department of Pathology at Training and Research Hospital from 2016 to 2018.

Immunohistochemical staining method

In immunohistochemical stainings, all biopsy sections were stained with anti-H. pylori mouse monoclonal antibody (Novocastra TM Liquid Mouse Monoclonal Antibody Helicobacter pylori, Product Code: NCL-L-Hpylori, Clone: ULC3R, Leica Biosystems Newcastle Ltd., Newcastle, United Kingdom), using a fully automated immunohistochemistry slide staining system (Leica BonD-MaX, Leica Biosystems) according to the manufacturer's recommendations.

Ethical considerations

The study was in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was received from the local Human Non-invasive Clinical Research Ethics Committee (No: 216-1). The informed consent was not requested, since the study was retrospective and the data were analyzed anonymously.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 6.05 (GraphPad Software, Inc., CA, USA). The comparisons of categorical variables were analyzed with chi-square test. A P value <0.05 was considered statistically significant.

Results

Demographic and histopathological data are summarized in Table 1. The study group consisted of 118 female, 92 male patients (mean age 52 ± 18). Inflammation was seen in all cases. One hundred thirty-eight patients had chronic inactive, 72 patients had chronic active gastritis (Table 1, Figure 1). Fifty-five patients had chronic atrophic, 155 patients had chronic non-atrophic gastritis (Table 1, Figure 1). Activity was present in 72 cases, atrophy was present in 55 cases, and intestinal metaplasia was present in 40 cases according to the updated Sydney system for grading and classification of chronic gastritis [9]. While H. pylori was found as negative in all cases with H&E and Giemsa stainings, in 60 of all these cases, H. pylori was found as positive with anti-H. pylori IHC staining. Furthermore, H. pylori was found as negative with anti-H. pylori IHC staining in 150 cases (Table 1, Figure 2-4). When chronic inactive and active gastritis cases were compared for anti-H. pylori IHC staining status, significant difference was found (P < 0.0001, X2: 83.70; Table 2, Figure 1). Positive IHC staining was higher in the chronic active gastritis than chronic inactive gastritis. However, no significant difference was found between chronic atrophic and non-atrophic gastritis for IHC staining status (P= 0.197, X2: 1.665; Table 2, Figure 1). In addition, we compared cases according to the grades of inflammation and activation for IHC staining status, we found significant differences (P < 0.0001, X2: 88.75; Table 3). The rate of positive IHC staining was higher in cases with mild inflammation and mild activity. In cases with negative activity and mild or moderate inflammation, the rate of positive IHC staining was low (Table 3, Figure 5).

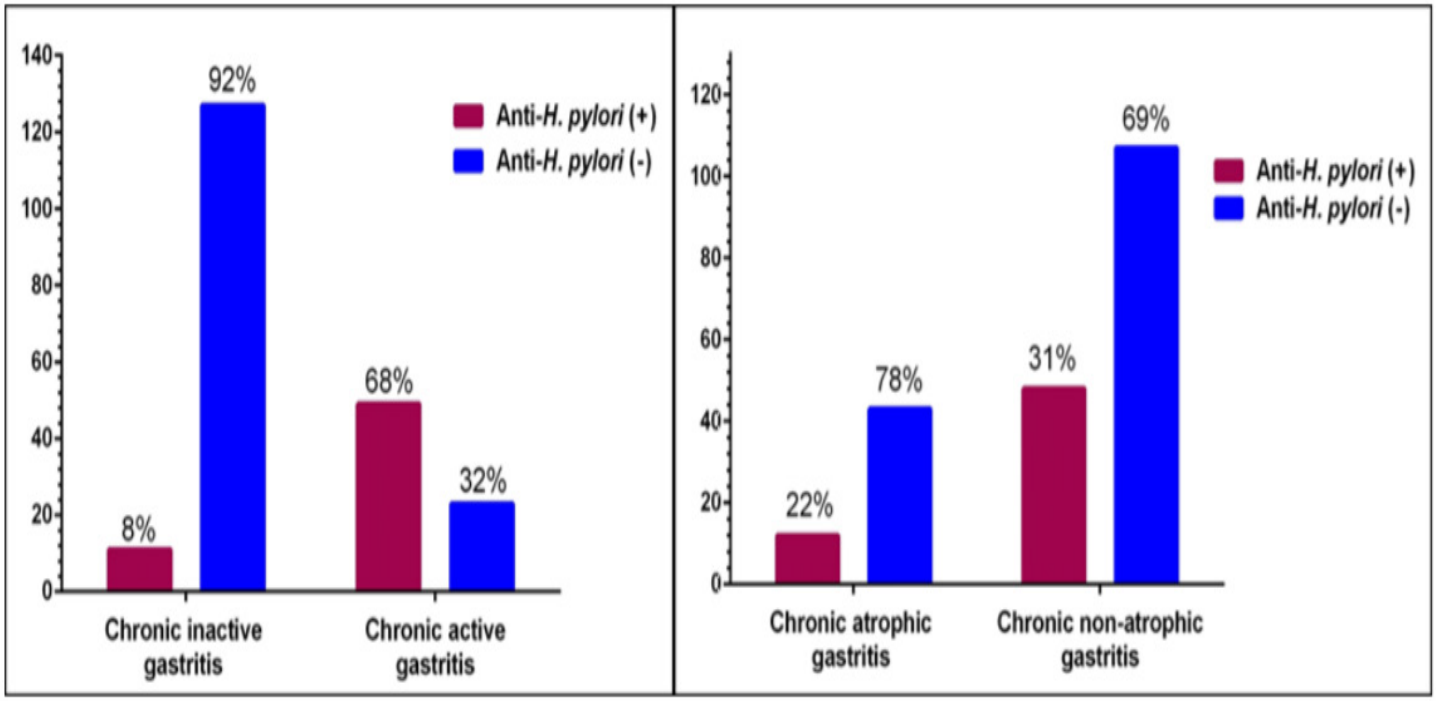

Figure 1. The comparison graphs of chronic inactive and chronic active, chronic atrophic and chronic non-atrophic gastritis cases for anti-H. pylori immunohistochemical staining status

Table 1. Demographic and histopathological data of chronic gastritis

Parameters (n=210)	**n (%)**
Age (years)	52 ± 18
Gender	
Female	118 (56)
Male	92 (44)
The site of endoscopic gastric biopsy	
Antrum	132 (63)
Corpus	28 (13)
Antrum + corpus	50 (24)
Type of chronic gastritis	
Chronic inactive	138 (66)
Non-atrophic	99 (71)
Atrophic	39 (28)
Chronic active	72 (34)
Non-atrophic	56 (78)
Atrophic	16 (22)
Grading and classification of chronic gastritis (Sydney System)	
Inflamation (+)	210 (100)
Activity (+)	72 (34)
Atrophy (+)	55 (26)
Intestinal metaplasia (+)	40 (19)
Immunohistochemical staining (Anti-H. pylori)	
H. pylori (+)	60 (29)
H. pylori (-)	150 (71)
Grade of H. pylori positivity	
H. pylori (+)	51 (85)
H. pylori (++)	9 (15)

Table 2. The comparison of chronic inactive and chronic active, chronic atrophic and chronic non-atrophic, intestinal mataplasia (+) and (-) gastritis cases for anti-H. pylori immunohistochemical staining status

	Anti-H. pylori (+) n (%)	**Anti-H. pylori (-) n (%)**
Chronic inactive gastritis	11 (8)	127 (92)
Chronic active gastritis	49 (68)	23 (32)
P < 0.0001, X2: 83.70		
Chronic atrophic gastritis	12 (22)	43 (78)
Chronic non-atrophic gastritis	48 (31)	107 (69)
P= 0.197, X2: 1.665		
Intestinal metaplasia (+)	10 (25)	30 (75)
Intestinal metaplasia (-)	0 (0)	170 (100)

Table 3. The comparison of graded and classified chronic gastritis cases for anti-H. pylori immunohistochemical staining status

Grading of chronic gastritis	**Anti-H. pylori (-) n (%)**	**Anti-H. pylori (-) n (%)**
Inflammation +	123 (94)	8 (6)
Activation -		
Inflammation ++	4 (57)	3 (43)
Activation -		
Inflammation +	18 (35)	34 (65)
Activation +		
Inflammation ++	5 (25)	15 (75)
Activation +		
P < 0.0001, X2: 88.75		

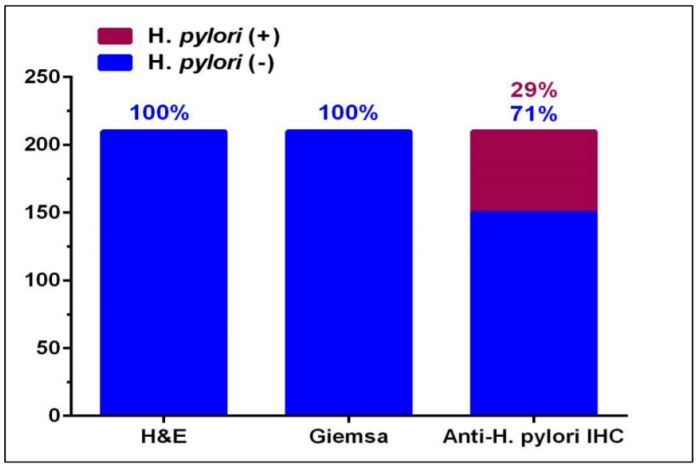

Figure 2. Detection ratios of H. pylori by H&E, Giemsa, and immunohistochemical stainsDiscussion

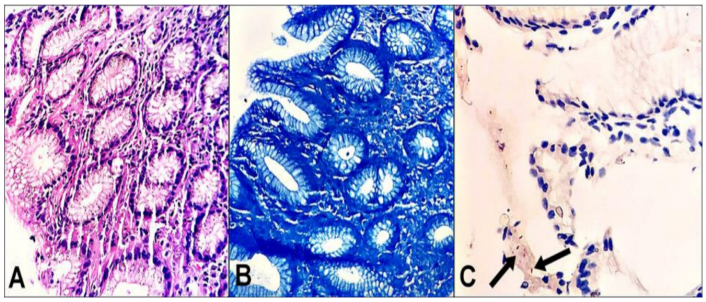

Figure 3. A. A case of chronic active gastritis in which the organisms cannot be detect with H&E staining (H&E X 40), B. A case of chronic active gastritis in which the organisms cannot be detect with Giemsa staining (Giemsa X 40), C. A case of chronic active gastritis in which the organisms can be detect with IHC staining (IHC X 100).

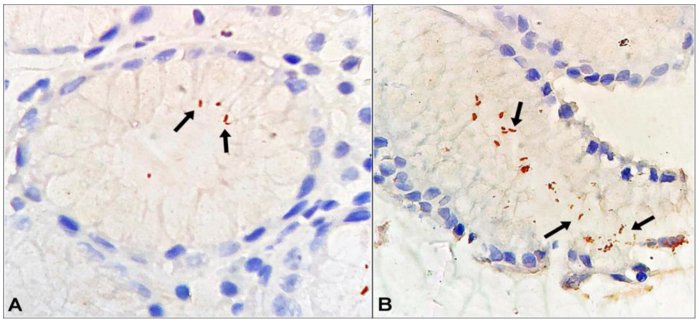

Figure 4. Gram-negative, spiral-shaped H. pylori microorganisms between superficial mucosa and glands stained by anti-H. pylori immunohistochemical staining method A. H. pylori (+), B. H. pylori (++), (IHC X 40).

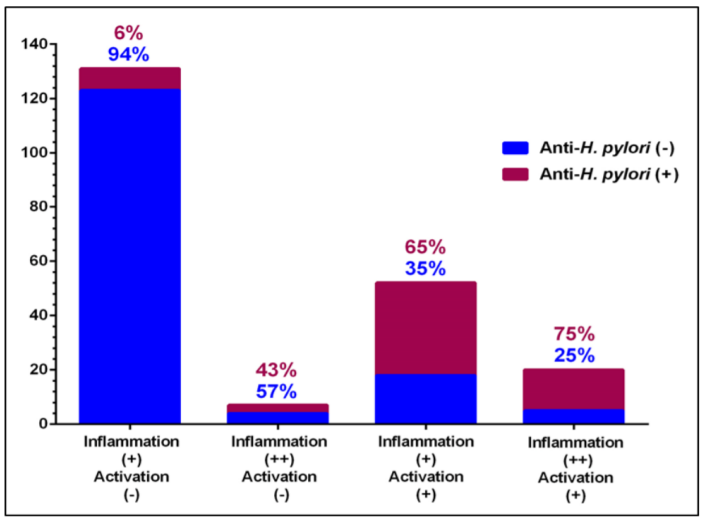

Figure 5. The comparison graph of graded and classified chronic gastritis cases for anti-H. pylori immunohistochemical staining status

Several methods, including H&E staining, special stainings, and IHC stainings, are used for the identification of H. pylori in gastric biopsy specimens. Although IHC staining is considered as the most sensitive and specific method, there are controversial opinions about the necessity of routine IHC stain [10,11]. In this retrospective study, we investigated whether anti-H. pylori IHC staining method is a definitive diagnostic tool for H. pylori false negative chronic gastritis cases in which previously undetected microorganism with both H&E and Giemsa staining techniques. Our findings showed that microorganism was detected with IHC staining in 60 of 210 cases. Whereas, no microorganism could been detected at initial examinations with H&E and Giemsa stainings in all cases. Considering the important role of H. pylori in the etiology of gastric cancer, 29 % H. pylori positivity is a considerable percentage and false negative results were prevented by using IHC staining method.

H. pylori causes chronic inflammation in the gastric mucosa, and the presence of chronic gastritis is most frequently suggestive for H. pylori infection. About 50% to 80% of H. pylori-positive gastritis samples display chronic active gastritis, less than 10% of samples display chronic inactive gastritis [12,13]. In concordance with the current findings, Smith and colleagues (2012) recommended the use of IHC staining for chronic active or inactive gastritis cases with suspicious histological structures in which H. pylori cannot be identified by H&E staining [14]. Cutler et al. (1995) reported that the presence of chronic antral inflammation are most accurate parameter for diagnosis of H. pylori infection [15]. In a study by Wang et al. (2010), the IHC positivity rate was 73.5% (50/68) in chronic active gastritis, 5.3% (4/76) in chronic inactive gastritis [16]. In our study, chronic inflammation was seen in all cases and 66% of these was chronic inactive, 34% was chronic active gastritis. In 11 of 138 cases with chronic inactive gastritis and in 49 of 72 cases with chronic active gastritis, H. pylori was identified with IHC staining, but microorganism could not identified with H&E and Giemsa staining methods. Therefore, we suggested that even though activity is negative, when chronic inflammation is present and no H. pylori is identified with H&E and Giemsa stainings in gastric biopsy samples, IHC staining should be applied to avoid from false negative result possibility.

When the bacteria are abundant, comparisons among between various routine histological stains and IHC stains show very little difference with regard to sensitivity and specificity [17]. In case of application of eradication therapy, therapeutic agents may cause the alterations in the characteristics of H. pylori infection. Bacteria are present in smaller numbers in patients who receive eradication therapy, and in this cases, sensitivities of histological stains, including H&E and Giemsa, decrease compared with those of IHC due tosmaller numbers of bacteria [18,19]. Goldstein (2002) studied 105 patients with dyspepsia who had the H. pylori eradication therapy before endoscopy. It has been found that the most common morphologic feature associated with H. pylori infection was moderate chronic inactive gastritis, which was found most often in patients who had received recent H. pylori eradication therapy. The author suggested that the pathologists should consider the association between these medications and chronic inactive gastritis with rare H. pylori. Thus, in the chronic inactive gastritis, IHC should be preferred instead of histological stains to the accurate identification of H. pylori [20]. In another study (2015) including 79 cases, H. pylori was detected in 49 (62%) cases. Routine H&E and Giemsa stains detected bacteria in 26 (32.9%) cases. On the other hand, IHC staining detected additional 29 cases including very few bacteria. In these 29 cases, H. pylori was not detected initially by H&E and Giemsa stains [21]. In our study, when bacterial density was graded according to the IHC staining, grade was 1 (+) in 51 of 60 cases and grade was 2 (++) in 9 of 60 cases with H. pylori positive. Consequently, 85% of our cases with H. pylori positive consisted from biopsy samples included low density bacteria and only IHC staining could detected these small amount of bacteria.

Atrophic gastritis, which is often accompanied by intestinal metaplasia, significantly increases the risk of developing gastric cancer. Atrophy and intestinal metaplasia are closely associated with long term gastritis caused by H pylori infection [22]. The association between H. pylori infection and the development of atrophic gastritis as well as intestinal metaplasia significantly supports the role of this infection in gastric carcinogenesis [23]. Approximately, two-thirds of atrophic gastritis patients have H. pylori infection [24]. In a study (2000), atrophic gastritis were found in 52 (25%) of 207 outpatients referred for gastroscopy as consistent with our findings. In 77 of 207 patients, H. pylori was detected on histological examinations and 31 (40%) of these had atrophic gastritis [25]. If H. pylori is not eradicated, the infection and associated gastritis can cause loss in gastric glands and intestinal metaplasia [26]. Thus, it is important to determine the presence of H. pylori in patients with atrophic gastritis and intestinal metaplasia [27]. However, atrophic and metaplastic changes occurred in gastric mucosa commonly give histological negative results for bacteria in a high percentage of endoscopic biopsy samples and microorganism is frequently undetectable with conventional histological staining techniques, including H&E and Giemsa, in most cases, even though serologic tests are positive for infection. The reason for this failure in the detection of the bacteria is more likely related to the low density of bacterial colonization in atrophic and as well as metaplastic gastric mucosa [28-30]. In a study (2002) on patients showing histologic characteristics of chronic gastritis with atrophy, in 40.5% of cases, H&E and Giemsa stain underestimated the true prevalence of current H. pylori infection in comparison with serum IgG antibody determination

[31]. Ohkuma et al. (2000) found significant relationship between H. pylori infection and the gastric atrophy and intestinal metaplasia [32]. In our study, atrophic gastritis was found in 55 (26%) of 210 cases. H. pylori was identified by IHC staining in 12 (22%) of 55 cases with chronic atrophic gastritis, whereas, no bacteria was detected in all of 55 cases with atrophic gastritis by H&E and Giemsa stain. In addition, 40 of 55 atrophic gastritis cases also had intestinal metaplasia and in 10 of these, H. pylori was detected with IHC method.

Consequently, depending on the results of this study, we suggest that when bacteria cannot detected with H&E and Giemsa stains, the IHC staining is a definitive diagnostic tool for detection of H. pylori to avoid from false negative results in the following cases: If chronic non-active or active gastritis cases with histological features suggestive H. pylori is present, but bacteria cannot detected with routine histological stains; when intestinal metaplasia or gastric atrophy is present because of H. pylori density is low in metaplasic areas.

Conclusion

When large numbers of H. pylori are present in the gastric biopsy samples, the H. pylori can be usually identified with H&E or Giemsa stains. However, in cases of chronic gastritis suggestive of H. pylori infection, when H. pylori cannot be seen by routine histological stains, the IHC staining method is a definitive diagnostic tool for identification of H. pylori to avoid from false negative results.

Conflict of interest

The authors declare that there are no conflicts of interest.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Ethical approval was received from the local Human Non-invasive Clinical Research Ethics Committee (No: 216-1).

Sirin Kucuk ORCID: 0000-0002-8552-2101
Ersoy Ercihan ORCID: 0000-0002-3690-5886
Mehmet Gundogan ORCID: 0000-0002-3890-5396
Canan Sakar ORCID:0000-0003-0990-2863
Asli Ucar Uncu ORCID: 0000-0003-1503-3385
Bulent Mizrak ORCID: 0000-0003-1813-9564

References

1. Galiatsatos P, Wyse J, Szilagyi A. Accuracy of biopsies for helicobacter pylori in the presence of intestinal metaplasia of the stomach. Turk J Gastroenterol. 2014;25:19-23.
2. Forman D, Newell DG, Fullerton F, et al. Association between infection with helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. BMJ. 1991;302:1302-5.
3. Parsonnet J, Friedman GD, Vandersteen DP, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med. 1991;325:1127-31.
4. International Agency for Research on Cancer. IARC monographs on the evaluation of the carcinogenic risks to humans. Schistosomes, liver flukes and Helicobacter pylori. Vol. 61. Lyon: International Agency for Research on Cancer; 1994. p. 177.
5. Barbosa AJA. Detection of H. pylori in endoscopic gastric biopsies: a routine research that goes far beyond the laboratory limits. J Bras Patol Med Lab. 2015;51:70-71.
6. Geller SA. Small organism, many challenges. Hum Pathol. 1996;27:1-4.
7. Eyzaguirre E, Haque AK. Application of immunohistochemistry to infections. Arch Pathol Lab Med. 2008;132:424-31.
8. Marzio L, Angelucci D, Grossi L, et al. Anti-Helicobacter pylori specific antibody immunohistochemistry improves the diagnostic accuracy of Helicobacter pylori in biopsy specimen from patients treated with triple therapy. Am J Gastroenterol. 1998;93:223-6.
9. Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161-1181.
10. Jonkers D, Stobberingh E, de Bruine A, et al. Stockbrügger R.Evaluation ofimmunohistochemistry for the detection of Helicobacter pyloriin gastric mucosal biopsies. J Infect. 1997;35:149-54.
11. Ashton-Key M, Diss TC, Isaacson PG. Detection of Helicobacter pylori in gastric biopsy and resection specimens. J Clin Pathol. 1996;49:107-11.
12. Yang Y, Xiong W, Wang S, et al. Factors associated with detection of helicobacter pylori in gastric biopsies: A case-control study of 396 Biopsies. Appl Immunohistochem Mol Morphol. 2018;26:345-50.
13. Batts KP, Ketover S, Kakar S, et al. Appropriate use of special stains for identifying Helicobacter pylori: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society. Am J Surg Pathol. 2013;37:e12-22.
14. Smith SB, Snow AN, Perry RL, et al. Helicobacter pylori: to stain or not to stain? Am J Clin Pathol. 2012;137:733-8.
15. Cutler AF, Havstad S, Ma CK, et al. Accuracy of invasive and noninvasive tests to diagnose Helicobacter pylori infection. Gastroenterology. 1995;109:136-41.
16. Wang XI, Zhang S, Abreo F, et al. The role of routine immunohistochemistry for Helicobacter pylori in gastric biopsy. Ann Diagn Pathol. 2010;14:256-9.
17. Anim JT, Al-Sobkie N, Prasad A, et al. Assessment of different methods for staining Helicobacter pylori in endoscopic gastric biopsies. Acta Histochem. 2000;102:129-37.
18. Sepulveda AR, Patil M.Practical approach to the pathologic diagnosis of gastritis. Arch Pathol Lab Med. 2008;132:1586-93.
19. Tajalli R, Nobakht M, Mohammadi-Barzelighi H, et al. The immunohistochemistry and toluidine blue roles for Helicobacter pylori detection in patients with gastritis. Iran Biomed J. 2013;17: 36-41.
20. Goldstein NS. Chronic inactive gastritis and coccoid Helicobacter pylori in patients treated for gastroesophageal reflux disease or with H pylori eradication therapy. Am J Clin Pathol. 2002;118:719-26.
21. Patnayak R, Reddy V, Jena A, et al. Utility of immunohistochemistry in demonstrating Helicobacter pylori. Onc Gas Hep Rep. 2015;4:4-7.
22. Kuipers EJ, Uyterlinde AM, Peña AS, et al. Long-term sequelae of Helicobacter pylori gastritis. Lancet. 1995;345:1525-8.
23. Kuipers EJ, Klinkenberg-Knol EC, Vandenbroucke-Grauls CM, et al. Role of Helicobacter pylori in the pathogenesis of atrophic gastritis. Scand J Gastroenterol Suppl. 1997;223:28-34.
24. Annibale B, Negrini R, Caruana P, et al. Two-thirds of atrophic body gastritis patients have evidence of Helicobacter pylori infection. Helicobacter. 2001;6:225-33.
25. Oksanen A, Sipponen P, Karttunen R, et al. Atrophic gastritis and Helicobacter pylori infection in outpatients referred for gastroscopy. Gut. 2000;46:460-3.
26. Kuipers EJ. Helicobacter pylori and the risk and management of associated diseases: gastritis, ulcer disease, atrophic gastritis and gastric cancer. Aliment Pharmacol Ther. 1997;11 Suppl 1:71-88.
27. Ley C, Mohar A, Guarner J, et al. Helicobacter pylori eradication and gastric preneoplastic conditions: a randomized, double-blind, placebo-controlled trial. Cancer Epidemiol Biomarkers Prev. 2004;13:4-10.

28. Karnes WE Jr, Samloff IM, Siurala M, et al. Positive serum antibody and negativetissue staining for Helicobacter pyloriin subjects with atrophic body gastritis. Gastroenterology. 1991;101:167–74.

29. Lee JY, Kim N.Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med. 2015;3:10.

30. Kokkola A, Rautelin H, Puolakkainen P, et al. Diagnosis of Helicobacter pylori infection in patients with atrophic gastritis: comparison of histology, 13C-urea breath test, and serology. Scand J Gastroenterol. 2000;35:138-41.

31. Testoni PA, Bonassi U, Bagnolo F, et al. In diffuse atrophic gastritis, routine histology underestimates Helicobacter pylori infection. J Clin Gastroenterol. 2002;35:234-9.

32. Ohkuma K, Okada M, Murayama H, et al. Association of Helicobacter pylori infection with atrophic gastritis and intestinal metaplasia. J Gastroenterol Hepatol. 2000;15:1105-12.