https://doi.org/10.15218/zjms.2022.019

Clinical manifestation of *Helicobacter pylori* infection and its association with gastric adenocarcinoma in gastritis patients in Erbil, Kurdistan Region, Iraq

Received: 05/09/2021 Accepted: 04/01/2022

Tavga Hushiar Salaim[1] Salah Tofik Jalal Balaky[1]* Rafal Al-Rawi[2] Saman Salah eldeen Abdulla[3]
Ahang Hasan Mawlood[1] Nazar M.T. Jawhar[4] Abdulla Delmany[5]

Abstract

Background and objective: *Helicobacter pylori* can be regarded as one of the most common causes of chronic gastritis, which affects more than half of the world's population. This study aimed to assess the presence of *H. pylori* in patients with gastritis and its association with gastric inflammation and adenocarcinoma.

Methods: The presence of *H. pylori* was detected by rapid urease test and histopathological tests using biopsy specimens. Data were analyzed using the GraphPad Software Statistical Package.

Results: The mean age of patients ± SD was 47.41 ± 18.13 years. The age range was 13 to 90 years. Results showed a significant association between the intensity of *H. pylori* and inflammation (P = 0.001). The more the intensity of *H. pylori*, the more severe the inflammation was noticed. Patients with high intensity of *H. pylori* had positive lymphoid aggregates. The *H. pylori* positive for the rapid urease test and the hematoxylin and eosin (H&E) staining test were 95.2% and 96.3%, respectively. *H. pylori* infection was detected in more than 85% of patients with gastric adenocarcinoma.

Conclusion: Histopathology and rapid urease tests are reliable diagnostic tools for detecting *H. pylori*. Results revealed a significant association of chronic active gastritis, mucosal lymphoid follicle formation, and adenocarcinoma with *H. pylori* infection.

Keywords: *Helicobacter pylori*; Histopathology; Gastric adenocarcinoma; Gastritis.

Introduction

Significant progress has been noticed in gastroduodenal disease principles and management since 1982 when *Helicobacter pylori* (*H. pylori*) was first discovered.[1,2] The pathogen can be regarded as one of the most common causes of chronic gastritis, affecting more than half of the worldwide population. *H. pylori* chronic gastritis increases the risk of other gastrointestinal-related disorders such as gastric peptic ulcer, gastric NonHodgkin lymphoma, and gastric adenocarcinoma. Histopathological examination using hematoxylin and eosin stain is considered the best standard method for detecting *H. pylori*. Nevertheless, other staining methods (e.g., Giemsa stain) have been used for better visualization.[3] The chronic gastritis Sydney grading system and its revised Houston version are widely used for the diagnosis, in which the infection was classified based on the activity of neutrophils and eosinophils in lamina propria, gastric glands, and chronic inflammatory infiltrates (e.g., lymphocytes), the intensity of *H. pylori*, glandular atrophy, and intestinal metaplasia.[4] The most documented risk factor for gastric cancer progression was

[1] Department of Medical Microbiology , College of Health Sciences, Hawler Medical University, Erbil, Iraq.
[2] Department of Clinical Analysis , College of Pharmacy, Hawler Medical University, Erbil, Iraq.
[3] Branch of Oral Diagnosis and Oral Medicine, College of Dentistry, Hawler Medical University, Erbil, Iraq.
[4] Department of Pathology, Medical College, Ninevah University, Mosul, Iraq.
[5] Department of Medicine, College of Medicine, Hawler Medical University, Erbil, Iraq.
Correspondence: salah.balaky@hmu.edu.krd

https://doi.org/10.15218/zjms.2022.019

H. pylori infection. Gastric cancer is a serious cancer-related cause of death worldwide. By producing inflammatory reaction, the pathogen can lead to chronic active gastritis and atrophic gastritis.[5] The elevated relation of *H. Pylori* infections with precancerous gastric nodes led to the WHO identifying the pathogen as a class I carcinogen in 1994. It has also been documented that infections caused by *H. pylori* have contributed to a 2- to 3-fold rise, estimated at 75% of the worldwide gastric cancer risk.[6] This study documented the histopathological changes in gastric mucosa induced by *H. pylori* colonization and its relation with clinical presentation, endoscopic findings, and gastric adenocarcinoma.

Methods

This study was conducted in the laboratory of PAR Private Hospital, Erbil, Iraq, from January 2018 to October 2019. A total of 188 patients were included of any age and gender with gastritis symptoms. A questionnaire regarding the age, gender, and whether they were taking any dyspepsia-related medications was included. Rapid Urease Test (RUT) is a rapid diagnostic test for the diagnosis of *H. pylori*. The basis of the test is the ability of *H. pylori* to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and carbon dioxide. A biopsy of gastric tissue was placed into a medium containing urea and a pH indicator. When the bacterial urease splits the urea, the liberated ammonia will increase the pH; this is recognized by a color change in the test indicator. Three biopsy specimens from the antrum and the corpus were taken by endoscopy and sent for histopathologic evaluation. In the histopathological unit, the biopsy specimens of patients were fixed in 10% buffered formalin for at least 12 hrs and then embedded in paraffin wax. Hematoxylin and Eosin (H and E) staining was performed on the tissue sections of each case. Sections for each specimen were de-paraffinized and hydrated in descending grades of alcohol, cut in sequential 4-µm sections. The sections were then stained with H and E stain to determine the presence of *H. pylori* and gastritis. The *H. pylori* was clearly detected as curved bacilli on the surface of the gastric epithelial cells. The slides were evaluated by a histopathologist and assigned to each morphological variable. Histopathology was used to confirm gastritis and neoplastic conditions found during endoscopy. Patients with *H. pylori* gastritis were divided into three groups according to the *H. pylori* severity status of infections (high, moderate, and low). Scattered organisms covering less than one-third of the surface are regarded as mild colonization; large clusters or a continuous layer over two-thirds of the surface are graded as severe; intermediate numbers are mentioned as moderate colonization severity.

The normal number of gastric mucosal mononuclear cells in the lamina propria was defined as a maximum of 2 to 5 lymphocytes, plasma cells, and macrophages per high-power field (×40 objective). Mild chronic inflammation was defined as a mild increase of inflammatory infiltration, predominantly plasma cells, within the lamina propria in a patchy, loose distribution without destruction or involvement of epithelium using ×10 objective lens to identify mononuclear clusters. Dense lymphoplasma cell infiltration of the lamina propria with or without lymphoid follicles, easily identifiable on x4 objective lens, with infiltration and destruction of epithelium, was regarded as severe chronic inflammation. Intermediate status was mentioned as moderate degree. The activity of gastritis was defined as neutrophilic infiltration of the lamina propria, pits, or surface epithelium.

Atrophic changes were defined as the loss of specialized glands from either the antrum or corpus. The metaplastic epithelium was recognized morphologically by the presence of goblet cells, absorptive cells, and cells resembling colonocytes.[7]

https://doi.org/10.15218/zjms.2022.019

Ethical consideration

Consents from the study participants were obtained verbally, and the study protocol was reviewed and approved by The Scientific and Research Ethics Committee of the College of Health Sciences, Hawler Medical University.

Statistical analysis

Data were analyzed using GraphPad Software. The Chi-square test of association was used to compare proportions. Fisher's exact test was used when the expected count of more than 20% of the cells of the table cells was less than 5. A *P* value of ≤0.05 was considered statistically significant.[8]

Results

The total number of the studied sample was 188 patients with gastritis. The mean age of patients ± SD was 47.41 ± 18.13 years. The age range was 13 to 90 years, and the median was 46 years. The largest proportion (39.4%) of the sample was in the age group 30-49 years, and more than half (53.2%) of the sample were males (Table 1).

The main histological changes were neutrophils (78.2%) and lymphoid aggregate (77.7%). The inflammatory changes were moderate in severity in 68.1% of the patients, as shown in Table 2. The findings showed a significant association between the intensity of *H. pylori* and inflammation (*P* = 0.001). The more the intensity of *H. pylori*, the more severe the inflammation was noticed. All those with high intensity of *H. pylori* had positive lymphoid aggregate, compared with 85.9% and 73.4% among those with moderate and low-intensity *H. pylori,* respectively (*P* = 0.003). No significant association was detected between the intensity of *H. pylori* with metaplasia (*P* = 0.394), and none of the patients with high-intensity *H. pylori* had glandular atrophy, compared with 1.4% and 12.8% among those with moderate and low-intensity of *H. pylori* (*P* = 0.010). Regarding the presence of neutrophils, the highest rate was seen among the high intensity of *H. pylori* samples (*P* = 0.0012).

Around two-thirds (64%) of the patients presented with chronic epigastric pain, 20% with dyspepsia and acidity, and 14.3% presented with iron deficiency anemia. No significant association was detected between the intensity of *H. pylori* with chronic epigastric pain (*P* = 0.333), dyspepsia and acidity (*P* = 0.365), and iron deficiency anemia (*P* = 0.190), as presented in Table 3.

Table 1 Age and gender distribution of the studied sample

Parameter	No.	(%)
Age (years)		
10-29	35	(18.6)
30-49	74	(39.4)
50-69	58	(30.9)
70-90	21	(11.2)
Mean (±SD)	47.41	(±18.13)
Gender		
Male	100	(53.2)
Female	88	(46.8)
Total	188	(100.0)

https://doi.org/10.15218/zjms.2022.019

Table 2 Histological changes by *H. pylori* intensity

Parameter	*H. pylori* intensity						
	Low		Moderate		High		
	No	(%)	No	(%)	No	(%)	*P* value
Inflammation							
Mild	32	(34.0)	16	(22.5)	1	(6.3)	
Moderate	61	(64.9)	53	(74.6)	14	(87.5)	
Severe	1	(1.1)	2	(2.8)	1	(6.3)	0.0011*
Lymphoid aggregate							
Positive	69	(73.4)	61	(85.9)	16	(100.0)	
Negative	25	(26.6)	10	(14.1)	0	(0.0)	0.003*
Metaplasia							
Positive	16	(17.0)	18	(25.4)	4	(25.0)	
Negative	78	(83.0)	53	(74.6)	12	(75.0)	0.394†
Neutrophils							
Present	70	(74.5)	57	(80.3)	16	(100.0)	
Absent	24	(25.5)	14	(19.7)	0	(0.0)	0.0012*
Glandular atrophy							
Positive	12	(12.8)	1	(1.4)	0	(0.0)	
Negative	82	(87.2)	70	(98.6)	16	(100.0)	0.010*
Total	94	(100.0)	71	(100.0)	16	(100.0)	

*By Fisher's exact test. †By Chi-square test.

Table 3 Prevalence of symptoms by *H. pylori* intensity

Parameter	*H. pylori* intensity						
	Low		Moderate		High		
	No.	(%)	No.	(%)	No.	(%)	*P* value
Chronic epigastric pain							
No	35	(37.2)	22	(31.0)	8	(50.0)	
Yes (64%)	59	(62.8)	49	(69.0)	8	(50.0)	0.333
Acidity and burning sensation							
No	78	(83.0)	55	(77.5)	11	(68.8)	
Yes (20%)	16	(17.0)	16	(22.5)	5	(31.3)	0.365
Iron deficiency anemia							
No	77	(81.9)	65	(91.5)	13	(81.3)	
Yes (14.3%)	17	(18.1)	6	(8.5)	3	(18.8)	0.190
Total	94	(100.0)	71	(100.0)	16	(100.0)	

https://doi.org/10.15218/zjms.2022.019

It is evident in Table 4 that there was no significant association between the severity of *H. pylori* with the endoscopic findings (*P* = 0.733). The *H. pylori* positive test results for the RUT and the H&E staining test were 95.2% and 96.3%, respectively (*P* = 0.626), as presented in Table 5.

The accuracy of the RUT was tested against the H&E staining test (as a gold standard). Table 5 presents the accuracy indicators of the RUT. Data showed that the sensitivity and specificity of RUT were 98.3% and 85.7%, respectively.

Table 6 shows that the prevalence of adenocarcinoma was associated with positive *H. pylori*, as 12 (85.71%) cases out of the 14 adenocarcinoma cases were positive for *H. pylori*, and just two cases of adenocarcinoma showed negative *H. pylori*. Although none of the patients severely infected with *H. pylori* had adenocarcinoma, compared with 78.57% of those with low *H. pylori* infection, statistical analysis showed a highly significant (*P* = 0.007) association between the prevalence of adenocarcinoma and *H. pylori* intensity (Table 6).

The most common type of adenocarcinoma was moderate to poorly differentiated intestinal adenocarcinoma (50%), then the well-differentiated intestinal adenocarcinoma (42.9%).

Table 4 Endoscopic findings by *H. pylori* intensity

Endoscopic findings	*H. pylori* Low		Moderate		High		
	No.	(%)	No.	(%)	No.	(%)	*P* value
Nodular	8	(8.5)	9	(12.7)	1	(6.3)	
Non erosive	36	(38.3)	31	(43.7)	7	(43.8)	
Erosive	50	(53.2)	31	(43.7)	8	(50.0)	0.733
Total	**94**	**(100.0)**	**71**	**(100.0)**	**16**	**(100.0)**	

Table 5 Accuracy of RUT compared with the H&E test

Parameter		H&E test for *H. pylori* Positive	Negative	Total
RUT	Positive	178	1	179
	Negative	3	6	9
Total		181	7	188
Sensitivity%	**Specificity%**	**PV+ %**	**PV- %**	**Agreement %**
(98.3)	(85.7)	(99.4)	(66.7)	(97.9)

Table 6 Prevalence of adenocarcinoma by *H. pylori* infection

Parameter	Prevalence of adenocarcinoma Negative		Positive		Total		
	No.	(%)	No.	(%)	No.	(%)	*P* value
Prevalence of *H. pylori*							
Negative	5	(2.87)	2	(14.29)	7		
Positive	169	(97.13)	12	(85.71)	181		0.087*
Total	**174**	**(100)**	**14**	**(100)**	**188**	**(100)**	
***H. pylori* intensity**							
Negative	5	(2.87)	2	(14.29)	7		
Low	83	(47.70)	11	(78.57)	94		
Moderate	70	(40.23)	1	(7.14)	71		
High	16	(9.20)	0	(0.00)	16		0.007*
Total	**174**	**(100)**	**14**	**(100)**	**188**	**(100)**	

*By Fisher's exact test.

https://doi.org/10.15218/zjms.2022.019

Discussion

The prevalence of *H. pylori* differed both between and within countries, with high rates of infection being associated with low socioeconomic status and high densities of people. When endoscopy is clinically indicated, the test of the first choice is the RUT on an antral-biopsy specimen. In the current study, *H. Pylori* colonizing gastric mucosa was identified by routine histological examination using hematoxylin and eosin stain in a total of 188 cases and confirmed by RUT, which showed positive results for 181 out of the 188 cases. The present study indicated that the infection rate among male patients was 53.2% compared with 46.8% in female patients, while the most common age affected by *H. pylori* gastritis was between 30-49 years. Histologically, moderate chronic inflammatory infiltrate (lymphocyte infiltration) was found in the majority of *H. pylori* infections with a significant association. Our analysis revealed that the low density of *H. pylori* was highest (111 cases) compared to 17 and 63 cases for high and moderate density infections, respectively. *H. pylori* caused chronic gastritis in all colonized subjects.

A study in Kurdistan, Iraq, indicated that the prevalence of *H. pylori* in the age group between 41- 50 years was 51.2%, followed by patients with age >61 years (46.5%). The infection rate among females was (40.7%), compared with the rate among males (38.2%). The study concluded that the prevalence of H. *pylori* in Erbil city was high, and the infection occurred at different stages of life.[9]

In our study, 146 cases showed lymphoid aggregate, and half of the cases had lymphoid follicles formation, which showed significant relation with *H. pylori* infection. It is believed that the presence of lymphoid aggregate and lymphoid follicles in the gastric mucosa was conspicuous features of *H. pylori*-associated chronic gastritis as lymphoid tissue hyperplasia is a specific immunological reaction to *H. pylori* infection and is considered to be a risk factor for the development of low-grade lymphoma (Tumor of Mucosa Associated Lymphoid Tissue).[10]

The present study showed a significant association between *H. pylori* infection and acute inflammatory infiltrate (neutrophils), indicating the infection activity. Alam et al. also reported that activity was very commonly seen in acute *H. pylori* infection.[11] The present study showed 14 cases with gastric adenocarcinoma, most commonly moderate to poorly differentiated intestinal-type adenocarcinoma, which showed significant association with *H. pylori* gastritis. Furthermore, individuals with chronic *H. pylori* infection had an increased risk of acquiring adenocarcinoma,[12,13] as the pathogen indicated as a carcinogen associated with gastric adenocarcinoma. *H. pylori* does not just lead to peptic ulcer disease, atrophic gastritis, gastric adenocarcinoma, and MALT (mucosa-associated lymphoid tissue) lymphoma.[14]

Patients with current *H. pylori* infection have a higher risk of developing gastric cancer than patients with past infection or eradication history of *H. pylori* because the eradication of *H. pylori* reduced the risk of gastric cancer. It was reported that the successful treatment of *H. pylori* decreases the risk of developing gastric cancer by approximately three-fold.[15]

Conclusion

The histopathology and RUT tests are reliable invasive diagnosis for *H. pylori*. It was noticed that *H. pylori* are seen more in young and middle age group male patients living in Erbil, Kurdistan. *H. pylori* was observed more in moderate chronic active gastritis patients and more than two third of patients with gastric adenocarcinoma. There was a significant association between the prevalence of adenocarcinoma and *H. pylori* intensity. A significant association of chronic active gastritis, mucosal lymphoid follicle formation, and adenocarcinoma with *H. pylori* infection was found.

Funding

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Rajan A, Ganguli P, Pathak N, Ranjan A, Thapa R, Tripathi S. Correlation of serology with morphological changes in gastric biopsy of *H. pylori* infection. International Journal of Research in Medical Sciences. 2017;26; 5(5):1851–7. https://doi.org/10.18203/2320-6012
2. Abu-Sbeih RS, Hawari AD, Hassawi DS, Al-Daghistani HI. Isolation and detection of Helicobacter pylori from patients suffering from peptic ulcer using biochemical tests and molecular techniques. American Journal of Biochemistry & Biotechnology. 2014;1:10(1):58. https://doi.org/10.3844/ajbbsp.2014.58.68
3. Marcela SB, Rivelle DP, Alfredo JA. Pathology Histological identification of H. pylori stained by hematoxylin-eosin and Giemsa: review for quality control. J Bras Patol Med Lab. 2015;51:2. https://doi.org/10.5935/1676-2444.20150019
4. Taha MM, Hassan SI, Ibrahim HA, Malek G A. Helicobacter pylori chronic gastritis updated Sydney grading in relation to endoscopic findings and H. pylori IgG antibody: diagnostic methods. J Microsc Ultrastruct. 2016;4(4):167–74. https://doi.org/10.1016/j.jmau.2016.03.004
4. Kurt O, Ozturk K, Altun B. Association between Helicobacter pylori and gastric cancers. Gut and Liver. 2017;11(2):312–3. https://doi.org/10.5009/gnl15316
5. Amieva M, Peek RM. Pathobiology of Helicobacter pylori-induced gastric cancer. Gastroenterology. 2016;150(1):64–78. https://doi.org/10.1053/j.gastro.2015.09.004
6. Hamid RGB, Mehdi G, Parvin A, Arash D, Mohamad ASR. Correlation between the Intensity of *Helicobacter pylori* Colonization and Severity of Gastritis. Gastroenterol Res Pract. 2017;2017:8320496. https://doi.org/10.1155/2017/8320496
7. GraphPad Software, Inc.2018.7825 Fay Avenue, Suite 230, La Jolla, CA 92037 USA. (Accessed 2018, at https://www.graphpad.com/scientific-software/prism/).
8. GraphPad Software, Inc.2018.7825 Fay Avenue, Suite 230, La Jolla, CA 92037 USA. (Accessed 2018. at https://www.graphpad.com/scientific-software/prism/).
9. AL-Mashhadany DA, Layla QI, Ahmed MZ. Seroprevalence of helicobacter pylori among human in Erbil governorate, Kurdistan region, Iraq. RJLBPCS. 2018;4(2):268–80. https://doi.org/10.26505/DJM.18014880818
10. Bhavna GVS, Neena S, Ajit S, Vineeta M. Histopathological analysis of chronic gastritis and correlation of pathological features with each other and with endoscopic findings. Pol J Pathol. 2012;63(3):172–8. https://doi.org/10.5114/pjp.2012.31501
11. Sulaiman KM. Cytogenetic study of stomach cancer in Erbil City. ZANCO Journal of Pure and Applied Sciences. 2016;28(4):56–65. http://doi.org/10.21271/ZJPAS.28.4.9
12. Nocturne G, Pontarini E, Bombardieri M, Mariette X. Lymphomas complicating primary Sjögren's syndrome: from autoimmunity to lymphoma. Rheumatology 2019; (Oxford, England). https://doi.org/10.1093/rheumatology/kez052
13. Abbas H, Niazi M, Makker J. Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma of the Colon: A Case Report and a Literature Review. The American Journal of Case Reports. 2017;18:491–97. https://doi.org/10.12659/AJCR.902843
14. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. On behalf of the European Helicobacter and Microbiota Study Group and Consensus panel. Management of *Helicobacter pylori* infection—the Maastricht V/Florence Consensus Report. 2016. https://doi.org/10.1136/gutjnl-2016-312288
15. Omer K, Kadir O, Battal A. Association between *Helicobacter pylori* and Gastric Cancers. Gut and Liver. 2017;11:2312–3. https://doi.org/10.5009/gnl15316