The Effect of *Helicobacter pylori* on Gastric Cancer Treated with Adjuvant Chemotherapy After Curative Resection

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Abstract *Helicobacter pylori* has been associated with diverse pathologies of varying severity. We investigated the *H. pylori* infection status and its association with the pathologic features and clinical outcomes in stage III gastric cancer patients treated with adjuvant therapy after curative resection. Between 2004 and 2009, the records of 76 consecutive patients were retrospectively reviewed. *H. pylori* infection was confirmed by examination of pathological specimen. The relationship between *H. pylori* and the clinicopathological features was analyzed by Fisher exact test, Student's *t* test, and Kaplan-Meier method. Of the 76 patients, 16 patients (21.1 %) were confirmed for *H. pylori* infection. The median age was 59 years. Twenty-three patients received chemotherapy and remainder received chemoradiotherapy. *H. pylori* status did not correlate with the clinicopathologic features. It was greater in non-neoplastic tissue than the tumor tissue (21.1 vs 7.9 %). Median follow-up was 21 months. During this period, 88.2 % patients had experienced tumor recurrence, and 85.5 % patients had died. Recurrence was observed in 87.5 % patients and in 88.3 % patients in *H. pylori*-positive and *H. pylori*-negative patients, respectively ($P=0.92$). Disease-free survival was 28.4 ± 7.9 months and overall survival was 31.5 ± 7.4 months in *H. pylori*-positive patients compared with 28.3 ± 3.7 and 33.2 ± 3.4 months, respectively, in *H. pylori*-negative patients. *H. pylori* infection status did not have effect on the overall or disease-free survival ($p=0.85$ and $P=0.86$),

respectively. *H. pylori* status might not be useful as a prognostic and predictive factor for clinical outcomes.

Keywords *Helicobacter pylori* · Gastric carcinoma · Prognosis · Survival

Introduction

Helicobacter pylori is considered the most common etiologic agent for infection-related cancers, which represent 5.5 % of the global cancer burden [1]. It is so common that more than 50 % of the global population harbors the bacterium. *H. pylori* infects the gastric mucosa and causes chronic inflammation that often persists for years. In addition, it also leads to genetic and epigenetic changes resulting in genetic instability [2]. Among infected individuals, approximately 10 % develops peptic ulcer disease, 1–3 % progresses to gastric cancer, and 0.1 % develops mucosa-associated lymphoid tissue lymphoma [3]. After the relationship between *H. pylori* infection and development of gastric cancer has been defined by both epidemiological and clinical prospective studies, *H. pylori* was classified as a group 1 carcinogen for human by International Agency for Research on Cancer in 1994 [4]. Although many studies over the past two decades have revealed the strong correlation between *H. pylori* and gastric cancer development, the effect of *H. pylori* status on clinical outcome of gastric cancer patients has not been well documented, especially according to the stage. Currently, the available data have suggested that the influence of *H. pylori* infection on the progression and clinical outcome of gastric cancer is still obscure [5–11]. In this study, we investigated the *H. pylori* infection status and its association with the pathologic features and clinical outcomes in stage III gastric cancer patients treated with adjuvant therapy after curative resection.

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Patient and Methods

Ethical Consideration

This study was approved by the Institutional Review Board of Ankara Oncology Education and Research Hospital.

Patient Selection

Between 2004 and 2009, the records of 76 consecutive patients who had a curative resection followed by postoperative chemotherapy or chemoradiotherapy for the treatment of stage III gastric adenocarcinoma (according to the American Joint Committee on Cancer, 7th edition [12]) were retrospectively reviewed. Patients who had a previous gastric resection or had other coincident malignancies and those with Siewert type I cardia adenocarcinoma were excluded from the study. All patients underwent subtotal/total gastrectomy with D II lymphadenectomy with curative intent and all received chemotherapy or chemoradiotherapy postoperatively. Chemotherapy regimen was 5-fluorouracil and leucovorin or epirubicin, cisplatin, and 5-fluorouracil. Chemoradiotherapy consist of 45 Gy of regional radiotherapy (1.8 Gy/day 5 days/week) for 5 weeks, in addition with 5-fluorouracil and leucovorin regimen. We analyzed clinicopathological features including age, sex, tumor location, type of gastrectomy, histological type of the tumor, Lauren classification, tumor size, T stage, N stage, lymphovascular and perineural invasion, adjuvant treatments, type of patterns of metastasis, and survival outcomes. All patients' surgical specimens re-evaluated for *H. pylori* infection. Patients were grouped according to the presence of HP infection and potential differences in clinical and pathologic characteristics between the two groups of patients were investigated.

Histopathologic Examinations

All resected gastric specimens were fixed in neutral-buffered 10 % formalin. After tissue processing overnight, all tissues were embedded in paraffin and cut into 4- μ m sections. The sections were stained with hematoxylin–eosin for histology and Giemsa for detecting *H. pylori*. Histopathologically, analyses were established both in neoplastic and non-neoplastic areas of the gastric specimen by one pathologist who was unaware of the patient's clinical information. The histopathological findings of gastritis in non-neoplastic areas including glandular atrophy, intestinal metaplasia, inflammation, and *H. pylori* density were analyzed using the visual analog scale of the Updated Sydney System [13]. The density of *H. pylori* infection was graded as negative (normal) or positive (mild, moderate, marked) in neoplastic and non-neoplastic areas. Patients were regarded as morphologically negative for

H. pylori if not detected in both areas. Otherwise, they were regarded positive for *H. pylori*.

Follow-up

Follow-up evaluation included complete medical history and physical examination, chest radiography, and laboratory tests, including complete blood cell count, blood urea nitrogen, creatinine, liver function tests, and tests for tumor markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). The patients were reviewed every 3 months for 2 years, every 6 months for next 3 years, and yearly thereafter. Abdominal ultrasonography or computed tomography scan and chest radiography every 6 months until 5 years after the operation and yearly thereafter and endoscopy of the upper digestive tract once annually were performed. In case of a relapse, all patients were restaged to detect disease at other sites. Recurrences were documented by clinical or radiologic assessment or both and were categorized as locoregional, systemic, or combined.

Statistical Analysis

The primary end point of the study was disease-free survival (DFS) and overall survival (OS). The disease-free survival was calculated from time of diagnosis to disease recurrence or last follow-up. Overall survival was measured from the date of tumor resection to death or to the last date the patient was known to be alive. Descriptive statistics were used to calculate frequencies and percentages for all variables involved. The association between *H. pylori* infection status and clinicopathological features was compared using the Fisher exact test or the Pearson chi-square test. The independent sample Student's *t* test was used for comparison of age at time of surgery and tumor size. The Kaplan-Meier method was used to estimate the effect of *H. pylori* status on DFS and OS. The differences between the survival curves were tested by using the log-rank test. *P* values of <0.05 were considered statistically significant. All analyses were carried out using SPSS software (version 21.0; SPSS Inc, Chicago, IL).

Result

Sixteen out of 76 patients (21.1 %) had *H. pylori* infection in stage III gastric carcinoma.

The median age of the enrolled patients was 59 years, with a range of 25–79 years. Stage distribution was as follows: 31.6 % of patients in stage IIIA, 23.7 % in stage IIIB, and 44.7 % in stage IIIC. *H. pylori* infection was not related to the level of serum CEA and CA 19-9 preoperatively (data not shown). All patients had undergone surgery with curative intent either with total (43 patients, 56.6 %) or subtotal

gastrectomy (33 patients, 43.4 %). Twenty-three patients received 5-fluorouracil-based regimen, and the remainder received chemoradiotherapy.

H. pylori status did not correlate with the clinicopathologic factors of gastric adenocarcinoma (Table 1). It was greater in non-neoplastic tissue than the tumor tissue (21.1 vs 7.9 %), and only six patients (7.9 %) had *H. pylori* infection in both non-neoplastic tissue and tumor tissue. In terms of relationship between *H. pylori* status and histopathologic findings of gastritis, positive *H. pylori* infection had no correlation with inflammation, atrophy, and intestinal metaplasia (Table 2).

The median follow-up was 21 (6–102) months. During the follow-up period, 67 patients (88.2 %) had experienced locoregional recurrence or distant metastasis, and 65 patients (85.5 %) had died of gastric cancer-related complications. Recurrence were showed as follows: locoregional in three patients, both locoregional and systemic in five patients, and the remainder in systemic recurrence. Recurrence was observed in 14 patients (87.5 %) and in 53 patients (88.3 %) in *H. pylori*-positive and *H. pylori*-negative patients, respectively ($p=0.92$).

Disease-free survival was 28.4 ± 7.9 months and overall survival was 31.5 ± 7.4 months in *H. pylori*-positive patients compared with 28.3 ± 3.7 and 33.2 ± 3.4 months, respectively, in *H. pylori*-negative patients. *H. pylori* infection status did not have a significant effect on the overall or disease-free survival ($p=0.85$ and $p=0.86$), respectively.

Discussion

In the present study, *H. pylori* status did not correlate with survival in gastric cancer patients. Additionally, *H. pylori* status could not predict recurrence in patients with gastric cancer. The clinicopathological features of *H. pylori*-positive patients were also compared with those of the group of patients with negative *H. pylori* status. “Statistical analysis” revealed that *H. pylori* status was not identified to be significantly associated with the clinicopathologic factors of gastric adenocarcinoma.

In contrast to our study, a few recent studies have supported the role of *H. pylori* infection in gastric cancer prognosis. After Lee et al. [14] reported the significant association between seronegative *H. pylori* status and poor outcome in 128 resected gastric cancer patients in univariate analysis, three large prospective studies investigated the prognostic role of *H. pylori* infection [10, 11, 15]. First, Meimarakis et al. [15] identified *H. pylori* status as an independent beneficial prognostic factor for overall and relapse-free survival in gastric cancer patients with curative resection, especially in early stages. The other prospective study by Marrelli et al. [10] indicated that negative *H. pylori* status was an independent prognostic factor of poor prognosis in patients with gastric

cancer. A recent study by Wang et al. [11], in which they investigated the prognostic impact of *H. pylori* status on the prognosis of patients undergoing curative resection for gastric cancer in Chinese prospective cohort, showed that *H. pylori* positivity was a beneficial prognostic indicator, independent of other clinicopathologic variables.

The different findings between current and abovementioned recent studies might be attributed to several points. First, unlike previous studies, our study did not include patients with early gastric cancer; all patients are in stage III and received adjuvant chemotherapy or chemoradiotherapy as a routine treatment. In contrast, most of the patients have received no adjuvant treatment in the studies of Marrelli and Meimarakis et al. Second, different *H. pylori* detection methods have been used. Although there are many methods used to detect the *H. pylori*, including culture, histopathological diagnosis, urease test, real-time polymerase chain reaction (PCR), serological analysis, and urea breath test, none of the methods of *H. pylori* detection is perfect. There is also no universally accepted standard diagnostic test for *H. pylori*. In concordance with many previous studies in the literature, we used only histopathological diagnosis for *H. pylori* detection [8, 11, 16]. Since the combination of two or more methods might increase the sensitivity and specificity of a diagnosis of *H. pylori* infection to define the *H. pylori* status, Marrelli et al. used the combination of methods PCR for *vacA* gene and serologic analysis, and Meimarakis et al. used bacterial culture, histological analysis, and serology. Nevertheless, we could not use the serologic test and PCR analysis due to the retrospective nature of our study. However, conventional serological analysis for *H. pylori* infection may not be an appropriate method for detecting a relationship between *H. pylori* and gastric cancer prognosis. It might be considered as the most accurate method for determining previous *H. pylori* infection, but not all of them are associated with gastric cancer [17]. In addition, it has been reported that 14 % of PCR-positive gastric cancer patients demonstrated negative serology for *H. pylori* [10]. On the other hand, *H. pylori* may be localized in or around the tumor and carcinogenesis related to *H. pylori* is considered to be localized rather than systemic in gastric cancer patients [16]. As a result, histopathological diagnosis and molecular methods may be likely to be most successful in detecting a relationship between *H. pylori* infection and gastric cancer prognosis.

The reasons why negative *H. pylori* status is associated with poor outcomes in gastric cancer patients remain to be defined. To date, several possible explanations are suggested to explain the correlation between a positive *H. pylori* status and a better prognosis. According to one of them, improved prognosis in gastric cancer patients with positive *H. pylori* status may be the result of local immune response to *H. pylori* infection [15, 18]. As a gram-negative bacterium, *H. pylori* stimulates the production of many inflammatory

Table 1 The association between clinicopathologic features and *H. pylori* infection status

		<i>H. pylori</i>		
		Negative 60 pts	Positive 16 pts	<i>P</i> values
Age (year)		57.2±11.4	58.6±11.0	0.66
Gender	Male	42 (70.0 %)	9 (56.3 %)	0.29
	Female	18 (30.0 %)	7 (43.7 %)	
Tumor location	Upper third	9 (15.0 %)	1 (6.3 %)	0.58
	Middle	28 (46.7 %)	10 (62.4 %)	
	Lower third	21 (35.0 %)	5 (31.3 %)	
	Whole	2 (3.3 %)	0 (0.0 %)	
Resection	Total gastrectomy	33 (55.0 %)	10 (62.5 %)	0.59
	Subtotal gastrectomy	27 (45.0 %)	6 (37.5 %)	
Pathology	Adenocarcinoma	44 (73.3 %)	13 (81.2 %)	0.51
	Signet ring cell carcinoma	16 (26.7 %)	3 (18.8 %)	
Lauren classification	Intestinal	41 (68.3 %)	10 (62.5 %)	0.65
	Diffuse	19 (31.7 %)	6 (37.5 %)	
Tumor size	Mean±SD	6.4±3.1	6.1±2.9	0.71
Tumor invasion	T2	2 (3.3 %)	0 (0.0 %)	0.38
	T3	6 (10.0 %)	1 (6.3 %)	
	T4	52 (86.7 %)	15 (93.7 %)	
N stage	N1	14 (23.3 %)	3 (18.8 %)	0.94
	N2	16 (26.7 %)	6 (37.4 %)	
	N3	30 (50.0 %)	7 (43.8 %)	
Stage	Stage IIIA	20 (33.3 %)	4 (25.0 %)	0.68
	Stage IIIB	13 (21.7 %)	5 (31.2 %)	
	Stage IIIC	27 (45.0 %)	7 (43.8 %)	
Lymphovascular invasion	Absent	16 (26.7 %)	3 (18.8 %)	0.51
	Present	44 (73.3 %)	13 (81.2 %)	
Perineural invasion	Absent	21 (35.0 %)	5 (31.3 %)	0.77
	Present	39 (65.0 %)	11 (68.7 %)	

mediators such as cytokines which may promote the development of cellular and humoral immune response mainly of the type-1 T helper cell which contributes to elevated antitumor immunity [19, 20]. Improved immune response against the tumor improves survival rates of gastric cancer patients with *H. pylori* infection. In addition, it has been assumed that because *H. pylori* components mimic specific receptors or

surface molecules on gastric epithelial cells, autoantibodies could induce a cross-reaction against gastric cancer cells [21].

However, several authors have raised doubts regarding the true prognostic value of *H. pylori* status, suggesting that *H. pylori* negativity may be simply related to more advanced tumor stage [22, 23]. When the disease is far advanced, parietal cells in the gastric mucosa are destructed and the lumen of

Table 2 *H. pylori* and chronic gastritis (according to Sydney classification) status in pathologic assessment of the surgical specimen

		<i>H. pylori</i>		
		Negative 60 pts	Positive 16 pts	<i>P</i> values
Inflammation	Positive	44 (73.3 %)	13 (81.2 %)	0.57
	Negative	16 (26.7 %)	3 (18.8 %)	
Atrophy	Positive	25 (41.7 %)	5 (31.3 %)	0.42
	Negative	35 (58.3 %)	11 (68.7 %)	
Intestinal metaplasia	Positive	42 (70.0 %)	10 (62.5 %)	0.59
	Negative	18 (30.0 %)	6 (37.5 %)	

the stomach becomes an alkaline environment, which is unfavorable for *H. pylori*. The organism is destroyed and the patient becomes *H. pylori* negative [22]. Actually, it was reported that the rates of histologically detected *H. pylori* positivity in tumor tissue was higher for early-stage gastric cancer compared to advanced gastric cancer [24]. It is therefore not surprising that *H. pylori*-positive patients whose disease is at a less advanced stage show higher survival rates than *H. pylori*-negative patients with gastric cancer. Because the cohort consisted of patients with advanced (stage III) gastric cancer, the abovementioned scenario might be the reason of relative lower rate of *H. pylori* positivity in our study. In the present study, the rate of *H. pylori* positivity was identified as 21 % in gastric cancer patients. This value is lower than both the rates reported in most studies [7, 10] which vary from 17.5 to 86.2 % and expected rates in Turkey where *H. pylori* infection is highly prevalent [25]. It might also explain the reason of the rate of *H. pylori* positivity was higher in non-neoplastic tissue than in tumor tissue in current and most studies in the literature [9, 11].

On the other hand, there are only a few studies suggested that *H. pylori* infection is also an independent prognostic factor in locally advanced and metastatic gastric cancer [8, 16]. One of them had a non-homogenous patient group which includes stages IB, II, IIIA, IIIB, and IV and two groups of patients receiving different adjuvant chemotherapy regimens in contrast to our study. It reported *H. pylori*-negative status was associated with poor outcome in all stages except stage IIIB. The others included only inoperable advanced gastric cancer patients and also did not use any chemotherapy regimen. The difference in survival rates of these studies may be due to a better response to different chemotherapy regimen in *H. pylori*-positive patients.

The cohort included the patients with gastric cancer in same stage and received similar adjuvant treatment. This differs the current study from other studies in the literature. The current study was limited by a relatively small number of patients from single institution and retrospective nature, with consequent methodological limitations. Only histopathological method for *H. pylori* diagnosis was used. Because of the nature of the disease, survival in advanced gastric carcinomas is limited. This may be the reason that the effect of *H. pylori* status in gastric cancer prognosis might not be identified.

Conclusion

H. pylori status does not seem to be associated with survival in stage III gastric cancer patients treated with gastrectomy. Data from the current study suggest that *H. pylori* status might not be useful as a prognostic and predictive factor for clinical outcome of this group of patients. It should be noticed that this study consists of small number of patients. Considering

previous reports and our study, the effect of *H. pylori* status on survival, especially in advanced stage gastric cancer, has to be validated with further studies. Associations and mechanism of *H. pylori* infection have not yet been defined sufficiently well to guide the clinician in treatment decisions.

Conflict of Interest All authors have declared no conflict of interest.

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