RESEARCH ARTICLE

Is *Helicobacter pylori* a Poor Prognostic Factor for HER-2 SISH Positive Gastric Cancer?

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Abstract

Background: Helicobacter pylori (*H. pylori*) is one of the risk factors for gastric cancer (GC). Any prognostic effect of HER-2 status in gastric lymph node metastasis in *H. pylori* positive cases is unknown. Materials and Methods: A total of 74 patients, 47 (64%) male, and 27 (34%) female, who had subtotal or total gastrectomy and also positive lymph nodes, were included in the study. Age range was 29-87 years, and median age was 58 years. HER-2 expression was assessed in both gastric resection samples and lymph node material with carcinoma metastasis of the same patient by immunohistochemistry (IHC) and silver in situ hybridization (SISH) methods. *H. pylori* status was examined in gastric materials of all patients. Relationships between HER-2 status in gastric cancers and lymph nodes and *H. pylori* status were investigated. Results: *H. pylori* was positive in 40 cases (54%), and negative in 34 (46%). While in the primary tissues of *H. pylori* positive cases, SISH positivity for HER-2 was observed in 13 cases (86%), SISH negativity was observed in 2 (14%), in metastatic lymph nodes 21 cases (72%) were SISH positive and 8 cases (28%) were SISH negative (P=0.005 and P=0.019, respectively). Initial CEA values were high in 18 cases (78%) with positive *H. pylori* and in 5 cases (22%) with negative *H. pylori* (P=0.009). While SISH data of patients were negative in 59 cases (80%) and positive in 15 cases (20%) in primary tissues, they were negative in 56 cases (75%) and positive in 18 cases (25%) in lymph nodes. Discrepancy between primary tissue and lymph node results was detected in 3 cases, in which SISH was negative in the primary tissue and HER-2 expression was positive in the lymph nodes. Conclusions: Clinical progression was poor in *H. pylori* positive cases with HER-2 negativity in primary gastric tissue, but HER-2 positivity in the lymph nodes. SISH positivity can be expected in *H. pylori* positive cases, and it may be predicted that these cases can benefit from trastuzumab treatment.

Keywords: *H. pylori* - immunohistochemical - HER-2 - silver in situ hybridization - gastric cancer

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Introduction

Relationship between *H. pylori* and gastric carcinogenesis has been known for a long time. Correa's model (Correa et al., 1990) is only one of hypotheses about this issue. According to this, there is a long and thin way to development of cancer starting from chronic superficial gastritis in subjects with positive *H. pylori*. In 1994, the World Health Organization has accepted that *H. pylori* was a carcinogen bacterium especially in development of intestinal type gastric cancer (Neubauer et al., 1997). Superficial gastritis observed at the baseline causes accumulation of leukocytes on lamina propria, and especially leukocyte invasion of neck epithelium of glands. As a result, genomic damage is occurred in DNAs of cells, and gastric carcinoma is developed in time (Wagner et al., 1997). Although they are not independent from each other, three genes, which have been named as cytotoxic Vac A (causing vacuolization in cells during cancer development), cytotoxin associated gene A (Cag A) and Ice A may reflect degrees of risks in different gastric diseases.

HER-2 status is a molecule, which has a key role in development and progression of many cancers, and it is related to poor prognosis. For example, while HER-2 molecule expression is amplified at 20% in breast cancer (Marx et al., 2009), this amplification or protein overexpression is detected as 7-34% in gastric cancer (Bozzetti et al., 2011). HER-2 overexpression is related to short survival in gastric cancer (Sawaki et al., 2012). HER-2 expression in lymph node gains importance because it may work in a clonal proliferation different from the primary due to possible intra-tumoral heterogeneity in a GC case, which has metastasized to gastric lymph nodes. Lymph node expression state should be defined because poor prognoses of patients are related to HER-2 expression of tumor cells, which have metastasized to gastric lymph nodes.

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It has been known that *H. pylori* has a major role in EGFR receptor expression in gastric mucosal cells (Coyle et al., 1999). Changes like growth, proliferation and differentiation are observed with EGF receptor expression. This overexpression may cause development of gastric cancer. There are performed studies, which have indicated that EGF and EGFR levels were decreased in cells with *H. pylori* eradication (Coyle et al., 1999). The limited number of studies in the literature has investigated HER-2 status both in gastric and its metastatic tissues. However, the relationship of this status with *H. pylori* has not been investigated. In our study, effects of HER-2 status in *H. pylori* and its effects on prognosis have been investigated in the primary and in metastases.

Materials and Methods

A total of 74 patients between years 2009 and 2012 followed up by our outpatient clinic were included in the study. Patients with subtotal or total gastrectomy and who had positive lymph node at the same time were included in the study. Patients were made up of the ones with stage II-IV diseases, without distant metastasis, and 47 of them were male (64%), and 27 of them (34%) were female. Age range was 29-87 years, and median age was 58 years. Tumor localizations were 30 at the antrum, 23 at the corpus, and 21 at the cardia. Samples of all patients were obtained from gastric resection and lymph node dissection materials. HER-2 expression was examined in both gastric and carcinoma metastasis of lymph node material of the same patient by IHC and SISH methods. *H. pylori* status was determined in all patients. *H. pylori* was obtained histologically from gastric tissue. Relationships between *H. pylori* and gender, tumoral localization, lymphovascular invasion (LVI), perineural invasion (PNI), CEA, CA 19-9, and histological subtype were revealed in patients. Again, relationship of HER-2 status and *H. pylori* in the gastric and lymph node was detected.

Statistical analysis

Patient and tumor characteristics were defined descriptively as median and percentage. HER-2 status changes were expressed as percentages in primary tumors and lymph node metastasis. HER-2 expression was compared in primary tumor and lymph nodes by correlation tests. Absolute HER-2 status changes were tested between clinical/pathological characteristics and lymph node-primary tissue by regression analysis. HER-2 status in primary tumor, and HER-2 changing state between primary tissue and lymph node were examined by Chi square test. Disease free survival and overall survival were calculated by Kaplan-Meier method. Changed/unchanged HER-2 state of subgroups were compared in survival by log rank test. Planned all statistical analyses were performed by SPSS statistical program (SPSS 16.0, SPSS Inc. Chicago, Illinois).

Results

A total of 74 patients, who were followed up by our outpatient clinic, were included in the study. Age range was 29-87 years, and median age was 58 years. While 47 of patients (64%) were male, 27 (34%) were female; and 48 of samples (65%) were intestinal type, and 26 (35%) of them were diffuse type of pathology. Baseline clinical and pathological characteristics of patients enrolled in the study are summarized in Table 1. Patient distributions according to TNM classification were 9 (12%) in stage IIa; 20 (28%) in stage IIb; 16 (21%) in stage IIIa; 12 (16%) in stage IIIb; and 17(23%) in stage IIIc. While *H. pylori* positivity was detected in 40 cases (54%), *H. pylori* negativity was detected in 34 cases (46%). Initial CEA values were high in 18 cases (78%) with positive *H. pylori*, whereas in 5 cases (22%) with negative *H. pylori* (P=0.009). Initial CA 19-9 values were high in 7 cases (53%) with positive *H. pylori*, and in 6 cases (47%) with negative *H. pylori* (P=0.9). According to *H. pylori* status, no statistical significance was present for gender, and tumor location (P=0.099 and P=0.7, respectively). In the primary tissue, 13 (86%) cases were SISH positive, 2 (14%) cases were SISH negative among *H. pylori* positive ones (P=0.005); in metastatic lymph nodes 21 (72%) cases were SISH positive, whereas 8 (28%) cases were SISH negative (P=0.019). Again according to *H. pylori* status, there was no statistically significance between lymphovascular invasion, perineural invasion and tumoral histology (P=0.1, P=0.2, P=0.5, respectively). Relationships between primary tissue-lymph node SISH status and *H. pylori* was shown table 2. *H. pylori* was detected positive in 3 cases, in which primary tissue was SISH negative, whereas metastatic lymph node was SISH positive. Overall survivall of according to *H. pylori* and

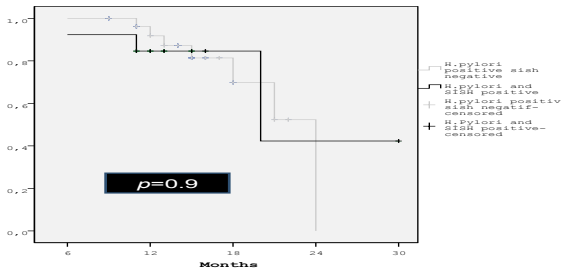

Figure 1. Overall Survivall of According to *H. pylori* and SISH Status

Table 1. Clinical and Pathological Characteristics of Patients

			No.	%
Gender	Male		47	64
	Female		27	36
Age, range			58 (29-87)	
Surgery type	H. pylori	Positive	40	54
		Negative	34	46
		Total gastrectomy	45	60
		Subtotal gastrectomy	29	40
	Tumor localization	Antrum	30	40
		Corpus	23	31
		Cardia	21	29
	Histological type	Intestinal	48	65
		Diffuse	26	35
	Stage	IIa	9	12
		IIb	20	28
		IIIa	16	21
		IIIb	12	16
		IIIc	17	23

Table 2. Relationships between Primary Tissue- lymph Node SISH Status and *H. pylori*

		Primary tissue		Lymph node	
		SISH (-)	SISH (+)	SISH (-)	SISH (+)
General (n/%)		59 (80)	15 (20)	56 (75)	18 (25)
H. pylori	Positive (n/%)	2 (14%)	13 (86%)	8 (28%)	21 (72%)
	Negative (n/%)	32 (94%)	2 (6%)	26 (76%)	8 (24%)
Histology	Diffuse	21	5	20	6
	Intestinal	38	10	36	12
Stage	Stage II	22	7	19	10
	Stage III	37	8	37	8
Localization	Antrum	24	4	24	4
	Corpus	17	7	14	10
	Cardia	18	4	18	4

*Histology subtype of primary SISH(-) cases: p 0.87, stage of primary SISH (-): p 0.5, localization of primary SISH (-): p 0.39, histology subtype of primary SISH (+): p 0.1, stage of primary SISH (+): p 0.5, localization of primary SISH (+): p<0.05, H. pylori positive of primary SISH (+) cases: p=0.005, H. pylori negative of primary SISH (-) cases: p=0.005

SISH status was shown Figure 1.

Discussion

H. pylori is a microorganism, whose infection has been defined in etiology of gastric cancer development. It has been defined in 95% of cases with duodenal ulcers and 70-80% of cases with gastric ulcers. Concomitance of *H. pylori* and gastric cancer has been reported at different rates from different studies. *H. pylori* antibody was defined in controls in 47% and 69% in cases with gastric cancer by Forman; in 61% of controls and in 84% of gastric cancers cases by Parsonnet; and in 76% of controls and in 94% of gastric cancer patients by Nomura (Parsonnet et al., 1991; Nomura et al., 1991; Forman, 1995). Some molecular structures like oncogene and cellular growth factors are present in normal cells. Mutations, which may occur in them, may contribute in tumor development period. On the other hand, there are also some genes with tumor suppressive effects, and as the results of mutations, it is known that these may also have a role in tumor development.

Although it was reported that there was no difference in *H. pylori* seroprevalence between majorities of intestinal and diffuse types of cancer cases, there were also studies in which significantly higher rates were defined in intestinal type (80-90%) than the diffuse type (30%) cancer cases (Bhandari and Crowe, 2012).

H. pylori increases mucosal cellular proliferation. This proliferation has been shown in the *H. pylori* infected mucosa by nucleolar organizer regions (NOR) and by high expression rate of proliferated cellular nuclear antigen (PCNA). When *H. pylori* is eradicated, cellular proliferation markers are regressed.

In some studies investigating relationship between *H. pylori* infection and p53, p53 expression was shown to be increased more at the regenerative zone of gastric pit than normal mucosa. p53 immunoreactivity and mRNA were defined in proliferative and apoptotic regions in incomplete intestinal metaplasic glands. After *H. pylori* eradication, p53 immunoreactivity has returned the normal mucosal value. *H. pylori*-related gastritis, known to cause DNA damages, has played a role in development of intestinal type cancer by causing genetic defects probably including p53 mutations (Hıbı et al., 1997). E-cadherin is a protein responsible for cellular-basal membrane and cell-to-cell adhesions. E-cadherin protein loss in tumors has caused invasive progression of relatively benign and rapid progression of metastatic tumors. Mutations occurred at E-cadherin gene may cause the basement for poorly differentiated and diffuse tumors. *H. pylori* infection shows a close relationship with E-cadherin protein loss (Terres et al., 1998).

Epidermal growth factor receptors: EGF receptor family contains four genes coding for four homologous epidermal growth factor receptors (Yarden, 2001). All of receptors are localized in cellular membrane, and they are present in various tissues. They simply code for a transmembrane glycoprotein named as HER-2 protein or receptor.

Protein overexpression mechanisms of HER-2/neu and high amplifications of HER-2/neu in gastric adenocarcinoma have similar appearances with the ones emphasized in breast cancer cases (Kono et al., 2002). Majority of studies conclude in that gastric adenocarcinoma cases with c-erbB-2 protein overexpression had lower survival rates than those without c-erbB-protein overexpression, and also c-erbB-2 overexpression was a poor prognostic factor (Nakajıma et al., 1999; Allgayer et al., 2000).

Finally, nearly in line with all of the literature knowledge, HER-2/neu overexpression is known to be the poor prognosis indicator in especially intestinal type of gastric adenocarcinoma. In the latest stages of studies, HER-2/neu overexpression mechanisms and high level amplification of c-erbB-2 in gastric adenocarcinoma were similar in appearance with the emphasized ones in breast cancer cases, and gastric adenocarcinoma cases with c-erbB-2 amplification were underlined to be potential candidates for human monoclonal antibody trastuzumab (Takehana et al., 2002).

H. pylori is known to play a major role in EGF receptor expression in gastric mucosal cells (7). Changes like growth, proliferation and differentiation are observed with EGF receptor expression. This overexpression can cause gastric cancer development (7). This expression is ended up with HER-2/neu (cerbB-2) oncogene activities. No relationship between *H. pylori* and c-erbB-2 overexpression was detected in studies, which were conducted on EGFR expression or c-erbB-2 (HER-2/neu) oncogene activity in gastric cancer patients (Wang et al., 2002a; 2002b; Pryczynicz et al., 2009).

Coyle et al. (1999) measured EGF and EGFR protein levels by the flow cytometry method, and they observed that tissues were infected by *H. pylori*. Protein overexpression was shown to be decreased by bacterial eradication. Naturally, this is one of many mechanisms causing gastric cancer development. Also Wong et al. (2000) measured EGF and EGFR mRNA levels by using ELISA. It was observed that both EGF and EGFR mRNA levels were increased by *H. pylori* infection, and decreased after eradication. These studies indicated that EGF and EGFR levels were increased in subjects with *H. pylori*, and eradication had the vital importance.

H. pylori eradication should be performed to prevent gastric cancer development. In one of studies investigating relationship of *H. pylori* status and prognosis, negative *H. pylori* cases were defined to have poor prognosis (Marrell et al., 2009). Again in another study, similarly negative *H. pylori* gastric cancer cases were reported to have poor prognosis, and it was recommended that that group of patients should be closely followed up after the surgery (Kang et al., 2012). Similarly, Meimarakis et al. drew also attention to poor prognosis in *H. pylori* negative cases (Meimarakis et al., 2006). In our study, HER-2 state in gastric and lymph nodes were investigated, and HER-2 state in gastric and lymph nodes showed concordance generally. However, when primary tissues from three patients did not show HER-2 expression, metastatic lymph nodes showed the expression. Interestingly, these three cases were *H. pylori* positive. Of these three patients, one has been still receiving adjuvant treatment; one has presented with metastasis in a short time; and the last one was dead. Therefore, importance of *H. pylori* eradication has been emphasized again. HER-2 status evaluation in metastatic lymph node is not a routine test in gastric cancer cases. According to our results, metastatic lymph nodes may be HER-2 positive, and this condition, contrary to the above mentioned studies, *H. pylori* positive cases may have poor prognosis due to possible HER-2 positivity in the lymph nodes.

Again in our study, initial CEA values were statistically significant in *H. pylori* positive patients. However, this significance was not detected for CA 19-9. CEA values in *H. pylori* negative cases were not statistically significant. This condition also indicates that *H. pylori* eradication has the vital importance for patients. However, limitations of our study are short duration of follow up period and small sample size. Further large scale studies are required in the future.

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