Serum *Helicobacter pylori* IgG and IgA levels in patients with gastric cancer

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The association between *Helicobacter pylori* and gastric cancer has been debated in the last decade and evidence for such a causal relationship has been claimed. This study aimed to detect the seroprevalence of *Helicobacter pylori* in patients with gastric cancer and compare it to the other cancer patients. In addition, the value of IgG and IgA in *Helicobacter pylori* detection was compared in patients with gastric cancer. Consecutive gastric and other cancer patients treated between 1999–2001 were prospectively studied. Serum *Helicobacter pylori* IgG and IgA levels were determined. Serological tests revealed IgA and IgG positivity as 53.9% and 50.9%, respectively, while 74.5% had positive results for either IgA or IgG. Serum IgA positivity was significantly higher in gastric cancer group compared to control group (p=0.02). In contrast, serum IgG positivity did not show a significant difference in both groups and either IgG or IgA seropositivity was significantly higher in patients with gastric cancer compared to control patients (p=0.04). This study revealed a higher seroprevalence of *Helicobacter pylori* in gastric cancer patients and IgA was a better predictor of *Helicobacter pylori* seropositivity in gastric cancer patients.

Key words: Helicobacter pylori, IgA, IgG, gastric cancer.

The role of *Helicobacter pylori* (HP) in duodenal ulcers and gastritis has been established in the previous studies. Eradication of the bacteria is a requisite to decrease the recurrence rate in these patients. The association between HP and gastric cancer has been debated in the last decade. In the previous studies, evidence for such a causal relationship has been claimed [6, 19, 20]. In the light of these studies, WHO accepted HP as a type I carcinogen in 1994 indicating its role in gastric cancer [13]. In a recent meta-analysis, the risk of developing gastric cancer in HP positive individuals was reported to increase two-fold [5].

Various methods have been used to demonstrate the presence of HP in the previous studies [4, 6, 8, 11, 14, 17, 19, 20, 25]. Histological, serological and urea breath tests and bacterial cultures are some of them. There were great variations in the study designs and the formation of the control groups as well [2, 3, 17, 25]. This study aimed to detect the seroprevalence of HP in patients with gastric cancer using both IgG and IgA and compare it to the patients with other cancers. In addition, the value of IgG and IgA in HP detection was compared in patients with gastric cancer.

Patients and methods

In this study, consecutive patients with gastric cancer treated between 1999–2001 were prospectively included. During the same period, patients treated with the diagnosis of other cancers were included to form the control group excluding those with the history of dyspeptic complaints, blood transfusions or gastric resection and treated for gastritis or peptic ulcer disease. Patients with various cancers were chosen as the control group in order to decrease the possible variations observed in the immune response of the cancer patients and healthy controls that may affect the serological test results. Patients' age, gender, socioeconomic level, family history of gastric cancer and the diagnosis of those other than gastric cancer were recorded. In patients with gastric cancer, diagnosis was established with upper gastrointestinal system endoscopy and biopsy. Tumor location was determined during endoscopy or histological examination. Patients were staged according to tumor, node, metastases 1997 classification system using plain chest

radiography, abdominal ultrasonography and/or tomography [26]. Patients without any distant metastases were operated with a curative intent. Palliative procedures were performed for the patients with intraoperatively detected locally unresectable disease. Type of the operation and the histopathologic findings were recorded as well.

Patients' serum samples were obtained during routine blood withdrawal at admission. They were immediately centrifuged and the serum was stored at –20 °C until further use. Serum HP IgG and IgA levels were determined with enzyme-linked immunosorbent assay (ELISA) method. Commercial kits were used as described in the manufacturer's manual (Immuno-Biological Laboratories, Hamburg, Germany). Accepted positive values for serum IgG and IgA levels were greater than 20 U/ml and 15 U/ml, respectively.

For the comparison of the variable frequencies in different groups, chi-square or Fisher's exact test was used as appropriate. Mean values of different variables were compared with Student's t test. Statistical analyses were performed with SPSS 9.0 statistical software package (SPSS Inc, Chicago, IL). P value <0.05 was accepted as significant.

Results

Ninety-four patients with gastric cancer and 71 patients with other cancers were eligible and included in the study. Eighty-four male (50.9%) and 81 female patients with a median age of 55 (range 16–84) formed the study group. The number of patients from low and high socioeconomic backgrounds were 76 (46.1%) and 89, respectively. The diagnosis of the patients in the control group was as follows: 27 breast cancers, 12 colorectal cancers, 8 thyroid cancers, 6 sarcomas and pancreas cancers each, 5 malignant melanomas, two oesophagus, biliary and hepatic cancers and one squamous cell skin cancer. The comparison of demographic properties of the patients with gastric cancer and other cancers is depicted in Table 1. Although two groups were comparable in terms of age and socioeconomic level, there were significantly more females in the control group due to patients with breast cancer.

Table 1. Demographic properties of patients in gastric cancer and control groups

	Gastric cancer	Control	p
Age	56.7 ± 13.4	52.4 ± 15.2	0.06
Gender			
Male	60	24	<0.0001
Female	34	47	
Socioeconomic level			
Low	48	28	0.16
High	46	43	

Family history was positive in 12 patients (12.8%) with gastric cancer. Tumor localization was distal to cardia in 81 (86.2%) and in the cardia in 13 patients with gastric cancer. Staging of patients with gastric cancer revealed 2 in stage IB, 7 in stage II, 18 in stage IIIA, 14 in stage IIIB and 53 in stage IV. Totally, 41 patients (43.6%) had less than stage IV disease. Surgery was not performed in 20 patients. Total and subtotal gastrectomy were performed with a curative intent in 29 (31%) and 26 (28%) patients, respectively. Palliative gastroenterostomy and only simple laparotomy were performed in 7 and 12 patients, respectively. According to Lauren classification, 78 patients (83%) had diffuse and 16 patients had intestinal type tumors.

Serological tests revealed that 89 patients (53.9%) had positive and 76 patients had negative IgA results in the entire group. On the other hand, IgG was found as positive and negative in 84 (50.9%) and 81 patients, respectively, while 123 patients (74.5%) had positive results for either IgA or IgG and 42 patients had negative results for both IgA and IgG. Median serum IgG and IgA levels for seropositive patients were calculated as 40 U/ml (range 20-300 U/ml) and 35 U/ml (range 15–150 U/ml), respectively. Comparison of IgG or IgA seropositive and seronegative patients are shown in Table 2. When IgG or IgA seropositive patients were compared to seronegative patients in terms of age, gender, socioeconomic level, tumor location, stage of disease, family history of gastric cancer, type of surgery and tumor type, no significant difference was established between IgA positive and negative patients. In contrast, IgG seropositive patients had more non-cardia tumors compared to seronegative patients (p=0.038). Other properties were comparable for IgG positive and negative patients.

Distribution of IgG and IgA seropositivity in gastric cancer and control groups are shown in Table 3. Serum IgA levels were positive in 61.7% and 43.7% of the patients in gastric cancer and control groups, respectively. There were significantly higher number of seropositive patients in gastric cancer group compared to control group (p=0.02). In contrast, serum IgG positivity was similar in both groups (51.1% vs 50.7%) (p=1.0). When either IgG or IgA seropositivity was taken into account, HP seropositivity was significantly higher in patients with gastric cancer compared to control patients (p=0.04). When cardia tumors were excluded from the analysis, serum Ig A levels were still found to be significantly higher in patients with gastric cancer compared to control group (p=0.017).

In patients with gastric cancer, seropositive and seronegative patients for Ig G or Ig A were compared in terms of age, gender, socioeconomic level and family history of gastric cancer. Results of this comparison are shown in Table 4. No significant difference was detected between seropositive and seronegative patients with gastric cancer.

Table 2. Comparison of demographic and clinicopathologic properties of IgG and IgA seropositive and seronegative patients

	IgG		IgA		IgG or IgA	
	Positive	Negative	Positive	Negative	Positive	Negative
Gender						
Male	48	36	48	36	66	18
Female	36	45	41	40	57	24
Family history						
Positive	7	5	8	4	9	3
Negative	41	41	50	32	67	15
Socioeconomic level						
Low	41	35	39	37	55	21
High	43	46	50	39	68	21
Stage						
IB	2	–	2	–	2	–
II	3	4	3	4	6	1
IIIA	6	12	14	4	16	2
IIIB	8	6	9	5	11	3
IV	29	24	30	23	41	12
Tumor location						
Non-cardia	45	36*	51	30	66	15
Cardia	3	10	7	6	10	3
Surgery						
No surgery	13	7	13	7	15	5
Laparotomy	7	5	6	6	10	2
Gastroenterostomy	3	4	3	4	4	3
Subtotal gastrectomy	16	10	20	6	23	3
Total gastrectomy	9	20	16	13	24	5
Histologic type						
Diffuse	40	38	47	31	62	16
Intestinal	8	8	11	5	14	2

*p=0.038

Table 3. Distribution of IgG and IgA seropositive patients in gastric cancer and control groups

	Gastric cancer	Control	p	p*
IgA				
Positive	58	31	0.02	0.017
Negative	36	40		
IgG				
Positive	48	36	1.0	0.62
Negative	46	35		
IgA or IgG				
Positive	76	47	0.04	0.04
Negative	18	24		

*p values calculated without cardia tumors

Table 4. Demographic properties of seropositive and seronegative patients in gastric cancer group*

	IgG		IgA		IgG or IgA	
	Positive	Negative	Positive	Negative	Positive	Negative
Gender						
Male	37	23	34	26	49	11
Female	21	13	14	20	27	7
Socioeconomic level						
Low	28	20	28	20	38	10
High	30	16	20	26	38	8
Family history						
Positive	8	4	7	5	9	3
Negative	50	32	41	41	67	15

*p values calculated with chi-square test for this table are not significant.

Discussion

A possible causal relation between gastric cancer and HP has been indicated in this study. In patients with gastric cancer, HP seropositivity was shown to be significantly higher compared to patients with other cancers. Besides, serum HP IgA levels were found as more accurate than IgG in determining HP infections in patients with gastric cancer. Although serum IgG levels were higher in gastric cancer group, this difference did not reach statistical significance.

Advanced age, lower socio-economic level and family history of HP positivity have been previously reported as risk factors increasing HP incidence [4, 5, 22]. When HP positive and negative patients were compared regarding age, socio-economic level and family history of gastric cancer in this study, no significant difference was detected between gastric cancer and control groups. In addition, HP positive and negative patients in the gastric cancer group were also comparable in terms of age, socio-economic level and family history. In contrast, the number of female patients was significantly higher in the control group compared to gastric cancer group. This difference was due to the patients with breast cancer in the control group. However, gender was not previously found as a factor affecting HP positivity and this difference was not expected to change the study results [5, 10].

In the previous studies on HP and gastric cancer, usually control groups comprised of either healthy subjects or patients with other benign diseases or patients with dispeptic complaints, but without gastritis and gastroduodenal ulcers. In addition, in prospective nested case-control studies, HP positive and negative healthy subjects were followed to detect the incidence of gastric cancer cases. On the other hand, immune response is subjected to various changes in patients

with solid tumors. Since serological tests depend on the immune response of the individual to different antigenic stimuli, a control group formed by the patients with other cancers is a better option for the study design. For this reason, patients with various cancers, but without any dyspeptic complaints or gastroduodenal disease formed the control group in the current study. Similarly, two previous studies had control groups consisting of the patients with other cancers [17, 25].

Various direct and indirect methods have been previously utilized for the detection of HP. Microbiological cultures, urea breath test, histological and serological tests are among the most widely used. Although histologic detection and culture were known as the "gold standard", HP tends to eradicate from gastric mucosa especially in patients with atrophic gastritis and gastric cancer [8, 14, 24]. In these situations, hostile environment in the stomach endangers its vitality. As the integrity of the mucosa disappears, HP could no longer stay alive in the stomach. Hence, utilizing histological detection methods to verify the presence of HP in gastric cancer underestimates its frequency. Besides, sampling errors occuring during the biopsies might affect the results in histological studies. Previous studies have given credit to serological tests for their longer positivity. The patients treated due to gastritis and peptic ulcers showed high levels of HP immunoglobulins even after histologic eradication of HP [16]. Serological tests, due to their longer positivity, will be more useful in depicting a relation between HP and gastric cancer. On the other hand, HP seropositivity lasts 6–12 months after its eradication from the gastric mucosa and this still indicates an underestimation of real HP positivity when serological tests are used in cross-sectional studies [16]. Despite this underestimation, HP seropositivity was much higher in gastric cancer group in the current study.

In the previous studies, higher incidence of HP positivity has been reported in patients with gastric cancer. In nested case-control studies, the risk of gastric cancer increased 2.8–6.0 fold [1, 6, 19, 20]. A recent meta-analysis revealed a two-fold increase in gastric cancer risk for HP positive individuals despite the heterogeneity of the evaluated studies [5]. When only the nested case-control studies were considered the risk of developing gastric cancer reached three and six-fold in patients with non-cardia tumors and serum collected >10 years before the gastric cancer development, respectively [10]. Similarly a meta-analysis considering only serological studies reported an increased gastric cancer risk of three-fold for HP seropositive individuals [12].

In this study, IgA was shown to be better in predicting HP seropositivity in gastric cancer patients. Similarly, Aromaa et al [1] found significantly higher levels of IgA compared to IgG in gastric cancer patients. Longer half-life of IgA might be the reason for this difference [16]. In contrast, various studies found IgG as a better predictor of HP seropositivity compared to IgA [2, 25, 27]. Buruk et al [3] in a previous histological study from our institution showed a higher incidence of HP in gastric cancer patients compared to patients with peptic ulcer and gastritis. Incidence of HP was higher in gastric cancer and control groups in the current study (80.8% vs.74% and 66.2% vs.60%, respectively) [3]. This difference could result from the different methods used for HP detection. In another Turkish study, serum cagA levels rather than IgG levels were reported as significantly more positive in gastric cancer patients [9]. Recently newly defined HP antigens such as cagA, vacA and iceA were reported to predict the virulence of HP [21, 24]. Detection of higher HP positivity utilizing these antigens could be due to their stronger immunostimulant effect rather than implying a higher virulence to the organism.

Previous studies reported an increased risk of non-cardia and intestinal type gastric carcinoma attributable to HP infection. In a meta-analysis, the risk of gastric cancer increased from approximately 2.7 to 3-fold when the cardia tumors were excluded [10]. However, the significant relation between HP and gastric cancer did not change excluding the cardia tumors in this study. Only serum IgG positivity was found to be significantly higher in non-cardia tumors ($p=0.038$). HP was thought to cause gastric cancer through the steps of atrophic gastritis and intestinal metaplasia [29]. Hence, intestinal type tumors were expected to be more in HP positive gastric cancer patients [5]. However, no significant difference in HP positivity was detected between intestinal and diffuse type tumors in this study as reported in a recent meta-analysis and previous studies [10, 19, 20, 25, 27]. As the gastric cancer advances, HP positivity is expected to decrease. Previously, higher HP seropositivity was reported in stage III/IV gastric cancer patients [15]. No significant association was present between HP positivity and disease stage in this study.

HP could not be the only factor causing gastric cancer. Previous studies indicated that all HP positive individuals did not later develop gastric cancer. Most probably HP acts as a promoter leading to cell proliferation in the gastric mucosa. Other genetic and dietary mutagens cause DNA damage during cell proliferation. Recent studies, in support of this view, have associated HP to various signal transduction pathways that stimulate cell growth and proliferation [18, 30]. Besides, sanitation and dietary factors in the childhood and individual's acid secreting capacity may determine the faith of HP infection [7]. Poor nutrition in the childhood may lead to a decrease in acid secretion and increase the chance of HP infection. As a result, a suitable environment for gastric cancer development will occur.

In conclusion, HP is thought to cause gastric cancer in the light of the current and previous studies. Almost all of the studies show heterogeneity in the selection of the control groups and the methods used. A better study design could be the detection of HP positive individuals and their follow-

up without any treatment [28]. Gastric cancer cases observed will give the closest estimation to the true incidence. Such a study takes a long time and causes ethical issues. Until then, serological tests, especially using IgA, would be a better way of detecting HP seropositivity in gastric cancer patients.

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