Helicobacter pylori Infection and Intestinal Metaplasia among Healthy Adolescents

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Background/Aims: The purpose of our study was to investigate the change in incidence of intestinal metaplasia (IM) in healthy, young adults over 10 years.

Materials and Methods: Urease test and histopathology by endoscopic biopsies were performed from volunteers between 1995 and 2005. Histopathological grade was assessed using the updated Sydney System.

Results: In total, 714 subjects with a median age of 22.4 years were enrolled. *Helicobacter pylori* was observed at the antrum and body in 44.5% and 35.1%, respectively. IM limited to the antrum was present in 1.1% of the subjects. The degree of IM correlated negatively with age ($P=0.04$) but there was no correlation with *H. pylori* levels or the degree of chronic or active gastritis. Compared to the beginning of the study period, the positivity rate at the end of the study period dropped to 45%. IM incidence did not change over the 11-year study period, whereas *H. pylori*-positivity and the frequency of chronic and active gastritis in the antrum and body dropped significantly over this period ($P<0.05$).

Conclusions: This result suggests that other factors, besides chronic *H. pylori* infection or degree of gastritis, may contribute to the progression of atrophy to IM in healthy, young adults. (Korean J *Helicobacter* Up Gastrointest Res 2015;15:243-248)

Key Words: *Helicobacter pylori*; Intestinal metaplasia

INTRODUCTION

Helicobacter pylori is infected from early in life and persistent infection can cause chronic gastritis, intestinal metaplasia (IM) and adenocarcinoma.^{1,2} A widely accepted hypothesis is that most cases of gastric adenocarcinoma develop after a cascade of multifactorial changes including atrophic gastritis, complete IM followed by incomplete IM, and dysplasia.³ Thus, IM is considered to be a gastric precancerous lesion.⁴

In Korea, while the gastric cancer-associated mortality has decreased over the past 10 years, it remains high.⁵ Since the incidence of atrophic gastritis in children correlates with gastric cancer in the elderly,⁶ it is of interest to

perform endoscopic analyses on young people. However, most studies investigating the association between *H. pylori* infection and precancerous or cancerous lesions have focused on patients with gastrointestinal disease. Therefore, we evaluated the relationship between *H. pylori* infection and gastric IM in healthy Korean adolescents without gastrointestinal disease over 11 years.

MATERIALS AND METHODS

1. Subjects

Between 1995 and 2005, healthy volunteers with consents underwent gastroduodenal endoscopy (Olympus GIF-XP 20 or NP-30; Olympus, Tokyo, Japan) at the Department of Pediatrics of the Gyeongsang National University Hospital (GNUH). The volunteers included first-year medical students, faculty members, residents, and research assistants. Among them, healthy adolescents under the age of 30 years were included.

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2. Methods

From 693 of the 714 volunteers who were enrolled, gastric endoscopic biopsies of both the gastric antrum and body were taken. In the remaining 21, biopsies of only the antrum were taken. A clinical diagnosis of *H. pylori* infection was obtained by urease tests and histopathology. Urease tests were performed immediately after endoscopic procedure. Biopsy specimens were incubated in 2% urea broth (urea 20 g/L, phenol red 0.04 g/L, KH₂PO₄ 0.2 g/L, NaCl 0.5 g/L, pH 6.8) and if a change in color was noted in the following 48 hours, the biopsy was deemed to be urease-test positive. The histological slides were stained with H&E and the results were interpreted using the Updated Sydney System by an experienced pathologist under unawareness of the volunteers' clinical informations.⁷ All histopathological slides were

prepared and donated by the National Biobank of Korea, which is an arm of GUNH, after its Institutional Review Board reviewed the research protocols (GUNHIRB-2009-007). Analysis of the results of histological and urease tests were performed retrospectively.

3. Statistical analysis

The Wilcoxon rank sum test was used for two-group comparisons and the Kruskal-Wallis test for multiple-group comparisons. The Spearman rank correlation test was used to test the association between two variables. Statistical significance was set at a *P* value of <0.05 (IBM SPSS Statistics ver. 23.0; IBM Co., Armonk, NY, USA).

RESULTS

In total, 714 volunteers (male 464 and female 250)

Table 1. Gender Distribution and Median Ages of the Volunteers Divided according to the Study Year

Year	Female		Male		Total	
	n	Median age (yr)	n	Median age (yr)	n	Median age (yr)
1995	8	23.6	25	22.8	33	22.8
1996	11	21.5	27	21.9	38	21.8
1997	19	21.5	62	22.6	81	22.2
1998	26	22.3	34	22.4	60	22.4
1999	25	21.8	53	22.4	78	22.0
2000	29	22.1	41	23.0	70	22.5
2001	24	21.9	46	22.4	70	22.0
2002	29	21.8	45	22.5	74	22.1
2003	35	22.1	35	22.2	70	22.2
2004	24	22.2	43	23.0	67	22.7
2005	20	21.8	53	22.8	73	22.3
Total	250	21.9	464	22.5	714	22.4

Table 2. Demographic Characteristics, Urease Test Results, and Histological Findings of Eight Volunteers with Intestinal Metaplasia

Volunteer No.	Year	Sex	Age (yr)	Degree of IM	Urease test		Histological findings in antrum				Histological findings in body			
					Antrum	Body	Chronic	Active	<i>H. pylori</i>	Follicle (n)	Chronic	Active	<i>H. pylori</i>	Follicle (n)
1	1996	Male	21	Mild	—	+	Mild	Normal	Normal	0	Mild	Normal	Normal	0
2	1996	Male	20	Marked	+	+	Moderate	Mild	Normal	0	Moderate	Normal	Normal	0
3	1997	Male	20	Moderate	—	—	Moderate	Normal	Normal	0	Mild	Normal	Normal	0
4	2001	Male	27	Mild	+	+	Marked	Moderate	Marked	1	Moderate	Mild	Mild	1
5	2003	Male	27	Mild	+	+	Marked	Mild	Mild	1	Moderate	Mild	Normal	0
7	2005	Female	23	Moderate	+	+	Moderate	Mild	Moderate	0	Moderate	Mild	Mild	1
8	2005	Female	21	Marked	—	—	Mild	Normal	Normal	0	Mild	Normal	Normal	1

IM, intestinal metaplasia; Chronic, chronic gastritis; Active, active gastritis; *H. pylori*, density of *Helicobacter pylori*. The degree of intestinal metaplasia did not correlatesignificantly with the urease-test results, the degree of chronic or acute gastritis, the density of *H. pylori*, or the number of follicles, but it correlated negatively with age ($r = -0.729, P = 0.04$).

Fig. 1. Incidence of intestinal metaplasia (IM) and *Helicobacter pylori* histological and urease-test positivity rates over the 11-year study period.

were enrolled in this study (Table 1). The median age was 22.4 years (range, 19.9~29.9 years). When the subjects were divided according to the study year, their gender distribution and median ages did not change significantly (Table 1).

Histology of the gastric antrum and body revealed that IM was only observed in the antrum in eight of the 714 subjects (1.1%) (Table 2). The proportion of IM did not change significantly over the 11-year study period (Fig. 1). The degree of IM did not correlate with the density of *H. pylori* in either the antrum or body. It also did not correlate with the degree of chronic or active gastritis in the antrum and body. However, the degree of IM correlated negatively with age ($r = -0.729$, $P = 0.04$; Fig. 2). Atrophic gastritis was not observed in either the antrum or the body in any of the subjects.

Overall, 44.5% and 35.1% of the antrum and body biopsies were histologically positive for *H. pylori* infection, respectively. Table 3 shows the antrum data of the subjects divided according to study year, namely the number of subjects with (1) positive and negative urease-test results; (2) normal, mild, moderate, or marked chronic gastritis, active gastritis, *H. pylori* density, and IM; and (3) 0, 1, 2, or ≥ 3 follicles. Table 4 shows the equivalent body data of the subjects. In the antrum, histological *H. pylori* positivity rates over the 11-year period remained rela-

Fig. 2. Age distribution of subjects with mild, moderate, and marked gastric intestinal metaplasia. There was a negative correlation between intestinal metaplasia severity and age ($P = 0.0379$).

tively steady over the first eight years until 2003, when it started dropping sharply. Compared to the positivity rate at the beginning of the study period (42.4%), the positivity rate at the end of the study period (23.3%) represents a drop of 45% (Fig. 1). In the body, histological *H. pylori* positivity rates showed a similar trend, with the rate in 2005 being 12% lower than the rate in 1995 (Fig. 1). Similarly, urease test positivity rates in the antrum and body dropped by 30% and 45%, respectively (Table 3, 4).

There was also a significant decrease over the 11-year study period in terms of the degree of chronic and active gastritis in the antrum and body ($P < 0.05$). Moreover, the number of follicles in the antrum also decreased significantly over time ($P < 0.05$).

DISCUSSION

In the present study, the overall proportion of IM in healthy, young adults during the 11-year study period was 1.1%. This proportion is lower than the prevalence of IM in patients with gastrointestinal symptoms.⁸⁻¹⁰ It is also lower than the prevalences of IM in unselected infected subject populations, which range between 10 and 60%.¹¹ The low proportion of IM in the present study can be explained by the fact that we investigated healthy, young adults regardless of whether they were infected with *H. pylori*. IM is widely believed to be one of several possible outcomes of chronic *H. pylori* infection. However, we

Table 3. Numbers of Subjects, Divided according to Study Year, Who Have Positive and Negative Urease-test Results and Various Histopathological Findings in the Gastric Antrum

Year	Result of urease test ^a				Chronic inflammation ^b				Active inflammation ^c				<i>H. pylori</i> density ^d				Intestinal metaplasia				Number of follicles ^e			
	Negative	Positive	Normal	Mild	Moderate	Marked	Normal	Mild	Moderate	Marked	Normal	Mild	Moderate	Marked	Normal	Mild	Moderate	Marked	0	1	2	≥3		
1995	14	19	0	18	11	4	18	15	0	0	19	12	0	2	33	0	0	0	20	6	3	4		
1996	13	25	0	13	16	9	15	22	1	0	17	12	5	4	36	1	0	1	22	8	7	1		
1997	30	51	0	30	34	17	33	42	6	0	33	22	16	10	80	0	1	0	43	22	14	2		
1998	24	36	0	27	18	15	26	29	5	0	28	16	12	4	60	0	0	0	36	16	7	1		
1999	24	54	0	30	31	17	30	39	9	0	43	26	9	0	78	0	0	0	38	23	14	3		
2000	22	48	0	33	28	9	34	23	12	1	39	17	9	5	70	0	0	0	49	13	6	2		
2001	13	57	0	28	20	22	28	25	17	0	31	15	15	9	69	1	0	0	42	19	7	2		
2002	29	45	0	35	30	9	34	33	7	0	35	20	13	6	74	0	0	0	53	14	3	4		
2003	6	64	0	46	18	6	46	22	2	0	48	19	1	2	68	2	0	0	57	9	3	1		
2004	36	31	0	43	15	9	45	19	3	0	47	16	3	1	66	0	1	0	48	9	7	3		
2005	44	29	0	54	12	7	54	12	7	0	56	11	3	3	72	0	0	1	57	10	5	1		
Total	255	459	0	357	233	124	363	281	69	1	396	186	86	46	706	4	2	2	465	149	76	24		
(%)	(35.7)	(64.3)	(0.0)	(50.0)	(32.6)	(17.4)	(50.8)	(39.4)	(9.7)	(0.1)	(55.5)	(26.1)	(12.0)	(6.4)	(98.9)	(0.6)	(0.3)	(0.3)	(65.1)	(20.9)	(10.6)	(3.4)		

Significant differences: ^a $P<0.0001$; ^b $P<0.0001$; ^c $P<0.0001$; ^d $P<0.0001$; ^e $P=0.0002$.

Table 4. Numbers of Subjects, Divided according to Study Year, Who Have Positive and Negative Urease-test Results and Various Histopathological Findings in the Gastric Body

Year	Result of urease test ^a			Chronic inflammation ^b				Active inflammation ^c				<i>H. pylori</i> density ^d				Number of follicles			
	Negative	Positive		Normal	Mild	Moderate	Marked	Normal	Mild	Moderate	Marked	Normal	Mild	Moderate	Marked	0	1	2	≥3
1995	12	21		0	22	10	0	26	6	0	0	24	4	4	0	24	3	2	3
1996	10	28		0	21	16	0	25	12	0	0	25	10	0	2	29	6	2	0
1997	18	63		1	40	37	2	46	31	3	0	40	29	9	2	56	21	3	0
1998	22	38		0	32	26	1	33	25	1	0	32	17	8	2	46	12	1	0
1999	16	62		0	46	29	2	37	38	2	0	45	27	4	1	64	13	0	0
2000	17	53		0	44	20	1	37	27	1	0	44	18	3	0	55	8	2	0
2001	11	59		0	34	27	3	30	31	3	0	34	22	6	2	50	13	1	0
2002	20	46		0	43	26	3	41	28	3	0	41	21	5	5	61	10	1	0
2003	8	62		0	48	17	2	48	18	1	0	56	10	1	0	51	16	0	0
2004	37	30		0	47	20	0	45	21	1	0	52	14	1	0	54	12	1	0
2005	47	26		0	58	13	2	56	14	3	0	57	12	4	0	64	9	0	0
Total	226	488		1	435	241	16	424	251	18	0	450	184	45	14	554	123	13	3
(%)	(31.7)	(68.3)		(0.1)	(62.8)	(34.8)	(2.3)	(61.2)	(36.2)	(2.6)	(0.0)	(64.9)	(26.6)	(6.5)	(2.0)	(79.9)	(17.7)	(1.9)	(0.4)

Significant differences: ^a $P<0.0001$; ^b $P=0.013$; ^c $P=0.0008$; ^d $P<0.0001$.

observed that the yearly incidence of IM did not change during the 11-year study period despite the fact that the *H. pylori*-positivity rates decreased markedly. Similarly, studies in Turkey and Hong Kong did not find a significant relationship between *H. pylori* positivity and IM.^{9,10} Poor correlation between IM and *H. pylori* positivity in the previous and present studies is possible because *H. pylori* usually disappears in the human stomach after IM.¹² Moreover, of the eight volunteers who had IM, there was no correlation between the degree of IM and the degree of *H. pylori* colonization, chronic gastritis, or active gastritis. In addition to *H. pylori*, host and other factors such as bile reflux, smoking and diet can contribute to develop IM^{13,14} but we were unaware of the volunteers' informations including smoking habits, diet and family histories. Intestinal epithelium in gastric antrum in 2- to 17-year-old children was reported with no evidence of *H. pylori* infection and chronic gastritis and the gastric intestinal epithelium in the children has different clinical implication from gastric IM in adults with chronic *H. pylori* infection and chronic inflammation.¹⁵

Long-term follow-up studies and animal experiments have confirmed that chronic active gastritis can progress to atrophic gastritis, IM, and finally to dysplasia.^{3,8} Thus, it has been suggested that atrophic gastritis and IM provide the milieu in which the true precancerous lesion, dysplasia, can develop.³ Notably, atrophic gastritis correlates strongly with *H. pylori* infection, active and chronic inflammation in the gastric mucosa of *H. pylori*-infected children who live in countries with a high incidence of gastric cancer.⁶ Another study has shown that the incidence of IM in patients with *H. pylori*-associated gastritis increases with age.¹⁶ In addition, the severity of IM according to age and the extent of the metaplastic changes becomes more accentuated.^{10,16} However, it should be noted that cancer patients occasionally exhibit IM that is not associated with concomitant atrophy.¹⁷ Similarly, none of the volunteers with IM in the present study exhibited concomitant definitive atrophic gastritis. Other reports have also observed IM in non-atrophic mucosa and have recommended that such cases be subjected to further examination to determine whether IM is present as an isolated patch within non-atrophic mucosa.^{17,18} These

observations contradict the notion that the inevitable sequence of changes is atrophy-metaplasia-dysplasia-carcinoma. Thus, it remains unclear whether IM will always progress to gastric carcinoma. Indeed, it has been suggested that the extent of gastric mucosal atrophy may signify the risk of the intestinal type of gastric carcinoma better than the presence or type of IM.¹⁷ Clinical implications of IM in the present study are inconclusive yet because we did not perform follow up examinations and the types of IM were not differentiated. However, in other aspect, IM in the present study has a possibility of pre-cancerous lesion because all of the volunteers with IM was older than previous children's report¹⁵ and accompanied with chronic gastritis on pathologic review (Table 2).

Why the healthy, young volunteers with IM in the present study lacked concomitant definitive atrophic gastritis is not clear. However, IM can arise when the gastric mucosa suffers various etiopathogenetic injuries while it is regenerating, such as *H. pylori* infection, bile reflux, or drugs.^{13,14,18,19} However, since there was no correlation between *H. pylori* infection and IM in our study, injuries induced by agents other than *H. pylori* may explain why the relatively well-nourished and healthy, young medical students in our study population had IM in the absence of definitive gastric atrophy. Notably, we also found that IM severity correlated negatively with age. Why this is so is not clear. While the IM observed in a 9-year-old child was explained by early *H. pylori* infection,⁶ this explanation cannot be applied here because of the lack of correlation between *H. pylori* infection and IM.

The discrepancy of positive rates between histopathologic and urease test was observed in the present study (Fig. 1), which might be caused by patchy distribution of *H. pylori* in gastric mucosa²⁰ and longer duration for reading of urease test. We observed urease tests for 48 hours. Although reading time is different according to the type of urease test, sensitivity increases over time.²¹

There were several limitations in the present study. The first limitation was the cases with IM did not undergo follow-up evaluations. The rate with which IM progresses to dysplasia, per 100 person-year, is 2.1 for subjects under the age of 40 years as compared to 4.0 for older persons.²² However, none of the volunteers participating in the

present study have reported the development of gastric cancer yet. The second was sampling errors may occur because gastric intestinal metaplasia often occurs in a patchy pattern. Gastric biopsy specimens were taken at the angulus and mid-body of stomach. The third was that the study population was relatively small of 714 volunteers, the inclusion per year varied between 33 and 78 subjects. The forth was that the histological detection of *H. pylori* was based on the H&E alone. However, the sensitivity of the H&E for diagnosis of *H. pylori* infection was similar to that of other special stains, such as Giemsa and Warthin-Starry silver stains.²³

The present study is unique because it investigated how the *H. pylori*-positivity and IM rates in healthy, young Korean adults changed between 1995 and 2005. While the *H. pylori*-positivity rates of the healthy, young volunteers changed significantly over the 11-year period, the incidence of IM did not change. In conclusion, the proportion of IM in healthy, young Korean adults was very low and did not reflect changes in the prevalence of *H. pylori* infection. This suggests that other factors may contribute to the development of IM in healthy, young adults and the progression of gastric atrophy to IM.

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