Original Article

Association between Body Mass Index and Gastric Cancer Risk According to Effect Modification by *Helicobacter pylori* Infection

Jieun Jang, BS[1,2,3]
Eun-Jung Cho, PhD[4]
Yunji Hwang, PhD[1,2,3]
Elisabete Weiderpass, MD, PhD[5,6,7,8,9]
Choonghyun Ahn, MD[1,2,3]
Jeoungbin Choi, MD[1,3]
Soung-Hoon Chang, MD, PhD[10]
Hai-Rim Shin, MD, PhD[11]
Min Kyung Lim, PhD[12,13]
Keun-Young Yoo, MD, PhD[1,14]
Sue K. Park, MD, MPH, PhD[1,2,3]

*A list author's affiliations appears at the end of the paper.

Correspondence: Sue K. Park, MD, MPH, PhD
Department of Preventive Medicine,
Seoul National University College of Medicine,
103 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: 82-2-740-8338
Fax: 82-2-747-4830
E-mail: suepark@snu.ac.kr

Received March 27, 2018
Accepted November 4, 2018
Published Online November 21, 2018

*Jieun Jang and Eun-Jung Cho contributed equally to this work.

Purpose
Few studies investigated roles of body mass index (BMI) on gastric cancer (GC) risk according to *Helicobacter pylori* infection status. This study was conducted to evaluate associations between BMI and GC risk with consideration of *H. pylori* infection information.

Materials and Methods
We performed a case-cohort study (n=2,458) that consists of a subcohort (n=2,193 including 67 GC incident cases) randomly selected from the Korean Multicenter Cancer Cohort (KMCC) and 265 incident GC cases outside of the subcohort. *H. pylori* infection was assessed using an immunoblot assay. GC risk according to BMI was evaluated by calculating hazard ratios (HRs) and their 95% confidence intervals (95% CIs) using weighted Cox hazard regression model.

Results
Increased GC risk in lower BMI group (< 23 kg/m^2) with marginal significance (HR, 1.32; 95% CI, 0.98 to 1.77) compared to the reference group (BMI of 23-24.9 kg/m^2) was observed. In the *H. pylori* non-infection, both lower (< 23 kg/m^2) and higher BMI (≥ 25 kg/m^2) showed non-significantly increased GC risk (HR, 10.82; 95% CI, 1.25 to 93.60 and HR, 11.33; 95% CI, 1.13 to 113.66, respectively). However, these U-shaped associations between BMI and GC risk were not observed in the group who had ever been infected by *H. pylori*.

Conclusion
This study suggests the U-shaped associations between BMI and GC risk, especially in subjects who had never been infected by *H. pylori*.

Key words
Stomach neoplasms, Body mass index, *Helicobacter pylori*, Cohort studies, Case-cohort, Effect modification

Introduction

Globally, gastric cancer (GC) holds the fourth and fifth rank of cancer incidence in males and females, respectively and is the third leading cause of cancer death in both sexes worldwide [1]. With the combination of high incidence and poor survival, GC is one of main contributor to the cancer burdens worldwide [2]. Korea is one of the countries showing highest GC incidence rate (age-standardized rate [ASR],

35.8 per 100,000 persons in 2014) and mortality rate (ASR, 9.7 per 100,000 persons in 2014) in the world [3]; therefore, verifying etiologic factors of GC is a great concern.

In recent decades, increase in obesity is a noticeable epidemiologic phenomenon in worldwide [4] and obesity has been considered as an attributable factor of several types of cancer incidence, such as colorectal, breast, endometrial, kidney, esophageal, and gastric cardia cancers [5]. Based on elevated gastric cardia cancer incidence coinciding with the increase in obesity, a number of studies conducted in West-

ern populations showed positive association between high body mass index (BMI) and gastric cardia cancer incidence [6-8]. In a recent meta-analysis, summary relative risk (SRR) of gastric cardia caner in obese group (BMI ≥ 30 kg/m²) was reported as 1.82 (95% confidence interval [CI], 1.32 to 2.49) compared to normal weight group (BMI of 18.5-24.9 kg/m²). However, evidence on association between obesity and gastric non-cardia cancer (SRR, 1.00; 95% CI, 0.87 to 1.15) was not found in the same study [9]. Controversially, recent large scaled cohort studies reported elevated GC risk in low BMI group (BMI < 22 kg/m²) [10] and significantly decreased risk of gastric non-cardia cancer in population with high BMI (≥ 23 kg/m²) than low BMI group (< 20.32 kg/m²) [11], hence further verification of relationship between BMI and GC is still needed.

The proportion of gastric cardia cancer is higher in the Western population than amongst Asians [12,13]. Contrary to the Western population, gastric non-cardia cancer comprises a larger proportion in the East Asian populations, and particularly in the Korean population, gastric non-cardia cancers are responsible for over 95% of total GC [14].

Moreover, the prevalence of *Helicobacter pylori* infection, a group I carcinogen defined by the International Agency for Research on Cancer (IARC) [15], is higher in Asian countries compared to Western countries. The East Asian strains of *H. pylori* were also reported to be more virulent to gastric carcinogenesis than the Western strains of *H. pylori* [16]. Several studies reported the inverse association between *H. pylori* infection prevalence and BMI level [17]. These results infer that underweight population probably have high risk of gastric non-cardia cancer due to high prevalence of *H. pylori* infection. However, previous studies did not in fact measure *H. pylori* infection status as a potential confounder on the association between gastric non-cardia cancer risk and BMI; thus the association between BMI and gastric non-cardia cancer according to *H. pylori* infection is not well-characterized.

Based on the differences in gastric non-cardia cancer incidence rates, prevalence of *H. pylori* infection and its strains, and the proportions of the population with extremes of BMI between the Asian countries and Western countries, we realized the necessity to focus on the association between low BMI and GC risk in Asia. Hence, we carried out a study (1) to assess GC risk according to BMI while controlling the potential confounding effect of *H. pylori* infection, (2) to investigate the potential effect modification by *H. pylori* infection on the association between BMI and GC risk, (3) to evaluate potential difference in association between *H. pylori* and GC risk according to BMI level in the case-cohort study which is based on the Korean Multicenter Cancer cohort (KMCC).

Materials and Methods

1. Data and study subjects

This study was designed within the population-based prospective KMCC which is constructed to assess risk factors related to cancer incidence and mortality in Korea. The KMCC participants were recruited from the four urban and rural areas (Haman, Chungju, Uljin, and Pohang) between 1993 and 2004. All participants signed an informed consent form [18].

This study was conducted in two observational study designs, a prospective cohort study and a case-cohort study. We tried to assess *H. pylori* infection status in order to adjust potential confounding effect of *H. pylori*. Because entire cohort was too large to test *H. pylori* infection status of all subjects, we choose case-cohort study design which is efficient in the aspect of reducing time and cost by selecting subset of entire cohort subjects.

Flowchart of subject selection on total cohort and case-cohort study is shown in the S1 Fig. After excluding the subjects without information on height or weight measures, 19,016 subjects were defined as the total cohort study population and 485 incident GC cases were identified in the total cohort study subjects. Plasma samples for assessing *H. pylori* infection status had been collected from 9,402 subjects including 332 incident GC cases among 19,016 total cohort subjects. We stratified these 9,402 subjects with plasma sample according to sex and age (10-year interval). Then, we randomly sampled 24% of subjects in each strata to constitute the subcohort by reflecting sex and age structure of the KMCC. After excluding subjects with insufficient or poor condition of plasma sample, a total of 2,200 subjects (about 23%) were defined as eligible subjects for subcohort in this study. Number of GC incident cases within the subcohort and outside of the subcohort were 67 and 265 cases, respectively.

We assessed *H. pylori* infection status and IgG seropositivity to *H. pylori* virulence factors of eligible subjects for subcohort (n=2,200) and GC incidence cases outside of the subcohort (n=265). There were seven subjects whose *H. pylori* infection status or IgG antibody to virulence factors were not measured due to insufficient sample volume or inadequate sample condition. Hence, a total of 2,193 subjects were defined as the subcohort population after excluding subjects whose *H. pylori* infection status or IgG antibody to virulence factors were not measured. Finally, a total number of 2,458 case-cohort subjects were denoted by integrating the subcohort subjects (n=2,193) and GC incident cases outside of the subcohort (n=265).

2. Outcome assessment

Incident GC cases in the KMCC were ascertained through the record linkage between the KMCC data and the national cancer registry data. Death certificate only cases were also confirmed through linking the national death certificate of the National Statistical Office to the KMCC data. A total of 508 incident GC cases among the total KMCC participants were identified until December 31, 2013. After excluding GC cases without information on height or weight, 485 GC cases were left in the total cohort study. We measured *H. pylori* infection status of case-cohort study subjects. Because we could not assess *H. pylori* infection status of 153 GC cases due to insufficient volume or unstable condition of plasma samples, a total of 332 GC cases with information of *H. pylori* infection status were included in the case-cohort study.

3. Exposure assessment: BMI

Information on individual characteristics such as demographic factors, lifestyle factors, and past medical history was collected using a standardized interview-based questionnaire. Anthropometric indices such as height, weight, waist and hip circumferences were also measured by the study team. Height, the perpendicular distance between the top of head and the heel after taking off shoes and standing with their back against the wall straightly, and weight of study subjects were measured by skilled investigators on the same day of questionnaire interview at the cohort enrollment. Measurements of height and weight were conducted twice and the mean value was used in this study.

BMI (kg/m^2) was computed in accordance with its definition: weight (kg) divided by square of the height (m^2). Because World Health Organization (WHO) BMI classification was proposed to reflect risk for mortality and non-communicable diseases related to death, such as type 2 diabetes and cardiovascular disease [19], other BMI criteria might be more informative to indicate GC risk. Recent cohort studies also reported significant change in GC risk in group with BMI around of 23.0 kg/m^2, hence we applied modified criterion of BMI in several ways as follows:

(a) < 18.5, 18.5-22.9, 23-24.9 (reference), 25-29.9, and ≥ 30 kg/m^2
(b) < 23, 23-24.9 (reference), and ≥ 25 kg/m^2
(c) < 18.5, 18.5-22.4, 22.5-24.9 (reference), 25-27.4, and ≥ 27.5 kg/m^2
(d) < 22.5, 22.5-24.9 (reference), and ≥ 25 kg/m^2
(e) < 23 kg/m^2 and ≥ 23 kg/m^2 (reference)

When we apply BMI category (a) to analysis, too small number of GC cases were included in one category. Thus, we made BMI category (b) by combining each of two lower and two higher BMI groups than reference group as one group. Because small number of GC cases were included in the reference group of BMI category (a) and (b) in the case-cohort study, we conducted sensitivity analysis by slightly changing criteria of BMI category (a) as BMI category (c). BMI category (d) was also determined by combining each of two lower and two higher BMI groups in category (c) as one BMI group.

4. Exposure assessment: Measurement of *H. pylori* infection status

Specimens including serum, plasma, buffy coat, packed red blood cell, and spot urine were also collected from the KMCC participants and stored in standardized stable condition (–70°C for blood samples and –20°C for spot urine samples) [18]. Cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) has been known for virulence factors of *H. pylori*. CagA and VacA have been reported that these virulence factors are associated with cellular responses related to carcinogenesis such as excessive cell proliferation, inhibition of apoptosis, and excessive inflammatory responses [20]. Hence, we assessed seropositivity of CagA and VacA IgG along with *H. pylori* infection status.

Because plasma sample collected at cohort enrollment were the sole specimen to assess *H. pylori* infection status and the invasive endoscopy (a gold standard of *H. pylori* test) and a noninvasive reliable Urea Breath test was not easy to apply in the setting of the community population group, we used immunoblot assay, the Helico Bolt 2.1TM (MP Biomedicals Asia Pacific, Singapore), to test *H. pylori* infection and seropositivity on CagA and VacA IgG. The validity of Helico Blot 2.1 was fairly good for sensitivity, 99%; for specificity, 98% [21].

Procedure for immunoblot assay is as follows: nitrocellulose strips were diluted with 2 mL of diluted wash buffer and were incubated for 5 minutes at room temperature on a rocking platform. The plasma samples were collected at baseline survey and frozen at –70°C until analysis; 2 mL of blotting buffer was added to each well containing 2 μL of plasma or positive/negative controls. After incubating for 1 hour at room temperature on a rocking platform, the plates were washed three times with 2 mL of diluted wash buffer allowing 5 minutes soak on the rocking platform between each wash. Two milliliters of working conjugate solution was added to the wells and was incubated for 1 hour at room temperature. After aspirating the conjugate and washing three times, 2 mL of substrate solutions was added. Plates were shaken at room temperature for 15 minutes and washed three times, the same as above. Each strip was interpreted according to the recommended criteria in manufacturer's instruction.

5. Statistical analysis

Basic characteristics between group with BMI < 23 kg/m^2 and group with BMI ≥ 23 kg/m^2 were compared by conducting the Pearson's chi-square test for categorical variables and Student t test for numerical variables in both total cohort and case-cohort study.

Differences in the mean of age, height, weight, and cohort follow-up time (year) along with frequency of sex, GC cases, education years (< 12 years vs. ≥ 12 years), ever smoking, more or equal to 400 cigarettes (yes vs. no), alcohol drinking (ever vs. never drinker), history of gastritis, *H. pylori* ever infection (yes vs. no), CagA-secreting *H. pylori* infection (positive vs. negative) between the low and high BMI groups were considered.

Hazard ratios (HRs) and their 95% CIs of GC incidence according to BMI level in the total cohort population were calculated by using the Cox proportional hazards regression models. We included age, sex, and year at cohort enrollment in the Cox hazard regression model and variables showing statistically significant differences between low and high BMI groups (education, cigarette smoking, and alcohol drinking) were also considered for adjustment in the total cohort study.

In case of the case-cohort study, GC risks according to BMI level or *H. pylori* infection were computed using the weighted Cox proportional hazard model. *H. pylori* infection status was additionally adjusted in the weighted Cox proportional haz-

ard regression model when GC risk according to BMI level was evaluated. We used the Barlow's weighting method in the case-cohort study [22]. Weight of all GC cases in the case-cohort study were assigned as 1 and weight of controls were assigned as inverse of sample fraction defined as the proportion of subcohort controls within the total cohort controls.

We also conducted stratified analysis by *H. pylori* infection status to assess effect modification by *H. pylori* on the association between BMI and GC risk. Subgroup of individuals with *H. pylori* infection was further considered according to CagA and VacA virulence factors (group with CagA-secreting *H. pylori* infection, CagA+; group with both CagA-secreting *H. pylori* and VacA-secreting *H. pylori* infection, CagA+ VacA+).

We also assessed differences in GC risk according to *H. pylori* infection among BMI categories (< 23 kg/m^2, 23-24.9 kg/m^2, and ≥ 25 kg/m^2) to investigate effect modification by BMI on the association between *H. pylori* and GC risk.

Sensitivity analyses for cardia and non-cardia GC development were performed. Non-cardia GC category included GC cases with unspecified locations and missing T-code since non-cardia GC consisted of more than 95% of total GC cases in Korean population [14].

All statistical analysis was two-sided with alpha error of 5% and were performed using SAS software ver. 9.4 (SAS Institute Inc., Cary, NC).

Table 1. General characteristics of cohort and case-cohort study subjects within the Korean Multicenter Cancer Cohort (KMCC), 1993-2004

Characteristic	Total cohort (n=19,016)			Case-cohort (n=2,458)		
	Low BMI[a] (n=8,645)	High BMI[a] (n=10,371)	p-value	Low BMI[a] (n=1,168)	High BMI[a] (n=1,290)	p-value
Age (yr)	53.6±16.5	53.8±12.4	0.28	56.8±14.7	54.1±12.1	< 0.01
Height (cm)	158.0±8.9	157.1±8.8	< 0.01	158.9±9.0	157.8±8.9	< 0.01
Weight (kg)	52.0±7.1	64.1±8.7	< 0.01	52.6±7.2	64.5±8.8	< 0.01
Follow-up (yr)	12.9 (9.9-16.5)	12.4 (9.9-16.4)	0.93	12.9 (7.4-15.4)	13.2 (10.4-17.3)	< 0.01
Male	3,905 (45.2)	3,714 (35.8)	< 0.01	650 (55.7)	554 (43.0)	< 0.01
GC cases	277 (3.2)	208 (2.0)	< 0.01	193 (16.5)	138 (10.8)	< 0.01
Education ≥ 12 yr	1,122 (13.1)	1,456 (14.2)	0.04	139 (11.9)	190 (14.8)	0.04
Ever smokers[b]	3,670 (43.1)	3,244 (31.7)	< 0.01	594 (51.1)	470 (36.8)	< 0.01
Ever alcohol drinkers	3,759 (44.4)	4,124 (40.4)	< 0.01	568 (48.7)	550 (43.0)	< 0.01
History of gastritis	1,600 (21.7)	1,788 (20.1)	0.78	160 (21.3)	152 (19.2)	0.31
Helicobacter pylori–infected	-	-		996 (85.3)	1,108 (85.9)	0.66
CagA+*H. pylori*-infected	-	-		952 (81.5)	1,061 (82.3)	0.63

Values are presented as mean±standard deviation, median (IQR) for continuous variables and number (%) for category variables. GC, gastric cancer; CagA, cytotoxin-associated gene A. [a]Low BMI indicates < 23.0 kg/m^2; high BMI indicates ≥ 23.0 kg/m^2, [b]Ever smoking is defined as past and current smoking more or equal to 400 cigarettes.

Table 2. Association between BMI and GC risk in the total cohort and case-cohort study within the KMCC, 1993-2004

BMI (kg/m²)	GC development			Non-cardia GC development[b]		
	Person-year	No.	HR (95% CI)[a]	Person-year	No.	HR (95% CI)[a]
Total cohort (n=19,016)						
< 18.5	10,903	29	1.36 (0.89-2.08)	10,903	29	1.41 (0.92-2.17)
18.5-22.9	100,613	248	1.38 (1.08-1.76)	100,559	239	1.38 (1.08-1.77)
23.0-24.9	57,776	90	1.00	57,758	87	1.00
25.0-29.9	67,418	107	1.13 (0.85-1.50)	67,395	105	1.15 (0.86-1.53)
≥ 30.0	8,515	11	1.18 (0.63-2.20)	8,515	11	1.21 (0.64-2.27)
< 18.5	10,903	29	1.36 (0.89-2.08)	10,903	29	1.41 (0.92-2.17)
18.5-22.9	100,613	248	1.38 (1.08-1.76)	100,559	239	1.38 (1.08-1.77)
23.0-24.9	57,776	90	1.00	57,758	87	1.00
25.0-27.4	48,060	76	1.11 (0.82-1.51)	48,038	74	1.12 (0.82-1.53)
≥ 27.5	27,873	42	1.18 (0.81-1.70)	27,873	42	1.21 (0.84-1.75)
< 23.0	111,516	277	1.38 (1.08-1.75)	111,462	268	1.38 (1.08-1.77)
23.0-24.9	57,776	90	1.00	57,758	87	1.00
≥ 25.0	75,933	118	1.14 (0.86-1.50)	75,910	116	1.15 (0.87-1.52)
Case-cohort (n=2,458)						
< 18.5	1,156	23	1.22 (0.74-2.01)	1,156	23	1.28 (0.77-2.12)
18.5-22.9	12,259	170	1.33 (0.99-1.79)	12,227	163	1.32 (0.98-1.79)
23.0-24.9	7,208	62	1.00	7,204	60	1.00
25.0-29.9	8,547	71	1.08 (0.77-1.52)	8,524	69	1.08 (0.76-1.53)
≥ 30.0	903	6	1.01 (0.43-2.36)	903	6	1.02 (0.44-2.39)
< 18.5	1,156	23	1.22 (0.74-2.01)	1,156	23	1.28 (0.77-2.12)
18.5-22.9	12,259	170	1.33 (0.99-1.79)	12,227	163	1.32 (0.98-1.79)
23.0-24.9	7,208	62	1.00	7,204	60	1.00
25.0-27.4	6,094	51	1.11 (0.76-1.61)	6,071	49	1.10 (0.75-1.61)
≥ 27.5	3,356	26	1.01 (0.63-1.61)	3,356	26	1.03 (0.65-1.65)
< 23.0	13,415	193	1.32 (0.98-1.77)	13,383	186	1.32 (0.98-1.78)
23.0-24.9	7,208	62	1.00	7,204	60	1.00
≥ 25.0	9,450	77	1.07 (0.77-1.51)	9,427	75	1.08 (0.76-1.52)

BMI, body mass index; GC, gastric cancer; KMCC, Korean Multicenter Cancer Cohort; HR, hazard ratio; CI, confidence interval. [a]Adjusted for age, sex, year of recruitment, education years (< 12 years vs. ≥ 12 years), ever smoking more or equal to 400 cigarettes (yes vs. no), and alcohol drinking status (yes vs. no), [b]Non-cardia GC including unspecified cases (9% of total GC cases). The results for cardia GC development were not presented due to non-estimation of parameter estimators and their 95% CIs by sparse cardia GC cases (n=14).

6. Ethical statement

This study protocol was approved by the institutional review boards of Seoul National University Hospital (H-01-10-084-002 and H-1310-082-528) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Results

1. Characteristics of study subjects

The general characteristics were presented in Table 1. Subjects in the high BMI group were more likely to be women, have a high level of education compared to subjects in the

Table 3. Association with body mass index for GC risk by *Helicobacter pylori* infection in the case-cohort study within the KMCC, 1993-2004

BMI (kg/m²)	GC development			Non-cardia GC development[a]		
	Person-year	No.	Stratification HR (95% CI)[b]	Person-year	No.	Stratification HR (95% CI)[b]
Never *H. pylori* infected						
< 23.0	2,017	12	10.82 (1.25-93.60)	2,017	12	12.07 (1.34-108.39)
23.0-24.9	1,071	1	1.00	1,071	1	1.00
≥ 25.0	1,358	7	11.33 (1.13-113.66)	1,345	6	9.95 (0.91-108.31)
Ever *H. pylori* infected						
< 23.0	11,398	181	1.26 (0.93-1.70)	11,366	174	1.26 (0.93-1.71)
23.0-24.9	6,137	61	1.00	6,133	59	1.00
≥ 25.0	8,091	70	1.03 (0.71-1.40)	8,082	69	1.05 (0.72-1.44)
***H. pylori*+CagA+**						
< 23.0	10,886	174	1.26 (0.93-1.72)	10,855	167	1.26 (0.92-1.72)
23.0-24.9	5,895	59	1.00	5,891	57	1.00
≥ 25.0	7,762	68	1.12 (0.72-1.65)	7,753	67	1.03 (0.72-1.48)
CagA+VacA+						
< 23.0	8,494	126	1.46 (1.01-2.11)	8,468	120	1.44 (0.99-2.10)
23.0-24.9	4,866	41	1.00	4,862	40	1.00
≥ 25.0	6,397	51	1.14 (0.75-1.72)	6,388	50	1.14 (0.75-1.74)

GC, gastric cancer; KMCC, Korean Multicenter Cancer Cohort; BMI, body mass index; CagA+, cytotoxin-associated gene A (CagA)–secreting *H. pylori* infection; CagA+VacA+, both CagA-secreting *H. pylori* and vacuolating cytotoxin A (VacA)–secreting *H. pylori* infection. [a]Non-cardia GC including unspecified cases (9% of total GC cases). The results for cardia GC development were not presented due to non-estimation of parameter estimators and their 95% CIs by sparse cardia GC cases (n=14), [b]Adjusted for age, sex, year of recruitment, education years (< 12 years vs. ≥ 12 years), ever smoking more or equal to 400 cigarettes (yes vs. no), and alcohol drinking status (yes vs. no).

low BMI group. A relatively low proportion of ever smokers and ever drinkers were found in the high BMI group compared to the low BMI group. In general, we also confirmed the consistent results in both total cohort and case-cohort population. Additional difference of age in the total cohort and difference of follow-up period in the case-cohort study were found.

2. GC risk according to BMI level in the total cohort study

GC risk according to BMI categories in the total cohort is presented in Table 2. There was an increased GC risk in groups with BMI < 18.5 kg/m² or of 18.5-22.9 kg/m² compared to the reference group (BMI of 23-24.9 kg/m²) in the total cohort study (HR, 1.36; 95% CI, 0.89 to 2.08 for group with BMI < 18.5 kg/m² and HR, 1.38; 95% CI, 1.08 to 1.76 for group with BMI of 18.5-22.9 kg/m², respectively; however, it was non-significant in group with BMI < 18.5 kg/m².

In contrast, there was no statistically significant associations between BMI ≥ 25 kg/m² and GC risk (HR, 1.13; 95% CI, 0.85 to 1.50 for group with BMI 25-29.9 kg/m² and HR,

1.18; 95% CI, 0.63 to 2.20 for group with BMI ≥ 25 kg/m², respectively).

3. GC risk according to BMI level in the case-cohort study

We also assessed GC risk according to BMI with additional adjustment of *H. pylori* infection in the case-cohort study (Table 2). Similar results to the total cohort study on the association between BMI and GC risk were observed in the case-cohort study. Compared to the reference group (BMI of 23-24.9 kg/m²), group with BMI of < 18.5 kg/m² or 18.5-22.9 kg/m² were associated with increased GC risk (HR, 1.22; 95% CI, 0.74 to 2.01 for group with BMI < 18.5 kg/m² and HR, 1.33; 95% CI, 0.99 to 1.79 for group with BMI of 18.5-22.9 kg/m², respectively), although the associations were not statistically significant.

4. Effect modification by *H. pylori* infection on associations between BMI and GC risk in the case-cohort study

Further, we performed analysis on the association between

Table 4. The association between *Helicobacter pylori* infection and GC risk by BMI level in the case-cohort study within the KMCC, 1993-2004

	GC development			Non-cardia GC development[a]		
	Person-year	No.	Stratification HR (95% CI)[b]	Person-year	No.	Stratification HR (95% CI)[b]
BMI < 23.0 kg/m²						
Never infected	2,017	12	1.00	2,017	12	1.00
Ever infected	11,398	181	2.04 (1.13-3.70)	11,366	174	1.99 (1.10-3.62)
H. pylori+CagA+	10,886	174	2.06 (1.14-3.74)	10,855	167	2.01 (1.11-3.65)
CagA+VacA+	8,494	126	2.03 (1.11-3.72)	8,468	120	1.97 (1.08-3.62)
BMI 23.0-24.9 kg/m²						
Never infected	1,071	1	1.00	1,071	1	1.00
Ever infected	6,137	61	10.52 (1.44-76.67)	6,133	59	9.98 (1.37-72.78)
H. pylori+CagA+	5,895	59	10.38 (1.42-75.70)	5,891	57	9.84 (1.35-71.84)
CagA+VacA+	4,866	41	12.17 (1.63-90.02)	4,862	40	11.59 (1.55-86.69)
BMI ≥ 25.0 kg/m²						
Never infected	1,358	7	1.00	1,345	6	1.00
Ever infected	8,091	70	1.73 (0.78-3.82)	8,082	69	2.02 (0.86-4.72)
H. pylori+CagA+	7,762	68	1.80 (0.81-4.00)	7,753	67	2.11 (0.90-4.94)
CagA+VacA+	6,397	51	1.73 (0.76-3.93)	6,388	50	2.03 (0.85-4.87)

GC, gastric cancer; BMI, body mass index; KMCC, Korean Multicenter Cancer Cohort; HR, hazard ratio; CI, confidence interval; CagA+, cytotoxin-associated gene A (CagA)–secreting *H. pylori* infection; CagA+VacA+, both CagA-secreting *H. pylori* and vacuolating cytotoxin A (VacA)–secreting *H. pylori* infection. [a]Non-cardia GC including unspecified cases (9% of total GC cases). The results for cardia GC development were not presented due to non-estimation of parameter estimators and their 95% CIs by sparse cardia GC cases (n=14), [b]Adjusted for age, sex, year of recruitment, education years (< 12 years vs. ≥ 12 years), ever smoking more or equal to 400 cigarettes (yes vs. no), and alcohol drinking status (yes vs. no).

BMI and GC risk stratified by *H. pylori* infection status and CagA-secreting *H. pylori* infection status (Table 3). BMI < 23 kg/m² and ≥ 25 kg/m² were associated with increased GC risk (HR, 10.82; 95% CI, 1.25 to 93.60 for BMI < 23 kg/m² and HR, 11.33; 95% CI, 1.13 to 113.66 for BMI ≥ 25 kg/m², respectively) amongst subjects without *H. pylori* infection. In contrast, there were no statistically significant increase in GC risk in the group with BMI < 23 kg/m² and ≥ 25 kg/m² who were *H. pylori* positive (HR, 1.26; 95% CI, 0.93 to 1.70 for BMI < 23 kg/m² and HR, 1.03; 95% CI, 0.71 to 1.40 for BMI ≥ 25 kg/m², respectively).

Because only one GC cases were included in the reference group, we conducted sensitivity analysis after changing reference group as subjects with BMI of 22.5-24.9 kg/m² (S2 Table). Similar U-shaped association between BMI and GC risk in subjects who were not infected by *H. pylori* was found even when we changed reference group. No statistically significant differences in GC risk by BMI categories in subjects positive for *H. pylori* infection were observed.

5. Difference in association between *H. pylori* infection and GC risk according to BMI level in the case-cohort study

GC risk according to *H. pylori* infection status in stratified group by BMI level was also conducted (Table 4). In the group with BMI < 23 kg/m², *H. pylori* infection was associated with about 2-fold higher GC risk (HR, 2.04; 95% CI, 1.13 to 3.70). Elevated GC risk according to *H. pylori* infection was also observed in group with BMI of 23-24.9 kg/m² (HR, 10.52; 95% CI, 1.44 to 76.67). In contrary, there was no statistically significant difference in GC risk by *H. pylori* infection in group with BMI ≥ 25 kg/m² (HR, 1.73; 95% CI, 0.78 to 3.82). Even when we used other BMI categories (< 22.5, 22.5-24.9, and ≥ 25 kg/m²), similar patterns on the association between *H. pylori* infection status and GC risk were observed.

6. Sensitivity analysis by GC anatomical location

In cardia GC analysis, the risk estimates were not calculated due to sparse GC cases (only 14 cases in total subjects), and thus the results were not shown in this paper. Only the

results for non-cardia GC development were additionally presented in the Tables 2-4. As the result, the associations between BMI and GC risk in subgroup population without cardia GC cases, were persistent.

Discussion

About 1.3-fold increased GC risk in population with low BMI (< 23 kg/m²) was found in both total cohort and case-cohort studies, but the association was marginally significant in the case-cohort study. In the case-cohort study, BMI levels showed U-shaped association with GC risk, especially in the *H. pylori* non-infected group: the risk of GC was higher in two different groups such as lower BMI group (< 23 kg/m²) or higher BMI group (≥ 25 kg/m²) compared with the reference BMI group (23-24.9 kg/m²).

A recently published large-scale cohort study in UK reported a reverse-J-shaped association between BMI and GC for the first time in Western studies [10]. This study including 5.24 million subjects and 3,337 incident GC cases reported an increased risk of GC in those with low BMI (< 20 kg/m²) as well as in obese subjects. Results from China cohort study also reported that high BMI (≥ 23.31 kg/m²) compared to low BMI (< 20.32 kg/m²) was associated with reduced gastric non-cardia cancer risk rather than increased risk, implying significantly more elevated GC risk in low BMI group [11].

Our findings were analogous with those results above in terms of demonstrating increased GC risk in population with low BMI. However, significant associations between BMI and GC risk results were obtained only in *H. pylori* non-infected persons in our study. This suggests that *H. pylori* has a very strong effect on the development of GC, whereas BMI can play a modest role in the development of GC, as compared to *H. pylori* infection.

The link with gastric non-cardia cancer risk in obese population has not been fully established, and the explanation to support increased GC risk in populations with low BMI has so far been limited.

However, the increased risk of GC in obese people can be explained by biological changes, such as an increase in chronic inflammation and a decrease in immune response in obese people [23,24], which lead to contributing the chronicization of inflammatory conditions and consequently go to gastric carcinogenesis. This can be induced into carcinogenesis regardless of *H. pylori* infection. The increased risk of GC in people with low BMI can be explained by the following mechanisms. Smokers are more likely to have low BMI and those with low BMI are more likely to have multiple nutrient deficiencies [25]. These conditions lead to reduction of natural immunity, and in particular, the decrease of micronutrients such as vitamins A, C, and E can cause the limit of antioxidant activity, which can lead to an increase of inflammatory reaction due to oxidative stress [26,27].

Our results show that the U-shaped association of BMI with GC risk in non-infected individuals is more evident than in *H. pylori* infected individuals. The risk of GC in all *H. pylori* infected individuals has already risen even in the reference group (people with BMI of 23-24.9 kg/m²) at the baseline risk of GC, due to *H. pylori* infection itself, and thus, the GC risk of the high-risk groups (those with BMI < 23 kg/m² or those with BMI ≥ 25 kg/m²) does not seem to rise much compared to the reference group. Conversely, in the *H. pylori* non-infected individuals, the effect of BMI itself on GC risk appears to be clearly demonstrated because major carcinogenic factor, *H. pylori* infection, are not involved. In other words, the *H. pylori* infection, a big carcinogenic factor, may obscure the risk of GC by BMI in infected individuals due to "Umbrella effect" by infection, whereas the effect of BMI on GC risk is clearly expressed in non-infected individuals because of no "Umbrella effect" by infection. This may be due to the fact that the effect of BMI is too weak for the development of GC compared to the effect of *H. pylori* infection.

This study has some limitations. First, the number of incident GC cases was relatively small, limiting the statistical power in stratified analysis. Though we found U-shaped relationship between BMI and GC risk in subjects not infected by *H. pylori*, only one GC case was included in the reference group. Therefore, point estimate of GC risk by BMI in *H. pylori* non-infected group might be overestimated. Second, total energy expenditure or nutritional status was not considered, because of insufficient information on food consumption. Third, BMI information was based on height and weight measured at the time of enrollment, only, and information of BMI change during follow-up time could not be taken into account. Fourth, the results in the *H. pylori* IgG Ab test is limited because if the IgG Ab test is positive, it does not mean an active infection because it is seen as positive in both past and current infections. Fifth, in this cohort, 4.1% GC cancer cases were detected by death certificate only (DCO) (=22 DCO/485 total GC cases). Of total 463 GC cases after excluding DCO cases, GC cases with no T-Code were 26 (5.7%) and those with unspecified anatomical location (C169) were 66 (16.3%). A prior paper presenting the classification cardia and non-cardia GC in hospital setting reported the unspecified GC cases were 25% (n=50/200 cases) [28]. The DCO occurs due to the gap between the coverage years of cancer registration data and those of death certificate data. This KMCC data was first linked to cancer registry, death certificate, and health insurance data (with confirmation using medical record survey) in 2001. We found that those

who were identified as confirmed cancer cases in the secondary data in 2001 were not confirmed in recent data. The reason is because each institution had limited the retention year of data. The other reason of DCO is due to incompleteness of secondary data, such as the year of the application of the secondary data source. In estimating the cancer incidence and mortality rates reported by the WHO Globocan, the completeness of the data is evaluated by the DCO (%) of each country (http://gco.iarc.fr/).

This study has several strengths. It was designed as a prospective cohort study and a case-cohort study. Both studies are based on prospectively collected data, thus time sequence between exposure and outcome is considered in these study designs, which minimizes reverse causation. Matching between cases and controls was not carried out in the case-cohort study design; thus, effects of age or sex on development of disease could be studied on the incidence of disease in the study population. Unlike most previous published studies on the association between BMI and GC which were conducted in Western population, this study was conducted on an East Asian population. Therefore, this study was appropriate to assess the association between BMI and gastric non-cardia cancer. *H. pylori* infection status, a potential confounder, was also adjusted in the case-cohort study.

In conclusion, this study suggests that BMI levels are associated with GC risk in a U-shaped fashion, especially in population without *H. pylori* infection.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Author Details

[1]Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, [2]Cancer Research Institute, Seoul National University, Seoul, [3]Department of Biomedical Science, Seoul National University Graduate School, Seoul, [4]Department of Public Health, Graduate School, The Catholic University of Korea, Seoul, Korea, [5]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, [6]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, [7]Department of Research, Cancer Registry of Norway – Institute of Population-Based Cancer Research, Oslo, Norway, [8]Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, [9]Faculty of Medicine, University of Helsinki, Helsinki, Finland, [10]Department of Preventive Medicine, Konkuk University, Chungju, Korea, [11]Non-communicable Disease and Health Promotion, Western Pacific Regional Office, World Health Organization, Manila, Philippines, [12]Department of Cancer Control and Population Health, Graduate School of Cancer Science & Policy, National Cancer Center, Goyang, [13]Cancer Risk Appraisal and Prevention Branch, National Cancer Control Institute, National Cancer Center, Goyang, [14]The Armed Forces Capital Hospital, Seongnam, Korea

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