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Impact of alcohol drinking on gastric cancer development according to *Helicobacter pylori* infection status

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Background: *Helicobacter pylori* are major carcinogen of gastric cancer, but the associations among gastric cancer, *H. pylori* infection status, and alcohol consumption are not fully described. This study aimed to clarify how *H. pylori* infection status affects the association between alcohol consumption and gastric cancer risk.

Methods: We selected 949 case–cohort participants from the 18 863 Korean Multi-center Cancer Cohort (KMCC) populations. Gastric cancer incidence inside and outside of the subcohort were 12 and 254 cases, respectively. Seropositivities for CagA, VacA, and *H. pylori* infection were determined by performing immunoblot assays. Weighted Cox regression models were used to calculate hazard ratios and 95% confidence intervals (CIs).

Results: Relative to non-drinking, heavy drinking (⩾7 times a week), and binge drinking (⩾55 g alcohol intake per occasion) showed a 3.48-fold (95% CI, 1.13–10.73) and 3.27-fold (95% CI, 1.01–10.56) higher risk in subjects not previously infected by *H. pylori*. There was no significant association between drinking pattern and gastric cancer risk in *H. pylori* IgG seropositive subjects. An increased risk for gastric cancer in heavy- and binge-drinking subjects were also present in subjects not infected by CagA- or VacA-secreting *H. pylori*.

Conclusions: Heavy and binge alcohol consumption is an important risk factor related to an increasing incidence of gastric cancer in a population not infected by *H. pylori*.

Gastric cancer is the fifth most common cancer and the third most common cause of cancer-associated death in the world (Ferlay *et al*, 2015). In South Korea, gastric cancer was the second most common cancer and the third most common cause of cancer-associated death in 2009 (Jung *et al*, 2014).

Gastric cancer is a multi-factorial disease (Kelley and Duggan, 2003), and *Helicobacter pylori* infection has been reported to be the most important aetiological factor for the development of non-cardia gastric cancer (Forman *et al*, 1991). However, in countries with a high prevalence of *H. pylori* infection, such as Korea and

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Japan, gastric cancer patients have *H. pylori* infection prevalence similar to that in control subjects (Tajima, 2002; Lunet and Barros, 2003; Shin *et al*, 2004). Therefore, the presence of *H. pylori* infection alone is not a sufficient basis for gastric cancer development (Tajima, 2002). In our previous study, an association between *H. pylori* infection and gastric cancer risk was not detected. However, seropositivity of the *H. pylori* virulence factor cytotoxin-associated gene A (CagA) immunoglobulin G (IgG) antibody was associated with an increased gastric cancer risk (Gwack *et al*, 2006).

Results in recent meta-analyses (Li *et al*, 2011; Tramacere *et al*, 2012) and in large cohort studies in China and Europe (Ji *et al*, 1996; Duell *et al*, 2011) indicate that alcohol consumption is a risk factor for gastric cancer. However, there is controversy over the aetiological aspects of the correlation between alcohol and gastric cancer, and the International Agency for Research on Cancer (IARC) has reported inconsistency in the association between alcohol consumption and gastric cancer risk (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans, 2010). Most previous studies have not considered *H. pylori* infection as a confounder or potential effect modifier of gastric cancer risk. Only two studies have evaluated the association between alcohol and gastric cancer by adjusting for (Duell *et al*, 2011) or stratifying by (Zaridze *et al*, 2000) *H. pylori* infection status. The study based on the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort analysed heavy alcohol consumption ($\geqslant$60 g per day) in relation to gastric cancer risk adjusted for *H. pylori* serostatus, but the results indicated no difference between adjusted (odds ratio (OR), 1.60; 95% confidence interval (CI), 0.91–2.82) or not adjusted (hazard ratio (HR), 1.65; 95% CI, 1.06–2.58) (Duell *et al*, 2011). The stratified study from Russia reported that vodka consumption increased the risk of gastric cancer regardless of *H. pylori* infection status. Vodka consumption increased gastric cancer risk 2.0-fold (95% CI, 1.2–3.1) and 2.3-fold (95% CI, 1.4–3.7) from that in non-drinking subjects without or with *H. pylori* infection, respectively (Zaridze *et al*, 2000). However, the effects of heavy- and binge-drinking alcohol consumption patterns in relation to *H. pylori* were not considered in Russian study.

Korea has been reported to have the highest gastric cancer incidence in the world (Ferlay, 2013). In 2007, about 60% of the Korean population was infected by *H. pylori*, although the prevalence of *H. pylori* infection has been decreasing (Yim *et al*, 2007). These noteworthy conditions in Korea indicate the importance of elucidating the association between alcohol consumption and gastric cancer in relation to *H. pylori* infection status in the Korean population.

In this study we hypothesised that *H. pylori* may be an effect modifier in the relationship between alcohol drinking and gastric cancer development, or alcohol drinking may be an independent risk factor for gastric cancer regardless of *H. pylori* infection. We thus investigated the association between alcohol-drinking patterns and gastric cancer risk within a prospective Korean Multi-center Cancer Cohort study (KMCC).

MATERIALS AND METHODS

Study participants and data collection. The study's subjects were recruited from the KMCC study, a population-based, prospective cohort study designed to investigate causes of cancer in Korea. From 1993 to 2004, the KMCC study recruited 19 688 voluntary participants from four urban and rural areas in Korea (Haman, Chungju, Uljin, and Pohang). All participants completed standardised questionnaires during personal interviews after providing informed consent. Information on individual characteristics, including lifestyle, medical history, and environmental exposure factors, was collected by using a detailed, standardised questionnaire. Biological samples (urine and blood) were also collected and stored under stable conditions (Yoo *et al*, 2002). Of the 19 688 participants in the KMCC study, we excluded those under 20 years old and those with no confirmed alcohol-drinking status. After those exclusions, 18 863 participants with information of alcohol consumption were identified and included in our study population. By 31 December 2006, among the study population there were 301 cases of gastric cancer, as defined by the International Statistical Classification of Diseases and Related Health Problems tenth Revision (ICD-10, C16). The cases were identified through computerised record linkage to the Korean national cancer registry and the national death certificate. The passive follow-up methods were reported to be 99% efficient, and completeness was assured (Cho *et al*, 2009).

Case–cohort population was derived from total KMCC participants. An 89% of total 19 688 members ($N=17\,522$) donated blood specimens and of them we selected 4% population ($N=701$) by age/sex stratified random sampling. After excluding subject not having plasma or serum, 695 were selected as subcohort members, including 12 gastric cancer cases inside of subcohort. In contrast, of total 301 gastric cancer cases, 268 cases donated their blood at the enrolment, and finally 266 gastric cancer cases were included in this study, after excluding subjects not having plasma or serum specimens ($N=2$). In summary, our case–cohort population consisted of total 949 participants: total 695 subcohort (including 683 healthy subjects and 12 gastric cancer cases inside of subcohort) and additional 254 gastric cancer cases outside of subcohort.

The study protocol was approved by the Institutional Review Boards of the Seoul National University Hospital (H-0110-084-002) and the National Cancer Center of Korea (C-0910-049-297 and C-0907-044-286).

Exposure assessment. Patterns of alcohol consumption were determined by reviewing the KMCC questionnaire items related to alcohol-drinking status (non-drinker, past drinker, or current drinker), total years of drinking, frequency of drinking alcohol in 1 week, and the amount of alcohol consumed on one occasion. These items were surveyed separately by type of alcohol beverage, as reported in the National Alcohol Survey (Greenfield *et al*, 2009).

Internationally, there is no consensus on the definition of heavy and binge drinking. The Substance Abuse and Mental Health Services Administration has suggested that heavy drinking involves consuming more than five drinks in a single episode on more than 5 days within 1 month (Substance of Abuse and Mental Health Services Administration, the Center Behavioral Health Statistics and Quality, 2014). The National Institute of Alcohol Abuse and Alcoholism defines binge drinking as alcohol consumption that produces a blood alcohol concentration over 0.08 g dl^{-1} with this concentration corresponding to drinking more than five drinks in men or more than four drinks in women within 2 h (National Institute on Alcohol Abuse and Alcoholism, 2004). Without a standardised definition of alcohol amounts for heavy or binge drinking, we developed consumption classification criteria based on the drinking patterns in the study population. Additional classification criteria were used to define different patterns of alcohol consumption.

With regard to drinking status, the study participants were divided into three groups: never drinkers, past drinkers, and current drinkers; the latter two were denoted as 'drinkers' in our analysis. Frequency of consumption was used to categorise the drinkers as non-drinker, drinking <4 times per week, drinking 4–6 times per week, and drinking $\geqslant 7$ times per week (Tables 1–3). Participants who drank $\geqslant 7$ times per week were defined as heavy drinkers.

We also classified the drinking frequency of drinkers as non-drinker, light drinker (alcohol-drinking frequency $\leqslant 2.5$ and $\leqslant 0.6$ times a week in men and women, respectively), moderate drinker (alcohol-drinking frequency between light and heavy drinkers), and heavy drinker (alcohol-drinking frequencies >7 and >3 times a week in men and women, respectively). These categories were based on the cutoff levels for the 80th and 40th percentiles of alcohol consumption frequency for both men and women.

In addition, the participants were asked to identify the type of alcoholic beverage consumed (soju, beer, gin, rice wine, etc.) and the average amount of alcohol per drink (in cc). The amount of pure alcohol intake in the different types of alcoholic beverage was calculated from the known percentage of ethanol in each alcoholic beverage: soju, 20%; beer, 4.5%; gin, 40%; rice wine, 6%; and other alcohol beverages common in Korea, 25%. To determine average alcohol-drinking dosage, the average alcohol consumption was categorised into quartiles based on the frequency distribution among the total cohort. The alcohol doses per single occasion were then presented as non-drinker, <25 g alcohol, 25–54.9 g alcohol, and $\geqslant 55$ g alcohol (Tables 1–3). Participants whose drinking dose was $\geqslant 55$ g alcohol per drinking episode were defined as binge drinkers in total cohort population. In Supplementary Tables, the average alcohol-drinking dose was classified by sex as non-drinking, low dose (<28 g alcohol and <4 g alcohol on a single occasion in men and women, respectively), intermediate dose (alcohol dose between low and high dose), and high dose ($\geqslant 120$ g alcohol and $\geqslant 29$ g alcohol on a single occasion in men and women, respectively). The high-dose category was denoted as a binge-drinking dose level.

Because U-shaped associations between alcohol-drinking dose or frequency and gastric cancer risk were observed in spline analysis and most of women in our study were non-drinkers or drank small amount of alcohol, alcohol drinking may seem protective factor of gastric cancer if we applied lower cutoff level in women. We also want to evaluate the association between absolute quantity of alcohol-drinking dose or frequency and gastric cancer, thus the same cutoff points were applied in both men and women. The participants' drinking pattern assessment was based on the year before interview, and we assumed that pattern would be maintained during follow-up time.

***H. pylori* infection, CagA, and VacA seropositivity.** The CagA protein (cytotoxin-associated gene A), a key toxin of *H. pylori*, is reported to be associated with the development of gastric cancer. The toxin produces an inflammation of the gastric epithelium (Wang *et al*, 2013). It has also been reported that certain *H. pylori* genotypes produce vacuolating cytotoxin A (VacA) protein, another multi-functional virulence factor of *H. pylori* that has been associated with an increase in gastric cancer risk (Ogiwara *et al*, 2009). Thus, in addition to recording *H. pylori* infection status, both CagA and VacA IgG antibody seropositivities were determined in the study participants.

Seropositivities were determined by using the Helico Blot 2.1 (MP Biomedicals Asia Pacific, Singapore) immunoblot assay. *H. pylori* IgG seropositivity according to the immunoblot assay determined at enrolment, which *H. pylori* seropositivity reflects individuals' current infection as well as ever infection in the past.

Statistical analysis. Average and s.d. values of possible confounding factors including age, height, weight, and body mass index (BMI) were calculated for each alcohol-drinking status (non-, past, and current drinkers). Differences in height, weight, and BMI between groups were compared by Student's *t*-tests. Differences in proportions by sex, smoking status (non-, past, and current smokers), history of gastric ulcer (no/yes), and education level (lower, middle, high school, community college, or higher) between groups were tested by χ^2 or Fisher's exact test.

To determine whether alcohol consumption is associated with a risk for gastric cancer, we conducted Cox regressions in the total cohort analysis and estimated HRs and 95% CIs in the models. Analyses were adjusted for age, sex, BMI, educational level, and smoking status in the total cohort. In addition, *H. pylori* infection status was adjusted in the case–cohort. The assumption that each predictor affected risk for gastric cancer proportionally over the entire follow-up period was examined by using graphical methods; the results indicated that the assumption was reasonable for all predictors considered in this study. The significance of the explanatory variables included in the Cox models was computed by the likelihood-ratio test. Tests for dose–response trends were assessed by fitting ordinal exposure variables as continuous terms.

We used weighted Cox proportional hazard regression models for case–cohort analysis, and each subject's weight was the inverse of their sample fraction to account for the differential sample proportion among the cases and the subcohort participants; that analysis was undertaken by using Barlow's method (Barlow *et al*, 1999). Gastric cancer cases were given a sample weight of 1 because they were sampled with certainty, whereas the subcohort participants were given a sample weight based on inverse selection probability.

We compared the Akaike's Information Criteria (AIC) in nonlinear spline and linear model for the relationship between alcohol consumption and gastric cancer and finally decide nonlinear cubic spline model to fit the relationship. The number and location of the knots used to fix the splines were created by following the recommendations of Harrell and Steyerberg (Harrell, 2001; Steyerberg, 2009).

Analyses were performed by using Stata version 12.1 and SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). A two-sided significance level of 0.05 was used.

RESULTS

A summary of the general characteristics of the study population is presented in Supplementary Table 1. On average, current and past drinkers were mostly men, were older, had lower BMI, and smoked more than non-drinkers.

Table 1 shows the alcohol-drinking-related HRs (95% CIs) for gastric cancer obtained from a multivariable analysis adjusted for age, sex, BMI, educational level, and smoking status for the total cohort. The results indicate a statistically significant association between long duration of alcohol drinking (HR, 1.49; 95% CI, 1.11–2.01), drinking frequency $\geqslant 7$ times per week (HR, 1.50; 95% CI, 1.08–2.07) and gastric cancer risk in the total cohort. In the subcohort population for which there was information on *H. pylori* status, the multivariable model results adjusted for age, sex, BMI, educational level, smoking status, and *H. pylori* were similar to those for the total cohort, although the associations between alcohol-drinking patterns and gastric cancer risk were not statistically significant.

Table 2 shows the alcohol-drinking-related HRs (95% CIs) for gastric cancer in the KMCC subcohort population with *H. pylori* IgG antibody seropositivity. In our analysis stratified for anti *H. pylori* IgG antibody status, drinking alcohol $\geqslant 7$ times a week resulted in a 3.48-fold higher risk of gastric cancer (95% CI, 1.13–10.73), and drinking frequency of at least seven times per week resulted in an HR of 3.48 (95% CI, 1.13–10.73) as compared with the non-drinking, non-*H. pylori*-infected group. An increased risk was also detected in the groups without CagA IgG- or VacA IgG-secreting *H. pylori* (Table 3). In particular, the increase in gastric cancer risk associated with drinking $\geqslant 7$ times a week was greatest in the group not infected by CagA-secreting *H. pylori* (HR, 11.31; 95% CI, 1.45–87.92) and 3.76 times (95% CI, 1.13–12.49) in the group not infected by VacA. However, there are no heterogeneous in HR for gastric cancer between

Table 1. The risk for gastric cancer in relation to alcohol-drinking status in the KMCC, 1994–2004

	Total cohort ($N=18\,863$)			Case–cohort[a] ($N=949$)		
	Person-years	No. of cases ($N=403$)	HR (95% CI)[b]	Person-years	No. of cases ($N=266$)	HR (95% CI)[b]
Drinking status						
Non-drinking	129 602	166	1 (reference)	91 801	107	1 (reference)
Past	10 143	52	1.45 (0.99–2.12)	7851	35	1.32 (0.83–2.08)
Current	75 039	185	1.21 (0.94–1.56)	55 555	124	1.02 (0.74–1.40)
Duration of alcohol drinking (year)						
Non-drinking	129 602	166	1 (reference)	91 801	107	1 (reference)
⩽10	19 585	23	1.25 (0.79–1.96)	1583	10	1.04 (0.54–2.00)
11–30	33 378	54	0.91 (0.64–1.31)	2773	27	0.95 (0.48–1.88)
31+	24 393	134	**1.49 (1.11–2.01)**	2375	86	1.30 (0.93–1.83)
Drinking frequency (times per week)						
Non-drinking	129 602	166	1 (reference)	91 801	107	1 (reference)
<4	40 198	80	1.24 (0.92–1.68)	28 766	48	1.50 (0.87–1.81)
4–6	13 048	24	1.00 (0.62–1.55)	9785	14	1.28 (0.36–5.16)
⩾7	16 684	76	**1.50 (1.08–2.07)**	13 170	56	1.35 (0.96–1.88)
Average alcohol-drinking dose (g per single occasion)						
Non-drinking	129 602	166	1 (reference)	91 801	107	1 (reference)
<25	39 754	93	**1.33 (1.01–1.77)**	24 132	48	1.22 (0.83–1.79)
25–54.9	19 296	26	1.14 (0.72–1.80)	7971	21	0.95 (0.56–1.60)
⩾55	15 768	51	1.36 (0.95–1.96)	16 867	44	1.05 (0.70–1.58)

Abbreviations: CI = confidence interval; HR = hazard ratio; KMCC = Korean Multi-center Cancer Cohort. The bold values indicate statistical significance at 95% confidence levels.
[a]Case–cohort subjects had the information of *H. pylori* infection.
[b]Adjusted for age, sex, body mass index (BMI), educational level, and smoking status in total cohort population; adjusted for age, sex, BMI, educational level, smoking status, and *H. pylori* infection in case–cohort population.

Table 2. The risk for gastric cancer in relation to alcohol-drinking status according to *H. pylori* antibodies in the KMCC case–cohort population with the information of *H. pylori* antibody, 1994–2004

	H. pylori (+) ($N=817$)			*H. pylori* (−) ($N=132$)		
	Person-years	No. of cases ($N=235$)	HR (95% CI)[a]	Person-years	No. of cases ($N=31$)	HR (95% CI)[a]
Drinking status						
Non-drinking	3021	95	1 (reference)	437	12	1 (reference)
Past drinker	549	30	1.32 (0.81–2.57)	84	5	1.36 (0.37–5.01)
Current drinker	3176	110	1.01 (0.72–1.48)	432	14	1.17 (0.44–3.12)
Duration of alcohol drinking (year)						
Non-drinking	3021	95	1 (reference)	437	12	1 (reference)
⩽10	318	10	1.95 (0.52–2.09)	28	0	0.84 (0.22–3.18)[b]
11–30	766	23	1.01 (0.60–1.70)	130	4	
31+	1761	75	1.17 (0.80–1.70)	230	11	1.65 (0.54–5.10)
Alcohol-drinking frequency (times per week)						
Non-drinker	3021	95	1 (reference)	437	12	1 (reference)
<4	1136	45	1.19 (0.80–1.78)	228	3	0.21 (0.03–1.74)
4–6	532	12	0.60 (0.31–1.14)	71	2	1.76 (0.31–9.96)
⩾7	1192	48	1.17 (0.78–1.77)	102	8	**3.48 (1.13–10.73)**[c]
Average alcohol-drinking dose (g per single occasion)						
Non-drinker	3021	95	1 (reference)	437	12	1 (reference)
<25	993	43	1.36 (0.92–2.02)	213	4	0.42 (0.10–2.64)
25–54.9	583	18	0.86 (0.49–1.51)	74	3	1.86 (0.40–3.93)
⩾55	1153	39	0.94 (0.61–1.46)	93	6	**3.27 (1.01–10.56)**[c]

Abbreviations: CI = confidence interval; *H. pylori* = *Helicobacter pylori*; HR = hazard ratio; KMCC = Korean Multi-center Cancer Cohort. The bold values indicates statistical significance at 95% confidence levels.
[a]Adjusted for age, sex, body mass index, educational level, and smoking status in case–cohort population.
[b]The results were combined due to few events among subpopulation.
[c]*P*-value for heterogeneity between two hazard ratio (95% CIs) in infection-positive and infection-negative groups was statistically significant ($P=0.047$ for drinking frequency in the groups of '⩾7 times per week'; $P=0.042$ for average alcohol-drinking dose in the groups of '⩾55 g per day').

H. pylori non-infected population, CagA (or VacA)-secreting *H. pylori* non-infected population (*P*-heterogeneity >0.3). In the ever infected by *H. pylori* group, none of the alcohol-drinking variables were significantly associated with gastric cancer. In the not infected by *H. pylori* group, the risk of gastric cancer increased according to average alcohol-drinking dose, and those consuming a high alcohol dose had a 3.27-fold higher risk of gastric cancer (95% CI, 1.01–10.56) than non-drinker. An increase in gastric cancer risk associated with high-dose alcohol consumption, that is, ⩾55 g of alcohol per single occasion, was also detected in groups not infected by CagA-secreting *H. pylori* and VacA-secreting *H. pylori* (HR, 6.06; 95% CI, 1.04–35.32 for the CagA; and HR, 4.44;

Table 3. The risk for gastric cancer in relation to alcohol-drinking status according to CagA- or VacA-secreting *H. pylori* antibodies in the KMCC case–cohort population with the information of *H. pylori* antibody, 1994–2004

	CagA (+) (*N* = 772)			CagA (−) (*N* = 85)			VacA (+) (*N* = 567)			VacA (−) (*N* = 126)		
	Person-years	No. of cases (*N* = 227)	HR (95% CI)[a]	Person-years	No. of cases (*N* = 16)	HR (95% CI)[a]	Person-years	No. of cases[a] (*N* = 171)	HR (95% CI)[a]	Person-years	No. of cases (*N* = 28)	HR (95% CI)[a]
Drinking frequency (times per week)												
Non-drinker	2794	92	1 (reference)	313	6	1 (reference)	2170	68	1 (reference)	410	11	1 (reference)
<4	1080	44	1.19 (0.79–1.78)	188	3	0.44 (0.05–4.19)	837	34	1.02 (0.63–1.66)	228	3	0.25 (0.03–2.06)
4–6	527	12	0.59 (0.30–1.13)	62	2	5.14 (0.53–49.95)	386	10	0.57 (0.28–1.87)	71	2	2.53 (0.42–15.37)
⩾7	1110	46	1.17 (0.77–1.78)	44	4	**11.31 (1.45–87.92)[b]**	779	37	1.17 (0.72–1.88)	102	7	**3.76 (1.13–12.49)[b]**
Average alcohol-drinking dose (g per single occasion)												
Non-drinker	2794	92	1 (reference)	313	6	1 (reference)	2170	68	1 (reference)	410	11	1 (reference)
<25	922	42	1.39 (0.93–2.07)	163	4	0.69 (0.11–3.87)	742	31	1.13 (0.70–1.82)	213	4	0.51 (0.10–2.53)
25–54.9	572	18	0.85 (0.49–1.50)	92	3	2.64 (0.43–16.59)	401	16	0.95 (0.51–1.77)	74	2	1.60 (0.31–8.33)
⩾55	1104	37	0.90 (0.57–1.40)	26	2	**6.06 (1.04–35.32)[b]**	753	29	0.90 (0.54–1.51)	93	6	**4.44 (1.13–17.39)[b]**

Abbreviations: CagA = cytotoxin-associated gene A; CI = confidence interval; *H. pylori* = *Helicobacter pylori*; HR = hazard ratio; KMCC = Korean Multi-center Cancer Cohort; VacA = vacuolating cytotoxin A. The bold values indicates statistical significance at 95% confidence levels.

[a]Adjusted for age, sex, body mass index, educational level, and smoking status in case–cohort population.

[b]*P*-value for heterogeneity between two hazard ratio (95% CIs) in infection-positive and infection-negative groups was statistically significant (for drinking frequency in the groups of '⩾7 times per week', $P = 0.033$ between CagA-positive and -negative groups and $P = 0.036$ between VacA-positive and -negative groups; for average alcohol-drinking dose in the groups of ⩾55 g per day', $P = 0.028$ between CagA-positive and -negative groups and $P = 0.036$ between VacA-positive and -negative groups).

95% CI, 1.13–17.39 for VacA). In the group infected by *H. pylori*, there were no statistically significant results for any of the different doses of alcohol consumed per day. The trend to an increased gastric cancer risk with increases in drinking frequency and average alcohol dose was persistent even when we applied different alcohol-drinking classifications (Supplementary Table 2). Although there is no gender-specific effect in the association between alcohol drinking and gastric cancer risk (*P*-heterogeneity of ORs between gender groups >0.1), most results were statistically significant in men group (table not shown).

Figure 1 presents the results for the associations between alcohol consumption and gastric cancer risk in only *H. pylori*-negative subjects based on the spline regression analyses. The results were obtained by using three (drinking frequency) or four knots (average alcohol-drinking dose) with the exclusion of extreme consumption values (last three values in each knot) and values for non-consumers. After comparing each obtained AIC_{spline} and AIC_{Linear} value, it was observed that use of the spline model resulted in only a very slight improvement in fit over that from a standard linear regression model (data not shown).

DISCUSSION

Our results showed that alcohol drinking for long periods of time (decades) and frequent drinking (daily) is associated with an increased risk of gastric cancer in the study cohort. However, this increased risk of gastric cancer by heavy or binge drinkers relative to non-drinkers was observed in only *H. pylori* non-infected subjects. Heavy drinking (i.e., drinking seven or more times a week) and binge drinking (i.e., alcohol consumption of ⩾55 g per occasion) were associated with 3.48-fold and 3.27-fold higher risks for gastric cancer compared with non-drinking among the *H. pylori* non-infected study population.

The associations between heavy or binge drinking and gastric cancer risk in the non-infection groups were not statistically heterogeneous, regardless of common or highly pathogenic *H. pylori*, classified by CagA or VacA. This association may be only conditioned by *H. pylori* non-infection. On the contrary, our finding of non-association in *H. pylori*-infected group may be due to any antimicrobial effect of *H. pylori* on high-dose alcohol-drinking condition. Our hypothesis is supported by several epidemiologic studies reported an antimicrobial effect of alcohol on *H. pylori* (Brenner *et al*, 1997, 1999; Ogihara *et al*, 2000). High alcohol consumption was significantly associated with a decrease in *H. pylori* infection. In spite of the negative relationship between alcohol intake and *H. pylori* infection, only two previous studies included information on *H. pylori* infection status when evaluating the association between alcohol drinking and gastric cancer risk (Zaridze *et al*, 2000; Duell *et al*, 2011). One of those studies presented results adjusted for *H. pylori* infection (Duell *et al*, 2011), whereas the other study, conducted in Russia, presented the results stratified by *H. pylori* infection status (Zaridze *et al*, 2000). In the Russian study, vodka consumption was associated with an increased risk of gastric cancer in both *H. pylori*-positive and *H. pylori*-negative subjects (Zaridze *et al*, 2000).

Our study showed dose- and frequency-dependent associations between alcohol drinking and gastric cancer risk, but only in subjects not infected by *H. pylori*. The difference in results between our study and the Russian study might be related to the different amounts of alcohol consumption in the two studies. Vodka drinking in the Russian study was classified as 'never' or 'ever', and the average intake of pure alcohol was not presented. In the absence of dose and frequency data, we were unable to compare directly the two studies results. The World Health Organization (WHO) has reported that from 2003 to 2005 the pure alcohol

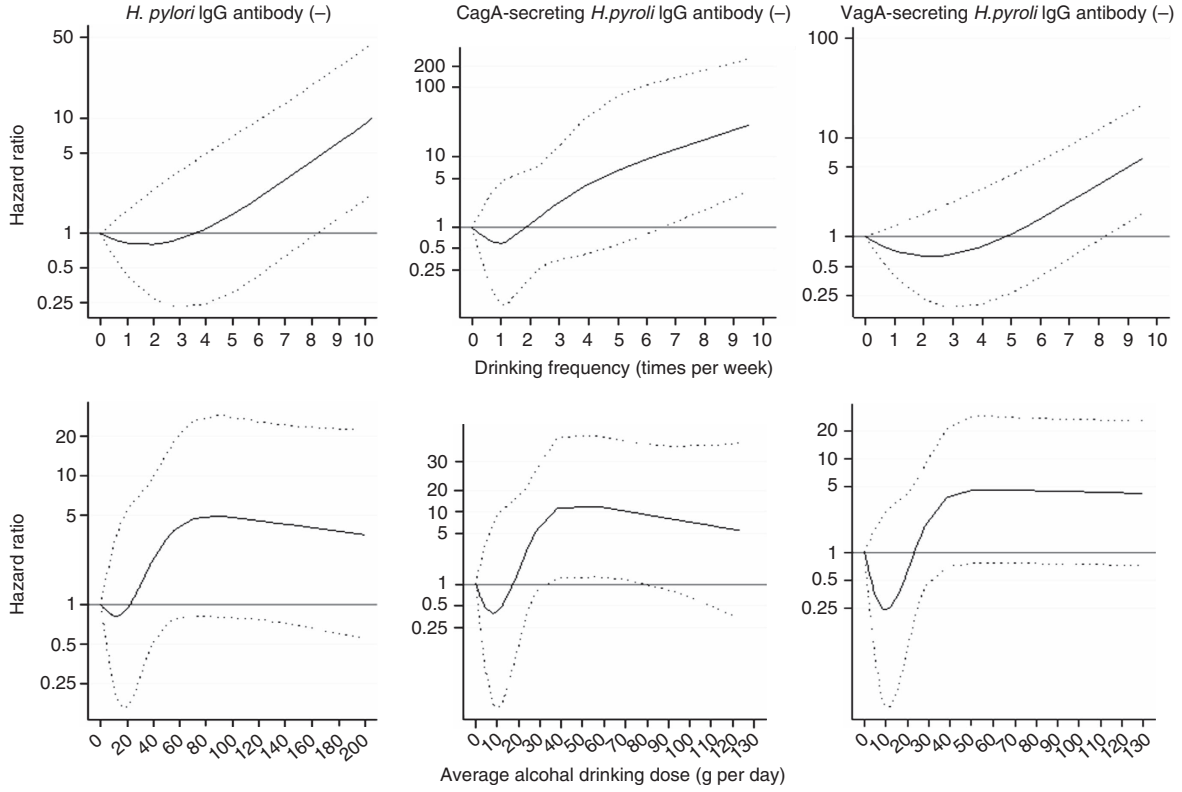

Figure 1. HRs (solid line) and 95% CIs for baseline alcohol consumption and gastric cancer risk assessed by using restricted cubic spline regression in KMCC cohort. The spline regression model excluded extreme consumption (top 1%, ⩾200 g per day; three cases). Weighted model (four knots) adjusted for age (1-year categories), sex, BMI, and smoking status.

intake per capita for those over 15 years old was 16.1 l in Russia and 12.3 l in Korea (World Health Organization, 2014). On the basis of those WHO report, we assume that the average alcohol dose in the Russian study was higher than that in our study, which may account for the difference in results.

Supposing some peoples with *H. pylori* infection (or highly pathogenic *H. pylori* infection) has gastric symptoms due to *H. pylori* or highly pathogenic *H. pylori*, individuals with gastric symptoms possibly decide to reduce or quit drinking alcohol. However, subjects infected with *H. pylori*, even with CagA-positive *H. pylori*, are generally asymptomatic and have no difference in symptom severity even in subjects with mild gastric symptoms (Bommelaer *et al*, 2001). In our cohort, the proportions of heavy drinking and binge drinking among *H. pylori*-infected peoples (20% and 21%, respectively) were higher than those in non-infected peoples (12% and 11%, respectively). On the basis of the research showing no difference of symptoms according to virulence of *H. pylori* and the evidence from our cohort population, we did conclude that our finding was not biased by changing alcohol habit due to *H. pylori* infection and gastric symptoms. An *in vitro* study reported by Brenner *et al* showed a relationship between *H. pylori* prevalence and alcohol drinking and included results that considered alcohol dose. In that study moderate drinking (alcohol consumption of 75–175 g per week) was associated with a decreased OR of *H. pylori* infection (OR, 0.60; 95% CI, 0.38–0.94), but the antimicrobial effect of alcohol consumption was lessened at higher alcohol dose level (Brenner *et al*, 2001). Those results suggest a U-shaped curve relationship between alcohol consumption level and prevalence of *H. pylori*. On the basis of that type of relationship, we conclude that the alcohol dose level in the Russian study was excessive and that dose level would not inhibit *H. pylori* infection efficiently, suggesting that the *H. pylori* infection status was similar in non-drinkers and drinkers. Under such conditions, the independent effect of alcohol on gastric epithelium may result in an increased risk of gastric cancer compared with the level of risk in non-drinking subjects. In our study, the alcohol consumption amounts for heavy and binge drinkers may be close to the level that can inhibit the infection of *H. pylori* efficiently. In a *H. pylori*-infected group, a decreased level of *H. pylori* infection derived from the effects of alcohol consumption may lessen the gastric cancer risk through the independent effect of alcohol as a carcinogen. This could result in no detectable difference in gastric cancer risk between drinkers and non-drinkers in a *H. pylori*-infected population. However, in a population that is not infected by *H. pylori*, the carcinogenic effects of alcohol may increase the risk of gastric cancer.

The mechanisms responsible for the carcinogenic effect of alcohol consumption on gastric cancer have not been fully elucidated. Thus far, some reports suggest that ingested alcohol can cause direct and indirect dose-dependent mechanical damage to the gastric epithelium (Chari *et al*, 1993; Knoll *et al*, 1998). It has been reported that alcohol intake increases acid secretion from the stomach, which leads to gastric mucosal damage (Chari *et al*, 1993). In addition, a high concentration of ingested alcohol has been associated with the generation of reactive oxygen species (ROS; e.g., peroxide, superoxide) as well as other free radicals, inorganic arsenic, preservatives, and additives (Zimmerman *et al*, 1995), which can produce alterations in hormonal balance and depletion of vitamin deposits, subsequently promoting carcinogenesis of gastric cancer (Blot, 1992). The direct processes that produce mucosal damage, such as increased acid secretion and ROS generation in the stomach, can induce an inflammatory environment in the stomach. The correlation between gastric inflammation and carcinogenesis of gastric cancer has been well described (Rakoff-Nahoum, 2006). Moreover, alcohol can have indirect effects on carcinogenesis of gastric cancer by conversion of

alcohol to metabolites. Alcohol is endogenously broken down into acetaldehyde, which can produce DNA strand breakage and abnormal binding to proteins, potentially leading to cancer development (Aberle *et al*, 2004).

Our study has two limitations. First, the number of gastric cancer cases and the number of study population members with *H. pylori* infection information were small, thus some groups were combined into one group for statistical analysis. Second, information on alcohol consumption was collected at enrolment and was not repeated measurements during the follow-up period. We assumed this baseline information reflected the routine alcohol-drinking pattern of study population throughout the follow-up period. Effects of changes in alcohol-drinking patterns on gastric cancer development were not evaluated. However, considering the latent period of gastric cancer is over 10 years and median follow-up time of our study was 8.4 years, the baseline information of alcohol drinking is still valid for this study.

Nevertheless, this study has some strength. First, this study is a case–cohort study and its data are derived from prospective cohort data that were collected considering the time sequence between exposure and outcome. Thus, we can use the data to assess the causality relative to other study designs and minimise the effects of recall bias. Second, the sensitivity and specificity of the immunoblot assay (Helico Blot 2.1) used to detect *H. pylori*, CagA IgG seropositivity, and VacA IgG seropositivity are very high (99%, 99%, 93% for sensitivity, and 98%, 90%, 88% for specificity, respectively) (Park *et al*, 2002). Third, our study classified the subjects' alcohol-drinking patterns according to duration, frequency, and dose of alcohol consumption. Analysis of these different aspects of alcohol consumption can indicate which of these aspects provide the best information on alcohol effects on gastric cancer risk.

In conclusion, alcohol drinking was associated with an increase in gastric cancer risk. The effects were exhibited in binge or heavy drinkers who were not previously infected by *H. pylori*. The associations between alcohol drinking and risk of gastric cancer were similar in subjects not infected by CagA- or VacA-secreting *H. pylori*. The study results can partly explain why an *H. pylori* not-infected group may have gastric cancer. Such results suggest that abstention from alcohol can lower the risk of gastric cancer, especially among subjects who are not infected by *H. pylori*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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