RESEARCH COMMUNICATION

A Case-Control Study of Gallbladder Cancer in Hungary

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Abstract

Our previous study indicated an association of chili pepper consumption with gallbladder cancer (GBC) in the presence of gallstones (GS) in Chile. We investigated whether or not a similar association was present in Hungary, where mortality from GBC is high and chili peppers are frequently consumed. In a case-control study, we compared 41 female GBC patients with GS and 30 gender and GS-matched hospital controls. Trained staff interviewed all subjects to determine socioeconomic status, family history, past history and life style habits (smoking, alcohol intake, dietary habits and elimination habits). Because mean ages differed significantly between the case and control groups, age-adjusted odds ratios (ORs) were calculated. A shorter education period (< 10 years / ≥16 years) was indicated to be a risk factor (age-adjusted OR (95%CI): 3.2 (1.2-8.7)). In addition, the intake of Hungarian hot pepper (yes / no) was found to be significantly higher in the GBC cases than in controls (age-adjusted OR (95%CI): 8.4 (2.3-30.4)). There were no differences between the case and control groups for other variables. Multivariate logistic regression analysis retained only Hungarian hot pepper consumption as a significant independent risk factor for GBC. Its age-adjusted OR was 16.2 (95%CI: 2.1-126.2), while there were no differences associated with low education, frequent consumption of fresh fruit and vegetables, low socioeconomic status or smoking. Hungarian hot pepper consumption was identified as a risk factor for GBC by multivariate logistic regression analysis.

Key Words: Gallbladder cancer - epidemiology - case-control study - Hungarian hot pepper - gallstones

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Introduction

We have been carrying out epidemiological studies of gallbladder cancer (GBC) for almost 19 years (Yamamoto, 2003). In the early stage of our epidemiological studies especially, world-wide comparison was limited to biliary tract cancer (BTC) since the demographic data on GBC for international comparison were available only as a part of the BTC statistics. According to the ICD code, BTC consists of gallbladder cancer (GBC) (code 156.0 ICD-9 or C23 ICD-10) and extra-hepatic bile duct cancer (EBC) (code 156.1, 156.2 and 156.9 ICD-9 or C24 ICD-10). An initial descriptive study was launched to allow international comparison of BTC mortality rates among 39 countries worldwide during the 1981-1986 period. It was disclosed that the five countries with the highest mortality in terms of standardized mortality ratios were Chile, Japan, the former Czechoslovakia, Hungary and the former East Germany for males, and Chile, Hungary, East Germany, Czechoslovakia and Japan for females (Chen, 1990).

Since BTC mortality rates in Japan were found to rank among the highest in the world in the 1980's, we later focused on GBC and EBC separately from BTC and conducted various descriptive as well as analytical studies on both in Japan (Yamamoto, 2003). Together with various epidemiological studies conducted in Japan, we have attempted to carry out collaborative studies on GBC in Chile, since our previous demographic analysis revealed BTC mortality in Chile to be the highest in the world for both males and females (Chen et al.,, 1990) and it was later demonstrated that BTC in Chile consists mainly of GBC.

Epidemiological studies in Chile suggested the presence of gallstones (GS) to be a definite risk factor for GBC. Thus, we proposed a multi-factorial causation hypothesis; i.e. GBC occurs when an unknown factor or factors interact(s) with the presence of GS. The subsequent ten-year effort to complete the research in Chile revealed associations of red chili pepper consumption, low socioeconomic status and longstanding GS with GBC in Chile (Serra et al., 2002).

This finding prompted us to conduct another collaborative study on GBC in Hungary for two reasons;

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first, the mortality rates for GBC in Hungary are also among the highest in the world and second, Hungarian hot pepper is widely consumed in Hungary. Our three year study of GBC in Hungary finally yielded a clue suggesting that Hungarian hot pepper consumption is related to the high Hungarian GBC rate. We report here the details of our case-control study on GBC in Hungary.

Materials and Methods

Based on the multi-factorial causation hypothesis, we endeavored to detect a risk factor or factors working together with GS by employing a case-control study design for GBC. The study was carried out in Hungary from 2003 to 2006. Prior to the start of this investigation, our protocol was submitted to the Ethics Committee of Niigata University School of Medicine and the Committee of the Hungary National Institute of Oncology. Approval was from both on the condition of obtaining informed consent from all participants. We enrolled 41 patients with GBC and GS whose diseases had been diagnosed at the institute or its related hospitals in Hungary, who provided informed consent, and we also sought gender and GS matched hospital controls free of malignant neoplasm. Since all GBC patients with accompanying GS were females, the controls were also selected from among female patients whose diseases had been diagnosed as GS only at these institute or hospitals who volunteered to participate in the present study. We ultimately enrolled 30 controls who signed the informed consent documents. We could not match age as the third matching due to the limit number of controls. This case-control study was thus a gender- and GS-matched study.

All subjects were interviewed by trained staff in the Hungarian National Institute of Oncology. Due to a Hungarian law, contacts with patients with malignant neoplasm are limited to physicians in charge and other health personnel are not allowed to contact with such patients. Interviews were conducted after obtaining permission from the physicians in charge. It was therefore not possible for the interviewers to be blinded as to whether the subject they interviewed was a case or a control.

Questionnaires used in the interviews included information concerning socioeconomic status, family history, past history and life style habits, such as smoking, alcohol intake, dietary habits and elimination patterns. Socioeconomic status was evaluated according to the Graffar index (Graffar, 1956). This index has five categories from I (the lowest) to V (the highest), in which education, occupation, household and neighborhood characteristics have been taken into consideration. This index was used mainly for comparative purposes, as we had already used it in the previous case-control study conducted in Chile. Family and past histories focused on infectious disease, GS and cancers in the subject herself and first degree relatives. Dietary habits meant long-term dietary intake history of more than 30 food items. In particular, consumption of Hungarian hot pepper, Hungarian sweet pepper, fresh fruits and vegetables were asked about in detail, since these have been reported to confer risk (Serra, 2002) and be protective (Moerman, 1995) as regards GBC, respectively. Intestinal evacuation was classified as daily evacuation, diarrhea or constipation.

Statistical analyses of questionnaires were done using SPSS 14.0J for Windows. Frequencies were obtained for all variables in the questionnaires, and a reference was established for each variable. The variables of most interest were consumptions of Hungarian hot pepper, Hungarian sweet pepper, vegetables and fresh fruits. Cross-tabulations for each potential risk factor versus case-control status were done and odds ratios (OR) and 95% confidence intervals (95%CI) were calculated. Next, variables were retained, if the 95%CI did not contain the value of 1, for multivariate logistic regression analysis. In logistic regression analysis, the dependent variable was a binominal variable representing the presence or absence of GBC. A set of explanatory variables were chosen based on the significance of the calculated OR and in reference to epidemiological importance as risk factors of GBC.

Results

Table 1 shows the characteristics of the study subjects by age. Average ages of the case and control groups were 67.1±9.0 and 54.0±11.6 years, respectively, and there was a significant difference between the two groups ($p<0.001$). This significance evidently resulted from the difference in age distribution between the case group that skewed towards older ages and the control group which had younger subjects. There was a significant difference ($p < 0.001$) in percentages of those who were 70 years of age or older between the two groups. Medical histories of diseases for the controls were investigated to reveal no malignancies. In addition to GS that all controls had, we found two cases of gastric ulcer, a case of duodenal ulcer, four cases of hepatitis, three cases of ulcerative colitis, one case of pulmonary tuberculosis and a case of parasitosis.

The results of the analyses of relationships between the 12 variables examined and the occurrence of GBC are shown in Table 2. Because mean ages differed significantly between the case and control groups, age-adjusted ORs were calculated in addition to ORs (Table 2).

A shorter education period (≤10 years/≥16 years) was indicated to be a risk factor (age-adjusted OR (95%CI): 3.2 (1.2-8.7)). In addition, consumption of Hungarian

Table 1. Proportion of Study Subjects by Gender and Age

Variable	Cases	Controls	p
Gender			
Female	n=41(100%)	n=30 (100%)	
Age (years)			
<40	0 (0.0%)	3 (10.0%)	
40 - 49	0 (0.0%)	6 (20.0%)	
50 - 59	10 (24.4%)	13 (43.3%)	
60 - 69	15 (36.6%)	2 (6.7%)	< 0.001*
Average age	67.1 ±9.0 ys	54.0 ±11.6 ys	< 0.001#

* p value for the chi-square test; # p value for the t- test

Table 2. Risk Factors for Gallbladder Cancer in Hungary

Variable	Cases	Controls	OR#	95%CI	Age-adjusted OR#	95%CI
Education period (years)						
≥16	3	8	Ref		Ref	
11-15	15	16	2.5	0.5-11.2	3.1	0.8-11.5
≤10	23	5	12.3	2.3-63.4	3.2	1.2-8.70
Socioeconomic status*						
High	25	22	Ref		Ref	
Low	14	1	12.3	1.5-101.4	5.4	0.6-49.0
Duration of biliary colic (years)						
≤12	37	29	Ref		Ref	
≥12	4	1	3.1	0.3-29.6	6.7	0.6-76.9
Parity (pregnancies)						
≤5	36	25	Ref		Ref	
>5	3	2	1.0	0.2-6.7	0.8	0.1-6.80
Constipation						
No*	36	14	Ref		Ref	
Yes	1	3	0.1	0.0-1.4	0.6	0.0-8.00
Obesity (BMI)						
≤24.9	13	13	Ref		Ref	
25.0-29.9	9	11	0.8	0.3-2.4	1.5	0.4-5.00
≥30.0	19	13	61.0	0.3-3.6	0.8	0.3-1.80
Typhoid fever (self-reported)						
Negative	40	30	Ref		Ref	
Positive	1	0	1.0	0.9-1.0	-	-
Smoking						
No	30	19	Ref		Ref	
Yes	11	10	0.7	0.3-2.0	1.0	0.3-3.50
Intake of Hungarian sweet pepper						
No	3	7	Ref		Ref	
Yes	38	23	3.9	0.9-16.4	4.0	0.7-22.3
Intake of Hungarian hot pepper						
No	11	24	Ref		Ref	
Yes	30	6	10.9	3.5-33.8	8.4	2.3-30.4
Vegetables (per week)						
< 3 days	5	6	Ref		Ref	
3-7 days	36	24	1.8	0.5-6.6	3.8	0.8-18.7
Fresh fruits (per week)						
<2 days	4	4	Ref		Ref	
2-7 days	36	26	1.4	0.3-6.1	1.5	0.3-8.9

*Socioeconomic status: high (Graffar index IV~V), low (I~III) #OR:odds ratios; age-adjusted ORs: calculated using logistic regression analysis Diarrhea/normal /ex-smokers

hot pepper (yes/no) was found to be significantly higher in GBC cases than in controls (age-adjusted OR (95%CI): 8.4 (2.3-2.3-30.4)). When the frequency of Hungarian hot pepper consumption was categorized as none, 1-4 days/week and ≥5 days/week, the age-adjusted ORs of the latter two against the first were 12.8 (2.3-71.8) and 2.5 (1.1-5.5), respectively. Thus, no evident dose-response relationship was obtained. On the other hand, consumption of Hungarian sweet pepper for GBC cases was not higher than that for controls.

The age-adjusted OR for frequent consumption of fresh fruits (2-7 days per week / < 2 day/week) was 1.5 (95%CI: 0.3-8.9), not a statistically significant value. The frequent intake of vegetables (3-7 days per week / < 3 day per week) was not significantly protective according to the age-adjusted OR of 3.8 (95%CI: 0.8-18.7). In regard to other variables, there were no differences between the case and control groups.

Table 3. Odds ratios (ORs) and adjusted ORs of major variables of interest in logistic regression analysis

Risk factor	OR	Age-adjusted OR	Multi factor-adjusted OR
Hungarian hot pepper consumption			
(yes/no)	10.9	8.4	16.2
	(3.52-33.8)*	(2.34-30.4)	(2.09-126.2)
n	41 vs 30	41 vs 30	38 vs 23
Education period (years)			
(≤10 />10)	6.1	3.9	10.4
	(1.95-19.3)	(1.10-14.0)	(0.48-227.8)
n	41 vs 29	41 vs 29	38 vs 23
Fresh fruits (days per week)			
(2-7 /<2)	1.4	1.5	6.3
	(0.32-6.05)	(0.29-8.92)	(0.27-149.7)
n	40 vs 30	40 vs 30	38 vs 23
Vegetables (days per week)			
(3-7/<3)	1.8	3.8	16.9
	(0.49-6.57)	(0.75-18.7)	(0.96-297.5)
n	41 vs 30	41 vs 30	38 vs 23
Socioeconomic status[1]			
(low /high)	12.3	5.4	1.0
	(1.50-101.43)	(0.59-49.0)	(0.02-47.45)
n	39 vs 23	39 vs 23	38 vs 23
Smoking			
(yes[2]/ no)	0.7	1.0	1.6
	(0.26-2.04)	(0.30-3.51)	(0.18-13.78)
n	41 vs 30	41 vs 30	38 vs 23

*95%CI; n, cases vs controls; [1]high (Graffar index IV~V), low (I~III); [2]smokers+ex-smokers

Table 3 shows the results of multivariate logistic regression analysis. Variables employed in addition to age were Hungarian hot pepper consumption, education period, intakes of fresh fruits and vegetables, socioeconomic status and smoking. The only statistically significant variable was Hungarian hot pepper consumption (multi factor-adjusted OR (95%CI): 16.2 (2.1-126.2)), while no differences were found for low education, frequent consumption of fresh fruits and vegetables, low socioeconomic status or smoking.

Discussion

Hungary is reportedly ranked second worldwide among countries with high mortality rates from GBC (Chen, 1990), but in the present study it was difficult to obtain sufficient numbers of GBC cases due to the small population size (about 10 million people) of Hungary. Our efforts to enroll as many cases as possible for three years yielded 41 cases from affiliated hospitals for this analysis. It was also difficult to obtain the same number of controls as the cases by matching for the presence of GS, age and gender. Our controls numbered 30. The average age of the cases was significantly older than that of the controls. This limitation of the present study at the case and control selection stage was considered to make it difficult to compare the data between the two groups and draw conclusions from the comparisons. We must also have to consider the effects of information bias resulting from the design feature that did not allow interviewers to be blinded to case-control status, which would be difficult to mask. Systematic errors might occur in estimating the effects of risk factors of interest in the results of the present study.

Another issue we should discuss is our finding that there was no dose-response relationship between GBC and Hungarian hot pepper consumption, which finding decreased the strength of the association. One possible reason is the categorizing method of Hungarian hot pepper consumption based on frequency, not dose per week. The other is an interview bias resulting from the fact that interviewers in charge of the two study groups were not the same, although all interviewers were trained in the same manner beforehand.

The multi factor-adjusted OR for Hungarian hot pepper consumption was calculated to be larger than both of the crude and age-adjusted ones. In the calculation of the multi factor-adjusted OR, 3 and 7 missing data arouse in the case and control groups, respectively. Out of them, the number of missing data without Hungarian hot pepper consumption in controls was 5, which number was higher than one missing data in the GBC cases. A selection bias due to this uneven proportions of missing data without Hungarian hot pepper consumption of the two study groups is considered to enhance in part the multi factor-adjusted OR.

Notwithstanding, it is noteworthy that the consumption of Hungarian hot pepper was identified as a risk factor for GBC when the age variable was adjusted in the multivariate logistic regression analysis. This finding is consistent with our previous observation of red chili pepper consumption being related to the occurrence of GBC in Chile (Serra, 2002).

In case-control studies, hot chili pepper consumption seemed to be a common risk factor in Chile (Serra, 2002) and Hungary. It was not found to be a risk factor in Japan (Yamamoto, 2003), however. We should pay attention to the link between GBC and hot chili pepper consumption regardless of the inconsistency of this association.

As to the possibility of hot chili peppers being a risk factor for cancer, we conducted a survey of the literature. Notani and Jayant (1987) used a case-control study design to assess the role of dietary factors including hot chili peppers in cancers of the oral cavity, pharynx, esophagus and larynx. The use of hot chili peppers doubled or tripled cancer risk at all sites. Lopez-Carrillo et al. (1994) reported the risk of gastric cancer for those who consumed chili peppers to be 5.49-fold greater than that of those not consuming peppers.

To our knowledge, Bornstein (1970) was the first to suggest consuming strong seasonings with various chilies among Mexican people in the southwestern US to be related to GBC. Serra et al. (2002) confirmed the role of red chili pepper consumption in association with GBC in Chile.

There has been controversy concerning the carcinogenicity of chili peppers, particularly that of capsaicin, a primary pungent substance in chili peppers (Surh, 2002). Surh and Lee (1955) noted that the activities of capsaicin and chili extracts constitute sort of “a double-edged sword”; that is, they are carcinogenic but also chemoprotective against some chemical carcinogens and mutagens. Identification of carcinogens in chili peppers, specifically whether or not capsaicin, mycotoxins or unknown chemicals are carcinogenic, is another major objective for future research.

Although there is major concern regarding the association between hot chili peppers and GBC, we should first optimize a research protocol by enrolling an adequate number of cases and matched controls in Hungary. We give high priority to further epidemiological studies aimed at confirming the strength and consistency of the association discussed above, and then to laboratory studies for identifying the actual carcinogenic substances in hot chili pepper.

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