Indian J Med Res 154, November 2021, pp 699-706
DOI: 10.4103/ijmr.IJMR_201_19

Quick Response Code:

Risk factors for gallbladder cancer development in northern India: A gallstones-matched, case–control study

Kumudesh Mishra[1], Anu Behari[1], Pooja Shukla[1], Yasuo Tsuchiya[2], Kazuo Endoh[3], Takao Asai[4], Toshikazu Ikoma[5], Kazutoshi Nakamura[2] & Vinay Kumar Kapoor[1]

[1]Department of Surgical Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, [2]Division of Preventive Medicine, Niigata University Graduate School of Medical & Dental Sciences, Departments of [3]Health & Nutrition & [4]Clinical Engineering & Medical Technology, Niigata University of Health & Welfare, Niigata & [5]Department of Medical Technology & Clinical Engineering, Faculty of Health & Medical Technology, Hokuriku University, Kanazawa, Japan

Received February 3, 2019

***Background & objectives*: A high incidence of gallbladder cancer (GBC) is observed in northern India. This study was aimed to identify the factors involved in developing GBC in this region.**

***Methods*: A gallstones-matched, case–control study was conducted in northern India. Ninety nine patients with GBC and gallstones (33 men and 66 women, mean age of 51.4 yr) comprised the case group, while 99 patients with cholelithiasis (40 men and 59 women, mean age of 45.7 yr) comprised the control group. All participants were interviewed to complete 183 questionnaire items that included 105 food items. Potential risk factors were identified using a multivariate analysis adjusted for age and sex. Significant risk factors were identified using a stepwise logistic-regression analysis.**

***Results*: Age (≥50 yr), education (illiterate), socioeconomic status (≤below poverty line), bowel habits (≤once a day), hypertension history, hypotensive drug use, non-vegetarian diet, use of firewood for cooking, tap water drinking, hand pump water drinking and high consumption of coffee and sweets were identified as the potential risk factors. In women, factors included menarche (<13 yr), number of pregnancies (≥3 pregnancies) and parity (≥3 babies). Of these factors, age, education, bowel habits, tap water drinking and multiple pregnancy and/or multiparity were identified as significant risk factors, whereas a high consumption of coffee and sweets or hypotensive drug use and/or hypertension history were protective factors.**

***Interpretation & conclusions*: Poor bowel habits and drinking unsafe water appear to be the main risk factors for developing GBC. These are, however, modifiable factors which are capable of decreasing the risk of GBC in the north Indian population.**

Key words Gallbladder cancer - India - multivariate analysis - questionnaires - risk factor - stepwise procedure

Gallbladder cancer (GBC) is ranked 25th among all cancers, about 1,15,949 GBC cases worldwide with 74887 (females) and 41062 (males) showed a remarkable gender and geographic variation[1].

The incidence of GBC in Delhi, north India (4.1/100,000 men, 9.5/100,000 women), has been reported to be approximately 4-6 times higher compared with that in Bengaluru, South India (1.4/100,000 men, 1.7/100,000 women)[2]. Recent projections estimate that by 2025, the number of patients with GBC in India will reach approximately 9.8 and 11.2 per cent among men and women, respectively, across all patients worldwide[1]. Furthermore, two predictions regarding the future incidence of global GBC have been put forth. First, that the incidence of GBC in Chile will soon decrease significantly because of countermeasures implemented against typhoid fever between 1977 to 1990. Second, if no specific intervention is implemented to ameliorate GBC risk factors in India, it will replace Chile as the country with the highest GBC incidence and mortality[3]. Therefore, all possible measures should be urgently undertaken to reduce the incidence of GBC in India, especially in the northern region.

In the 1970s, Niigata Prefecture had the highest incidence of GBC for both sexes in Japan[4]. Researchers hypothesized a multifactorial cause, including the presence of gallstones or cholecystitis in addition to region-specific environmental factors. The results of epidemiological studies provided compelling evidence that conducting risk management, after identifying the modifiable risk factors, reduced GBC incidence and mortality in Niigata Prefecture[4]. Thus, the same methods may be applied in other countries exhibiting a high incidence of GBC.

The Indian Council of Medical Research (ICMR) identified several risk factors for GBC including ethnicity, gender, age, gallstones, chronic inflammation, genetic factors, gallbladder polyps and lifestyle[5]. However, specific risk factors for GBC in the northern region of India have not yet been identified. The ICMR has provided information on obesity control and diabetes, adopting a healthy diet rich in fruits and vegetables and exercising regularly as preventive measures for the development of GBC.

GBC is considered to be a multifactorial disorder. The presence of gallstones is thought to be the most important risk factor. Only 1-3 per cent of cholelithiasis patients develop GBC[6], and the frequency of Indian GBC patients with gallstones is reportedly 70-90 per cent[7]. This suggests that unknown factors in addition to gallstones are involved in GBC development. To identify additional risk factors in the north India population, a gallstones-matched, case–control study was conducted in GBC patients with gallstones and cholelithiasis patients.

Material & Methods

Study design: A 1:1 pair matched (GBC with gallstone and cholelithiasis patients), case–control study was conducted at the department of Surgical Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, India, from April 2016 to May 2017. In April 2016, the first patient with GBC and gallstones, diagnosed by histopathological examination, was enrolled. This patient enabled us to evaluate the validity of our questionnaire. By the end of May 2017, a total of 99 GBC patients with gallstones (38 men and 61 women) were enrolled. The 100 patients (44 men and 56 women) in the control group were selected randomly from patients who had a diagnosis of cholelithiasis by abdominal sonography during the same time period. However, of the 100 patients with cholelithiasis, one was excluded from the study because she was 14 yr old. A written informed consent was obtained from all patients to participate in this study before surgical intervention. As shown in Supplementary Table, patients were interviewed at the department of Surgical Gastroenterology, SGPGIMS using a questionnaire consisting of 183 items. This survey contained questions about the frequency of consumption of 105 food items including meat and fish (n=23), vegetables (n=18) and fruit (n=12). The participants were asked to indicate their average intake of each food during the year before receiving their definitive diagnoses. The frequency of food consumption was categorized into the following six groups: (5) daily, (4) more than twice per week, (3) weekly, (2) monthly, (1) rarely and (0) none. Consumption of a particular food was considered high if the participants were in categories three to five.

Sample size and statistical analysis: The sample size (99 pairs) was calculated based on Schlesselman's formula[8] and previously reported odds ratios (ORs) in north Indians (gallstones *vs*. GBC with gallstones, ORs = 2.1-3.8)[9]. The conditions were as follows: assumed OR = 2 or 3, proportion of exposure in controls = 50 per cent, power = 80 per cent, number of controls per case = 1 and significance level = 5 per cent.

Significant differences were calculated using a Chi-square test. When significant differences were observed in a univariate analysis, only then a

multivariate logistic regression analysis adjusted for age and sex was applied to re-evaluate the results. To detect significant risk or protective factors for GBC, Spearman's correlation coefficient was used along with a stepwise logistic regression analysis. ORs and 95 per cent confidence intervals (CIs) were calculated using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). A two-tailed $P<0.05$ was considered statistically significant. All procedures used in this study were approved by the Ethical Committee of the SGPGIMS, Lucknow, India and Hokuriku University, Ishikawa, Japan.

Results

The mean age among women (50.8 yr, range 30-75 yr) and both sexes (51.4 yr, range 30-75 yr) in the case group were significantly higher than that of women (44.2 yr, range 21-74 yr) and both sexes (45.7 yr, range 21-74 yr) in the control group, respectively. No significant difference in the mean age was found between the male patients (52.7 yr, range 30-71 yr) and the control group (47.9 yr, range 23-73 yr). In this study potential risk factors for GBC using a multivariate logistic regression analysis adjusted for age and sex were identified.

Table I shows the potential risk factors for GBC development in Northern India. Factors that were associated with increased GBC risk included age (>50 yr), low education level, poor socioeconomic status, jaundice, fever, weight loss, poor bowel habits, non-vegetarian diet, use of firewood for cooking, drinking tap water, drinking hand pump water, early menarche, multiple pregnancies and parity. Conversely, medical history of hypertension, use of hypotensive drugs and high consumption of coffee and sweets were associated with a significant reduction in GBC risk.

Table II shows the significant risk and protective factors for GBC in the population of northern India. Of the 18 potential factors identified in this study, jaundice, fever and weight loss were excluded from the analysis because these are well known variables indicating a pathological condition of GBC, as opposed to risk factors. In addition, three reproductive factors applied only to females, so these were calculated separately from the other 12 factors. The Spearman's rank correlation test was applied to 12 potential factors including age, education, economic status, bowel habits, hypertension history, use of hypotensive drugs, non-vegetarian diet, use of firewood for cooking, drinking tap water, drinking hand pump water, coffee and sweets consumption. The strength of the resulting correlation coefficients was assessed by the method reported by Evans[10]. The factors showing a strong or very strong positive correlation (r=0.60 and higher) were as follows: hypertension history *vs*. use of hypotensive drugs (r=0.88) and number of pregnancies *vs*. parity number (r=0.97). To avoid including both factors in the analysis, hypotensive drug use and number of pregnancies were selected as representative factors. To identify significant risk factors, ORs and 95 per cent CIs were calculated using a stepwise logistic regression analysis. Three reproductive factors were evaluated using the same method. The results indicated that age (≥50 yr), low education level, poor bowel habits, drinking tap water and multiple pregnancies and/or multiparity were the risk factors for GBC. In contrast, hypotensive drug use, hypertension history and high consumption of coffee and sweets were identified as protective factors.

Discussion

In this pair matched (for gallstones), case–control study, advanced age, low education level, bowel movement once a day or less and drinking tap water were all associated with increased GBC risk. In women, multiple pregnancies and/or multiparity also represented an elevated risk of GBC. In contrast, high consumption of coffee and sweets and the use of hypotensive drugs and/or hypertension history were associated with reduced GBC risk.

Advanced age is a well known risk factor for not only GBC but also other cancers. Our findings indicate that the frequency of GBC cases in patients aged 50 yr and older (63/99, 63.4%) is significantly higher than that of controls (58/99, 58.6%). A recent study in India reported that the mean age of GBC patients at diagnosis is 60.1 yr for men and 57.2 yr for women, with a peak age of 70-74 yr in both sexes[11]. Other reports have found that the mean or median age of Indian GBC patients at diagnosis ranges from 50 to 55 yr[12] similar to the findings in our study (52.7 yr for men, 50.8 yr for women), which suggests that advanced age is a risk factor for GBC.

At least two studies conducted in India reported a significant positive association of illiteracy with GBC risk[13,14]. A study conducted in Gwalior, a central region of India, revealed that the highest incidence of GBC was found among illiterates and accounted for 51.3 per cent of the total patients (relative risk, 1.49; 95% CI, 1.31-1.70)[13]. Another hospital-based case–control study conducted in

Table I. Potential actors for gallbladder cancer development among North Indians

Factors assessed	Case	Control	Unadjusted analysis OR (95% CI)	Multivariate analysis[#] OR (95% CI)
Age (yr)				
<50	36	41	1 (ref)	1 (ref)
≥50	63	58	2.5 (1.4-4.4)[††]	2.6 (1.5-4.7)[***]
Education				
Literate	48	72	1 (ref)	1 (ref)
Illiterate	51	27	2.8 (1.5-5.2)[†††]	2.8 (1.3-5.7)[**]
Economic status				
≥ Low	61	85	1 (ref)	1 (ref)
≤ BPL	38	14	3.8 (1.9-7.8)[†††]	3.3 (1.6-6.8)[**]
Jaundice symptom				
No	78	94	1 (ref)	1 (ref)
Yes	21	5	5.1 (1.8-13.9)[††]	6.6 (2.3-19.1)[***]
Fever symptom				
No	85	96	1 (ref)	1 (ref)
Yes	14	3	5.3 (1.5-19.0)[††]	4.4 (1.2-16.2)[*]
Weight loss				
No	49	94	1 (ref)	1 (ref)
Yes	50	5	19.2 (8.4-44.0)[†††]	19.6 (7.1-54.3)[***]
Bowel habits				
>1/d	24	53	1 (ref)	1 (ref)
≤1/d	56	27	4.6 (2.3-9.0)[†††]	5.1 (2.5-10.2)[***]
Hypertension history				
No	96	80	1 (ref)	1 (ref)
Yes	3	19	0.1 (0.04-0.4)[†††]	0.1 (0.02-0.2)[***]
Taking hypotensive drugs				
No	96	77	1 (ref)	1 (ref)
Yes	3	22	0.1 (0.04-0.3)[†††]	0.1 (0.02-0.2)[***]
Non-vegetarian				
No	84	96	1 (ref)	1 (ref)
Yes	15	3	5.7 (1.6-20.1)[††]	4.8 (1.3-17.7)[**]
Using firewood for cooking				
No	21	39	1 (ref)	1 (ref)
Yes	78	60	2.4 (1.3-4.7)[††]	2.0 (1.1-4.0)[**]
Drinking tap water				
No	10	25	1 (ref)	1 (ref)
Yes	89	74	3.0 (1.3-6.9)[††]	4.1 (1.8-9.6)[***]
Drinking hand pump water				
No	24	43	1 (ref)	1 (ref)
Yes	75	56	2.4 (1.3-4.5)[††]	2.2 (1.2-4.2)[**]
Menarche age (yr)				
≥13	23	35	1 (ref)	1 (ref)

Contd...

Factors assessed	Case	Control	Unadjusted analysis OR (95% CI)	Multivariate analysis[#] OR (95% CI)
<13	42	23	2.8 (1.3-6.0)[†]	3.0 (1.4-6.5)[**]
Number of pregnancy				
0-2 pregnancies	9	26	1 (ref)	1 (ref)
≥3 pregnancies	56	33	4.9 (2.0-11.8)[†††]	2.3 (1.03-5.1)[*]
Number of parity				
0-2 babies	10	27	1 (ref)	1 (ref)
≥3 babies	55	32	4.6 (2.0-11.0)[†††]	3.3 (1.3-8.1)[*]
Coffee/consumption				
0-2	91	71	1 (ref)	1 (ref)
3-5	8	28	0.2 (0.1-0.5)[†††]	0.3 (0.1-0.6)[**]
Sweets/consumption				
0-2	44	28	1 (ref)	1 (ref)
3-5	55	71	0.5 (0.3-0.9)[†]	0.5 (0.3-0.9)[**]

P [†]<0.05, [††]<0.01, [†††]<0.001 for unadjusted analysis. *P* [*]<0.05, [**]<0.01, [***]<0.001 for multivariate analysis. [#]Adjusted for age and sex. Food intake frequency was categorized into the following six groups: (5) daily, (4) more than twice per week, (3) weekly, (2) monthly, (1) rarely and (0) none. High consumption of food was indicated if participants were in categories 3 to 5. OR, odds ratio; CI, confidence interval; BPL, below poverty line

Table II. Significant risk and protective factors for gallbladder cancer development in North Indians

Factor	OR	95 per cent CI	*P*
Age (≥50yr)	1.06	1.03-1.09	0.001
Low education level	3.19	1.54-6.61	0.002
Poor bowel habits	3.15	1.39-7.14	0.006
Tap water drinking	4.74	1.89-11.9	0.001
Multiple pregnancy and/or multiple parity	5.58	2.07-15.0	0.001
High coffee consumption	0.33	0.13-0.88	0.027
High sweets consumption	0.48	0.24-0.98	0.043
Taking hypotensive drugs and/or hypertension history	0.01	0.02-0.30	<0.001

OR and 95 per cent CI were calculated using a stepwise logistic regression analysis

Delhi reported that illiteracy was significantly associated with GBC (OR=8.00, *P*<0.001)[14]. In the present study, the frequency of cases involving illiteracy (51/99, 51.5%) was significantly higher than that of the controls (27/99, 27.3%) similar to the study from Gwalior. The correlation between education level and socioeconomic status (r=0.42, *P*<0.001) was moderately positive, suggesting that education level may be affected by the socioeconomic status.

In previous studies, infrequent bowel movement or constipation has been shown to be associated with an increased risk of GBC in Chile and Japan[15,16]. To our knowledge, however, no study has so for reported an association between the two in the Indian population. Therefore, this is the first report to show an association between bowel habits and GBC risk in India. Several factors for constipation have been identified including lack of dietary fibre, physical activity, water intake and poor bowel habits[17]. Further studies are, however, needed to clarify which variable is associated with infrequent bowel movements in the population of northern India.

An important finding in this study was that drinking tap water was associated with increased risk of GBC. Tap water is usually safe to drink, but illness may occur from ingesting poorly-chlorinated tap water containing microorganisms. We also performed a water-quality survey for several water sources including the Ganges

River, public tap water, public well water and the well water used at home by GBC patients living in or near Varanasi in north India. The bacterial concentration in the tap water in Varanasi was lower than the detectable limit, but the well water at the homes of three of four patients was contaminated with bacteria. In Varanasi, chlorine was detected at approximately 3 mg/l indicating a relatively safe tap water supply[18]. To date, an association between *Salmonella typhi* and *Helicobacter pylori* infections and GBC risk has been reported[19] although the results are inconsistent, these bacteria were detected in either gallbladder tissue or bile in GBC patients. Our recent study has demonstrated that no significant difference in plasma *H. pylori* antibody titre was found between patients with either GBC or cholelithiasis, thus suggesting that there is no positive association between the two in northern India[20]. In contrast, plasma *S. typhi* antibody titres in patients with GBC were significantly higher compared with cholelithiasis patients (unpublished data). A positive association between *S. typhi* and *H. pylori* infections and GBC risk has been reported in the Indian population[21]. The *S. typhi* infection rate in India was reported to be high, mainly in young children[22]. These results suggest that this bacterium may be involved in the development of GBC in the Indian population.

In women, reproductive factors including multiparity are associated with increased GBC risk, even in Indians[13,23,24]. As shown in Table I, women who had more than three pregnancies had an approximately 2.3 times higher GBC risk compared with women who had fewer pregnancies (P=0.010). Compared with women who had given birth to fewer than three children, women with three or more children showed a 3.3 times higher GBC risk (P=0.010). Parity or parity number has been associated with increased GBC risk[25-27], and parity is a risk factor for gallstone formation[25]. Blood concentrations of estrogen and progesterone are involved in gallstone formation and hence are considered as risk factors. Estrogen receptors have been reported to affect gallstone formation and subsequent GBC development[26]. Also the incidence of GBC in women is reportedly higher than that observed in men[27]. The incidence of constipation in patients aged 50 yr and older is also higher in women than in men[28]. Therefore, female hormones may contribute to the development of GBC.

A number of factors reduce the risk of GBC including coffee and sweets in the present study. Previous studies have examined the association between coffee consumption and GBC risk[29-31]. Although findings have been inconsistent, at least two studies conducted in India and Japan reported a protective effect of coffee consumption with GBC risk respectively[9,30]. However, Tyagi *et al*[31] reported that no significant difference was found between GBC patients and healthy relatives of these patients, and coffee consumption increased GBC risk. Since the correlation between socioeconomic status and coffee consumption was shown to be significant, though weakly positive (r=0.25, P<0.001), the patients who consume coffee may be of relatively high socioeconomic status. To our knowledge, no study has reported a significant association between sweets consumption and GBC risk in Indians, however, the reason for this could not be determined. In the present study, sweets consumption correlated only weakly with hypertension history (r=0.23, P=0.012). Since the consumption of sweets was higher among women as compared to men, another female-related factor may be involved in the reduced risk. In addition, the frequency of cases involving hypotensive drug use was significantly lower than that in the controls. Although we could not identify the reason for the protective effect of a hypotensive drug on GBC development, methyldopa has been reported to be toxic to C-1300 neuroblastoma cells *in vivo*[32].

The present study was not without its limitations. First, we were unable to include healthy subjects because of technical difficulty. We conducted a 1:1 (gallstones-matched) case–control study. Our findings identified the risk factors that may explain how GBC develops through a multifactorial process beginning with the presence of gallstones. However, a study intended for patients with GBC or cholelithiasis including healthy subjects will provide more clear evidence to support our findings. A second limitation is that we used a semi-quantitative questionnaire to collect dietary information from patients. Although this method was validated previously, the results may be subject some bias caused by the surveyor's skills. Third, we divided the patients into two categories based on bowel movement frequency: once a day or less, or twice a day or more[33]. Our findings indicated that frequency of bowel movements in the case group (70%) was significantly lower than that in the control group (34%). However, more detailed information on frequency of bowel movements may be needed to clarify the association between constipation or infrequent bowel movements and GBC development. Normally, the frequency of normal bowel movement

for adults ranges from three times a week to three times a day.

In conclusion, our results show that advanced age, low education level, bowel movement once a day or less, drinking tap water and multiple pregnancy and/or multiparity are associated with an increased risk of GBC. In contrast, high consumption of coffee and sweets and hypotensive drug use and/or hypertension history were inversely associated with GBC risk. Among these factors, poor bowel habits and drinking tap water that is poorly chlorinated are considered to be the modifiable risk factors for preventing GBC. Although our findings require further confirmation, GBC incidence in the population of northern India may be reduced by improved bowel habits and availability of clean drinking water.

***Acknowledgment*:** Authors acknowledge Edanz Group *(https://en-author-services.edanzgroup.com/)* for editing a draft of this manuscript.

***Financial support & sponsorship*:** This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI (grant number 16K09080).

***Conflicts of Interest*:** None.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; *71* : 209-49.
2. Indian Council of Medical Research. National Centre for Disease Informatics & Research, National Cancer Registry Programme, Report of National cancer registry Program 2020. *Comparison of cancer incidence and patterns of all Population Based Cancer Registries* Available from: *https://ncdirindia.org/All_Reports/Report_2020/resources/Chapter5ComparisonofcancerincidenceandpatternsofallPopulationBasedCancerRegistries.pdf*, accessed on December 20, 2020.
3. Ferreccio C. *Salmonella typhi* and gallbladder cancer. In: Khan AA, editor. *Bacteria and cancer.* New York: Springer; 2012. p. 117-37.
4. Yamamoto M. Epidemiological studies on the distribution and determinants of biliary tract cancer. *Environ Health Prev Med* 2003; *7* : 223-9.
5. Indian Council of Medical Research. Review of Published Data. In: *Consensus Document for Management of Gallbladder Cancer*; 2014. p. 14-36.
6. Murthy NS, Rajaram D, Gautham M, Shivraj N, Pruthvish S, Georg PS, *et al*. Trends in incidence of gallbladder cancer – Indian scenario. *Gastrointest Cancer* 2011; *2011* : 1-9.
7. Gupta S, Kori C, Kumar V, Misra S, Akhtar N. Epidemiological study of gallbladder cancer patients from north Indian Gangetic planes – A high-volume Centre's experience. *J Gastrointest Cancer* 2016; *47* : 27-35.
8. Schlesselman JJ. *Case-control studies, 6.6 sample size and power for pair-matched studies*. Oxford, NY: Oxford University Press; 1982. p. 160-2.
9. Jain K, Sreenivas V, Velpandian T, Kapil U, Garg PK. Risk factors for gallbladder cancer: A case-control study. *Int J Cancer* 2013; *132* : 1660-6.
10. Evans JD. *Straightforward statistics for the behavioral sciences*. Pacific Grove: Brooks/Cole Publishing; 1996.
11. Malhotra RK, Manoharan N, Shukla NK, Rath GK. Gallbladder cancer incidence in Delhi urban: A 25-year trend analysis. *Indian J Cancer* 2017; *54* : 673-7.
12. Dutta U, Bush N, Kalsi D, Popli P, Kapoor VK. Epidemiology of gallbladder cancer in India. *Chin Clin Oncol* 2019; *8* : 33.
13. Barbhuiya MA, Singh TD, Poojary SS, Gupta S, Kakkar M, Shrivastav BR, *et al.* Gallbladder cancer incidence in Gwalior district of India: Five-year trend based on the registry of a regional cancer center. *Indian J Cancer* 2015; *52* : 430-7.
14. Panda D, Sharma A, Shukla NK, Jaiswal R, Dwivedi S, Raina V, *et al.* Gall bladder cancer and the role of dietary and lifestyle factors: A case-control study in a North Indian population. *Eur J Cancer Prev* 2013; *22* : 431-7.
15. Endoh K, Nakadaira H, Yamazaki O, Yamamoto M, Tajima K, Serra I, *et al.* Risk factors for gallbladder cancer in Chilean females. *Nihon Koshu Eisei Zasshi* 1997; *44* : 113-22.
16. Yagyu K, Lin Y, Obata Y, Kikuchi S, Ishibashi T, Kurosawa M, *et al.* Bowel movement frequency, medical history and the risk of gallbladder cancer death: A cohort study in Japan. *Cancer Sci* 2004; *95* : 674-8.
17. Dinning PG, Zarate N, Hunt LM, Fuentealba SE, Mohammed SD, Szczesniak MM, *et al.* Pancolonic spatiotemporal mapping reveals regional deficiencies in, and disorganization of colonic propagating pressure waves in severe constipation. *Neurogastroenterol Motil* 2010; *22* : e340-9.
18. Ikoma T, Asai T, Endoh K, Tsuchiya Y. Drinking water survey to elucidate risk factors in a region with high incidence of gallbladder cancer in India. *JAMT* 2018; *67* : 760-5.
19. Kanthan R, Senger JL, Ahmed S, Kanthan SC. Gallbladder cancer in the 21st century. *J Oncol* 2015; *2015* : 967472.
20. Tsuchiya Y, Mishra K, Kapoor VK, Vishwakarma R, Behari A, Ikoma T, *et al.* Plasma *Helicobacter pylori* antibody titers and *Helicobacter pylori* infection positivity rates in patients with gallbladder cancer or cholelithiasis: A hospital-based case-control study. *Asian Pac J Cancer Prev* 2018; *19* : 1911-5.
21. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: Geographical distribution and risk factors. *Int J Cancer* 2006; *118* : 1591-602.
22. Balaji V, Kapil A, Shastri J, Pragasam AK, Gole G, Choudhari S, *et al.* Longitudinal typhoid fever trends in India from 2000 to 2015. *Am J Trop Med Hyg* 2018; *99* : 34-40.
23. Kumar JR, Tewari M, Rai A, Sinha R, Mohapatra SC, Shukla HS. An objective assessment of demography of gallbladder cancer. *J Surg Oncol* 2006; *93* : 610-4.

24. Khan I, Panda N, Banerjee M, Das R. Epidemiological factors in gall bladder cancer in eastern India – A single centre study. *Indian J Surg Oncol* 2013; *4* : 67-72.

25. de Bari O, Wang TY, Liu M, Paik CN, Portincasa P, Wang DQ. Cholesterol cholelithiasis in pregnant women: Pathogenesis, prevention and treatment. *Ann Hepatol* 2014; *13* : 728-45.

26. Chen A, Huminer D. The role of estrogen receptors in the development of gallstones and gallbladder cancer. *Med Hypotheses* 1991; *36* : 259-60.

27. Unisa S, Jagannath P, Dhir V, Khandelwal C, Sarangi L, Roy TK. Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. *HPB* (*Oxford*) 2011; *13* : 117-25.

28. Choung RS, Locke GR 3rd, Schleck CD, Zinsmeister AR, Talley NJ. Cumulative incidence of chronic constipation: A population-based study 1988-2003. *Aliment Pharmacol Ther* 2007; *26* : 1521-8.

29. Jain K, Sreenivas V, Velpandian T, Kapil V, Garg PK, Risk factors for gallbladder cancer: a case-control study. *Int J Cancer* 2013; *132* : 1660-6.

30. Kato K, Akai S, Tominaga S, Kato I. A case-control study of biliary tract cancer in Niigata Prefecture, Japan. *Jpn J Cancer Res* 1989; *80* : 932-8.

31. Tyagi BB, Manoharan N, Raina V. Risk factors for gallbladder cancer: A population based case-control study in Delhi. *Indian J Med Paediatr Oncol* 2008; *29* : 16-26.

32. Chelmicka Schorr E, Sportiello MG, Otten GR, Arnason BG. Toxicity of methyldopa (Aldomet) to mouse neuroblastoma cells *in vivo*. *Cancer Res* 1983; *43* : 3504-6.

33. Serra I, Yamamoto M, Calvo A, Cavada G, Báez S, Endoh K, *et al*. Association of chili pepper consumption, low socioeconomic status and longstanding gallstones with gallbladder cancer in a Chilean population. *Int J Cancer* 2002; *102* : 407-11.

For correspondence: Dr Yasuo Tsuchiya, Division of Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
e-mail: troof441@gmail.com

Supplementary Table. Questionnaire items collected for analysis
1. Patient characteristics
Age, sex, date and place of birth, resident place and year, blood type, civil status, religion, race
2. Disease history
Gastric ulcer, duodenal ulcer, infectious hepatitis, typhoid fever, intestinal parasites, ulcerative colitis, pulmonary tuberculosis, cholelithiasis, obesity, height, weight
3. Antecedents of biliary symptom
Alimentary intolerance, biliary colic, jaundice, fever, weight loss, oral cholecystogram and ecography data, date of medical diagnosis of gallstones, others
4. Antecedents of other diseases
Cancer, diabetes mellitus, cardiovascular disease, arterial hypertension, dyslipidemia, previous operations
5. Medicines used
Estrogen, diuretics, hypotensive drugs, methyl dopamine, insulin, oral hypoglycemic agents, others
6. Family disease history
Cancer, diabetes mellitus, cholelithiasis, liver diseases, cardiovascular disease
7. Reproductive factors
Menarche, menopause, pregnancies, hormonal treatment, anticonceptivos
8. Intestinal evacuation
9. Occupational history
Agriculture, mining, industry, construction, office worker, house wife, others
10. Socioeconomic status
11. Classification of occupations (ISCO-08)
12. Schooling
13. Alcohol consumption and cigarette smoking
14. Alimentary habit
Breakfast, lunch, tea time, dinner meals per day (all the time and last 15 yr)
15. Vegetarianism or non-vegetarianism
16. Foods intake frequency
Meats and fish (cattle, viscera, lamb, chicken, pig, fish), chili (red, yellow), eggs, beans, tubers, vegetables (tomato, cabbage, lettuce, celery, fluke, carrot, Swiss chard, beetroot, cauliflower, sheaths, broccoli, onion bean, pea, radish, cucumber, small pepper, others), fruits (papaya, banana, orange, tangerine, lemon, grapefruit, mango, peach, watermelon, pear, tune, grape), cereals (rice, wheat, corn, quinoa, semolina, noodle), bread, greases, sugars, beverages, condiments, infusions, dairy
17. Water for drinking and cooking
Tap water, well water, bottled mineral water, others
18. Type of cooking
Firewood, charcoal, dried manure, liquid gas, electricity
ISCO, International Standard Classification of Occupations