RESEARCH ARTICLE

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Plasma *Helicobacter pylori* Antibody Titers and *Helicobacter pylori* Infection Positivity Rates in Patients with Gallbladder Cancer or Cholelithiasis: a Hospital-Based Case-Control Study

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Abstract

Objective: Gallbladder cancer is the commonest gastrointestinal cancer in northern Indian women. Some studies have examined the association between *Helicobacter pylori* infection and gallbladder cancer risk, but findings have been inconsistent. We aimed to examine the association between *H. pylori* infection and gallbladder cancer in Indian people. **Materials and Methods:** We conducted a hospital-based case-control study including 100 gallbladder cancer patients with gallstones who were 32 to 79 years old (cases; 72 women and 28 men), and 100 cholelithiasis patients aged 14 to 75 years (controls; 65 women and 35 men). All patients had a diagnosis of gallbladder cancer or cholelithiasis at the Sanjay Gandhi Post Graduate Institute of Medical Sciences in Lucknow having a high gallbladder cancer incidence in northern India, from May 2014 through July 2017. Plasma samples were collected from all patients before surgical treatment. Plasma *H. pylori* antibody titer was measured by the latex agglutination method and an autoanalyzer. *H. pylori* infection was defined as antibody titer ≥10 U/mL. Plasma antibody titers and *H. pylori* infection positivity rates were compared between cases and controls. **Results:** Mean plasma antibody titers (standard deviation, range) were 11.1 U/mL (11.6, 0–78) in cases and 13.6 U/mL (23.0, 1–164) in controls. *H. pylori* infection positivity rates were 41% and 42% in cases and controls, respectively. No significant differences in antibody titers or *H. pylori* infection positivity rates were found between cases and controls. **Conclusions:** We found no evidence of *H. pylori* infection as an important risk factor for gallbladder cancer in Indian people.

Keywords: Autoanalyzer- gallbladder cancer- *Helicobacter pylori*- latex agglutination assay

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Introduction

According to recent global cancer statistics, gallbladder cancer ranks 20th among all cancers, thus it is a relatively uncommon cancer (International Agency for Research on Cancer, 2017). However, previous studies have reported a high incidence of gallbladder cancer in specific countries (Chile, Bolivia, and India) or confined areas (southern Chile and northern India) (Wistuba and Gazdar, 2004; Andia et al., 2008). Thus, the development of gallbladder cancer is suggested to be associated with environmental and genetic factors.

India has a high incidence of gallbladder cancer, especially in the northern states of Punjab, Uttar Pradesh, Bihar, West Bengal, and Assam (Kapoor and McMichael, 2003; Nandakumar et al., 2005). Some risk factors for the development of gallbladder cancer in Indian people have been identified (Khan et al., 2013; Mhatre et al., 2016). According to a guideline provided by the Indian Council of Medical Research (2014), ethnicity, sex, age, gallstones, chronic inflammation, genetic factors, gallbladder polyps, and lifestyle factors are risk factors for gallbladder cancer in Indian people. However, the onset mechanism for gallbladder cancer in Indian people has not been sufficiently explained by these disclosed factors alone. Although cholelithiasis or presence of gallstones is the strongest risk factor for gallbladder cancer (Randi et al., 2006; Ishiguro et al., 2008), less than 3% of patients with cholelithiasis develop gallbladder cancer (Hundal and Shaffer, 2014) and 69–100% of Indian patients with gallbladder cancer have gallstones (Khan et al., 2013; Indian Council of Medical Research, 2014). These data

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suggest not only gallstones but also other factors are associated with developing gallbladder cancer.

Some studies have reported an association between infections and gallbladder cancer risk (Kumar et al., 2006), but findings have been inconsistent because of differences in methodology and samples used. Some researchers reported that *Helicobacter pylori* (*H. pylori*) infection is associated with increased risk of gallbladder cancer (de Martel et al., 2009; Mishra et al., 2010), however, another study did not observe such association (Bohr et al., 2007). At least three studies have reported an association between *H. pylori* infection and gallbladder cancer in Indian people (Sharma et al., 2007; Mishra et al., 2011; Mishra et al., 2013). *H. pylori* DNA was detected in gallbladder tissue of gallbladder cancer patients, but *H. pylori* DNA detection rates did not differ between gallbladder cancer patients and cholelithiasis patients (Mishra et al., 2011).

A serological test to measure serum or plasma *H. pylori* antibody titer has been developed. This test is useful for population-based *H. pylori* screening and treatment program because it is non-invasiveness, convenient, and inexpensive (Inui et al., 2017). However, the test cannot differentiate between *H. pylori* infection in the gallbladder and infection in other organs, such as the stomach, liver, and biliary epithelium. Although the antibody titer is not specific for the gallbladder, Deeba et al., (2010) reported that the titer in cholelithiasis and cholecystitis patients is significantly higher than that in healthy subjects. According to this evidence, we hypothesized that serum or plasma *H. pylori* antibody titer in gallbladder cancer patients with gallstones would be higher than that in cholelithiasis patients because the presence of gallstones is a major risk factor for gallbladder cancer. To our knowledge, no study has examined the association between *H. pylori* infection and gallbladder cancer risk using serological tests. Therefore, we conducted a hospital-based case-control study to clarify the role of *H. pylori* infection in the development of gallbladder cancer in Indian people.

Materials and Methods

Subjects and plasma collection

We conducted a hospital-based case-control study to evaluate the association between *H. pylori* infection and gallbladder cancer risk in Indian patients from May 2014 through July 2017. A total of 100 gallbladder cancer patients with gallstones (cases) and 100 cholelithiasis patients (controls) participated in this study. All patients had a diagnosis of gallbladder cancer or cholelithiasis at Sanjay Gandhi Post Graduate Institute of Medical Sciences in Lucknow in northern India, a high incidence area, from May 2014 through July 2017. Informed consent was obtained from all participants for the use of plasma samples. This study was approved by the Ethical Committees of Sanjay Gandhi Post Graduate Institute of Medical Sciences and Niigata University of Health and Welfare (No. 17809-170517). Plasma samples were collected from all participants before surgical treatment.

Measurement of plasma H. pylori antibody titer

Plasma *H. pylori* antibody titer was measured using a commercial kit (LZ Eiken *H. pylori* antibody; Eiken Chemical Co. Ltd., Tochigi, Japan) and an autoanalyzer (BM 9130, JEOL Ltd., Tokyo, Japan). *H. pylori* infection was defined as plasma antibody titers ≥10 U/mL according to the kit's manual (Eiken Chemical Co., Ltd., 2015).

Statistical analysis

All statistical analyses were performed using Stata 14 software (StataCorp LLC, College Station, TX, USA). Differences in mean ages and antibody titers between cases and controls were analyzed by the chi-square test or Fisher's exact test. To compare differences in female proportions and *H. pylori* infection positivity rates of cases and controls, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using two-way contingency table analysis. P values <0.05 (two-tailed) were considered statistically significant.

Results

Table 1 shows participant characteristics. The proportion of female patients was 72% in cases and 65% in controls, which was not significantly different (P = 0.29). No significant difference was found in mean age between male cases (52.6 years; standard deviation [SD], 11.2; range, 33–73) and controls (47.1 years; SD, 12.5; range, 23–73), P = 0.07. However, significant differences in mean age were observed between female cases (53.2 years; SD, 11.3; range, 32–79) and controls (43.7 years; SD, 14.0; range, 14–75), P<0.001, or between both sexes (cases: 53.0 years; SD, 11.2; range, 32–79 vs. controls: 44.9 years; SD, 13.5; range, 14–75), P<0.001.

Table 2 shows plasma *H. pylori* antibody titers of cases and controls by sex and age. No significant difference was found in mean antibody titer (standard deviation) between cases and controls by sex; women: 10.7 U/mL (12.5) vs. 15.9 U/mL (27.8) (P = 0.15); men: 12.1 U/mL (9.2) vs. 9.4 U/mL (7.4) (P = 0.21). Additionally, no significant difference was observed in mean antibody titer between cases and controls by age, and antibody titer was not increased with older age.

Table 3 shows *H. pylori* infection positivity rates evaluated based on antibody titers. No significant differences in *H. pylori* infection positivity rates were observed between cases and controls grouped according to women only (P = 0.32), men only (P = 0.18), or both sexes (P = 0.89).

Table 1. Participant Characteristics

Characteristic	Cases	Controls	P value
No. of participants	100	100	
No. of female patients	72	65	0.29
Mean age			
Women (SD), years	53.2 (11.3)	43.7 (14.0)	<0.001
Men (SD), years	52.6 (11.2)	47.1 (12.5)	0.07
Both sexes (SD), years	53.0 (11.2)	44.9 (13.5)	<0.001

SD, standard deviation; P values show statistical significance between cases and controls

Table 2. Mean Plasma *Helicobacter pylori* Antibody Titers by Sex and Age

	Cases		Controls		P value
	No.	Mean (SD)	No.	Mean (SD)	
Women					
≤39	11	17.8 (17.4)	24	22.7 (41.6)	0.71
40 – 49	14	8.9 (6.6)	16	16.8 (18.8)	0.14
50 – 59	22	9.4 (8.9)	18	7.7 (8.6)	0.54
60≤	25	9.7 (14.7)	7	11.7 (10.6)	0.74
Total	72	10.7 (12.5)	65	15.9 (27.8)	0.15
Men					
≤39	6	11.7 (9.7)	9	6.2 (2.8)	0.13
40 – 49	2	21.5 (10.6)	9	8.9 (8.9)	0.11
50 – 59	10	10.6 (9.3)	12	10.0 (8.6)	0.88
60≤	10	11.9 (9.1)	5	14.8 (4.4)	0.52
Total	28	12.1 (9.2)	35	9.4 (7.4)	0.21
Both sexes					
≤39	17	15.6 (15.1)	33	18.2 (36.0)	0.78
40 – 49	16	10.4 (8.0)	25	14.0 (16.2)	0.43
50 – 59	32	9.8 (8.9)	30	8.6 (8.5)	0.60
60≤	35	10.3 (13.2)	12	13.0 (8.4)	0.52
Total	100	11.1 (11.6)	100	13.6 (23.0)	0.32

Plasma H. pylori antibody titers are expressed as U/mL; SD, standard deviation.

Discussion

This hospital-based case-control study demonstrated that plasma *H. pylori* antibody titer in cases is similar to that in controls. Furthermore, no significant difference was found in *H. pylori* infection positivity rates between cases and controls. Our data suggest that *H. pylori* infection does not play a significant role in the development of gallbladder cancer in Indian people.

Kanthan et al., (2015) proposed 4 broad categories of risk factors for gallbladder cancer: patient demographics, gallbladder abnormality, patient exposure, and *Salmonella* and *Helicobacter* infections. Previous studies have reported the association between *H. pylori* infection and gallbladder cancer risk, but findings have been inconsistent (de Martel et al., 2009; Mishra et al., 2010). Of the 3 studies including Indian patients with gallbladder cancer (Sharma et al., 2007; Mishra et al., 2011; Mishra et al., 2013), one case-control study including gallbladder cancer and cholelithiasis patients (Mishra et al., 2011) reported no association between the two patient groups, although the other 2 studies reported the detection of *H. pylori* DNA from gallbladder tissue of cancer patients. Conflicting evidence on the association between *H. pylori* infection and gallbladder cancer risk might have been caused by differences in samples and methods used in each study. Culture, serological examination, histological examination, and PCR analysis are common techniques used to evaluate *H. pylori* infection. Of these methods, PCR analysis is the most sensitive for detecting *H. pylori* in human biological samples. In fact, as summarized in a recent review (de Martel et al., 2009), *H. pylori* DNA was detected in 7 of 8 studies using PCR methods, but not in 2 studies using culture methods.

A serological test for evaluating the presence or absence of *H. pylori* infection based on *H. pylori* antibody titer has recently been developed and used. This method is non-invasiveness, convenient, inexpensive, and mainly used as a gastric cancer screening method (Inui et al., 2017). In the present study, to reveal whether *H. pylori* infection is a risk factor for gallbladder cancer development in Indian people, we conducted a hospital-based case-control study of gallbladder cancer patients with gallstones and cholelithiasis patients. We used patients with gallstones as our cases because gallstones are a major risk factor for gallbladder cancer. We found that mean plasma *H. pylori* antibody titer and infection positivity rate in cases were not higher than those in controls. Our findings support the results of Mishra et al., (2011) that *H. pylori* infection does not play a role in gallbladder cancer development in Indian people. They conducted a case-control study including 54 gallbladder cancer patients, 54 cholelithiasis patients. However, of the 54 gallbladder cancer patients, only 40 (74%) had gallstones. To our knowledge, our study is the first gallstone-matched case-control study to examine the association between *H. pylori* infection and risk of gallbladder cancer in Indian people. Thus, further studies are required to confirm our findings.

The following limitations must be considered when interpreting our data. First, serological test results are

Table 3. *Helicobacter pylori* Infection Positivity Rates in Cases and Controls

	Helicobacter pylori infection					
	No.	Negative (%)	Positive (%)	OR	95% CI	P value
Women						
Controls	65	37 (57)	28 (43)	1 (ref)		
Cases	72	47 (65)	25 (35)	0.7	0.3 – 1.4	0.32
Men						
Controls	35	21 (60)	14 (40)	1 (ref)		
Cases	28	12 (43)	16 (57)	2.0	0.8 – 5.4	0.18
Both sexes						
Controls	100	58 (58)	42 (42)	1 (ref)		
Cases	100	59 (59)	41 (41)	1.0	0.5 – 1.7	0.89

H. pylori infection was defined as antibody titer ≥10 U/mL; OR, odds ratio; CI, confidence interval.

not specific to the gallbladder, therefore, data may reflect *H. pylori* infection in the gallbladder and other organs. Because *H. pylori* has been detected in the stomach, bile, liver, heart, and biliary epithelium (Lacy and Rosemore, 2001; Griniatsos et al., 2009), our data may indicate *H. pylori* infection in these organs. Moreover, no significant difference in *H. pylori* infection was reported between women and men (Singh et al., 2002; Shukla and Tewari, 2012; Adlekha et al., 2013), however, a significant difference among age groups has been reported (Singh et al., 2002; Shukla and Tewari, 2012). In the present study, no significant differences in *H. pylori* infection in cases and controls were observed by sex and age. Thus, *H. pylori* infection in organs other than the gallbladder is unlikely to have made a substantial contribution to the results. If *H. pylori* infection is a risk factor for gallbladder cancer, then mean plasma *H. pylori* antibody titers or infection positivity rates should be higher in cases than those in controls.

Second, we defined *H. pylori* infection as plasma antibody titers ≥10 U/mL in accordance with the kit's instructions. The sensitivity and specificity of this method for detecting *H. pylori* are 90–95% (Burucoa et al., 2013). In addition, a recent study conducted in Japan demonstrated that this kit has a gray-zone between 5.6 U/mL and 10 U/mL, where infected patients are detected (Inui et al., 2017). Therefore, our results may not accurately reflect *H. pylori* infection status. However, no significant difference in *H. pylori* infection positivity rate was found between cases and controls, even if we defined positive infection as antibody titers ≥6 U/mL (cases, 65%; controls, 62%; P = 0.66).

Third, *Helicobacter* species other than *H. pylori* have been detected from gallbladder tissue using PCR (de Martel et al., 2009; Hamada et al., 2009; Pandey et al., 2010). This evidence suggests *Helicobacter* species may be associated with gallbladder cancer risk, however, we were unable to reveal a role of these species in gallbladder cancer development. Further work will clarify the association between these species and gallbladder cancer risk.

Fourth, we selected both gallbladder cancer and cholelithiasis patients as study subjects. In a previous case-control study including cholelithiasis patients and healthy subjects, mean serum *H. pylori* antibody titer in cholelithiasis patients was significantly higher than that in healthy subjects (Deeba et al., 2010). Moreover, an association between *H. pylori* infection and gallstone formation has been reported (Kawaguchi et al., 1996; Lee et al., 2010). If plasma or serum *H. pylori* antibody titer for gallbladder cancer patients, cholelithiasis patients, and healthy subjects are measured, the role of *H. pylori* infection in gallbladder cancer development might be more clearly defined.

In summary, our data showed *H. pylori* infection is not related to increased gallbladder cancer risk in Indian people. While our findings require further confirmation, they provide evidence that *H. pylori* infection is not an important risk factor for gallbladder cancer in Indian people.

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References

Adlekha S, Chadha T, Krishnan P, et al (2013). Prevalence of Helicobacter pylori infection among patients undergoing upper gastrointestinal endoscopy in a medical college hospital in kerala, India. *Ann Med Health Sci Res*, **3**, 559-63.

Andia ME, Hsing AW, Andreotti G, et al (2008). Geographic variation of gallbladder cancer mortality and risk factors in Chile: a population-based ecologic study. *Int J Cancer*, **123**, 1411-6.

Bohr UR, Kuester D, Meyer F, et al (2007). Low prevalence of Helicobacteraceae in gall-stone disease and gall-bladder carcinoma in the German population. *Clin Microbiol Infect*, **13**, 525-31.

Burucoa C, Delchier JC, Courillon-Mallet A, et al (2013). Comparative evaluation of 29 commercial Helicobacter pylori serological kits. *Helicobacter*, **18**, 169-79.

de Martel C, Plummer M, Parsonnet J, et al (2009). Helicobacter species in cancers of the gallbladder and extrahepatic biliary tract. *Br J Cancer*, **100**, 194-9.

Deeba J, Sanjay S, Abida M, et al (2010). Helicobacter prlori in gallbladder disease. Biomed Res, **21**, 437-40.

Eiken Chemical Co., Ltd (2015). Instructions for use of LZ *H. pylori* antibody. http://www.eiken.co.jp/en/product/others/pdf/380240A_LZ-Hp%20Reag(V-IY05).pdf. Accessed September 18, 2017.

Griniatsos J, Sougioultzis S, Giaslakiotis K, et al (2009). Does Helicobacter pylori identification in the mucosa of the gallbladder correlate with cholesterol gallstone formation?. *West Indian Med J*, **58**, 428-32.

Hamada T, Yokota K, Ayada K, et al (2009). Detection of Helicobacter hepaticus in human bile samples of patients with biliary disease. *Helicobacter*, **14**, 545-51.

Hundal R, Shaffer EA (2014). Gallbladder cancer: epidemiology and outcome. *Clin Epidemiol*, **6**, 99-109.

Indian Council of Medical Research (2014). Consensus document for management of gallbladder cancer. Ansari Nagar, New Delhi – 110029, pp 11-6.

International Agency for Research on Cancer. GLOBOCON 2012: Estimated cancer incidence, mortality and preventive world wide in 2012. http://globocan.iarc.fr/Default.aspx. Accessed October 10, 2017.

Inui M, Ohwada S, Inui Y, et al (2017). Analysis of the gray-zone cutoff values of new serum Helicobacter pylori antibody kits using latex immunoassay. Nihon Shokakibyo Gakkai Zasshi, **114**, 1968-77. In Japanese with English abstract.

Ishiguro S, Inoue M, Kurahashi N, et al (2008). Risk factors of biliary tract cancer in a large-scale population-based cohort study in Japan (JPHC study); with special focus on cholelithiasis, body mass index, and their effect modification. *Cancer Causes Control*, **19**, 33-41.

Kanthan R, Senger JL, Ahmed S, et al (2015). Gallbladder Cancer in the 21st Century. *J Oncol*, **2015**, 967472.

Kapoor VK, McMichael AJ (2003). Gallbladder cancer: an 'Indian' disease. *Natl Med J India*, **16**, 209-13.

Kawaguchi M, Saito T, Ohno H, et al (1996). Bacteria closely resembling Helicobacter pylori detected immunohistologically and genetically in resected gallbladder

mucosa. *J Gastroenterol*, **31**, 294-8.
Khan I, Panda N, Banerjee M, et al (2013). Epidemiological factors in gall bladder cancer in eastern India-a single centre study. *Indian J Surg Oncol*, **4**, 67-72.
Kumar S, Kumar S, Kumar S (2006). Infection as a risk factor for gallbladder cancer. *J Surg Oncol*, **93**, 633-9.
Lacy BE, Rosemore J (2001). Helicobacter pylori: ulcers and more: the beginning of an era. *J Nutr*, **131**, 2789-93.
Lee J-W, Lee D-H, Lee JI, et al (2010). Identification of Helicobacter pylori in gallstone, bile, and other hepatobiliary tissues of patients with cholecystitis. *Gut Liver*, **4**, 60-7.
Mhatre SS, Nagrani RT, Budukh A, et al (2016). Place of birth and risk of gallbladder cancer in India. *Indian J Cancer*, **53**, 304-8.
Mishra RR, Tewari M, Shukla HS (2010). Helicobacter species and pathogenesis of gallbladder cancer. *Hepatobiliary Pancreat Dis Int*, **9**, 129-34.
Mishra RR, Tewari M, Shukla HS (2011). Helicobacter pylori and pathogenesis of gallbladder cancer. *J Gastroenterol Hepatol*, **26**, 260-6.
Mishra RR, Tewari M, Shukla HS (2013). Association of Helicobacter pylori infection with inflammatory cytokine expression in patients with gallbladder cancer. *Indian J Gastroenterol*, **32**, 232-5.
Nandakumar A, Gupta PC, Gangadharan P, et al (2005). Geographic pathology revisited: Development of an atlas of cancer in India. *Int J Cancer*, **116**, 740–54.
Pandey M, Mishra RR, Dixit R, et al (2010). Helicobacter bilis in human gallbladder cancer: results of a case-control study and a meta-analysis. *Asian Pac J Cancer Prev*, **11**, 343-7.
Randi G, Franceschi S, La Vecchia C (2006). Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer*, **118**, 1591-602.
Sharma V, Chauhan VS, Nath G, et al (2007). Role of bile bacteria in gallbladder carcinoma. *Hepatogastroenterology*, **54**, 1622-5.
Shukla HS, Tewari M (2012). Discovery of Helicobacter pylori in gallbladder. *Indian J Gastroenterol*, **31**, 555-6.
Singh V, Trikha B, Nain CK, et al (2002). Epidemiology of Helicobacter pylori and peptic ulcer in India. *J Gastroenterol Hepatol*, **17**, 659-65.
Wistuba II, Gazdar AF (2004). Gallbladder cancer: lessons from a rare tumour. *Nat Rev Cancer*, **4**, 695-706.