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
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

|               | No. | Mean (SD) | No. | Mean (SD) | P value |
|---------------|-----|-----------|-----|-----------|---------|
| ≤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

|               | No. | Mean (SD) | No. | Mean (SD) | P value |
|---------------|-----|-----------|-----|-----------|---------|
| ≤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 | Women |               |            | M | No. | Negative (%) | Positive (%) | OR | 95% CI  | P value |
|------------------------------|-------|---------------|------------|---|-----|--------------|--------------|----|---------|---------|
|                              | Cases |               |            |   | 65  | 37 (57)      | 28 (43)      | 1  | ref     |         |
|                              | Controls |               |            |   | 72  | 47 (65)      | 25 (35)      | 0.7 | 0.3 – 1.4 | 0.32    |
|                              | Men   |               |            |   | 35  | 21 (60)      | 14 (40)      | 1  | ref     |         |
|                              | Cases |               |            |   | 28  | 12 (43)      | 16 (57)      | 2.0 | 0.8 – 5.4 | 0.18    |
|                              | Both sexes |               |            |   | 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 \textit{H. pylori} infection in the gallbladder and other organs. Because \textit{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 \textit{H. pylori} infection in these organs. Moreover, no significant difference in \textit{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 \textit{H. pylori} infection in cases and controls were observed by sex and age. Thus, \textit{H. pylori} infection in organs other than the gallbladder is unlikely to have made a substantial contribution to the results. If \textit{H. pylori} infection is a risk factor for gallbladder cancer, then mean plasma \textit{H. pylori} antibody titers or infection positivity rates should be higher in cases than those in controls.

Second, we defined \textit{H. pylori} infection as plasma antibody titers \(\geq 10\) U/mL in accordance with the kit’s instructions. The sensitivity and specificity of this method for detecting \textit{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 \textit{H. pylori} infection status. However, no significant difference in \textit{H. pylori} infection positivity rate was found between cases and controls, even if we defined positive infection as antibody titers \(\geq 6\) U/mL (cases, 65\%; controls, 62\%; \(P = 0.66\)).

Third, \textit{Helicobacter} species other than \textit{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 \textit{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 \textit{H. pylori} antibody titer in cholelithiasis patients was significantly higher than that in healthy subjects (Deeba et al., 2010). Moreover, an association between \textit{H. pylori} infection and gallstone formation has been reported (Kawaguchi et al., 1996; Lee et al., 2010). If plasma or serum \textit{H. pylori} antibody titer for gallbladder cancer patients, cholelithiasis patients, and healthy subjects are measured, the role of \textit{H. pylori} infection in gallbladder cancer development might be more clearly defined.

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

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