

## The Frequency of *Helicobacter pylori* Infection in Gastric Biopsies of Patients with Gallbladder Stones

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### ABSTRACT

#### Background:

There were few studies reporting simultaneous presence of *Helicobacter pylori* (*H.pylori*) in stomach and gallbladder stones, most of which have been conducted on bile specimens or gallbladder tissues. Presence of a chronic inflammatory disease may contribute to gallstone formation through production of inflammatory cytokines. Chronic active gastritis due to *H.pylori* may contribute to lithogenesis in gallbladder. In this regard, the present study aimed to investigate the frequency and association of gastric *H.pylori* infection and gallstones in patients who were undergoing upper endoscopy in a teaching hospital.

#### Materials and Methods:

In this case-control study patients presenting with dyspeptic symptoms who underwent upper endoscopy during 2008-2012 in Shahid Beheshti Hospital of Babol, northern Iran were enrolled, 72 patients with and 136 patients without gallstones were assigned as the case and control groups, respectively. Patients with history of gastric surgery PPI, and antibiotic consumption four weeks before endoscopy were excluded from the study. Two biopsies were obtained from gastric antrum and body of each patient in both groups and examined histologically for gastritis and the presence of *H.pylori*. Then, endoscopic, histological and demographic features (age, gender, BMI, smoking, NSAID) in these two groups were compared. The SPSS software (version 18) was used for analysis. P-value of <0.05 was considered as statistically significant.

#### Results:

Fifty-two (72.2%) and 98(72.1%) of all patients with and without gallstone were female, respectively ( $p=0.7$ ). The mean age of patients with gallstone was  $51.8\pm 14$  years, while it was  $51.1\pm 15$  years in patients without gallstone ( $p= 0.9$ ). *H.pylori* was detected in gastric mucosa of 31(43.1%) and 45 (33.8%) cases with and without gallstone, respectively. Although *H.pylori* infection was higher in patients with gallstones, the difference was not statistically significant ( $p=0.19$ ). In the group with gallstones, 21(29.2%) patients revealed severe gastritis, and in those without gallstones, 17(12.5%) patients were found with severe gastritis. This difference was statistically significant ( $p=0.003$ , OR=2.88, CI:1.41-5.91).

#### Conclusion:

This study demonstrates that patients with severe *H.pylori* induced gastritis are likely to have gallstone in comparison with mild gastritis. The frequency of *H.pylori* infection in patients with gallstones is more than those without gallstones.

**Keywords:** *Helicobacter pylori*; Dyspepsia; Biopsy; Gallstone

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#### INTRODUCTION

Gastric infection due to *Helicobacter pylori* (*H.pylori*) was found worldwide and more than 50% of the world population were at risk(1). In developing countries, prevalence of *H.pylori* was up to 80% in adults before age 50. In developed countries, the incidence of infection was uncommon in children but approximately 20% of adults were positive for *H.pylori*(2). In northern Iran the prevalence of *H.pylori* infection was reported about 80% in

general population(3). Although the role of *H.pylori* in gastritis, peptic ulcer and gastric malignancy was well established, the possible association of *H.pylori* infection and cholelithiasis was highly controversial.

Recently, several researchers have already performed investigations regarding the association of *H.pylori* induced gastritis and other systemic diseases like inflammatory bowel disease (IBD), idiopathic thrombocytopenic purpura (ITP), coronary artery disease and thyroid gland disease(4-8). It was believed that chronic gastritis may lead to release of cytokines in bloodstream that may contribute to some extra gastric diseases such as cholelithiasis(9).

Gallstone is a common problem which leads to serious complications such as cholecystitis, pancreatitis and cholangitis. Many theories have been raised the etiology of gallstones. Whether the presence of an inflammatory disease in an organ contributes to gallstone formation through the production of inflammatory cytokines is not known. The relationship of *H.pylori* -induced chronic gastritis with gallstones has been displayed in some studies; most of these investigations have been through detecting the *H.pylori* infection in bile or gallbladder tissue after cholecystectomy(10-12). We assumed that chronic active *H.pylori*-induced gastritis may predispose lithogenesis through inflammatory cytokines. This case-control study was conducted to evaluate the frequency and association of the *H.pylori* infection in a group of patients with gallstone. The presence of *H.pylori* infection was documented by endoscopy and pathology report.

## MATERIALS AND METHODS

This case control study was conducted from 2008 to 2012; among 4885 outpatients undergoing upper endoscopy with dyspeptic symptoms in Shahid Beheshti Hospital, Babol University of Medical Sciences, Babol, northern Iran. In all patients undergoing endoscopy, informed consent was obtained and approved by the Ethics Committee of Babol University of Medical Sciences. One hundred fifty two patients who had gallstones on ultrasound were selected. Also, 212 patients with dyspepsia but no gallstone on ultrasound were matched by age, gender and BMI and were assigned as control group.

Patients with the history of gastrointestinal bleeding, acute cholecystitis, pancreatitis, taking antibiotic, Bismuth, and proton- pump inhibitor four weeks prior to endoscopy, history of hemolytic disease, cirrhosis, gastric and bile duct surgery and active

peptic ulcer were excluded from the study. Finally, 72 and 136 patients with and without gallstone selected, respectively, were enrolled. In all enrolled patient two biopsies were obtained during endoscopy from antrum and body of the stomach. In pathology department gastric specimens were stained with Hematoxylin and Eosin and modified Giemsa and examined for the severity of gastritis and the presence of *H.pylori*. The severity of gastritis was assessed based on Sydney classification(13). Moderate and severe gastritis were considered in a single group. Then, demographic characteristics (age, gender, BMI, smoking, NSAID consumption), endoscopic and histological findings of these two groups of patients were compared. Chi-square test was used, for comparison of qualitative variables and T-test was used for quantitative variables. SPSS software (version 18) was used for analysis. *P*-Value less than 0.05 was considered statistically significant.

## RESULT

Thirty-one (43.1%) out of 72 patients with gallstones and 45 (33.8%) out of 136 patients without gallstones were positive in terms of *H.pylori* infection. Although *H.pylori* infection was higher in patients with gallstones, the difference was not statistically significant ( $p=0.19$ ). Severity of gastritis was significantly different in these two groups of patients: 21 (29.2%) patients with gallstone revealed severe gastritis, and in those without gallstones, 17 (12.5%) patients were found with severe gastritis ( $p=0.003$ , OR=2.88, CI=1.41-5.91).

This study shows that 52 (72.2%) and 98 (72.1%) of all patients with and without gallstone, respectively, were females. The difference was not statistically significant ( $p=0.7$ ). The mean age of patients with gallstone was  $51.8 \pm 14$  years, (age range, 22 to 84 years), while it was  $51.1 \pm 15$  years in patients without gallstone (age range, 19 to 85 years), and no significant difference has been observed in terms of age distribution ( $p=0.9$ ).

The BMI was  $26 \pm 4.6$  and  $25 \pm 5$  in patients with and without gallstone respectively there was not significant difference ( $p=0.2$ ).

In terms of non-steroid anti-inflammatory drugs (NSAID), 13 patients (9.3%) with and 17 patients (12.5%) without gallstone had a history of NSAID consumption; however, the difference (lower consumption in the group with gallstone) was not statistically significant ( $p=0.4$ ).

The frequency of smoking was 7.8% in patients with and 5.2% in those without gallstone and not significantly different between the two groups ( $p=0.4$ ). The prevalence of gastritis in patients of both groups is summarized in "Table 1".

**Table 1:** Comparison between the severity of *Helicobacter pylori* infection and presence of gallstones

	patients with gallstone (72)	patients without gallstone (136)	p-Value	OR , CI
Mild gastritis	10 (13.9%)	28 (20.6%)	0.28	OR:0.65(CI:0.29-1.43)
Moderate and Severe gastritis	21 (29.2%)	17 (12.5%)	0.003	OR: 2.88(CI:1.41-5.91)
Total	31	45		

\*Patients with normal gastric histology are not included in this table.

## DISCUSSION

In the present study, the frequency of *H.pylori* infection was 43.1% in patients with gallstones, there was not significantly more than patients without gallstones; however, in a study in Munich, Germany, 15 (20.5%) out of 73 patients were *H.pylori* positive(14). There was not control group in this study for the comparison, and *H.pylori* infection and there was not accompanied by gallbladder dysfunction.

In another study in Shiraz, Iran, *H.pylori* in gallbladder of patients underwent cholecystectomy was detected in 18.1% of gallstones and 12.1% of bile samples. In control group (patients without gallstone) there was not cases of *H.pylori* reported(12).

In a study by Silva et al. in Brazil, *H. pylori* was detected in 31.3% of gallbladder tissue and 42.9 % of bile samples from 46 patients with cholelithiasis, but there was not *H.pylori* found among 18 patients of the control group (without gallstone), indicating shows that *H.pylori* infection may be involved in the pathogenesis of gallstones(15). Nonetheless, Chen et al., showed there was not significant difference in the frequency of *H.pylori* infection in patients with and those without gallstones(16). In a prospective study by Maurer et al., the relationship between *H.pylori* infection and gallstone formation has been investigated and it has been reported that *H.pylori* infection plays no role in the formation of cholesterol stones(17).

Griniatsos et al. showed, there was not significant difference in the prevalence of *H.pylori* infection between two groups of patients with and without gallstone in the gallbladder tissue(18).

In a new study by de Moricz et al. in Brazil, published in 2010, in which there was not control group, the prevalence of *H.pylori* infection in 68 gallbladder of the patients underwent cholecystectomy for chronic cholecystitis with cholelithiasis was 61.8% and 27.9% using PCR and Giemsa methods, respectively(19). Bulajic et al., revealed that there was not statistically significant difference was found in prevalence of

*H.pylori* infection in patients with bile duct disease(20). Our findings extend those of prior studies that evaluated the association between *H.pylori* infection and lithogenesis of gallbladder.

To the authors' knowledge this is a first study that has addressed the association of *H.pylori* infection and subsequent gastritis in the groups of patients with and without gallstone and is, hence, different from the above- mentioned studies since a proper control group has been used in our study design. Although *H.pylori*-induced gastritis has been significantly more severe in patients with gallstone in comparison to those without gallstone, there was not statistical difference has been found between these two groups in terms of the frequency of *H.pylori* infection; it can be due to small sample size in this study. It seems that more severe gastritis induced by chronic *H.pylori* infection leads to higher release of inflammatory cytokines in blood and induces some alterations in homostasis of bile in gallbladder. Some studies shows that chronic inflammation may interrupt mucin production in gallbladder. These cytokines contribute to the increase in the risk of lithogenesis(12,19,21). This study has some potential limitations. This case control study may not be able to complete control all confounding related to choledocholithiasis. We tried to match these two groups to reduce this effect. Even adjustment may not eliminate the all confounding factors.

This study showed that the frequency of *H.pylori* infection in patients with gallstone is more than those without gallstone, but not statistically significant. Finally, the exact influence of *H.pylori* infection and its virulence on the pathogenesis of gallstone is not fully understood. However, this is an evolving area with ongoing research and further studies with larger sample size are required.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest related to this work.

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