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The Relationship Between Cholecystitis and Presence of *Helicobacter pylori* in the Gallbladder

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Abstract

Background: As there is an established relationship between *Helicobacter pylori* infection and gastric and duodenal ulcers, there might also be a relationship between this infection and other pathologies of gastrointestinal system.
Objectives: Thus in this study we decided to investigate the relationship between *H. pylori* infection and cholecystitis.
Methods: In this case-control study, patients were divided into two groups: case group with cholecystitis or cholelithiasis and controls with normal gallbladder. In both groups, after removal of the gallbladder, the samples were sent to the pathology laboratory for further evaluation. The presence of *H. pylori* gene in gallbladder tissue was determined by PCR (Polymerase chain reaction), and the results were compared between the groups.
Results: A total of 84 studied patients with mean age of 45.19 $\pm$ 1.78, 27 were male (32.1%) and 57 were female (67.9%). Results showed that among the studied variables, only height (P value = 0.002) and BMI (P value = 0.001) were significantly different between the two groups.
Conclusions: Although correlation between the prevalence of bacteria in gallbladder with the incidence of gallstones is a controversial issue, but studies suggested that higher prevalence of *H. pylori* in patients with cholecystitis caused by gallstones can be a stimulating factor for the formation of gallstones.

Keywords: Cholecystitis, Cholelithiasis, Gallstone, PCR, *Helicobacter pylori*

1. Background

The presence of gallstones is a condition that often causes problems in the gastrointestinal system [1]. The incidence of gallstones depends on many factors including age, sex and race. According to autopsy results, the global distribution of gallstones varied from 11% to 36% a few decades ago [2, 3]. It is estimated that the prevalence of gallbladder disease particularly gallstone is 5 % to 21.9% among different populations [4]. Studies showed that the incidence of gallstones is almost three times greater in women than in men [5]. Conditions such as obesity, pregnancy, nutrition, Crohn's disease, terminal ileum resection, stomach surgery, hereditary spherocytosis, sickle cell anemia and thalassemia as well as genetic factors may affect the incidence and formation of gallstones [6-9]. It is estimated that at least 25 million people in the United States have gallstones and approximately one million new cases of cholelithiasis are reported each year [10, 11].

Gallstones are divided into two types of cholesterol and pigment stones [12]. Cholesterol stones are more common, but pigment stones are usually secondary to bacterial infection caused by biliary stasis. In other words, bacterial debris comprises the major part of pigment stones [7]. Bilirubin enterohepatic cycle plays an important role in the pathogenesis of gallstones [13]. Brown pigment stones are composed of calcium salts of non-conjugated bilirubin that have varying amounts of protein and cholesterol. Bilirubin mono- and diglucuronide can be deconjugated by endogenous β-glucuronidase. This enzyme is widely produced during chronic bacterial infections of the bile ducts [14].

So far, many factors such as gallbladder movement disorder and high cholesterol due to high-cholesterol diet, medications (e.g., ceftriaxone, and Clofibrate), increase in the activity of HMG-CoA reductase enzyme or increase in the liver absorption of cholesterol from the blood, have been suggested as the major contributors in the pathogenesis of this disease [15, 16]. Different studies have suggested that other factors such as bacterial infection with *Helicobacter pylori* can also be involved in the pathogenesis of gallstones [17, 18]. If such association is proven, the incidence of gallstones, cholecystitis and subsequent morbidity and mortality can be reduced significantly following the treatment of *H. pylori* infection. Therefore, this

case-control study was conducted to investigate the role of these bacteria in the formation of gallstones.

2. Methods

2.1. Patients and Sample Collection

In this case-control study, patients who underwent cholecystectomy were divided into case and control groups. Case group consisted of patients who underwent cholecystectomy due to cholecystitis or cholelithiasis and the control group consisted of patients who underwent this procedure for any other reason (including hydatid cyst or other abdominal pathologies). Participants included in this study were patients admitted to Imam Reza hospital of Mashhad from 2003 to 2012, who had laparoscopic surgery or laparotomy for any reason including cholecystitis, and their gallbladder had been removed. After pathological confirmation of acute or chronic cholecystitis, DNA (deoxyribonucleic acid) was extracted from gallbladder specimens. Then, PCR was performed on the samples using *H. pylori* PCR kit to identify samples that were positive for *H. pylori*. All patients with gallstones, acute, chronic and acalculus cholecystitis and patients who underwent cholecystectomy for any reason other than gallstones were included in this study. Patients were excluded if they had biliary malignancy.

2.2. Statistical Analysis

After data collection and initial processing, the data was recorded and analyzed using SPSS statistical software Ver. 16.0. Data was described using frequency tables and statistical indices (mean, median, and standard deviation). Statistical tests including Chi-square, T-test and nonparametric tests were also used to analyze data. Four variables including weight, height, BMI and duration of illness were analyzed and compared between the groups. In all statistical analysis, $P < 0.05$ was considered as significant. Based on similar studies the sample size of 37 was calculated for each group [19].

3. Results

Descriptive statistics results of the variables in the studied population are expressed in Table 1. As the table shows, the mean age of participants in the study was 45.19 $\pm$ 1.78. The mean age of participants was 46.17 $\pm$ 2.56 and 44 $\pm$ 2.47 in the cases and control group, respectively. Of the total 84 patients, 27 were male (32.1%) and 57 were female (67.9%). After pathological study of the samples, 46 out of 84 patients with acute or chronic cholecystitis were selected as cases. The remaining 38 patients who had cholecystectomy for any reasons other than cholecystitis or cholelithiasis were considered as the control group. After PCR amplification, 5 *H. pylori* infected samples were found. Of these, 4 patients were in case group (chronic cholecystitis) and one in the control group.

Comparison of the variables in the two groups showed that among the studied variables, only sex (P value = 0.001), height (P value = 0.002) and BMI (P value = 0.000) were significantly different between the groups (Table 1). But, none of these variables were significantly associated with the presence of *H. pylori* (P value > 0.05). The results of PCR test also showed that the mean of four examined variables including weight, height, BMI and duration of illness were not significantly different between the positive and negative samples. The results also showed that despite higher prevalence of *H. pylori* in the intervention group compared to the control group (4 versus 1), this difference was not statistically significant between the two groups.

Table 1. Statistics Results of the Variables in the Studied Population[a]

	Acute Cholecystitis (n = 16)	Chronic Cholecystitis (n = 30)	Controls (n = 38)
Male	3	4	20
Female	13	26	18
Age, y	53.68 $\pm$ 4.02	42.16 $\pm$ 3.09	44 $\pm$ 2.47
Weight, kg	73.62 $\pm$ 1.9	70.3 $\pm$ 1.33	69.28 $\pm$ 1.34
Height, cm	166.6 $\pm$ 1.03	167.1 $\pm$ 0.84	171.2 $\pm$ 0.98
BMI	26.55 $\pm$ 0.72	25.22 $\pm$ 0.5	23.61 $\pm$ 0.38
***H. pylori* (+)**	0	4 (13.3)	1 (2.6)

[a]Values are expressed as mean $\pm$ SD.

4. Discussion

Presence of *Helicobacter pylori* in inflamed gallbladder tissue may indicate contribution of these bacteria as an etiologic factor in the formation of gallstones. So far numerous studies using a variety of methods such as different methods of staining, western blot, PCR, immunohistochemistry and serological and histological analysis have been conducted to evaluate the role of bacterial species as an etiologic factor in the formation of gallstones. But the results are contradictory. Some studies clearly show a significant association between positive *H. pylori* test in the stomach and the presence of stones in the gallbladder [20-22]. Studies also suggest that infection by this organism in the gallbladder can be considered as an etiologic factor in the formation of gallstones, especially pigment stones [23].

On the other hand, the results of other studies have shown that there is not any significant relationship between *H. pylori* infection and chronic cholecystitis, or stone formation in the gallbladder [24-26].

Helaly and colleagues showed that *H. pylori* is present in almost 40.9% of samples in patients with chronic calcular cholecystitis [23]. Similarly, Yakooband and colleagues demonstrated that 22 (25%) patients in case group and 5 (9%) in controls were positive for *H. pylori* [20]. Other findings were also indicative of the positive association between *H. pylori* infection and cholecystitis, where the association varied from 7% to more than 55% [21, 22]. In a study by Arismendi - Morillo and colleagues using Giemsa and hematoxylin - eosin staining to detect the bacteria, it was shown that only 6% of the chronic cholecystitis cases were positive for *H. pylori* [24]. In a similar study, Bostanoglu and colleagues showed that no samples were positive for Helicobacter species among the 47 gallstone associated cholecystitis cases [25]. Also, study of Yucebilgili and colleagues showed that despite 22% of samples were positive for bacteria, no significant association was found between *H. pylori* infection and cholelithiasis [27]. Our findings showed that although the number of positive cases for *H. pylori* in chronic cholecystitis group was more than the control group, the difference was not statistically significant. The results of this study also indicated that there is a significant difference in the mean height and BMI between the case and control groups. Higher height in the control group and higher BMI in the case group may indicate greater prevalence of obesity in this group of patients; therefore, suggesting that obesity can be considered as a risk factor for cholelithiasis. Consistent with this study, the results of many other studies also indicate that despite the lack of significant results, *H. pylori* can be considered as an etiologic factor in the formation of gallstones [27, 28]. Insignificancy of the results can be mainly due to the low number of patients; therefore, for more accurate assessment of the prevalence of *H. pylori* in gallstones associated cholecystitis, it is recommended to conduct a multicentric study with a larger sample size.

Although the significant correlation between the prevalence of the bacteria in the gallbladder with the formation of gallstones is a controversial issue, higher incidence of *H. pylori* in patients with gallstones associated cholecystitis can make it an important factor for the formation of gallstones.

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Footnotes

Authors' Contribution: This study was designed and conducted by Mohammadreza Motie as the executive author. Data were gathered and analyzed by Hadi Abbasi. Manuscript draft was written by Hadi Abbasi, Arash Arianpoor and Alireza Rezapanah and was edited by Mohammadreza Motie and Bahram Memar. The English writing was proofed by Mohammadreza Motie and Arash Arianpoor.

Conflict of Interest: Mohammadreza Motie, Bahram Memar, Alireza Rezapanah, Hadi Abbasi and Arash Arianpoor declare that they have no conflict of interest.

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