Does Helicobacter Pylori Identification in the Mucosa of the Gallbladder Correlate with Cholesterol Gallstone Formation?

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ABSTRACT

Objective: Helicobacter pylori (H pylori) represents a potential initiator of cholesterol crystallization and it has been proposed that it is related to gallstone formation. In this study, any possible association between the H pylori identification in the mucosa of gallbladder and cholesterol gallstone formation was evaluated.

Methods: Gallbladders containing pure or mixed cholesterol gallstones (cholelithiasis group, n = 89) and gallbladders without gallstones (control group, n = 42) were submitted to standard histopathological examination for H pylori detection, as well as to nested polymerase chain reaction amplification for H pylori DNA detection.

Results: Helicobacter pylori was identified in the gallbladder’s epithelium in four patients with cholelithiasis and in two patients in the control group by histology. In all the cases which were found to be H pylori positive by histological examination, H pylori DNA were also detected. No correlation between gallstone formation and H pylori detection in the biliary epithelium was found. A higher incidence of acute inflammation in the cholelithiasis (22.5% vs 9.5%, p = not significant [ns]) and in the H pylori positive groups (33% vs 17.6%, p = ns) were histologically detected. A higher incidence (10% vs 0%), p = ns) of H pylori in gallbladders with gallstones and acute inflammation, compared to gallbladders with acute inflammation but without gallstones, was noticed.

Conclusion: Helicobacter pylori is detectable in low frequency in the mucosa of the gallbladder and it does not seem to act as a lithogenic component for cholesterol gallstone formation. Its higher incidence in gallbladders with gallstones and acute inflammation, suggests a possible accessory role in a subset of patients with cholelithiasis.

¿Existe una Correlación de la Identificación de Helicobacter Pylori en la Mucosa de la Vesícula Biliar con la Formación de Cálculos Biliares de Colesterol?

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RESUMEN

Objetivo: Helicobacter pylori (H pylori) representa un iniciador potencial de la cristalización del colesterol, y se ha propuesto que guarda relación con la formación del cálculo biliar. En este estudio, se evaluó cualquier posible asociación entre la identificación de H pylori en la mucosa de la vesícula y la formación del cálculo biliar de colesterol.

Métodos: Las vesículas que contienen cálculos biliares de colesterol puros o mixtos (grupo de colestítisis, n = 89) y vesículas sin cálculos biliares (grupo control, n = 42) fueron sometidas a un examen histopatológico estándar con el fin de detectar el H pylori descubrimiento, así como a la amplificación de la reacción en cadena de polimerasa para la detección de ADN H pilori.

Resultados: El Helicobacter pylori fue identificado mediante histología en el epitelio de la vesícula en cuatro pacientes con el colestítisis y en dos pacientes en el grupo de control. En todos los casos que

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resultaron ser H pylori positivo por el examen histológico, se halló también DNA H pylori. No se halló correlación ninguna entre la formación del cálculo biliar y la detección de H pylori en el epitelio biliar. Se detectó histológicamente una incidencia más alta de inflamación aguda en la colelitiasis (22.5% contra 9.5%, p = no significativo [ns]) y en los grupos H pylori positivos (33% contra 17.6%, p = ns). Se observó una incidencia más alta (10% contra 0%, p = ns) de H pylori en las vesículas con los cálculos biliares e inflamación aguda, en comparación con las vesículas con la inflamación aguda pero sin cálculos biliares.

Conclusion: Helicobacter pylori es detectable en baja frecuencia en la mucosa de la vesícula y no parece actuar como un componente litogénico en la formación del cálculo biliar de colesterol. Su mayor incidencia en las vesículas con cálculo biliar e inflamación aguda, hace pensar en un posible papel auxiliar en un subconjunto de pacientes con colelitiasis.

INTRODUCTION
Helicobacter pylori (H pylori) is implicated in the pathogenesis of gastric and duodenal ulcer (1) and has been proposed as a risk factor for gastric cancer development (2, 3). Helicobacter pylori infection has also been associated with various extragastric diseases, including lesions of the gallbladder (4). Kawaguchi et al (5) first reported the presence of bacteria resembling H pylori in the gallbladder’s mucosa of a patient with gallstone cholecystitis and suggested a contribution between infection and gallstone formation. Following this initial report, H pylori has been identified in the bile (6–8), the liver (9) and the biliary epithelium (10, 11) of humans and its presence has been proposed as related to several hepatobiliary diseases. There is little doubt that bacteria, including H pylori, play a significant role in pigment gallstone formation (12). Bacterial genetic material has also been identified in pure and mixed cholesterol gallstones (13). Although, recent animal studies did not confirm any association between H pylori infection and cholesterol gallstone formation (14), clinical studies have reached contradictory results, providing data in favour of (7, 10, 15) or against (6, 11, 16–18) the possible role of H pylori as a predisposing factor for cholesterol gallstone formation.

There is a high prevalence of gallstone disease in Greece (19) and a high H pylori seroprevalence, ranging from 40% in age groups younger than 40 to 77% in those older than 60 years (20).

The aim of the present prospective case – control study was to investigate any possible association between H pylori identification in the gallbladder mucosa and cholesterol gallstone formation in a subset of patients with cholecystitis.

MATERIAL AND METHODS
The examined material consisted of 89 gallbladders containing one or more pure or mixed cholesterol gallstones. They originated from 22 male and 67 female patients with median age of 63 years (Interquartile Range, IR 51–73) who underwent cholecystectomy between April 2006 and March 2008 due to symptomatic gallstone disease. Among them, ten patients (n = 10) had emergency operations due to acute cholecystitis, unresolved after 48 hours conservative management with antibiotic administration. Patients with pigment gallstones or gallbladder polyps, as well as patients with a history of previous gastric surgery were excluded from the study.

Forty-two (n = 42) gallbladders without cholelithiasis constituted the control group. These were from patients with a median age of 58 years (IR 52–67) who underwent Whipple’s operation (n = 13) or pylorus-preserving pancreatoduodenectomy (n = 7) for pancreatic head cancer, as well as from patients who underwent different types of liver resection for hepatocellular carcinoma (n = 8) or metastatic colorectal cancer to the liver (n = 14).

The prevalence of H pylori infection in the population of the study was not different from its prevalence in the general Greek population, based on the origin, the gender and the age of the enrolled patients, as well as the current epidemiological data from Greece.

The study was conducted according to the declaration of Helsinki and informed consent was obtained from all patients. Gallstone subtyping was performed routinely by gross inspection after submission to the Pathology Department (21).

Gallbladder specimens were fixed in 10% buffered formalin solution and sampling was performed following established guidelines. Paraffin sections were stained with haematoxylin and eosin according to routine protocol. Screening for H pylori was carried out on Giemsa stain by two pathologists under an optical microscope (Olympus® BH2) while their presence was confirmed by additional silver stain (Warthin-Starry). Histological findings were grouped in two main diagnostic categories defined as chronic and acute on chronic cholecystitis. Acute inflammation was characterized by the presence of neutrophils.

H pylori DNA – PCR amplification. After the accomplishment of histopathological examination, the paraffin blocks were further submitted to polymerase chain reaction (PCR) amplification for H pylori DNA detection. In all cases, including those of the control group, DNA was extracted from the formalin-fixed paraffin-embedded (FFPE) gallbladder tissue using the NucleoSpin Tissue Kit.
(Macherey-Nagel, Germany), according to manufacturer’s instruction. To confirm the integrity of DNA, a 430 bp sequence in the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was amplified.

Nested PCR for \textit{H. pylori} was performed using primers of the 26-kDa species-specific antigen SSA gene (22, 23). Specifically, we used the outer-1 primer: 5’-TGGCGTGTCTATTGACAGCGAGC-3’ and outer – 2 primer: 5’-CTGTGTCATTTACCATCTACCAT-3’ that produced a PCR fragment of 303 bp, and the internal primers, internal-1: 5’-GAAAAAGGCAGTTATCGGTCA-3’ and internal-2: 5’-CAATCAAAAAAGCACCTCTCAAAG-3’ produced the final PCR fragment of 121 bp. The first PCR was performed under the following conditions: at 95 °C for 5 min, followed by 35 cycles at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 45 sec and a final extension at 72°C for 5 min. The second PCR was performed using as a template 1 µL of the first PCR reaction product. Cycling conditions of the second PCR were the same with the first ones, except the annealing temperature at 52°C for 30 sec. The amplification products were analyzed by electrophoresis in ethidium bromide-stained 2% agarose gel. Negative and positive controls were assayed in each experiment.

Univariate analyses between groups were determined by the Fisher exact test. \( p < 0.05 \), was considered statistically significant. All statistical calculations were performed using the SPSS (version 12-0) statistical programme.

\textbf{RESULTS}

In the examined material, the overall incidence of \textit{H. pylori} presence in the gallbladder mucosa was 4.5%. In particular, \textit{H. pylori} was identified by Giemsa stain (Fig. 1A) in four cases of cholelithiasis (4/89) and in two cases of the control group (2/42). In all six cases, \textit{H. pylori} presence was confirmed by the Warthin-Starry silver stain (Fig. 1B). \textit{Helicobacter pylori} micro-organisms were encountered locally and were usually low in number.

\textit{Helicobacter pylori} DNA was identified by PCR amplification in 6 out of the 131 cases; four in the cholelithiasis and two in the control group (Fig. 2). There was an exact match between the molecular and histologic findings; in all cases which were found to be \textit{H. pylori} positive by histologic examination, \textit{H. pylori} DNA was also detected.

As shown in Table 1, no correlation between gallstone formation and \textit{H. pylori} detection in the biliary epithelium was found. In the cholelithiasis group, the presence of \textit{H. pylori} correlated neither to the total number nor to the size of the gallstones (not shown). Although not statistically significant \((p = 0.074)\), a higher incidence of acute inflammation in the cholelithiasis group (22.5%), compared to the control one (9.5%), was histologically detected. Moreover, \textit{H. pylori} was detected in 2/20 (10%) patients with gallstones and acute inflammation. On the contrary, \textit{H. pylori} was not detected in any of the gallstone-free gallbladders with acute inflammation.

Comparative analysis between the \textit{H. pylori} (+) and \textit{H. pylori} (-) patients (Table 2) disclosed a marginally statistically significantly \((p = 0.049)\) higher incidence of \textit{H. pylori} in patients over 70-years old. A higher incidence of neutrophil infiltration in the \textit{H. pylori} (+) group (33%), compared to the \textit{H. pylori} (-) group (17.6%) was histologically detected, but the difference did not reach statistically significant levels \((p = 0.334)\). \textit{Helicobacter pylori} was not identified in the gallbladder mucosa, by either method, in any of the ten patients with clinically acute cholecystitis.
molecular techniques used for the detection of bile-resistant Helicobacter pylori. The ground theory is that colonization of the mucosa by Helicobacter pylori, as is the case with other bacteria, may cause chronic inflammation, impairing the acid secretion, reducing the solubility of calcium salts in the bile and increasing the risk of their precipitation in the lumen, thus favouring gallstone formation (29). The bacteria in pure and mixed cholesterol gallstones were located in the centre of the stone, suggesting that they facilitate formation of nidus and possible cholesterol precipitation (13).

Data from the present study strengthen the notion that Helicobacter pylori species are not a major contributor to pure and mixed cholesterol gallstone formation, since there was no difference in the colonization rate of the gallbladder mucosa between patients with and without cholelithiasis. However, the higher incidence of Helicobacter pylori in gallbladders with gallstones and acute inflammation, compared to gallbladders with acute inflammation but without gallstones (10% versus 0%), suggests a possible accessory role in a subset of patients with cholelithiasis.

The finding of the present study shows that no Helicobacter pylori was identified among the 10 patients with clinically acute cholecystitis, could be related not only to the therapeutic effect of the antibiotic therapy given to all these patients before cholecystectomy, but also to the overall low incidence of Helicobacter pylori presence in the gallbladder mucosa of this studied population as well as the central role of other bacteria in the acute inflammation process.

Moreover, a relative increased frequency of Helicobacter pylori colonization was found in the gallbladders of patients older than 70 years, a finding which may be explained by the increased prevalence of Helicobacter pylori infection in this age group (30), raising the possibility for Helicobacter pylori colonization of the biliary tree from the stomach.

According to our findings, Helicobacter pylori is detectable in a low frequency in the gallbladder mucosa and it does not seem to act as a lithogenic component in the context of pure or mixed cholesterol gallstone formation. The relative higher frequency of Helicobacter pylori in the biliary mucosa of patients older than 70 years, probably reflects the increased prevalence of the infection in this age group.

**REFERENCES**


