Case Report

The Association Between Helicobacter Pylori Infection and Liver and Biliary Tract Disorders

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ABSTRACT: Helicobacter Pylori (HP) persistently colonizes the stomach in about 50% of the globe population and it is the main risk factor for peptic ulcer, as well as for gastric adenocarcinoma and MALT gastric lymphoma. The treatment for HP revolutionized the management of the peptic ulcer disease, providing permanent healing in many cases. Preventing colonization of HP would be the primary prevention of gastric malignancy and peptic ulceration. At the same time, the presence of HP provides protection for some diseases (gastroesophageal reflux disease and its complications, esophageal adenocarcinoma, asthma), the eradication of the microorganism having negative repercussions. HP has an increasingly recognized role in other extragastric pathologies. Thus, immune thrombocytopenic purpura has improved after treating HP infection. There are controversial association with ischemic heart disease and cerebrovascular disease. The current article highlights an important association between HP infection and a range of hepatobiliary disorders such as biliary lithiasis (where even an etiological role is involved), cholestatic syndromes (primary sclerosing cholangitis and primary biliary cholangitis), chronic hepatitis B virus, chronic hepatitis C virus, with an evolution towards cirrhosis and hepatocellular carcinoma.

KEYWORDS: *helicobacter Pylori, hepatobiliary disorders, primary sclerosing cholangitis, primary biliary cholangitis, biliary litiasis*

Introduction

Helicobacter pylori (HP), a bacterium discovered in 1982 by Warren and Marshall, infects over 50% of the world's population. It is a gram-negative bacterium, producing urease, located in the mucus covering the gastric mucosa or between the mucus layer and the gastric epithelium. There are multiple factors that allow it to adapt to hostile environments; an important role is the production of urease which causes alkalization of the pH in the environment [1,2].

Epidemiology

The infection is contracted during childhood and over time it develops a typical, active gastritis that persists for the entire life without specific treatment [2].

The risk factors for those infected with HP include precarious socio-economic status, especially in numerous families living in agglomeration conditions, as well as the country of origin.

Thus, the prevalence differs depending on the country, being higher in the underdeveloped countries compared to the highly industrialized ones.

Most infected individuals are asymptomatic; 10-15% make ulcerative peptic disease during life. In addition, HP is a proven factor in the etiology of gastric cancer and MALT lymphoma.

In 1994 it was declared a first grade carcinogen by the International Agency for Research on Cancer (IARC).

HP infection increases 3-7 times the risk of peptic ulcer and gastrointestinal bleeding.

HP is found in 70-90% of patients with duodenal ulcer and 30-60% of cases with gastric ulcer; eradication of the microbe reduces the risk of relapse below 10%, while patients receiving only acid suppression treatment have a 70% relapse risk. HP causes gastric mucosal lesions as well as other complications by releasing mediators of inflammation.

Physiopathology

Although HP produces a systemic and mucosal humoral response, the production of antibodies does not lead to the eradication of infection.

The bacterium is very heterogeneous; the prognosis of HP infection depends on a combination of agent virulence and environmental factors that affect the distribution and severity of gastric inflammation and the level of acid secretion.

The virulence factors of HP that induce proinflammatory cytokine release or adhesion to epithelial cells explain the geographical differences in the incidence of the gastric cancer.

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Several of these virulence factors include:

- Cag PAI (cytotoxin-associated gene pathogenicity island) [3];
- Vac A (vacuolating cytotoxin A) [3];
- Blood group antigen-binding adhesion (Bab A) an external protein of HP that contributes to severe gastric mucosal lesions [4];

They are recognized as having a more severe prognosis. Other virulence factors include:

- HP neutrophil-activating protein (HP-NAP) is a neutrophil activating protein with a role in protecting against HP-DNA fragmentation by the oxidative stress [5];
- Cell-wall polysaccharide.

HP expressing the cytotoxin-associated gene A (Cag A) represents virulent strains having greater interaction with the human species.

Several genes of a genomic fragment that cause the Cag pathogenic islet encode components of a type IV islet that translocates Cag A into the host cells and affects cell growth and cytokine production. Cag A is a potent antigenic protein that is an integral part of the Cag PAI, being associated with a prominent inflammatory response, increasing the production of IL8 [2,3,6].

HP strains expressing active forms of Vac A or outer membrane-associated Bab A and Oip A (outer inflammatory protein A) proteins are similarly associated with increased disease risk compared to the strains lacking these proteins [6,7].

In addition to the local response, a systemic response is also induced in various extra gastric manifestations: haematological, cardiovascular, autoimmune diseases, respiratory disorders [8] and those of special interest in this work, liver and biliary tract diseases.

Case presentation

We present the case of a 35-year-old male who was hospitalized at University Emergency Hospital Bucharest for epigastric pain with sudden onset at a post-prandial hour, accompanied by dyspnea, sweating and nausea. He presented a history of heredocolateral ischemic cardiopathy (father with myocardial infarction) and recent stress. Vital signs were normal except for respiratory rate=28/min; however, D-dimers were in normal range and saturation of oxygen in peripheral blood SaO2 was 100%. Electrocardiogram revealed ST depression (1-2mm) in DII, DIII and V3-V6. Chest x-ray was normal. The patient received aspirin, clopidogrel, morphine intravenous, heparin and intravenous nitroglycerin.

In differential diagnosis, acute coronary ischemia, pericarditis, acute aortic dissection, acute vascular disease (acute mesenteric ischemia, systemic vasculitis, pulmonary embolism) were considered. Troponin T was negative and transthoracic echocardiography did not reveal signs of pericarditis or left ventricular hypokinesia. The coronarography showed normal epicardial coronary arteries and ejection fraction 55-60% without kinetic abnormalities of the left ventricular wall.

Evolving (after 24 hours), subxiphoid pain irradiated in the right hypochondrium, alkaline phosphatase increased by 50% and gamma-glutamyltransferase (GGT) increased five times, as well as total serum bilirubin. An abdominal ultrasound was performed to exclude acute cholecystitis or choledocholytiasis. This revealed gallstones without signs of acute cholecystitis (3mm thick wall, Murphy sign absent, no canalicular dilatation).

On day 3, Endoscopic Retrograde Cholangio-Pancreatography (ERCP) was performed, taking into account the signs of cholestasis, which did not detect dilatations of the ductus choledochus and intrahepatic bile ducts. The cystic channel was probably permeable by retrograde injection of biliary sludge by the contrast substance during ERCP. There was no biliary obstruction or choledocholithiasis. It was possible that a stone has passed spontaneously through the bile duct and the ultrasound and ERCP were normal, while GGT and serum bilirubin increased significantly. For this reason, laparoscopic cholecystectomy was decided to prevent another similar episode. During the intervention a very relaxed gallbladder, with a gangrenous wall, adherent to the epiploon (discordant with the ultrasound exam) was discovered. Surgical dissection was difficult and a drain was placed (unusual for laparoscopic cholecystectomy).

Cholesterol gallstones were found in the gallbladder, not pigment gallstones commonly found in hemolytic anemia or alcoholism. The patient did not have the usual clinical features in those with cholesterol lithiasis. This was a 35-year-old man with a 25 kg/m^2 BMI, non-obese or overweight, being known that cholesterol biliary lithiasis is found in 40-year-old, obese, fertile women.

It is known that HP may contribute to the formation of cholesterol. Since there is a growing literature on the association of cholelithiasis with HP and H. hepaticus infection, serological tests (Ac) have been performed to determine an old infection, thus

confirming the possible infectious etiology of cholesterol. These were positive. The drain was removed the next day after the intervention. The patient evolved normally.

The main hepatobiliary diseases involving HP

Considering the large number of people infected with the hepatitis B virus (over 350 million), 25-30% of whom develop to hepatic cirrhosis, hepatocellular carcinoma or death, and that 50% of the world's population is affected by HP, it is understood why more and more studies are being developed to demonstrate the association between HP infection and the evolution of chronic type B hepatitis. Thus, the studies of Fan et al. as well as Ponzetto et al. have shown that the percentage of HP infection is much higher in patients with chronic hepatitis B than in the healthy population [9]. The study of JuanWang et al., more complex, involving 4645 people, of which 2977 patients with chronic B-hepatitis and 1,688 healthy individuals (control group), had the purpose of demonstrating the role of HP infection in the evolution of hepatitis B and resulted in the recommendation of screening and HP eradication in these patients [9].

This study showed a series of associations:

- the much higher prevalence of HP infection in those infected with hepatitis B than in the healthy group, suggesting a strong association between HP infection and viral hepatitis B;
- the role of HP as a risk factor in the evolution of viral hepatitis B;
- the role of HP in the progression of chronic hepatitis B to fibrosis;
- increased prevalence of HP infection in patients with B virus cirrhosis and significantly higher in patients with severe cirrhosis with B-virus class C Child-Pugh Turcotte;
- the role of HP as a risk factor for hepatocellular carcinoma [9].

A multi-center, observer study, conducted over a two-year period and published in September 2015, from three Chinese hospitals that included 255 patients with hepatic cirrhosis compensated with B virus treated with nucleoside analogues (NA), showed the role of infection HP on **thrombocytopenia** in these patients. They were divided into three groups: the first group was that of the patients with compensated cirrhosis uninfected with HP who received only NA. The second group was that of patients with compensated cirrhosis and infected with HP who received NA and triple therapy to eradicate the bacterium. The third group was with patients infected with HP who received only NA.

It was found that, in those with HP infection, the platelet count was considerably lower than in non-infected patients with compensated cirrhosis, and during the two years of follow-up, by triple HP eradication therapy, the platelet count increased significantly in HP-cured patients than in the other two groups [10].

It can be concluded that the eradication of HP in patients with compensated cirrhosis with B virus and thrombocytopenic is beneficial and that a screening of HP infection should be performed in all cirrhotic B virus and thrombocytopenic patients.

The study by G. Esmat and collaborators in 2011 sought a link between hepatopathy caused by virus C (HVC) and HP infection. It was performed on 85 patients divided into five groups as follows:

- group **I** (control group **1**): 16 patients with chronic hepatitis C without histological activity;
- group **II**: 25 patients with chronical active hepatitis C;
- group **III**: 17 patients with HVC-associated cirrhosis;
- group **IV**: 16 patients with HVC-associated cirrhosis and hepatocellular carcinoma;
- group **V** (control group **2**): 11 patients suffering from gastro-duodenal and gallbladder disease, but negative for HVC

All eighty-five patients were sampled liver tissue samples that were tested by the **Polymerase Chain Reaction (PCR)** for the presence of the HP DNA Cag A gene. Besides this method there were also used biochemical, radiological and RT-PCR methods for HP RNA (11).

The presence of the HP Cag A gene was highest in the **4th** group (75%), 52.9% in the **3rd** group, and 32% in the **2nd** group compared to the control groups where the PCR positive for the Cag A gene was significantly lower [11].

The following differences were found in the METAVIR (F) staging: the HP Cag A gene was present in 28.2% of the cases of advanced fibrosis (F3 and F4) and only 5.9% in the cases of early fibrosis (F1 and F2) [11].

In the METAVIR staging activity (inflammation) score, the following differences were found: the presence of HP Cag A gene was in A1 cases of 15.3% and in A2 of 12.9% [11].

There were no percentage differences in the presence of the Cag A gene depending on the amount of viral RNA and the Child-Pugh Turcotte class in those with cirrhosis with C

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virus versus group IV where the presence of the Cag A gene was significantly elevated, but there may be a link between the presence of the Cag A gene and the evolution of the chronical hepatitis or cirrhosis in the presence or absence of hepatocellular carcinoma [11].

Although it was not possible to cultivate HP microorganisms from the liver, bile or gall bladder, Nilsson et al. found Helicobacter DNA in the liver of patients with **primary cholangiocarcinoma** and **hepatocellular carcinoma**. Failure cultivation HP from liver could be explained by using the frozen bile as a sample and the highly inhibitory effects of bile acid or body transformation in an adverse and biliary inhibitory environment, from the normal spiral form to an unstable coccoid, incompatible with life [12,13]. Another explanation might be that the presence of Helicobacter DNA could be the result of its propagation through the enterohepatic portal circuit [12,13].

In the same study, in a 23-year-old patient with **Wilson's disease** manifested by cirrhosis and who, in the evolution of the disease, developed a fulminant hepatitis, subsequently remitted, subjected to an exploratory laparoscopy, a microbe could be isolated having the morphological and enzymatic characteristics of HP (spiral, scarring, urease secretion as well as oxidase, catalase, alkaline phosphatase and glutamyl transpeptidase), cagA-negative but positive vacA, having a sequence similarity of RNA of 99.38% to that of HP. It should be noted that the patient presented a positive ureic respiratory test, from which the conclusion of the intense colonization of the patient's stomach with HP can be concluded [13].

Most likely, the bacterium has accessed the liver either retrograde from the duodenum or by haematogenic dissemination through the port system of the intestinal lumen [13].

Although it cannot be ruled out that a random association exists between the two pathologies (HP infection and Wilson's disease), there is still the possibility that the presence of HP infection may aggravate a pre-existing liver disease [13].

Gallstones are an important medical problem in their frequency and cost, which is an economic burden especially in North American and European countries. At the age of 60, it affects 20% of women, 10% of men. In the US, 1.200.000 cholecystectomies are performed annually, with the cost of 24 billion USD in 2017 for the treatment of the disease and its complications [14].

In the United States, the complications of this disease cause approximately 3,000 deaths per year, accounting for 0.12% of all deaths [15].

Gallstones can form anywhere in the liver and the biliary tract, 70-80% being cholesterol gallstones.

The risk factors associated with lithiasis are: age, female sex, pregnancy, weight loss and bariatric surgery, biliary sludge, total parenteral nutrition, various medications (estrogen, etc.) [15,16].

Of the systemic diseases that favor biliary litiasis, by far obesity occupies a prominent place (15).

Lately, more and more authors, on the basis of documented studies, have shown in patients with cholesterol lithiasis the more frequent discovery of HP and other Helicobacter species (H. bilis, H. hepaticus) both at the bile and bile tract level as well as at the level of calculus. On the basis of the latest studies, we can state that there is a series of evidence on the role of HP infection in the etiopathogenesis of cholesterol stones formation. Although some studies have not confirmed the association between HP and the formation of cholesterol stones, this may be explained by the precariousness of how they were performed. We can discuss three reasons for the failure to support these ideas:

- lack of properly chosen control groups [12];
- differences in the prevalence of HP infection from a geographic and socio-economic point of view in the countries where the studies were developed (30-50% in developed countries vs. 70-90% in poorly developed countries) [12,15];
- non-standardization of Helicobacter infection detection methods. Thus researchers from Taiwan obtained DNA fragments of Helicobacter species from patients with biliary lithiasis by PCR while authors from Germany, using the same method, did not achieve any similar results [12].

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver characterized by inflammation and fibrosis of the intrahepatic and /or extrahepatic biliary tract. Male/female ratio is 2/1.

The PSC evolution causes biliary tract damage and ultimately causing cholestasis, biliary cirrhosis, hepatocellular insufficiency and cholangiocarcinoma. Long-term monitoring of PSC patients has highlighted an increase in the incidence of biliary tract cancer, gallbladder and colon cancer that may be related to chronical inflammation and exposure to bile acids. With the exception of liver transplantation, no

medical, endoscopic or surgical therapy has determined improvement in survival [17].

PSC occurs in association with intestinal inflammatory disease, usually ulcerative colitis [17].

Etiology is unknown but genetic and immunological factors are probably involved. Of the other factors involved, the toxic bile can be remembered. This is manifested by the absence of phospholipids in the bile which not only causes bile acids toxicity but also the formation of an over-saturated cholesterol bile that facilitates the oxidation of bile epithelial cells.

Whether this is a primary lesion event or a secondary factor that leads to disease progression, it is a problem in the debate.

Primary biliary cholangitis (PBC), originally known as primary biliary cirrhosis, is a chronic cholestatic liver disease that typically affects middle-aged women: the women-to-men ratio is 9:1 [15,18,19].

Its pathogenesis includes a combination of environmental and genetic factors. Genetic studies suggest that human leukocyte antigen (HLA) and type 1 helper T cells (TH1/interleukin 12) are important in the destruction of bile ducts within this autoimmune cholangitis [20].

Finally, a large percentage of patients progresses to cirrhosis and require monitoring for hepatocellular carcinoma.

The study by Hans-Olof Nilsson and his team aimed to determine the existence of an association between Helicobacter species and PSC by investigating the existence of Helicobacter gene sequences on liver biopsies taken from these patients [18].

Note that the study also included patients with primary biliary cirrhosis, non-cholestatic hepatic cirrhosis (NCHC) and patients with normal liver (18) grouped as follows:

- 12 patients with primary sclerosing cholangitis of which 9 biopsies were positive for the Helicobacter genus;
- 12 patients with primary biliary cholangitis of which 11 biopsies were positive for the Helicobacter genus;
- 13 patients with non-cholestatic hepatic cirrhosis (6 with alcoholic cirrhosis, 4 with autoimmune hepatitis cirrhosis and 3 with cryptogenic cirrhosis) of which a single biopsy was positive for the Helicobacter genus;
- 10 patients with normal liver of which no biopsy was positive for the Helicobacter genus.

PCR analyses used primers for the following Helicobacter species: Helicobacter pylori (the gene encoding a 26-kDa specific protein and rRNA 16S), Helicobacter bilis, Helicobacter pullorum and Helicobacter hepaticus. It should be noted that no biopsy was compatible with the primers for H. bilis, H. pullorum or H. hepaticus.

Of the 24 patients with cholestatic disease (PSC and PBC), 20 patients representing 80% were positive for HP and the positivity for those with non-cholestatic and normal liver disease was 4% (1 out of 23 patients).

Thus, it was found, on the one hand, that patients with Helicobacter DNA sequences present in biopsies had higher bases of alkaline phosphatase and prothrombin time compared to those without Helicobacter DNA sequences [18]. One patient in the non-cholestatic liver cirrhosis group, with the Helicobacter sequence present, had the highest value alkaline phosphatase in this group. Later, on a microscopic biopsy review it was found that the histological structural model was actually suggestive of primary sclerosing cholangitis [18].

Also from Nilsson's study it is noted that there is a higher prevalence of this sequence of HP in patients with PSC and ulcerative colitis [18].

From this study there is no significant difference between the two cholestatic diseases (PSC and PBC) in relation to the frequency of this sequence of HP [18].

It is known HP vulnerability in vitro to the human bile free acids, such as deoxycholic and chenodeoxycholic acids. This explains the lack of hepatic colonization but does not explain the presence of HP in the faeces, nor the lack of reaction in terms of HP increase in antrum even under the conditions of a duodenal gastric reflux, the only justification in these situations being the adaptation of HP in vivo to bile acids [18].

The association of Helicobacter-positive patients with cholestatic liver disease is motivated by the higher alkaline phosphatase and prothrombin complex factors (consisting of coagulation factors II, VII, IX and X), and the lack of differences between serum bilirubin values in HP positive versus negative and differences in prothrombin complex factor levels in Helicobacter positive vs. Helicobacter negative, supports the association with cholestasis but not with severe hepatic impairment [18].

After initially considered optimal for the study to be made only for the patients with primary sclerosing cholangitis and those with primary biliary cholangitis associated with cholestatic inflammatory disease represent the control group, it was a surprise finding the relatively equal proportion of Helicobacter positive with both sclerosing cholangitis as well as biliary cholangitis. This suggests the lack of an etiological role of HP and rather a triggering and favoring role in the two cholestatic diseases [18].

It is known that HP Ig G antibodies are found more frequently in cirrhotic patients compared to

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those without cirrhosis. More recently, it was found that those with primary biliary cholangitis without gastric infection present anti-HP antibody titers; probably infected patients have gone through HP infection or have had a cross-reaction of antibodies against other Helicobacter species [18].

Conclusions

This paper wants to be a review of medical research performed correctly in terms of clinical and statistical which showed an association between HP infection and hepatobiliary disease (viral hepatitis with known consequences-cirrhosis and hepatocellular carcinoma) cholestatic syndromes (PSC and PBC) and especially biliary lithiasis in which the microorganism would also have an etiopathogenical role.

Regarding HP eradication only in patients with compensated cirrhosis with virus B and thrombocytopenia, triple therapy resulted in increased the number of platelets in patients cured of HP. In the case of the other hepatobiliary diseases associated with HP infection, further studies are needed to demonstrate the importance of finding and treating microorganism.

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