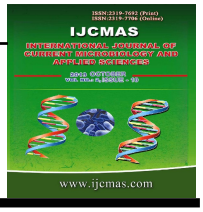
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 179-187**
http://www.ijcmas.com



Original Research Article

Gallbladder colonization by *Helicobacter pylori* in patients with symptomatic gall stone disease

Hayder M. Abdulnabi*

Department of Surgery, Kufa College of Medicine, Kufa University, Iraq
***Corresponding author e-mail:** hayderabdulnabi@yahoo.com*

Keywords

H.pylori; chole-cystectomy; gall stones; bile and stool.

A B S T R A C T

Bacterial infection is accepted as a precipitating factor in gallstone formation, and recent studies have revealed the presence of *H.pylori* in the hepatobiliary system; still causal relationship could not be established till now. This study aimed to detect the presence of *H.pylori* antigen in bile and stool of patient with gallstone. Also it evaluates the colonization of gallbladder by *H. pylori* in patients with symptomatic gallstone disease, and to find a possible causal relationship between them. The study enrolled (73) patients undergoing laparoscopic cholecystectomy for gallstones. Bile and stool samples were taken from all patients and subjected to rapid antigen detection test for *H.pylori* utilizing polyclonal anti *H.pylori* capture antibody meridian diagnostic kit (CTK biotech Inc.). The data were tested by applying chi-square at a level of significance (p< or = 0.05) using SPSS version19. *H.pylori* antigen was detected in the stool of 16 (21.9%) patients, 14 were females and 2 were males, and it was also detected in gallbladder bile of 14(19.2%) patients, 13 females and one male. A positive test was found in both bile and stool in 7(9.6%) of patients, all of them were females, the test was negative in both samples in 36(49.3%) of patients. It has been proposed that the presence of *H.pylori* antigen in the bile may represent an increased risk of gallstones formation. This study concluded that *H. pylori* antigen may be detected in the bile of many patients with gall stones. Consequently, gallbladder colonization by H. pylori might serve as initiating factor in development of gallstones. Nonetheless, whether eradication of *H. pylori* may or may not reduce future gallstone formation is yet not settled down.

Introduction

Helicobacter pylori are a gram-negative microaerophilic curved spiral bacterium, with a rapid corkscrew motility resulting from multiple polar flagella. It was identified in 1982 by Barry Marshal and Robin Warren, who found that it was present in patients with chronic gastritis and gastric ulcer (Blaser, 2006).

More than 50% of the world's population harbor *H.pylori* in their upper gastrointestinal. Infection is more

prevalent in developing countries, and incidence is decreasing in Western countries (Yamaoka and Yoshio, 2008; Brown,2000). Over 80% of people infected with *H.pylori* show no symptoms (Boyanovo, 2011).

Recent studies have revealed the presence of *H.pylori* in hepatobiliary system (Fox *et al*., 1998; Lin et al., 1995; Nilsson *et al.*, 2000; Nilsson *et al*., 2000; Rocha *et al.*, 2005). The presence of *H.pylori* DNA in gallstones was established by polymerase chain reaction (PCR) in several reports (Monstein *et al.*, 2002; Abayli *et al*., 2005). Together with the discovery of *H.pylori* antigen in bile juice (Lin *et al*., 1995; Monti *et al*., 1999; Neri *et al*., 2005) .This has led to the suggestion that Helicobacter species might be an etiological agent in gallstone formation.

Pathogenesis : Transmission of *H.pylori* is thought to be person to person by either the oro-oral or feco-oral routes (Brown, 2000) . The organism survives in the mucosal layer that coats the epithelium and causes chronic infection . Although it is non-invasive, it recruits and activates inflammatory cells as neutrophils, macrophages, and plasma cells. Urea that is normally filtered from plasma into GIT mucosal surfaces is broken down by urease enzyme into CO2 and ammonia. The latter is converted into ammonium by accepting (H+) which leads to neutralization of acidic media in the vicinity of organism ; the survival of *H.pylori* in the acidic media of stomach is dependent on urease. Ammonia also causes injury and potentiates effects of cytotoxins produced by H.pylori (Schreiber *et al.*, 2004; Petersen and Krogfelt, 2003; Liver *et al.*, 1998; Smoot, 1997). It has multiple flagella at one end which allow it to burrow and live deep beneath the mucosal layer closely adherent to the epithelial surface. *H. pylori* uses an adhesive molecule (BabA) to bind to the Lewis b antigen uniquely expressed by only gastric epithelial cells (as in stomach or Meckle's diverticulum ectopic gastric cells) or other epithelial cells which undergo gastric metaplasia as in duodenum metaplasia (Dumrese *et al.*, 2009).

Clinical significance:Patients with gallstones may be asymptomatic or presented with recurrent abdominal pain which has three notable characteristics, localization to right hypochondrium ,episodicoccurance and relationship to fatty meal. However some patients have atypical symptoms as vomiting, or chronic dyspepsia (Zaliekas and Manson, 2008; Heuman and Moore, 1996). A gallstone is a crystalline material formed within gallbladder by concretion of bile components, occasionally with amorphous materials from mucosal surfaces. On the basis of composition , gall stones can be divided into either cholesterol or pigment stones.

Cholesterol stones are single or multiple, varying in color from light yellow to dark green, usually their size range from small granules to large stones exceeding 3cm in diameter. They often have a tiny dark central spot. To be classified as such they must be at least 80% cholesterol by weight. The main two factors for cholesterol stones formation are:

A-the amount of cholesterol secreted by liver relative to lecithine and bile salt.

B-the degree of concentration and extent of bile stasis in gall bladder.

Pigment stones: contain <20% cholesterol and are dark because of the presence of calcium bilirabinate otherwise black and brown pigment stones have little in common and should be considered as separate entities.

Black pigment stones are usually small, brittle and black. They are formed by supersaturation of calcium bilirubinate, carbonate and phosphate, most often secondary to hemolytic disorders sush as hereditary spherocytosis and sickle cell disease.

Brown pigment stones are usually <1 cm in diameter, brownish-yellow, soft. They may form either in the gall bladder or in the bile ducts, usually secondary to bacterial infection caused by bile stasis.

Diagnosis

The current available option for diagnosis of *H.pylori* infection are mainly of two categories; invasive which require endoscopy and sometime tissue biopsy and non -invasive methods which include blood for detection of antibodies , stool antigen detection and carbon urea breath test in which the patient drink 14C or 13C-labeled urea. In the latter, the bacterium metabolizes urea producing labeled CO2, that can be detected in the breath of the patient (Table 1). However the most reliable methods is tissue biopsy through endoscopy with rapid urease test, histological examination and microbial culture[(23)].There is also a urine ELISA test with 90% sensitivity and 79% specificity.

Materials and Methods

A prospective cross sectional study was carried on in the general surgical department of AL-Sader teaching medical city hospital in Najaf, Iraq, during the period between March 2012 to August 2012.Inclusion criteria include any patient with gallstone(s) who is symptomatic and Those with atypical symptoms underwent esophagogastroduodenoscopy (OGD) examination and if negative, are scheduled for surgery. Those with asymptomatic gallstones or those undergoing cholecystectomy for reasons other than gallstone disease were excluded from the study.

A total of seventy three (73) patients (63 women and 10 men) who were diagnosed to have symptomatic gall stones, were enrolled in this study.

Age range was (28-63) with median age of 41 years. Routine demographic data had been collected from all patients with full clinical examination and routine preoperative evaluations. Stool specimen have been taken from all patients for rapid antigen detection test pre operativly. Patients were admitted at the same day of the surgery. Perioperative antibiotic in form of metronidazole 500mg and a third generation cephalosporin (ceftriaxone) 1g were given to all patients. Patients who are allergic to cephalosporin were given an aminoglycoside agent. All patients underwent laparoscopic cholecystectomy and cholecystic bile (2-3)ml was obtained during surgery and sent for *H.pylori* antigen test in the same day.

H.pylori Ag rapid test is a sandwich lateral flow chromatographic immunoassay. *H.pylori* antigens detection in the stool of all patients was also done. The trade name of the kit which were used in this study is CTK biotech inc. 10110 Mea rim rood Sandiego ,CA 92121; USA [e-mail : info @ctkbitech.com].

This study was approved by the ethics committee of each institution and

Table.1 Methods for the diagnosis of Helicobacter Pylori infection

Test	Advantages	Disadvantages
Non-invasive		
Serology	Rapid office kits available Good for population studies	Lacks sensitivity and specificity , and cannot differentiate current from postinfection
^{13}C-urea breath test	High sensitivity and specificity	Requires expensive mass spectrometer
Fecal antigen test	Cheap, specific (>95%)	Acceptability
Invasive (Endoscopic biopsy)		
Histology	Sensitivity and specificity	False negatives occur takes several days to process
Rapid urease tests	Cheap, quick specific (>95%)	Sensitivity 85%
Microbiological culture	'Gold standard' Defines antibiotic sensitivity	Slow and laborious Lacks sensitivity

informed consent was obtained from all patients .Differences between groups were statistically tested by applying chi-square test at a level of significance ($P \leq 0.05$) using SPSS version 19 software program .

Result and Discussion

In this study, a total of 73 patients diagnosed with symptomatic gallstones have been admitted for laparoscopic cholecystectomy where a sample from stool and from bile were collected and tested for the presence of *H.pylori* antigens for all patients. There were 63 female (86.3%) and 10 (13.7%) males with age ranging from 28-63 years, mean age 41 (SD11.3) years. Twenty three patients (31.5%) have positive *H. pylori* antigen in their stool samples, while 50 patients (68.5%) have negative test. Twenty one patients (28.8%) have positive *H. pylori* antigenin in their bile samples, while 52 patients (71.2%) have negative test. This shows the biliary colonization by *H. pylori* in patients with symptomatic gallstones, (Table 2).

Subgroup analysis revealed that sixteen patients (21.9%) have positive test for *H.pylori* antigen in their stool, but are bile-negative, and fourteen patients (19.2%) are positive for

Table.2 Results of HPSA test in bile and stool samples in patients who underwent laparoscopic cholecystectomy.

Sample	Antigen positive	Antigen negative	Total
Bile	21(28.8%)	52(71.2%)	73 (100%)
Stool	23(31.5%)	50(68.5%)	73 (100%)

Table.3 Subgroup analysis of patients with gallstones who underwent laparoscopic cholecystectomy according to their bile and stool HPSA results.

Result of HPSA test	Number of patients (n)	Percentage (%)
H.pylori antigen positive stool and negative bile samples	16	(21.9%)
H.pylori antigen negative stool and positive bile	14	(19.2%)
Both samples positive	7	(9.6%)
Both samples negative	36	(49.2%)
Total	73	(100%)

Chi-square = 46.448,DF (degree of freedom) = 3
P-value 0.0002

Table.4 Gender distribution of *H.pylori* antigen test in (73) patients underwent laparoscopic cholecystectomy for gall bladder disease

Sex of patients	Bile positive	Stool positive	Both positive	Both negative	Total
Female	13(17.8%)	14(19.2%)	7(9.6%)	29(39.7%)	63
Male	1(1.3%)	2(2.7%)	Zero(0%)	7(9.6%)	10
Total	14(19.2%)	16(21.9%)	7(9.6%)	36(49.3%)	73

P-value 0.449

H.pylori antigen in their bile, but are stool-negative. In contrast, only 7 patients (9.6%) revealed positive result in both specimens (stool and bile), with a P-value of 0.0002 which is highly significant (Table 3).

There was no correlation between the presence of *H.pylori* antigen in stool and bile with the sex of the patients with P-value =0.449 (Table 4).

This study showed the biliary colonization by *H. pylori* in patients with symptomatic gallstones was (28.8%), although it is an unusual anatomical site for H. pylori colonization. This is similar to Farshad *et al.,* (2004) who reported the presence of DNA but not antigen in 18.1% of gallstones and suggested that *H.pylori* infection may serve as initiating factor in development of gall stones (Farshad *et al*., 2004; Fox *et al*., 1998; Bulajic *et al.*, 1946; Sheta *et al*., Pandey, 2007; Figura *et al.,* 1998).

The role of *H.pylori* infection in formation of different types of gallstones is still unclear. Although human biliary system is thought to be sterile, this can be broken through an ascending infection via duodenal papillary sphincter and descending through portal system (Dye *et al*., 1978). Although the exact mechanism is not known, bacterial biofilm composed of glycocalyx is suggested to play a role as a nucleation factor. Changes of bile juice composition by beta-glucuronidase and phospholipase produced by bacteria, excessive mucin production of gall bladder epithelial cells triggered by lipopolysaccharides produced by bacteria and promotion of nucleation process through activation of immune system by bacterial itself (Stanley *et al.,* 1993).

In fact, there is no evidence of viable organism in the bile and biliary tract tissue and all recent published studies are based on DNA and antigen detection techniques. Nevertheless, positive presences of bacterial DNA and antigen in bile have been significantly associated with the presence of inflamed gallbladder and cholelithiasis (Kuroki *et al*., 2002).

It may be argued the same prototype of bacterium present in both intestine and cholecystic bile , therefore ; the intestine represent the source of biliary contagion. However, most patients were harboring the microorganism in their bile, but not their stool, This study may suggest that gastrointestinal infection with H. pylori may increase the risk for biliary colonization with *H.pylori* as the P-value is highly significant, this agreed with the study which had been done in southern Italy for detection of both the bacteria DNA and the specific antigen (*H.pylori* stool antigen) identified in the stools of 33 consecutive patients undergoing laparoscopic cholecystectomy for gall stones in Foggai University Hospital which concluded that *H.pylori* DNA and protein antigens may be found in gall bladder bile of patients with gall stones especially in the presence of a marked gastro-duodenal colonization by the bacterium. Nevertheless, it does not clarify whether bacterial DNA and/or protein antigens may be suggestive of the presence of viable organisms playing an active role in the pathogenesis of lithiasis and/or cholecystitis (Neri and Margiotta, 2005) .

However another explanation for findings in this study may be represented by the presence of residual material from

bacterium which has been damaged by bile.

It has been proposed that the presence of *H.pylori* in bile may represent an increased risk of gall stone formation (Figura *et al.*, 1998). A possible consequence of colonization by *H.pylori* is chronic inflammation of gall bladder mucosa ,which may impair gall bladder acid secretion and acidification of content ,reducing the solubility of calcium salts in the bile and increasing the risk of their precipitation in gall bladder lumen (Cetta, 1991). Together with the discovery of *H. pylori* in bile juice (Petersen and Krogfelt,2003), this has led to the suggestion that Helicobacter species are potential etiological agents in gallstone formation.

This study concluded that

1. *H. pylori* antigen may be detected in the bile of many patients with gall stones.
2. Gallbladder colonization by *H. pylori* might serve as initiating factor in development of gallstones.
3.Whether eradication of *H. pylori* may or may not reduce future gallstone formation is yet not settled down.

Recommendations

1.Further studies with larger samples of patients are needed to confirm a causal relationship between *H.pylori* infection and gallstone formation and other hepatobiliary diseases, especially if held in prospective way in asymptomatic patients who are harboring *H. pylori*, yet have normal gallbladder.
2-Although it is not cost-effective, use of PCR to detect *H.pylori* DNA in bile as well as in gallstones themselves is worthy to try in further studies.

From this available data it seems that H pylori stool test represents highly accurate diagnostic tool. In addition it is simple, noninvasive, cheap, and can be widely used , so it has the potential to become the preferred diagnostic tool for *H.pylori* infection.

References

Abayli, B., M. Serin et al. 2005. *Helicobacter* in the etiology of cholesterol gall stones Clin. Gastro. Enteral. 39:134-1370

Blaser, M. J., 2006." Indigenous microbes and the ecology of human diseases". EMBO reports 7 (10): 956–60. doi:10.1038/sj.embor.7400812. PMC 1618379.PMID 17016449.

Boyanovo, L., 2011. (editor) . Caister academic press. ISPN 978-1-904455.

Brown, L.M ., 2000. *Helicobacter pylori* - Wikipedia, the free encyclopedia a b c d e. . "Helicobacter pylori: epidemiology and routes of transmission". Epidemiol Rev 22 (2): 283–97.

Brown, L.M., 2000. "*H.pylori* epidemiology and route of tranxmission." Epidemiol. Review. 22(2):283-97.

Bulajic, M., et al. 2002. *Helicobacter pylori* and the risk of the benign and malignant biliary tract disease. Cancer . 95: 1946-1953.

Cetta, F..1991. The role of bacteria in pigment gallstone disease. Ann. Surg. 213:315-326.

Dumrese, C., Slomiank L, Ziegier U et al .2009."The selected Helicobacter cysteine-rich protein A causes adherence of human monocytes and differentiation into a macrophage-like phenotype." FEBS Lett.583(10):1637-43.

Dye, M., A. MacDonald and Smith, G. 1978. The bacterial flora of the biliary tract and liver in man. Br. J. Surg. 65::285-287.

Farshad, S., A. Alborzi and MalekHosseini, S.A. et al. 2004. Identification of *Helicobacter pylori* DNA in Iranian patients with gall stones. 132;1185-1189

Figura, N., F. Cetta, M. Angelico, et al. 1998. Most *Helicobacter pylori* re-infected patients have specific antibodies and genomic material in bile: is it a risk factor for gall stone formation? Dig. Dis. Sci .43:854-862.

Fox, G.J., F.E. Dewhrist, Z. Shen et al. 1998. Hepatic *Helicpbacter* species identified in the bile and gallbladder tissue from Chileans with chronic cholycystitis. Gastroenterol.114:755-763.

Fox, J.G., F.F. Dewbirst and Shen et al. 1998. *H.pylori* sp. Identified in bile and gallbladder. Gsrtointerol.114(4): 755-63.

Heuman, D.M., E.L. Moore and Vlahcevia, Z. R. 1996. Pathogenesis and dissolution of gallstones. In :Zakim D, Boyer TD, eds. Hepatology : A Textbook of Liver Disease. 1996. Third . Philadelphia, Pa: WB Saunders :376-417.

Kuroki, .T., K. Fakuda, K. Yamanouchi et al. 2002. *Helicobacter pylori* accelerates biliary epithelial cell proliferation activity in hepatolitheasis. Hepatogastoenterol. 49:648-51.

Lin, T.T., C.T. Yeh C.S Wu et al. 1995. Detection and partial sequence analysis of Helicopacter pylori DNA in the bile. Dig. Dis. Sci.40(10)2214-9.

Lin, T.T., C.T. Yeh, C.S. Wu and Liaw, Y.F.1995. Detection and partial sequence analysis of Helicopacter pylori DNA in the bile. Dig. Dis. Sci. 402214-2219.

Liver D, Arnqvist A, Ogren J etal (January 1998). "Helicobacter pylori Adhesion binding fucossylatedhistoblood group antigens revealed by retagging". Science 279(5349): 373-7.doi: 101126/science 2795349373.PMID 9430586.

Monstein, H.J., Y. Jonsson and Zodolesk, J. 2002. Identification of *Helicpbacter pylori* DNA in human cholesterol gall stones. Scand. J. Gastroenterol. 37:112-119.

Monti, J., M. Fay, C. Pangio, et al. 1999. Detection of *Helicobacter pylori* by PCR in gall bladder and bile stones. Acta.Gastroenterol.Latinoma. 29:251-253.

Neri, V. M., and Margiotta, V. 2005. University of Foggia, Italy.DNA sequences and proteic antigens of *H.pylori* in cholycystic bile and tissue of patients with gallstones. Aliment Pharmacol.Ther. 22:715-720.

Neri, V., M. Margiotta, et al. 2005. DNA sequences and proteic antigens of *H.pylori* in cholycystic bile and tissue of patients with gall stones. Aliment. pharmacolther. 22:715-720.

Nilsson, H.O., J. Taneera and Castedal. 2000. Identification of Helicpbacter pylori and other Helicobacter sp. By PCR ,hybridization and partial DNA sequencing in human liver sample from patient with primary sclerosing cholangitis. J. Clin.Microbiol. 38:1072-1076.

Nilsson, H.O., R.Mulchandanl and Trenberg, K.G. 2000. *Helicobacter* sp. Identified in liver from cellular carcinoma. Gastro entrol. 120(1):323-324.

Pandey, M.. 2007. Helicobacter species associated with possible increase in risk of biliary lithiasis and benign biliary disease. World. J. Surg.Oncol. 5:94.

Petersen, A.M., and Krogfelt, K.A, (2003). "*H.pylori* : an invading microorganism ? A review." FEMS Immunol . Med. Microbiol .36(3): 117-26.

Rocha, M., P. Avenaud and Menard et al. 2005. Association of Helicobacter sp. With hepatitis C cirrhosis. Gut. 54(3):396-401

Schreiber, S., M. Konradt, C.Groll et al. (2004). "The spatial orientation of *H.pylori* in the gastriv mucus." Proc. Natl. Acad. Sci. U.S.A 101(14):5024-9.

Sheta, E., A. Elfert, A. Amin et al. Molecular and immunhistochemical detection of *H.pylori* in patients with symptomatic gallbladder stones. Arab. J. Gastroenterolin.

Smoot, D.T., 1997. "How does Helicobacter pylori cause mucosal damage? Direct mechanisms". Gastroenterol. 113(6Suppl):S31-4 discussion S50. PMID 9394757.

Stanley, J., D. Linton, A.P. Burnens et al. 1993. *Helicobacter canis* sp. Nov., a new species from dogs: an intergrated study of phenotype and genotype. J. Gen. Micobiol. 139:2495-2504.

Stenstrom, B., A. Mendis and Marshall, B. 2008."*Helicobacter pylori.* The latest in diagnosis and treatment." AustFam Physician 37 (8):608-12.

Yamaoka, and Yoshio. 2008 . *Helicobacter pylori:* Molecular Genetics and Cellular Biology. Caister Academic Pr. ISBN 1-904455-31-X.

Zaliekas, J., and Manson J.L. 2008. Complications of gall stones : the Mirizzi syndrome, gall stone ileus, gall stone pancreatitis, complications of "lost" gall stone. SurgClin North Am. Dec. 88(6):1345-68, x. (Medline).