Gallbladder Colonization by H. Pylori in Patients with Symtomatic Gallstones Disease

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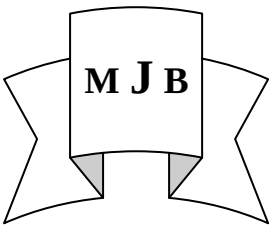

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Abstract

Background: 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.

Aim of the study: To evaluate the colonization of gallbladder by H. pylori in patients with symptomatic gallstone disease, and to find a possible causal relationship between them.

Patients and method: 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 at significance ($p< or = 0.05$) using SPSS version19.

Results and discussion: H.pylori antigen was detected in the stool of 16(21.9%) patients, 14 were females and 2 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.pylpri antigen in the bile may represent an increased risk of gallstones formation.

Conclusion: 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.

Key Words

H. Pylori, Patients, Gall Stone.

الخلاصة

ان الالتهابات الجرثومية للمرارة والقنوات الصفراء تعتبر عوامل مهمة لتكوين حصى المرارة وان الدراسات الحديثة بيّنت وجود الملوية البابية في كيس المرارة والصفراء ولكن لا توجد دراسة تؤكد وجود علاقة سببية موثقة لحد الآن.

هدف الدراسة: هذه الدراسة تهدف الى التثبت من وجود مولدات المضاد (الانتي جين) في الصفراء والبراز للمرضى المصابين بحصى المرارة.

المرضى والعمل: هذه الدراسة ادخلت (٧٣) مريضا اجريت لهم عملية رفع المرارة المنظارية بسبب حصى المرارة، في قسم الجراحة العامة في مدينة الصدر الطبية في النجف ومستشفى الغدير الاهلي في الفترة (من آذار ٢٠١٢ الى آب ٢٠١٢) وقد اخذت نماذج من المادة الصفراء لكيس المرارة والبراز لكل مريض حيث تم فحصها باستخدام مضاد الملوية البابية المتعدد المنتزع.

النتائج: لقد اظهرت هذه الدراسة بأن مولدات مضاد الملوية البابية كانت موجودة في براز ١٦(٢١,٩%)مريضا، ١٤ منهم اناث واثنان من الذكور، كما اظهرت وجوده في صفراء كيس المرارة ل١٤ مريضا(١٩,٢%) منهم ١٣ انثى ورجل واحد.

ان نتيجة الفحص كانت موجبة في كل من الصفراء والبراز ل٧ من المرضى(٩,٦%) بينما كان عدد المرضى الذين اظهرت النتائج سلبية الفحص عندهم في كل من الصفراء والبراز ٣٦ مريضا بنسبة(٤٩,٣%).

بناءً على هذه النتائج تضع هذه الدراسة افتراض وجود الملوية البابية في الصفراء عامل قد يساعد على تكوين حصى المرارة.

الاستنتاج: ان هذه الدراسة خلصت الى وجود مولدات المضاد الخاصة بالملوية البابية في صفراء المرضى المصابين بالحصى المرارية فضلا عن وجوده في براز نفس المرضى بالإضافة الى مرضى آخرين.

كما توصلت هذه الدراسة الى ان هذا الفحص البسيط، غير الغزوي، والدقيق الى حد كبير بحيث بيّنت الدراسة ان حساسية هذا الفحص البسيط والرخيص نوعمّا، وخصوصيته تصل الى ٩١,٣% و ٩٨% بالتعاقب.

Introduction

Helicobacter pylori (H.Pylori) is a gram-negative microaeroph-illiccurved 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 [1].

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[2-3]. Over 80% of people infected with H.pylori show no symptoms[4].

Recent studies have revealed the presence of H.pylori in hepatobiliary system [5-9]. The presence of H.pylori DNA in gallstones was established by polymerase chain reaction (PCR) in several reports [10-11].Together with the discovery of H.pylori antigen in bile juice[12-14].This has led to the suggestion that Helicobacter species might be 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 [15]. 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 CO_2 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[16-19] .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 [19].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 meckel'sdiverticulumectopicgastric cells) or other epithelial cells which undergo gastric metaplasia as in duodenum metaplasia[20].

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 [21,22]. 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.

1-Cholesterol stones aresingle 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 .

2-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.

A- 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.

B- 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 CO_2, 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.

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 spectrometer test	High sensitivity and specificity	Requires expensive mass
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

N.B: DNA Extraction of H.pylori by PCR from bile, stones and gall bladder tissue had been done in certain studies and was proved to be useful but it is costy and not available.

Patients and Methods

A prospective cross sectional study was carried on in the general surgical department of AL-Sader teaching medical city hospital and Al-Ghadeer private hospital in AL-Najaf during period between March 2012 to August 2012.Inclusion criteria include any patient with gallstone(s) who is symptomatic and seeking for surgery. 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. Two surgeons referred the patients and performed the surgery.

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 before doing laparoscopy. 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 underwentlaparoscopic cholecystectomy and cholecystic bile (2-3)mlwas 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. Enzymatic immunoassay (EIA) which detect H.pylori antigens in stool is used. It commonly used to detect H. pylori Ag in stool (HPSA is used as abbreviation), however it can detect the H. pylori antigen in various tissue and fluid samples contaminated or colonized by H. pylori (H.pylori stool antigen meridian diagnostic, Inc. Cincinnati USA).This test utilizes polyclonal anti-H.pylori capture antibodies absorption on micro-wells. it is clear that such test which detects bacterial antigen is identical to ongoing presence of pathogen .The trade name of kits 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 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 .

Results

In this study, a total of 73 patients diagnosed with symptomatic gallstones have been admitted to Al-Sader medical city and Al-Ghadeer private hospital in Al-Najaf , 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 their bile samples, while 52 patients (71.2%) have negative test. This shows the biliarycolonizationby 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 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). This indicates that gastrointestinal infection with H. pylori may increase the risk for biliary colonizationwith H. pylori with a P-value of 0.0002 which is highly significant (Table 3).

There was nocorrelation between the presence of H.pylori antigen in stool and bile with the sexof the patients with P-value =0.449 (Table 4).

Which agreed with another study done in department of surgical sciences university of Foggai Italy (33).

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 laparoscopiccholecystectomy 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

As shown in table (4) we did not find any correlation between the presence of H.pylori antigen in stool and bile with the sex of the patients.

Discussion

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 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 [24-29].

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[30].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 [31].

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, a positive presence of bacterial DNA and antigen in bile have been significantly

associated with the presence of inflamed gallbladder and cholelithiasis [32] 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 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[33].

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[29]. 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 [34]. Together with the discovery of H. pylori in bile juice [17], this has led to the suggestion that Helicobacter species are potential etiological agents in gallstone formation. Whether this indicates that eradication of H.pylori may or may not reduce future gallstone formation is yet not settled down.

Conclusion

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 HPSA 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.

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