



## Prevalence of Helicobacter pylori among patients with cholelithiasis

Batool ali khorsheed<sup>1</sup>, Sawsan M. Jabbar AL-Hasnawi<sup>2</sup>, Ali Nayyef Assi<sup>3</sup>

M.Sc. Student, Asst. Professor, Dr. Professor

<sup>1,2</sup>Dept. of Medical Microbiology, Kerbala college of medicine

<sup>3</sup>Dept. of surgical department, Thi-Qar medical college, (Dr. Professor)

University of Kerbala, Kerbala-Iraq

### Corresponding Author

Asst. professor Sawsan M. Jabbar AL-Hasnawi

Dept. of Medical Microbiology, College of Medicine, University of Kerbala, Kerbala-Iraq

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### ABSTRACT

**BACKGROUND:** Gallstones are one of the most prevalent digestive disorders. Helicobacter pylori (H. pylori) are a human pathogen affecting over 50% of the world population. Helicobacter pylori has a role in upper gastrointestinal disorders, including gallstones. So this study aimed to detect the association of H. pylori with gallstones, therefore developing a possible preventative strategy for gallstone formations by modification type of treatment.

**METHOD:** current study was conducted on 95 patients referred to the surgical clinic of Al-Husain General Hospital, Al Nasiriyah, Real-Time PCR technique was performed for in detection of Helicobacter pylori based on amplification UreC gene in gall bladder samples after cholecystectomy.

**RESULTS:** Out of the 95 patient's 31(33%) were positive for H. pylori UreC gene.

**CONCLUSION:** The presence of H. pylori in gallbladder mucosa might indicate a big risk for cholelithiasis. Real time PCR is a rapid reliable method for the detection of H. pylori DNA in gallbladder. This rapid molecular approach could help clinicians to effectively manage patients at high risk of developing gallstones at an earlier stage.

**Keywords:** cholelithiasis, cholecystectomy, Helicobacter pylori, PCR, prevalence.

### INTRODUCTION

Gallstones are one of the widely occurrent digestive disorders and considered to be one of the most frequent surgical ailments that the general surgeons encounter during their clinical practice .and Cholecystitis is the abrupt inflammation of your gallbladder. If this condition persists over time, such as for months, with continual attacks, or if there are recurrent problems with gallbladder function, it's known as chronic cholecystitis. Gallstone occurrence is a complicated development mediated by genetic and environmental factors. Until recently, the role of the immune system in the pathogenesis of gallstones was not considered a valid topic of research interest [1]. Symptoms of gallstone-related clinical conditions vary from nausea, vomiting and fatty dyspepsia to severe right hypochondrial and epigastric pain, jaundice, fever and shock. However being frequently seen, the diagnosis is not difficult and can be

established by a variety of diagnostic tools including ultrasonography, CT scan, ERCP, liver function tests and pancreatic enzymes[2] [3] . The hypothesis of the presence of H. pylori in the biliary epithelium of the patients with hepatobiliary ailments has been sporadically investigated,[4] [5]. At least half of are infected by H.pylori, making it the most widespread infection in the world, The prevalence of infection is one of the most common and the world's population he socioeconomic status and sanitation conditions; being fewer than 40% in the developed countries and more than 80% in developing countries. The problem is more complicated. H. pylori is implicated in the pathogenesis of gastric and duodenal ulcer and has been as a risk factor for gastric cancer development. Helicobacter pylori infection various different worldwide, depending proposed also been associated with extra gastric diseases, including lesions of the

gallbladder [6] [7]. The connection of gallstones with *Helicobacter pylori* has been researched but not clearly demonstrated. *H. pylori* is a Gram negative and microaerophilic that can lead to chronic gastritis, gastric and duodenal ulcers, gastric and pancreatic adenocarcinoma, and lymphoma of gastric mucosa-related lymphoid tissue (MALToma) [8]. The relationship of *H. pylori* with diseases of organs other than the stomach and duodenum has also been investigated and reported [9]. Literatures is replete with the suggestive evidence of *H. pylori* DNA components in bile, gallbladder tissue and/or cholesterol gallstones [10] [11]. However, Monstein et al [12]. reported that detecting bacterial DNA of *H. pylori* in cholesterol gallstone may indicate that *H. pylori* is a normal flora in the gallstone or, alternatively, the formation of cholesterol gallstone is maybe predisposed by the colonization of *H. pylori* in the biliary tract. More studies on cases and data of large number of patients having different hepatobiliary diseases should be performed in many research centers all over the world in order to verify the correlation of *Helicobacter* species with cholelithiasis [13]. Over the last decade, an escalating number of studies have reported the association of *H. pylori* infection with extra-digestive conditions [14]. lately, different authors have describe that DNA of bile tolerant *Helicobacter* spp. has been found in the human bile colonizing the biliary tract [15]. Majority of these studies have identified infection caused by *H. pylori* based on immunological assays and urease breath test (UBT), but rarely by using molecular approaches. Interestingly, no one could isolate any *H. pylori* using culture-based methods [14]. The association of gallstones with *Helicobacter pylori* has been investigated but not clearly demonstrated [16]. Pathways of *H. pylori* penetration into the bile have not been completely understood but there are possible routes of *H. pylori* migration and colonization in the biliary tract such as *H. pylori* translocation from the duodenum via sphincter of Oddi and/or its hematogenous spread to the liver and then excretion into the bile [17]. The present study was designed to elucidate the etiological association of *H. pylori* species with gallstone disease.

## Materials & METHODS

The type of the study is cross sectional descriptive study. From November - December 2018. Ninety-five consecutive samples after cholecystectomy were

collected from Al-Hussain Teaching Hospital in Nasiriyah, Iraq. The samples fresh for Real time PCR, with a diagnosis of chronic cholecystitis were enrolled in this study.

No antibiotics were given before surgery. Patients with acute cholecystitis and those who had used antibiotics before 2 weeks prior to cholecystectomy were excluded from the study.

### Ethical approval:

Ethical approval of this study was obtained from the Ethics Committee of in of Al-Husain General Hospital in Al Nasiriyah. Informed consent was also obtained from patient included in the study.

### Specimens processing:

Specimen were taken under sterile conditions in tightly closed sterile containers, transported to the laboratory for processing. The gall bladders tissue section was put in sterile Petri dishes and small pieces of tissues (25µg) were cut using sterile blades. Stored until PCR amplification.

### Molecular method using (quantitative real time PCR)

*H. pylori* DNA was extracted from prepared gallbladder tissue using gSYNCT<sup>TM</sup> DNA Extraction Kit (Geneaid Biotech Ltd. USA). The extracted DNA was stored at -20°C until PCR amplification. Amplification of DNA was carried out using NEXpro<sup>TM</sup> qPCR Master Mix (Probe) (Nexdiagnostics, Korea). *H. pylori* *UreC* gene Primer (F, AGCGTTGGCAGTGCTAAAAG and R, TTATAAGCCGCGCCATTAGC). probe (FAMTGTCATAGGGCGCTATATCGTGCA-BHQ1).

qPCR master mix was prepared by using (NEXpro<sup>TM</sup> qPCR Master Mix (Probe)) and this master mix done according to company instructions. After that, these PCR master mix component transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in Real-time PCR Thermocycler (BioRad . USA).

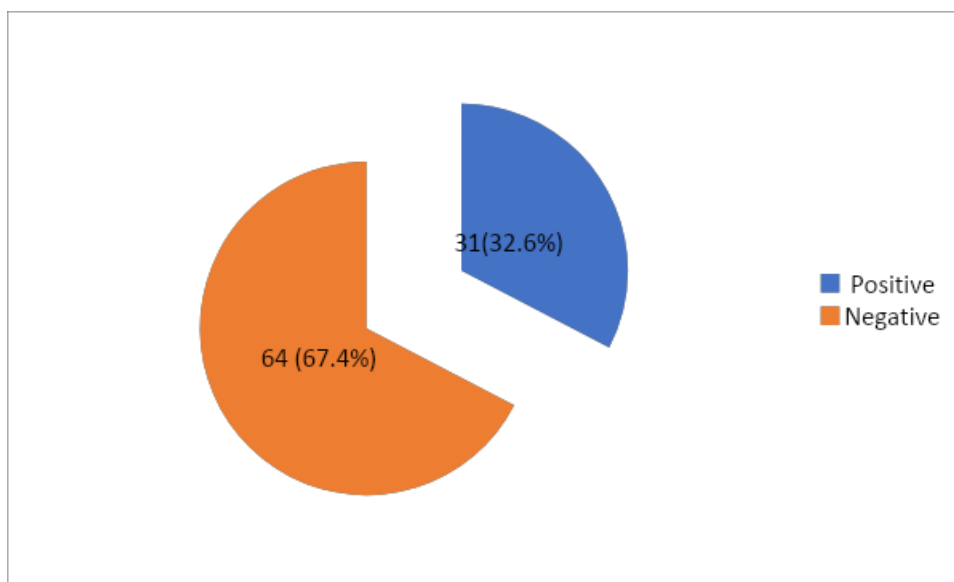
Real-Time PCR thermocycler conditions was set according to primer annealing temperature and qPCR TaqMan kit instructions by Biorad Real-Time PCR thermocycler system as Pre-Denaturation And HS-Taq enzyme activation at 95 °C for 10 min , 1 cycle . Denaturation 95 °C for 10 sec , Annealing 60 °C for 30 sec

Extension and Detection (Scan) 72 °C for 30 sec within 39 cycle. qPCR data analysis was performed by calculation the threshold cycle number (CT value).

**Result**

Study was conducted on 95 patients diagnosed with chronic calculous cholecystitis referred to the surgical clinic of Al-Husain General Hospital, Al Nasiriyah by using real time pcr .Molecular detection of h. pylori in gallbladder tissue

qPCR data analysis was performed by calculation the threshold cycle number (CT value) that presented the positive amplification for UreC gene in Real-Time PCR cycle number ,where real time pcr assay was run on DNA extracted From 95 chronic calculus cholecystitis bacterial DNA was detected in 31(32.6%) was positive while 64(67.4%) was negative as demonstrated in figure (1).positive result of real time pcr are illustrated in figure (2)



**Figure 1: Distribution of H. pylori among CCC**

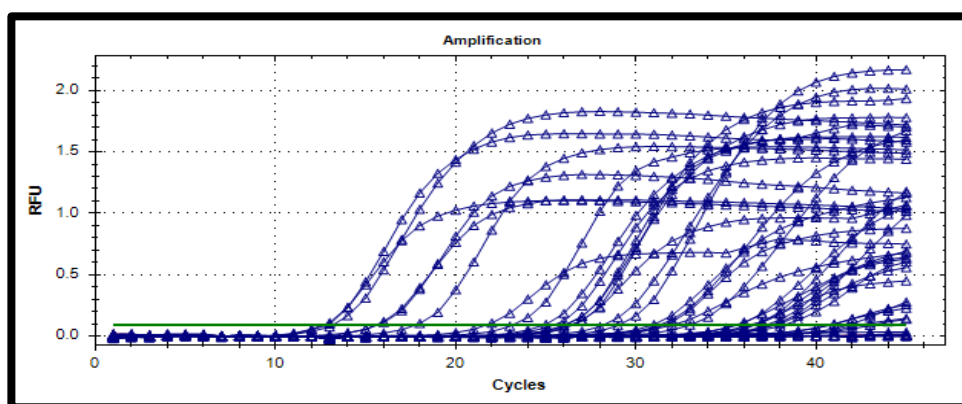


Figure (2): Real-Time PCR amplification plots of UreC gene in Helicobacter pylori based on TaqMan probe. Where, the positive amplification samples which shown cross up the threshold cycle number.

## Discussion

From ninety five chronic calculus cholecystitis patient (32.6%) was positive while (67.4%) was negative.

In this study we attempted to find a relation between *Helicobacter pylori* and gallstones formation. patients undergoing cholecystectomy for symptomatic gallbladder stones,, investigation by RT-PCR showed that the incidence of H.Pylori was 32.6% in gallbladder tissue .In Basra The results of Helicobacter detection in gallbladder specimens showed 25 (36.23%) positive gallbladder specimens from positive gallbladder specimens and the bile specimens appear 75% positive gallbladder specimens for Helicobacter more than other types of gallbladder specimens[18].

The presence of Helicobacter DNA was determined by nested polymerase chain reaction assay in Egyptian study, Helicobacter DNA was detected in the gallbladder tissue and bile of 28% and 18% respectively of the patients. [6].

A studying done in AL- Najaf 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 [19].

In Marmara University Hospital and Maltepe University Hospital, Istanbul, Turkey Overall 35 patients (37%) gallbladder mucosa tested positive for *H. pylori*. [16].

Another molecular investigation by RT-PCR showed that the incidence of H.pylori was 28.3%,26%and 3.3%in gallbladder tissue ,bile and stones samples respectively[7].

Again H. pylori DNA of helicobacter pylori was detected in stone samples of Iranian patients with gallstones [20].

Helicobacter species are commonly present in the gallbladder of patients with gallstone diseases and in controls, implying that Helicobacter infection alone may not play a significant role in the formation of gallstones. However, their results do not exclude the possibility of Helicobacter infection as a cofactor in the development of gallstones[16].

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