Kathmandu University Medical Journal (2009), Vol. 7, No. 2, Issue 26, 125-128

*Original Article*

# Study of *Helicobacter hepaticus* in gallbladders with cholelithiasis and its sensitivity pattern

**Pradhan SB**

Lecturer, Department of Pathology, Kathmandu Medical College & Teaching Hospital, Sinamangal, Nepal

**Abstract**

**Background**: Cholelithiasis is one of the most common digestive surgical disorders. The natural history of gallstone development is unknown. Bacteria are found in high concentration in bile and stone. It is difficult to ascertain whether bacterial infection of the bile arose before stone formation or vice versa.

**Materials & methods**: Prospective study was carried out in 30 cholecystectomy specimens of patients with cholelithiasis collected from 15th January 2005 to 24th July 2005 in Department of Pathology, Kathmandu Medical College Teaching Hospital. The samples were collected in a sterile vial containing 0.5% saline. A small piece of gallbladder was taken in Blood agar and Chocolate agar media for micro anaerobic culture at 37$^0$C. The growth of the organism was observed after 48 hours of incubation. Gram stain and various biochemical tests were performed for the confirmation of Helicobacter hepaticus and its sensitivity pattern was studied. Histologically Helicobacter hepaticus was demonstrated in formalin fixed tissue sections using Warthin Starry Silver stain.

**Results**: A total of 76.66% cases showed growth positivity. Nitrofurantoin was found to be the most sensitive drug (95.7%) for Helicobacter hepaticus followed by Ciprofloxacin (91.3%), Cephalaxin (91.3%), Certriaxone (91.3%), Ofloxacin (82.6%), Amikacin (65.2%) and Norfloxacin (60.9%). Helicobacter hepaticus was least sensitive to Amoxycillin (57%).

**Conclusion:** From this study, it is quite apparent that Helicobacter hepaticus infection is commonly associated (76.66%) with cholelithiasis. If the patients are treated with the sensitive antibiotics regularly and the infection, that is the number one cause for the precipitation of bile leading to the formation of gallstones, can be controlled, the incidence of cholelithiasis can be reduced so that the health care expenditure related to gallbladder disease can be reduced markedly.

**Key words:** Cholelithiasis Helicobacter hepaticus, WSS stain

Cholelithiasis is one of the most common digestive surgical disorders. The incidence is four times higher in women than in men with high prevalence among the age group of 30-40 years[1].

The natural history of gallstone development is unknown. Bacteria are found in high concentration in bile and stone. It is difficult to ascertain whether bacterial infection of the bile arose before stone formation or vice versa[2]. The incidence of gallbladder carcinoma is significantly high in Nepal comprising 2.63% out of all cholecystectomy specimens[3]. Several epidemiological factors have been studied and the presence of gallstone is a well established risk factor for gallbladder carcinoma[4,5]. However, what predisposes to the formation of gallstone is still unclear.

In a recent study, 82% of the gallbladder specimens were found to have Helicobacter hepaticus infection[3]. This might be the leading cause for the precipitation of the cholesterol that ultimately forms the gallstone. Helicobacter hepaticus can be detected from the gallbladder mucosa which is obtained from cholecystectomy specimen. Since cholelithiasis is common in Nepal and if it can be proved that Helicobacter species are associated with the causation of cholelithiasis and as the organisms are sensitive to certain drugs, the infection can be managed effectively and the incidence of cholelithiasis can be reduced so that the health care expenditure related to gallbladder disease can be reduced markedly.

### Aims and objectives

- To determine the incidence of Helicobacter hepaticus infection in gallbladder disease associated with gallstone.

**Correspondence**
Dr. Sailesh B. Pradhan
Lecturer,
Department of Pathology, KMC & TH
Kathmandu, Nepal.
E-mail: saibinita@yahoo.com

- To determine the sensitivity pattern of the Helicobacter hepaticus.

**Inclusion criteria**

All cholecystectomy specimens with gallstone.

**Materials and methods**

Prospective study was carried out in 30 cholecystectomy specimen. History was taken according to the proforma. Gallbladder specimens were received in a sterile vial containing 0.5% Normal Saline. A small piece of gallbladder was taken in Blood agar and Chocolate agar media for micro anaerobic culture at $37^0$C. The remaining portion of the gallbladder was immediately fixed in 10% formalin for the histopathological studies. The growth of the organism was observed after 48 hours of incubation. Gram stain and biochemical tests like Catalase, Oxidase and Urease test were also performed for the confirmation. For sensitivity, Amoxycillin, Amikacin, Ceftriaxone, Ciprofloxacin, Cephalaxin, Ofloxacin, Norfloxacin and Nitrofurantoin were used.

The sections made from the formalin fixed gallbladder specimens were stained with Haematoxylene and eosin stain along with Warthin Starry Silver stain for the demonstration of Helicobacter hepaticus.

**Results**

Out of 30 gallbladder specimens only 60% were found to have Helicobacter hepaticus infection with Warthin starry silver stain. (Table no. 1)

Out of total 30 cholecystectomy cases, 76.66% cases showed growth positivity. Colonies were gray white in color. Gram stain was done and found to be gram negative. Biochemical tests like Catalase, Oxidase and Urease tests were performed and all were found to be positive. (Table no. 2)

A total of 23 isolates were tested for antibiotic sensitivity. Nitrofurantoin was found to be the most sensitive drug for Helicobacter hepaticus and Amoxicillin the least. (Table no. 3)

**Table: 1** Frequency of Helicobacter hepaticus infection in Gallbladder.

| WSS stain | Cases | Percentage |
|---|---|---|
| **Positive** | 18 | 60% |
| **Negative** | 12 | 40% |
| **Total** | 30 | 100% |

**Table: 2** Growth pattern of Helicobacter hepaticus in Gallbladder

| Growth | Cases | Percentage |
|---|---|---|
| **Positive** | 23 | 76.66% |
| **Negative** | 07 | 23.33% |
| **Total** | 30 | 100% |

**Table: 3 Sensitivity pattern of Helicobacter hepaticus.**

| Drugs | Sensitive | Resistant | Total |
|---|---|---|---|
| Nitrofurantoin | **22(95.7%)** | 1(4.3%) | 23(100%) |
| Cephalaxin | **21(91.3%)** | 2(8.7%) | 23(100%) |
| Ciprofloxacin | **21(91.3%)** | 2(8.7%) | 23(100%) |
| Ceftriaxone | **21(91.3%)** | 2(8.7%) | 23(100%) |
| Ofloxacin | **19(82.6%)** | 4(17.4%) | 23(100%) |
| Amikacin | **15(65.2%)** | 8(34.8%) | 23(100%) |
| Norfloxacin | **14(60.9%)** | 9(39.1%) | 23(100%) |
| Amoxycillin | **13(57.0%)** | 10(43%) | 23(100%) |

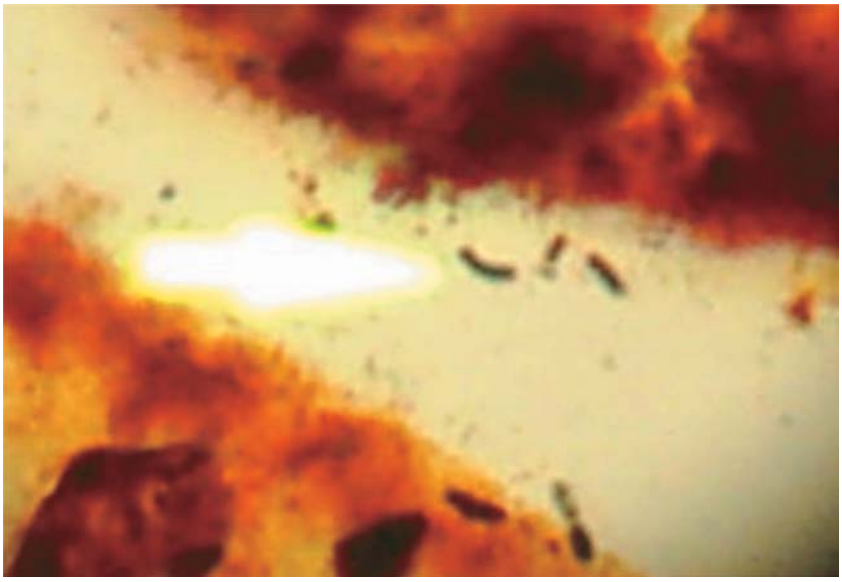

**Fig 1:** Helicobacter hepaticus in Gall Bladder- WSS Stain - x 100

**Discussion**

Cholelithiasis is one of the most common digestive surgical disorder. The incidence is four times higher in women than in men with high prevalence among the age group of 30-40 years[1].

Gallstone disease is common with the incidence ranging from 10% to 20% of the world population, 11% of the general population of the US[6]. In UK, it was found that around 5.5 million people are suffering from cholelithiasis[7]. In a study done by Shrestha et al [8] cholelithiasis was found in 49% of 2809 routine surgical procedure patients in Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. A total of 328 patients underwent cholecystectomy that comprised around 25% of all major surgical procedures done at TUTH, emphasizing the burden of health problem for the developing country like Nepal from the point of view of cost effectiveness, bed occupancy, morbidity and mortality.

Since the discovery of Helicobacter pylori, for which the Nobel Prize has been recently awarded to Barry J. Marshall and J. Robin Warren, many studies on the culture and sensitivity pattern of Helicobacter species have been done so that the number one cause for chronic gastritis and carcinoma stomach could be successfully treated. But the cause behind the reason for cholelithiasis has not been determined.

The natural history of gallstone development is unknown. Bacteria are found in high concentration in bile and stone. It is difficult to ascertain whether bacterial infection of the bile arose before stone formation or vice versa[2].

Bacterial infection may be one of the causes for the pathogenesis of gallstone formation as most gallstones are colonized by a bacterial biofilm[4]. In a study, Stewart et al[9] found bacteria in all brown pigment stones and Amin et al[10] have concluded a study by saying that gallstone formation is probably due to infection rather than super saturation as evidenced by predominance of pigment calcium stones.

In a recent study, Helicobacter hepaticus infection was found in 82% of the gallbladder specimens[3] which might be the number one cause for the precipitation of the cholesterol that ultimately forms the gallstone. Helicobacter hepaticus closely resembles Helicobacter pylori immunologically and genetically[11] but till date no association of these two organisms in causing diseases has been ascertained.

In the present study, 60% of the gallbladder specimens with gallstone were found to have Helicobacter hepaticus infection in contrast to 82% reported from TUTH[3] and 39% reported by Fox JG et al[12].The variation in the incidence could be due to the staining error, subjective error or medication taken by the patient prior to surgery.

Culture of the organisms was found to give better results (76.66%) as compared to demonstration of Helicobacter hepaticus in surgical specimens. Nitrofurantoin was found to be the most sensitive drug (95.7%) and Amoxicillin the least (57%).

From this study, it is quite apparent that Helicobacter hepaticus infection is commonly associated with

gallbladder diseases. If the patients are treated with the sensitive antibiotics regularly and the infection which is the number one cause for the precipitation of bile that leads to the formation of gallstone can be controlled, the incidence of cholelithiasis can be reduced so that the health care expenditure related to gallbladder disease can be reduced markedly.

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