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Original Article

Relation between gallbladder neoplasm and helicobacter hepaticus infection

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Abstract
Objective: The main objective of this study was to see the various histopathological changes in the gallbladder with cholelithiasis and to correlate them with Helicobacter hepaticus infection. **Methods:** A total of 380 cholecystectomy specimens were received during a study period from 2058/11/29 to 2059/11/4 at Department of Pathology, TU Teaching Hospital, Institute of Medicine. **Results:** Among 380 cases, 249 (65.53%) were found to have chronic cholecystitis, 52 (13.68%) cholesterolosis, 29 (7.63%) adenomyosis, 20 (5.26%) metaplasia, 15 (3.95%) low-grade dysplasia, 10(2.63%) malignancy, 4(1.05%) xanthogranulomatous change and 1(0.26%) carcinoma in situ. Out of these, 100 cases that were willing to provide gallbladder for study were taken as a study group. Sections were stained with Haematoxylin & Eosin for microscopic features and with Warthin Starry Silver stain for Helicobacter hepaticus. Among the study group, 43% cases were found to have chronic cholecystitis, 17% adenomyosis, 13% cholesterolosis, 9% low-grade dysplasia, 9% metaplasia, 7% malignancy, 1% carcinoma in situ and 1% xanthogranulomatous change. All the malignant cases were found to be Adenocarcinoma. Out of total 100 cases, 82% cases were found to have Helicobacter hepaticus infection. Only one out of 7 malignant cases (14.29%) was found to be negative for Helicobacter Hepaticus infection. Gallbladder neoplasm was found to be common in Nepal comprising 2.63%. Helicobacter hepaticus infection was found in 82% of gallbladders and it was found in 87.5% of malignant cases. Whether Helicobacter hepaticus that might be the number one cause for the gallstone formation that ultimately leads to malignancy or itself acts as a risk factor for the pathogenesis of carcinoma gallbladder is yet to be determined.

Key words: Helicobacter hepaticus, Helicobacter pylori, Warthin Starry Silver stain

Gallstone disease known as cholelithiasis is one of the most common digestive surgical disorder. The incidence of stone in general population of United State is 11%.[1] The incidence is four times higher in women than in men with high prevalence among younger age group (20-30 years).[2]

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 bile arose before stone formation or vice versa. [3] Bacterial infection may be one of the cause for the pathogenesis of gallstone formation as most gallstones are colonized by a bacterial biofilm.[3] In a study, Stewart et al [4] found bacteria in all brown pigment stones and Amin et al [5] have concluded a study by saying that gallstone formation are probably due to infection rather than super saturation as evidenced by predominance of pigment calcium stones.

Since the discovery of Helicobacter pylori in 1983 AD, more than 25 additional Helicobacter species have been isolated. Among them Helicobacter hepaticus was reported by peer-reviewed literature in 1994 AD. A microorganism resembling Helicobacter pylori was detected incidentally on pathologic specimen of the gallbladder mucosa [6,7,8] and has reported a possible association of Helicobacter hepaticus species with gallbladder disease. [7] Such an action is presumably enhanced by the presence of gallstone. Further more, bile resistant Helicobacter species are found in bile from patients with chronic cholecystitis and their presence is considered to be a risk factor for malignancy. In a study done by Fox JG et al [7], Helicobacter was found in 9 of 23 resected gallbladder tissues for cholecystitis. This organism was first identified in mice in 1992 [9] and subsequently characterized by ultra structural morphological examination, biochemical characteristics and 16 S rRNA genes sequence determined to be a new species related to helicobacter pylori, it was given the name Helicobacter hepaticus. [10]

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Helicobacter hepaticus is a gram negative, spiral shaped, microaerobic, motile bacterium, 0.2 to 0.3 μ m in diameter, 1.5 to 5 μ m long with one to several spiral, it has bipolar sheathed flagella (1 at each end) but lacks the periplasmic fibres that envelop the bacterial cells in other helicobacter species. Helicobacter hepaticus has strong urease activities, is oxidase and catalase positive, produce H_2S, reduces nitrate to nitrite and grows microaerobically at 37^0C but not at 25^0C or 42^0C. It is resistant to Cephalothin and Nalidixic acids but sensitive to Metronidazole. The species defining characteristics of organism, the nucleotide sequence of its 16S rRNA gene, has been used to develop diagnostic among based on polymerize chain reaction.[10]

Aims and objectives

1. To determine the incidence of carcinoma of gall bladder.
2. To determine the incidence of Helicobacter hepaticus infection in gallbladder.
3. To study the relationship of gall bladder carcinoma with Helicobacter hepaticus infection.

Materials and methods

A total of 380 cases of cholecystectomy specimens were received during a study period from 2058/11/29 to 2059/11/04 at TUTH. Among them 100 cases, whose were willing to provide gallbladder for study were selected. Detailed clinical history was taken according to the format. The resected specimens of gallbladder were formalin fixed. Detailed macroscopic examination of the specimen was done after complete fixation. The sections were stained with Haematoxylin & Eosin stain for microscopic examination and with Warthin Starry Silver stain for Helicobacter hepaticus.

Results

Table 1. Morphological changes in Gallbladder with cholelithiasis.

Type	Frequency	Percentage
Chronic Cholecystitis	249	65.53%
Cholesterolosis	52	13.68%
Adenomyosis	29	7.63%
Intestinal Metaplasia	20	5.26%
Dysplasia(low grade)	15	3.95%
Xanthogranulomatus	4	1.05%
Carcinoma in situ	1	0.26%
Malignancy	10	2.63%
Total	380	100%

Of the total malignant cases, all were diagnosed as Adenocarcinoma.
From these cases, 100 cases who had given the consent for the study were taken as a study group.

Table 2. Relation between age of the patients and GB malignancy.

No.	Age group	Frequency	Percentage
1	20-29 years	1	14.29%
2	30-39 years	_	_
3	40-49 years	_	_
4	50-59 years	1	14.29%
5	> 60 years	5	71.42%
Total		**7**	**100%**

There is no age bar for the incidence of carcinoma gallbladder however the incidence is more common after the age of 60 years.

Table 3. Relation between presences of Helicobacter hepaticus infection in GB in Malignant cases.

No	Helicobacter hepaticus	Frequency	Percentage
1	Positive	6	85.71%
2	Negative	1	14.29%
Total		7	100%

Helicobacter hepaticus infection was found in 82% of cholecystectomy cases and was found in 85.71% of malignant cases of gallbladder.

Discussion

Gallstone disease called as cholelithiasis is the most common digestive surgical disorder and account for an important part of health care expenditure. Cholelithiasis produces diverse histopathological changes in the gallbladder mucosa namely acute inflammation, chronic inflammation, glandular hyperplasia, granulomatous inflammation, cholesterolosis, metaplasia, dysplasia, carcinoma in situ and malignancy.

During the study period, 380 cholecystectomy specimens were received.

Out of total 380 cases, 97% of the specimens were found to have the features of chronic cholecystitis. Out of which, 65.53% of the cases were found to have chronic cholecystitis only and along with chronic cholecystitis 13.68% were found to have cholesterolosis, 7.63% adenomyosis, 5.26% intestinal metaplasia, 3.95% low grade dysplasia, 1.05% xanthogranulomatous changes and 0.26% carcinoma in situ. Malignancy was found in 2.63% of the cases. None of the specimens were found to have acute inflammation. . The reason could be due to the modern diagnostic facilities, health awareness and avoidance of cholecystectomy during an acute attack of cholecystitis.

In one retrospective study done by Shrestha et al [2] (1990 AD) in Nepal, 4.7% specimens were found to have the feature of acute inflammation. In the similar study done by Baig SL et al [11] in India (2000AD), 60% of the cases had only chronic cholecystitis, malignancy in 2.5% and none of the specimens were found to have acute inflammation. The findings are similar with our finding in this study.
Out of 380 cases of cholecystectomy specimens, 100 cases were analyzed along with their detail history, histopathological findings and Helicobacter hepaticus infection.

The incidence of gall bladder carcinoma was found to be high in Nepal comprising 2.63% with male to female ratio was 1:4. Poor economical condition, negligence and lack of knowledge might be the cause for turning into malignancy. Among the study group, 14.29% cases were found to have malignancy at the younger age group below 30 years and the youngest age at which the malignancy detected was 26 years. All the malignant cases were found to be adenocarcinoma: 42.85% well differentiated adenocarcinoma, 28.57% poorly differentiated adenocarcinoma, 14.29% moderately differentiated adenocarcinoma and 14.29% papillary adenocarcinoma

The reported incidence of carcinoma of the gallbladder in USA is 1-2.5 per 100,000 people (Lam, 1940; Brudette 1957; Strauch 1964).[12,13,14] The highest incidence is reported in women from Poland (23.1/100,000), followed by in Israel (13.8/100,000). [15] It is the most common biliary tract and third most common gastrointestinal tract malignancy comprising 4.4% of all malignancies in and around the Varanasi region of India (Shukla et al, 1985, Pandey et al, 2001).[16,17] Gallbladder cancer has been the number one cause of cancer mortality in Chilean women.[18]

Carcinoma of the gallbladder is predominantly a disease of elderly women with a peak incidence in the seventh decade. The reported age range at presentation in various series is 40-80 years. (Arninski 1949, Glenn & Heys 1954, Shieh et al 1981).[19,20,21] The mean age at presentation was 50 years with a range of 40-60 years. The reported men: female ratio ranges from 1:2 to 1:3.2.[17]

Carcinoma in situ was found in 0.26% cases which might be due to the chronic trauma, irritation and inflammation of the gallbladder mucosa caused by the presence of gallstones. It is a premalignant condition which may predispose to carcinoma. In one prospective study, Ojeda Vj el al [22] found carcinoma in situ in higher percentage which comprise 1.67%.

Helicobacter hepaticus infection was found in 82% of cholecystectomy cases. It might be the number one cause of cholelithiasis. Helicobacter hepaticus was found in 85.71% of malignant cases of gallbladder and absent in 14.29% of carcinoma gallbladder

associated with gallstone. Helicobacter hepaticus itself acts as risk factor for pathogenesis of carcinoma gallbladder or it acts as a nidus for the formation of gallstone that ultimately leads to malignancy has not been studied. In a study done by James GF et al [7] in Chile, where cancer of the gallbladder is the number one cause of the cancer mortality in Chilean women, 39.13% of gallbladder tissues were found to have Helicobacter hepaticus infection.

Approximate antibiotic regimen can successfully eradicate the infection of Helicobacter pylori in most of the patients, the complete resolution of mucosal inflammation and a minimal chance of recurrence of ulcers. It has not been tried for the eradication of Helicobacter hepaticus. Detection of Helicobacter pylori is relatively easy since gastric mucosa can be obtained from the endoscopic biopsy. However Helicobacter hepaticus can be detected from the gallbladder mucosa, which is obtained only from the cholecystectomy specimens. Helicobacter hepaticus is closely resembles to Helicobacter pylori immunologically and genetically [6]. Urea breath test, serological tests and PCR [10] can be used for the diagnostic purpose and if can be treated effectively, the incidence of cholelithiasis which is a major surgical problem can be reduced.

Whether Helicobacter hepaticus infection acts as a nidus for the formation of gallstone that ultimately leads to malignancy or Helicobacter hepaticus itself act as a risk factor for the pathogenesis of carcinoma gallbladder is yet to be determined.

Summary and conclusion

A total of 380 cholecystectomy cases were received during the study period from 2058/11/29-2059/11/04. Out of which 100 gallbladder specimens were taken for the analysis.

Chronic cholecystitis was found in 65.53% cholecystectomy specimens . Along with chronic inflammation, cholesterolosis was found in 13.68%, adenomyosis 7.63%, intestinal metaplasia 5.26%, low grade dysplasia 3.95%, xanthogranulomatous change 1.05% and carcinoma in situ in 0.26%. 2.63% cases were found to have malignancy and all were Adenocarcinoma

Gallbladder malignancy was found to be common in Nepal and comprises 2.63%. A total of 82% of the gallbladder were found to be infected with Helicobacter hepaticus and it was found in 85.71% cases of gallbladder malignancy. Whether Helicobacter hepaticus infection that acts as a nidus for the formation of gallstone that ultimately leads to malignancy or Helicobacter hepaticus itself act as a risk factor for the pathogenesis of carcinoma gallbladder is yet to be determined.

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