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Original Article

Pathology Section

Helicobacter pylori in Cholecystectomy Specimens- Morphological and Immunohistochemical Assessment

RASHMI PATNAYAK[1], VENKATARAMI REDDY[2], AMITABH JENA[3], SIVA GAVINI[4], ASHA THOTA[5], RUKAMANGADHA NANDYALA[6], AMIT KUMAR CHOWHAN[7]

ABSTRACT

Introduction: *Helicobacter pylori* (*H.pylori*) is associated with gastritis, peptic ulcer, gastric carcinoma and gastric lymphoma. Current literature describes presence of *H.pylori* in various extra-gastric locations and its association with many diseases. Apart from the conventional location of gastric and duodenal mucosa, *H.pylori* have been isolated and cultured from gallbladder.

Aim: Analysis of cholecystectomy specimens to detect *H.pylori* by means of immunohistochemical staining.

Materials and Methods: There were a total of 118 cholecystectomy specimens received in the Department of Pathology in three months duration. We have performed immunostaining for *H.pylori* in 45 consecutive cases of cholecystectomy specimen. Clinical and other investigational information were retrieved from the medical records department. For each case, routine Haematoxylin and Eosin stain was studied. Immunohistochemistry (IHC) was done using purified polyclonal *Helicobacter pylori* antiserum.

Results: Majority of the patients had undergone laparoscopic cholecystectomy for the presenting complaint of right hypochondrial pain. Multiple pigmented stones were present in majority (27/45) of them. Immunostain for *H.pylori* was positive in ten cases. Six of these cases had pigmented gall stones, two had stones not specified and in two of the cases there were no stones.

Conclusion: *Helicobacter pylori* is present in gall bladder and is commonly seen in association with stones. A more detailed study of cholecystectomy cases (both neoplastic and non-neoplastic) with serological, culture and molecular data of *H.pylori* is desirable to study the pathogenesis of cholecystitis, its association with gall stones and other gall bladder disorders.

Keywords: Gall bladder, Gastric biopsies, Immunostain

INTRODUCTION

Helicobacter pylori (*H.pylori*) is related to the pathogenesis of gastritis, peptic ulcer, gastric carcinoma and lymphoma of gastric mucosa-associated lymphoid tissues (MALToma) [1]. In recent literature, there is increasing description of *H.pylori* in extra-gastric locations and its association with many diseases [2]. Among other organs, apart from gastric and duodenal mucosa *H.pylori* have been isolated and cultured from gallbladder [1,3].

Routine Haematoxylin and Eosin (H&E) stain along with several special stains like warthin-starry and giemsa are used to detect *H.pylori* in histological sections of gastric biopsies. But their specificity and sensitivity vary greatly. *H.pylori* can be detected by immunohistochemical method in which anti *H.pylori* antibody reacts with somatic antigens of the whole bacteria [4-6].

AIM

We have undertaken the present study of cholecystectomy specimens to detect *H.pylori* by means of immunohistochemical staining.

MATERIALS AND METHODS

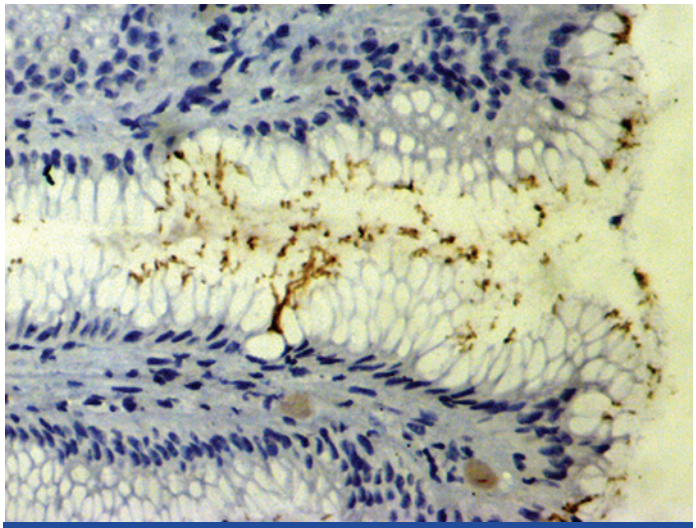
We received a total of 118 cholecystectomy specimens in the Department of Pathology in three months duration (September-December 2013). Since it is a retrospective study, we have not obtained institutional ethics committee approval. Majority of the patients had undergone laparoscopic cholecystectomy. We analysed 45 consecutive cases of cholecystectomy specimen where immunostaining for *H.pylori* was done. With the available quantity of immunomarker only 45 cases could be immunostained. In two cases, gall bladder was received along with Whipple's specimen for periampullary carcinoma, in one case there was choledochal cyst and one case was a carcinoma of stomach which showed extensive infiltration in different organs including the gall bladder. The specimens were fixed in 10% formal saline for 24 hours and then dehydrated in increasing concentrations of isopropyl alcohol. After this clearing of alcohol by xylene was done then it was impregnated and embedded in paraffin wax. Routine H&E staining was performed. Immunohistochemistry (IHC) was done using purified polyclonal *Helicobacter pylori* antiserum, (BioGenex). IHC was done on three- micron paraffin sections on 3-amino propyl ethoxy silane (APES) coated slides, using prediluted antibodies. The IHC detection system used was Polymer HRP (Horse Radish Peroxidase) method [6]. The slides were stained with 3, 3'-diaminobenzidine tetra hydrochloride (DAB) chromogen. They were counterstained with Haematoxylin and mounted [6]. Gastric mucosal tissue with *H.pylori* infection was taken as positive control. The data obtained was given in the form of simple percentages. Statistics was done by using Chi-square method. The data obtained was analysed using the SPSS 11.5 (Chicago, IL) statistical program.

RESULTS

There were a total of 45 cases where immunostaining for *H.pylori* was done. The mean age was 47±13.5 years (Min-20 and Max-77). Male:female ratio was 1:1.3. The most common complaint was pain in the region of right hypochondrium of variable duration from few days to months. In many cases (41/45) ultrasonogaphy detected the stones. Majority (27/45) showed multiple pigmented stones. Cholesterol stone was noted in three cases. Histopathological examination revealed chronic calculus cholecystitis with presence

of chronic inflammation comprising lymphocytes and plasma cells in most of the (37/45) specimens. Two specimens showed presence of eosinophils in addition to lymphocytes and plasma cells. Another two cases showed dense lymphocytic aggregates in the form of lymphoid follicles. One case showed infiltration of the gall bladder by adenocarcinoma of stomach and another three cases showed neutrophilic infiltration in combination with chronic inflammatory cells.

Immunostain for *H.pylori* showed positivity in ten cases [Table/Fig-1]. Six cases with *H.pylori* positivity had pigmented gall stones. Two cases showed positivity by *H.pylori* immunostain even without presence of stones [Table/Fig-2]. The p-value obtained was 0.429 which was not significant indicating that there was no significant association between presence of *H.pylori* and gall stones. Gall bladder mucosal ulceration was present in ten (22.2%) patients but none of them were associated with *H.pylori* infection. The cholecystectomy cases included in our study group were all benign in nature. There was no case of primary gall bladder carcinoma. The gall bladders received along with Whipple's specimen did not



[Table/Fig-1]: Mucosa of gall bladder showing presence of *Helicobacter pylori* by means of immunostain (Immunohistochemistry x 200).

Type of stones	Frequency (%)	*H.pylori* positive	p-value
No Stone	5(11.10)	2(40%)	0.429
Pigmented	27(60)	6(22.2%)	
Cholesterol	3(6.7)	0	
Stone not specified	10(22.2)	2(20%)	
Total	45(100)	10(22.2%)	

[Table/Fig-2]: Cholecystectomy specimen with/without stones and *H.pylori* immunostain.

reveal any malignancy. They did not reveal *H.pylori* either. One of our cases was a case of carcinoma stomach with extensive metastasis in different organs including gall bladder. This case did not show presence of *H.pylori* in gall bladder.

DISCUSSION

In Indian subcontinent, a high prevalence of *H.pylori* is reported in nearly all gastroduodenal diseases [6,7]. A South Indian study stated that *H.pylori* colonization is present in more than half of the Indian population [7]. In many cases it is present even without any associated abdominal symptoms [6,7].

Recently presence of *H.pylori* is frequently documented in various extra-gastric sites. The evidence of *H.pylori* colonization has been found in the gallbladder, ears, nose, skin, and even eyes [2]. Whether *H.pylori* is a primary cause of disease in these locations or colonizes only after other agents initiate disease is not known. In our study, we have used IHC staining to detect *H.pylori* in cholecystectomy specimens and tried to find if there is any association between presence of gall stones and *H.pylori* detection.

The list of diseases associated with *H.pylori* is also expanding day by day [2,8,9]. Ischemic heart disease, idiopathic thrombocytopenic purpura, iron-deficiency anaemia are to name a few from a long list [10]. There is sufficient evidence of presence of other *Helicobacter* species like "*H. heilmannii*" in the stomach, intestine, and biliary tract [2]. They appear to cause the same diseases as *H.pylori* [2]. *H.pylori* has a number of virulence factors which influence bacterial colonization and disease severity. One of the most important and best-studied virulence factor is CagA, encoded by cytotoxin-associated gene A (cagA) [2]. Another toxin, vacuolating toxin A (VacA), facilitates nutrient acquisition thereby improving the ability of *H.pylori* to colonize the gastric epithelium [2,11].

There are various invasive and non-invasive methods available for detection of *H.pylori*. The invasive tests include endoscopy and histopathological examination of biopsy specimen (using routine, special and immunohistochemical stains), culture and rapid urease test. The non-invasive techniques are serology, the urea breath test and detection of *H.pylori* antigen in stool [12].

There are several studies, from different parts of the world, which have documented the presence of *H.pylori* in gall bladder [13-15]. Zhou D et al., reported isolation of *Helicobacter pylori* from 20.55% of chronic cholecystitis specimens [16]. They had done culture, H&E and warthin-starry stain for *H.pylori*. They also have done IHC staining for inducible nitric oxide synthase (iNOS) and reactive oxygen species (ROS) and PCR for *Helicobacter* 16s rRNA gene in gall bladder mucosa for detection of *H.pylori* [16]. They concluded that *H.pylori* infection in gallbladder mucosa shows strong association with presence of *H.pylori* in stomach [16]. In their study patients with *H.pylori* positive gallbladders had higher incidences of premalignant lesions including adenomyomatosis and metaplasia and increased levels of iNOS [2,16].

The study by Chen DF et al., opined that levels of interleukin-1, 6 and 8 (IL-1, 6 and 8) in gallbladder mucosa homogenates were significantly higher in *H.pylori*-infected cholecystitis group than *H.pylori*-negative cholecystitis group and control group [1]. According to them, *H.pylori* participates in and aggravate cholecystitis, cause destruction of epithelial cells and atrophy of the gallbladder. They opined that *H.pylori* infection in the gallbladder may be one of the etiological factors leading to cholecystitis [1].

We have noted inflammatory cell infiltrates and fibrosis in most of the cases, the degree of which varied from case to case. The chronic inflammatory cell infiltrate included mostly lymphocytes admixed with plasma cells. In few cases, eosinophils and neutrophils were admixed with chronic inflammatory cells. There was no significant difference between *H.pylori* positive and negative cases as regard to type and amount of inflammatory infiltrate. We did not find metaplastic changes (intestinal and pyloric) in those cases which showed positivity for *H.pylori* immunostain. This can be explained by the hypothesis of Chen DF et al., that many epithelial cells of the gastrointestinal tract have receptors for *H.pylori* colonization factors [1]. Therefore, *H.pylori* can colonize the epithelial cells of the gallbladder mucosa even without gastric metaplasia [1].

Chen DF et al., observed that in 7.1% patients with chronic calculus cholecystitis, *H.pylori* was positive by immunohistochemistry using anti-*H.pylori* antibodies. They explained that this rather low level in gall bladder may be due to poisonous effect of unconjugated bile salts on *H.pylori*. *H.pylori* is sensitive to bile salts, and cannot live in an environment with bile salts [3].

In contrast, a study from Egypt by Helaly GF et al., showed 73.3% and 66.7% positivity among gall bladder neck and body biopsies respectively by immunohistochemistry. They observed a significant association between gastric and gall bladder *H.pylori* positivity. The authors have concluded that *H.pylori* infection may

be an etiological factor leading to cholecystitis. The source for gall bladder infection may be gastric colonization with *H.pylori*. *H.pylori* may act as a lithogenic component, especially in presence of pure pigmented gallstones [14]. Our study showed (10/45) 22% positivity for *H.pylori* by immunostain. Six of the cholecystitis cases which showed positivity for *H.pylori* had pigmented stones.

According to Apostolov E et al., there is high prevalence of *H.pylori* in gall bladder and liver. They had done *Helicobacter* DNA analysis and immunohistochemistry in support of their finding [15]. Because of this high prevalence they were skeptical about the infectious role of *Helicobacter* in patients with chronic cholecystitis [15].

These studies with contradictory results may have several explanations. Regional variations exist in humans as well as in *H.pylori* genotypes. The inconsistencies also may be due to the fact that *H.pylori* is a minor contributor or serves to exacerbate, rather than cause, a particular disease [2].

A recent Indian study noted the presence of *H.pylori* DNA in the gallbladder of carcinoma gallbladder patients. This study reported significant elevation of circulating levels of cytokines like interleukin 1-β(IL-1β) and tumour necrosis factor-α (TNF-α) in the *H.pylori*-positive gall bladder carcinoma patients [17].

A study from Pakistan demonstrated *H.pylori* DNA in cases of chronic cholecystitis and gall bladder carcinoma associated with cholelithiasis [13]. A Thai study suggested that *H.pylori*, especially the cagA-positive strains, may be involved in the pathogenesis of cholangiocarcinoma. The mechanism of pathogenesis may be due to increased biliary cell inflammation and proliferation [18]. There was significant correlation noted between cagA gene of *H.pylori* in patients with cholangiocarcinoma and cholelithiasis in their study [17].

In the present study there was no case of primary carcinoma of gall bladder. In one case the gall bladder was secondarily infiltrated by adenocarcinoma of stomach. Though six of our cholecystectomy cases with presence of pigmented stones also showed *H.pylori*, it was not significant statistically.

CONCLUSION

We found presence of *Helicobacter pylori* in gall bladder by means of immunohistochemical stain. Particularly, in gall bladders with pigmented stones, presence of *H.pylori* was detected. However, the limitation of our study is small sample size. A study with a larger sample size preferably including both non-neoplastic and neoplastic gall bladders and with relevant serological and molecular studies will help to arrive at a definite conclusion regarding the relationship between presence of *H.pylori* and various disorders of gall bladder.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.
2. Assistant Professor, Department of Surgical Gastroenterology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.
3. Additional Professor, Department of Surgical Oncology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.
4. Assistant Professor, Department of Surgical Gastroenterology, Sri Venkeateswara Institute of Medical Sciences, Tirupati, India.
5. Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.
6. Assistant Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.
7. Assistant Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rashmi Patnayak,
Assistant Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences,
Tirupati, Andhra Pradesh-517507, India.
E-mail: rashmipatnayak2002@yahoo.co.in

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