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Original Research Paper

Pathology

HELICOBACTER PYLORI INFECTION AND ITS ASSOCIATION WITH HISTOMORPHOLOGICAL CHANGES IN THE GALL BLADDER OF PATIENTS WITH CHRONIC CHOLECYSTITIS

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ABSTRACT Role of H.pylori in chronic gastritis, peptic ulcers, gastric carcinoma and malignant lymphoma of gastric mucosa associated lymphoid tissue (MALToma) is well established. However its role in gall bladder diseases is still not clear. The aim of this study is to find the prevalence of H.pylori in patients with symptomatic cholelithiasis and to assess its role in various morphological changes in gall bladders of the patients with chronic cholecystitis.

Our study comprised of 150 patients operated for chronic cholecystitis with cholelithiasis , with100 patients giving consent for gastric biopsies. Patients were divided into two groups depending on the presence and absence of H.pylori in gall bladder mucosa respectively. Histopathological changes were then assessed in both the groups and findings were analysed statistically. On comparing the morphological changes in gall bladders of patients in both groups, statistically significant difference were seen in mucosal hyperplasia (P=0.01926), mucosal metaplasia (P=0.01890) and in lymphocytic infiltrate (P=0.0307). Concomitant presence of H.pylori in gastric and gallbladder mucosa also showed positive correlation. As these lesions are believed to be key factor for the progress of numerous cancers, so the presence of H.pylori may be considered as potential risk factor for gallbladder cancers.

KEYWORDS : H.pylori, gall bladder, gastric, histomorphology

INTRODUCTION

Chronic cholecystitis is a disease prevalent world over, the commonest cause being cholelithiasis. It is one of the commonest causes of surgical intervention in patients throughout the world. [1] There is a marked geographic variation in prevalence of gallstones, which are present in 10-15% of the American adult population. [2] Cholesterol stones predominate in developed countries while pigment stones are more commonly seen in Asia.[3]

Two third of world population is infected with Helicobacter pylori (H.pylori).[4] Role of H.pylori in chronic gastritis, peptic ulcers, gastric carcinoma and malignant lymphoma of gastric mucosa associated lymphoid tissue (MALToma) is well established.[5, 6]

Oxidative stress induced by H.pylori through the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is considered to be an important cause of chronic inflammation, ulcer and cancer of the stomach.The role of H.pylori in gallbladder diseases is still not clear. A recent study has demonstrated the presence of H. pylori in the bile and gallbladders of more than 75% of patients with gallbladder cancer and 50% of patients with chronic cholecystitis.[7] Presence of H. pylori in biliary tract suggests that stomach is not the only organ to be affected by it.

The aim of this study is to find the prevalence of H.pylori in patients with symptomatic cholelithiasis, to assess its role in various morphological changes in gall bladders of the patients with chronic cholecystitis and to correlate H.pylori infection in gastric mucosa and gallbladder .

MATERIALS AND METHODS

One hundred and fifty patients presenting with symptomatic cholecystitis with cholelithiasis who underwent cholecystect omy in the Department of Surgery of our medical college were included in this study. Only hundred patients gave consent for upper gastrointestinal endoscopy before laproscopic cholecystectomy. Multiple biopsies were taken from antrum and corpus in these patients. Patients with acute cholecystitis, tumours, and patients who had received standard triple therapy for H.pylori eradication were excluded from this study.

Laparoscopic cholecystectomy was performed in all the patients after doing the routine investigations and ultrasonography. The resected gallbladders were fixed in 10%buffered formalin. 4μ sections were cut and then stained with Haematoxylin-eosin stain. Giemsa stain for H.pylori was done in all the cases and immunohistochemical stain with polyclonal antibody was done to confirm the presence of H.pylori wherever required.

Patients were divided into two groups depending on the presence and absence of H.pylori in gallbladder mucosa. Group I comprised of patients who stained positive for H.pylori in gallbladder mucosa with Giemsa and immunostain. Group II included the patients who were negative for H.pylori both by Giemsa and immunostain. Histopathological changes were then assessed in both the groups and findings were analysed by Pearson's chi-square test .

Chronic inflammation was diagnosed by the presence of mucosal erosions, predominantly mononuclear inflammatory infiltrate, fibrosis, or metaplastic changes.

Mucosal erosion and atrophy when present were graded as mild, moderate and severe.

Degree of inflammatory mononuclear infiltrate was categorised as mild (diffuse, not more than 10 inflammatory cells per high power field (HPF) in any layer), moderate (diffuse, between 11- 30 cells per HPF), and severe (diffuse, more than 31 cells per HPF or follicular).

Degree of fibrosis was graded as mild, moderate and severe according to uneven collagen deposition being ≤ 20%, between 20-70% and ≥ 71 % respectively.

Less than 70% of metaplastic epithelium in the whole material, in a discontinuous fashion was considered focal. Metaplasia was then divided into different types- pyloric, intestinal.

Thickness of the muscular layers were graded as mildly thickened (less than one third of the whole thickness), moderately thickened (one third to two thirds of the wall), and severely thickened (more than two thirds of the wall thickness). Histopathological evaluation of gastric mucosa was done with Haematoxylin and Eosin stain .Giemsa and immunohistochemical stain with polyclonal antibody was done to detect H.pylori.

RESULTS

Of the 150 patients in our study group, female: male ratio was found to be 6:1 with mean age of the patients being 40.91±13.28 yrs. There were no malignant lesions in the study group. Presence of H.pylori in gallbladder mucosa was seen in 63 patients who formed Group I (Fig 1, 2).

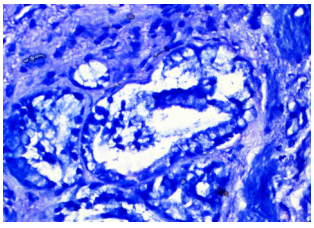


Fig1– Chronic cholecystitis with positive H.pylori in mucosal glands. (Giemsa, X 40)

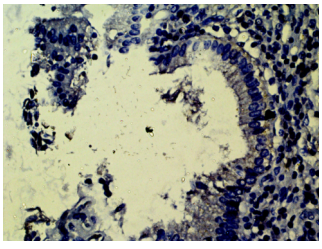


Fig. 2: Chronic cholecystitis with H.pylori on the mucosal surface.(Immunostaining, Polyclonal H .pylori antibody x40)

Group II comprised of 87 patients with no H.pylori seen in their mucosa. Mucosal erosions were the most common finding in both the study groups (Fig-3). It was seen in 93 patients [42(66.7%) in Group I and 51(58.6%) in Group II. This was further divided into mild, moderate and severe with most of the cases showing mild mucosal Presence of H.pylori in gallbladder mucosa was seen in 63 patients who formed Group I (Fig 1, 2). erosions in both groups. (Table 1)

Table 1: Histopathological findings in study groups

Histopathology	Degree	H.pylori status		Total N=150	P value
		Positive N= 63 (42%)	Negative N= 87 (58%)		
Mucosal Erosion	Absent	21 (33.3%)	36 41.4%	57	0.505
	Mild	24 (38.1%)	26 29.9%	50	
	Moderate	12 (19.0%)	20 23.0%	32	
	Severe	6 (9.6%)	5 5.7%	11	
Mucosal atrophy	Absent	42 66.7%	39 44.8%	81	0.045
	Mild	9 14.3%	15 17.2%	24	
	Moderate	5 7.9%	17 19.5%	22	
	Severe	7 11.1%	16 18.5%	23	
Mucosal hyperplasia	Absent	28 4.5%	63 72.4%	91	0.002
	Focal	30 47.6%	21 24.1%	51	
	Diffuse	5 7.9%	3 3.5%	8	
Mucosal dysplasia	Absent	54 85.7%	81 93.1%	135	0.137
	Low grade	9 14.3%	6 6.9%	15	
Mucosal metaplasia	Absent	39 61.9%	69 79.3%	108	0.019
	Present	24 38.1%	18 20.7 %	42	
Lymphoid infiltrate	Mild	27 42.9%	60 69.0%	87	0.005
	Moderate	24 38.1%	20 23.0%	44	
	Severe	12 19.0%	7 8.0%	19	
Muscular hypertrophy	Mild	33 52.4%	48 55.2%	81	0.470
	Moderate	21 33.3%	32 36.8%	53	
	Severe	9 14.3%	7 8.0%	16	
Fibrosis	Mild	36 57.1%	57 65.5%	93	0.405
	Moderate	18 28.6%	23 26.4%	41	
	Severe	9 14.3%	7 8.0%	16	

Mucosal atrophy was seen in 21(33.3%) patients in Group I and 48 (55.2%) patients in Group II.

Mucosal hyperplasia was present in 35 (55.5%) patients in Group I with focal hyperplasia seen in 30 (47.6%)cases and diffuse hyperplasia in 5(7.9%) cases.24(27.6%) patients in group II had mucosal hyperplasia with 21(24.1%) having focal and 3 (3.5%)with diffuse hyperplasia Metaplasia was seen in 21(33.3%) in Group I patients and 15 (17.2%) in Group II patients. Commonest metaplasia was pyloric type present in 12(57.1%) patients in Group I and 5(33.3%) patients in Group II. Intestinal metaplasia was seen in 3 patients in Group I and Group II each.

Dysplasia of low grade was seen in 9 (14.3%) patients in Group I and 6 (6.9%) patients in Group II.

Lymphoid infiltration was seen in all the cases with most of the patients having mild to moderate degree of mononuclear infiltration. Lymphoid follicle formation was seen in few cases. Muscular hypertrophy and fibrosis was present in all the patients with more than 50% of cases in both the groups were having mild hypertrophy as well as mild fibrosis.(Fig -3)

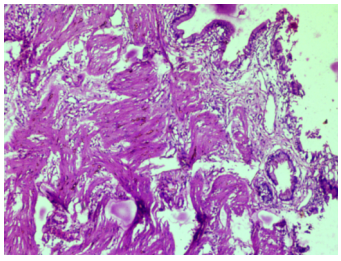


Fig 3- Ulcerated gall bladder mucosa with hypertrophy of muscle layer. (H&E, X40)

Statistically significant difference between the two groups were seen in mucosal hyperplasia (P=0.002)), mucosal metaplasia (P=0.019) as well as in lymphocytic infiltrate (P=0.005).

Out of 100 cases of gastric biopsies ,61 cases (61%) shows presence of H.pylori. In 35 patients h.pylori was positive in both gastric and gall bladder mucosa and 30 patients were negative for h.pylori in both gastric and gall bladder mucosa. Association of H.plori in gastric mucosa and gall bladder

mucosa was found to be stastically highly significant. (p=0.0007)(Table-2)

Table 2:Correlation of gallbladder mucosa and gastric mucosa with H.pylori infection

Histopathology		H. pylori status for Gastric Biopsy			P value
		Positive	Negative	Total	
H. pylori status for GB Biopsy	Positive	35	9	44	0.0007
	Negative	29	30	56	
	Total	61	39	100	

DISCUSSION

Eighty percent of the adult population in developing countries are infected with H.pylori. [8] ,[5] in 1996 were first to confirm the presence of H.pylori in gall bladder mucosa. Several studies have since then examined the relationship between the presence of H. pylori in the gallbladder and biliary diseases.

In Indian subcontinent, a high prevalence of H.pylori is reported in nearly all gastro-duodenal diseases.[9] Zhou D et al;2013[1], reported isolation of H. pylori from 20.5% of chronic cholecystitis specimens using culture, H&E, Warthin-starry stain, immunohistochemical staining and PCR for H.pylori.

In our study H.pylori was found in 42% of the patients with chronic cholecystitis with cholelithiasis. Helaly et al;2014 [10] in their study also showed the presence of H. pylori in almost 40.9% of samples in patients with chronic calcular cholecystitis. They also found a significant association between gastric and gall bladder H.pylori positivity. The source for gall bladder infection may be gastric colonization with H.pylori. They suggested that H.pylori may act as a lithogenic component, especially in presence of pure pigmented gallstones.

In our study out of hundred cases of gastric biopsy,61 cases were positive for H.pylori. 35 cases(35%) were positive for both gall bladder and gastric mucosa and 30 cases (30%) were negative for both gall bladder and gastric mucosa(Table -2). Positive correlation was seen between the concomitant presence of H.pylori in gastric and gallbladder mucosa. A study by Attaallah et al; 2013 found 43% of the patient showed H.pylori positivity in both gall bladder and gastric mucosa [11] Javaherzadeh et al;2016 reported 14% patients who were positive for H.pylori in gastric as well as gall bladder mucosa, this difference in result from our study may be due to different population group and people with different socio-economic status.[12]

Bulajic et al;2002[13] aimed to assess the association between H. pylori and biliary tumours and found a significant relationship between the presence of H.pylori in the stomach and gallbladder with age and the clinical diagnosis. The presence of this bacterium was 9.9 times more in patients with biliary carcinoma. This showed a strong association between the presence of H.pylori and biliary carcinoma. This may be due to release of several toxins and metabolites of recognised carcinogenic potentials by helicobacters. Gallstones along with toxins produceed by helicobacter can thus have a detrimental effect on gall bladder mucosa. [14, 15]

In our study, analysis of histological changes in the presence and absence of H.pylori in gall bladder mucosa was done. It was found that presence of mucosal hyperplasia and mucosal metaplasia show statistically significant correlation with H.pylori positivity in gall bladder mucosa. The presence of lymphoid infiltrate also showed significant correlation. The finding was found to be similar to the study by Hassani et al;2015 [16] who in there study analysed the presence of H.pylori with atypical histo-morphological changes such as metaplasia or the presence of lymphoid infiltration and displayed statistical correlation in analogy to patients lacking bacteria. The presence of lymphoid follicles can associated with development of MALT Lymphoma in stomach by infection with helicobacter spp . This is assumed to be due to stimulation of lymphocytes and the propagation of lymhocytic clones by helicobacter.

Patnaik R et al;2016 [17] however in their study found no significant difference between H.pylori positive and negative cases as regard to type and amount of inflammatory infiltrate. They also did not find metaplastic changes (intestinal and pyloric) in the cases showing positivity for H.pylori immunostain. The finding was explained by the hypothesis of Chen DF et al;2007 [18] that many epithelial cells of the gastrointestinal tract have receptors for H.pylori colonisation factors. Therefore, H.pylori can colonise the epithelial cells of the gallbladder mucosa even without gastric metaplasia.

Zhou et al., 2013[1] in their study showed that the patients with H.pylori positive gallbladders had higher incidences of premalignant lesions including adenomyomatosis and metaplasia and increased levels of iNOS (inducible nitric oxide synthetase). However, presence of hyperplasia and dysplasia did not show any significant correlation with H.pylori colonisation in gall bladder mucosa.

André de Moricz et al., 2010[15] emphasized that pre-neoplastic histological changes were found in the mucosa of the gallbladder, restricted to infection with Helicobacter spieces, for instance, intestinal metaplasia, hyperplasia, dysplasia, eosinophilic inflammation, and hyalinosis.

However, some of the studies failed to demonstrate any increase in risk of gallbladder carcinoma in the existence of H. bilis or H. pylori. [19,20].

In our study we found significant difference in mucosal hyperplasia, metaplasia, and lymphoid infiltrate between the two study groups. Though the presence of dysplasia was found to be higher in gallbladders with H.pylori infection as compared to the other group, but the difference was not statistically significant. This may be due to less number of cases of dysplasia in our study.

Thus we concluded that there is positive correlation between H.pylori infection in gastric and gall bladder mucosa . H.pylori increases various mucosal lesions in gallbladder, such as mucosal hyperplasia, metaplasia and increased lymphoid infiltrate. Chronic inflammation leads to DNA damage, cell death and enzymatic changes. As these lesions are believed to be key factors for the progress of numerous cancers, so the presence of H.pylori may be considered as potential risk factor for gallbladder cancers.However further studies with more cases of dysplasia and including gall bladder carcinomas and with various proliferative markers in H.pylori infected gall bladder mucosa is recommended.

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