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# A Study on Helicobacter pylori infection in patients with Calculous Cholecystitis

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***Abstract:***

***Background:*** *There has been alarming rise in the number of gallbladder calculi cases in Northeast India in past few years and this can be attributed to the fast changing life style and food habits. Helicobacter pylori, a gram negative bacillus has been recognised as a public health problem and approximately half of the world population has H.pylori infection. Recently, the bacterium has been implicated as a risk factor for various extra-intestinal diseases including hepatobiliary diseases ranging from calculous cholecystitis and primary biliary sclerosing cholangitis to gall bladder cancer and primary hepatic carcinomas.[5] Hence we conducted a study with the aim to see the association of H.pylori infection in gallbladder and stomach or duodenum in patients with calculous cholecystitis and to compare Gallbladder histology in those with Gallbladder H. pylori positive and negative groups.*

***Materials and Methods:*** *This Study was conducted in Departments of General Surgery and Pathology, NEIGRIHMS, Shillong for a duration of one and half years. Prior to cholecystectomy, all patients enrolled in this study underwent upper GI endoscopy with biopsy in order to see the infection status of H.pylori in their stomach and duodenum. Cholecystectomy was usually performed laparoscopic; the open cholecystectomies were those that were converted from laparoscopic to open; the biopsy from Gallbladder mucosa was subjected to urease test for H.pylori in the operation theatre. Rest of the Gallbladder was sent for histopathological examination to the Pathology department.*

***Results:*** *Out of 50 patients in the study, there were 10 male and 40 female patients, age ranged from 18 to 64 years (mean =38.3). Out of these 50 patients, 24 were diagnosed to be having H. pylori (46%) either in the stomach, duodenum or gallbladder. Out of the H.pylori positive cases, gallbladder positivity was found in 13 cases, gastric antral positivity in 24 cases and duodenal positivity in 12 cases. In those 13 cases of gallbladder positive for H. pylori infection , all (13/13) cases had H. pylori positive antrum and 6/13 cases had duodenum positivity for H.pylori and all (13/13) patients had positive finding on upper GI endoscopy.*

***Conclusion****: H.pylori in gallbladder is always associated with H.pylori in the stomach and this association was significant. The source of H.pylori in the gallbladder maybe the stomach. Unlike the significant association between gallbladder positivity and antral positivity for H.pylori , the same is not true for gallbladder and duodenal positivity. Chronic cholecystitis with pyloric metaplasia was noted in 2/13 cases (15.38%) and was associated with H.pylori positive gallbladders only, however the corelation was statistically insignificant.*

***Key Words****: H.pylori, cholelithiasis, UGIE, gallbladder, antrum,duodenum, urease test*

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## I. Introduction:

Cholecystitis is defined as inflammation of the gallbladder. About 90% of cases involve stones in the gallbladder (i.e., calculous cholecystitis) with the other 10% of cases representing acalculous cholecystitis.[1] There has been alarming rise in the number of gallbladder calculi cases in Northeast India in past few years and this can be attributed to the fast changing life style and food habits. *Helicobacter pylori,* a gram negative bacillushas been recognised as a public health problem and approximately half of the world population has

*H.pylori*infection. It causes chronic gastritis, peptic ulcer disease and gastric malignancies.[2,3] The prevalence is thought to be 80% in developing countries and 30–50% in developed countries.[4]

Recently, the bacterium has been implicated as a risk factor for various extra-intestinal diseases including hepatobiliary diseases ranging from calculous cholecystitis and primary biliary sclerosing cholangitis to gall bladder cancer and primary hepatic carcinomas.[5]

Hence we conducted a study with the aim to see the association of *H.pylori* infection in gallbladder and stomach or duodenum in patients with calculous cholecystitis and to compare Gallbladder histology in those with Gallbladder *H. pylori* positive and negative groups.

## II. Materials And Methods:

This Study was conducted in Departments of General Surgery and Pathology, NEIGRIHMS, Shillong for a duration of one and half years.

**Inclusion Criteria:** All patients with cholelithiasis proven on Ultrasonography and undergoing cholecystectomy

**Exclusion Criteria:** Patients with history of cholecystectomy. Patients on *H. pylori* eradication therapy within 6 months.Patients refusing cholecystectomy/managed conservatively. Patients with only CBD calculi but no cholelithiasis.Patients with acute calculus cholecystitis. Patients undergoing subtotal cholecystectomy.

**Procedure:**

Prior to cholecystectomy, all patients enrolled in this study underwent upper GI endoscopy with biopsy in order to see the infection status of *H.pylori* in their stomach and duodenum. The upper GI endoscopy was done in General Surgery department.

**Standard procedure of Upper GI endoscopy :**

The patients were advised to fast for 12 hours prior to the procedure and informed consent was taken from the patient. Oral 4% lignocaine spray was given to the patient 5-10 minutes before the procedure for the local anaesthetic effect. Only a few patients were given 2 mg midazolam intravenously for sedation depending on the preference of the consultants. The upper gastro-intestinal endoscopy was performed with flexible videoendoscope(Fujinon SN9G246A152) with patients in left lateral position.

**Fig 1: Flexible videoendoscope used for the study**

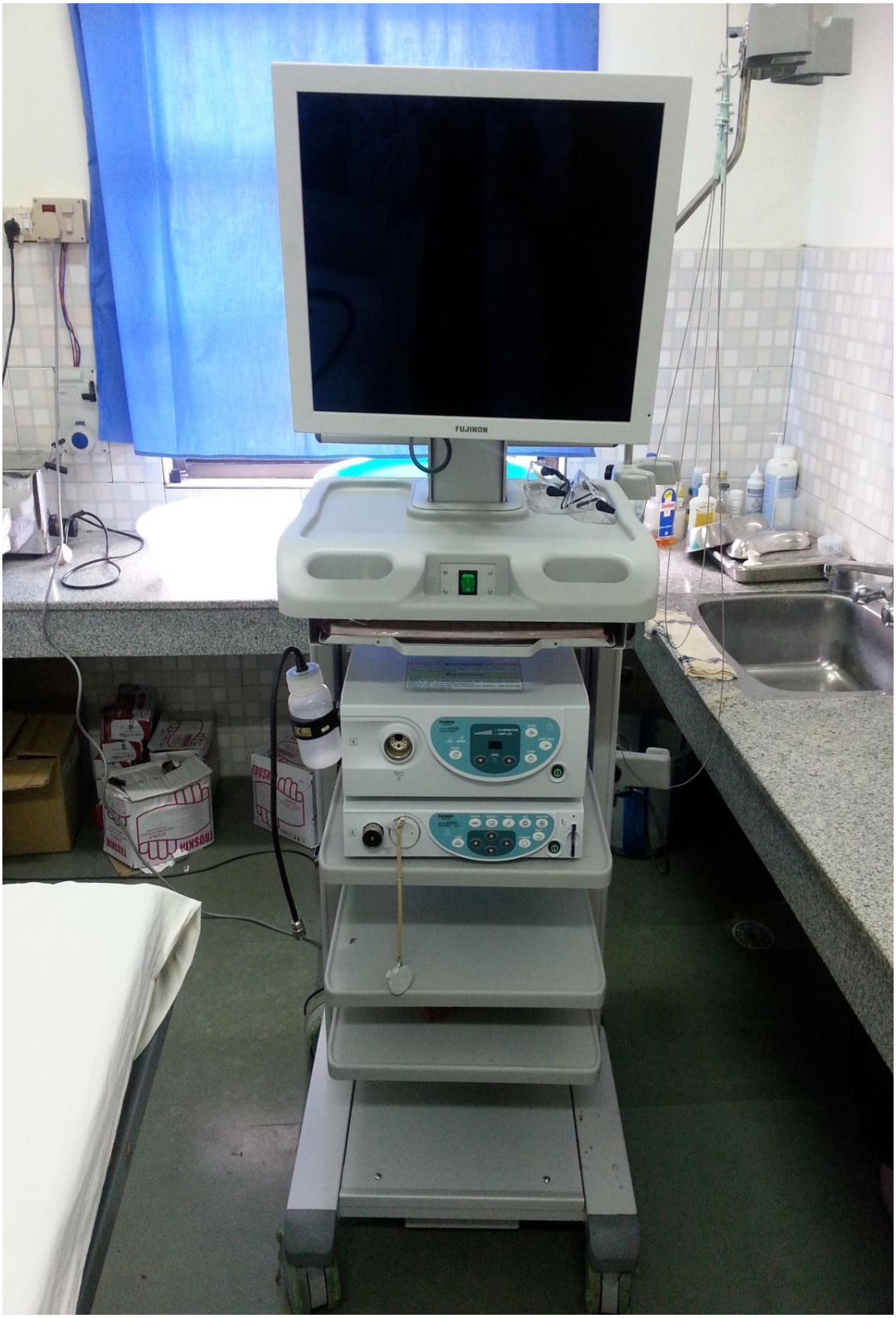


**Fig 2: Fujinon endoscopy machine used for the study**

On entering the stomach, presence of any ulcers or growth were looked for.Then the duodenum was entered up to its second part and any evidence of duodenitis or ulceration was looked for.

Two endoscopic biopsies each were taken, from the gastric antrum and other from 1st part of duodenum from diametrically opposite areas in both with and without ulceration.

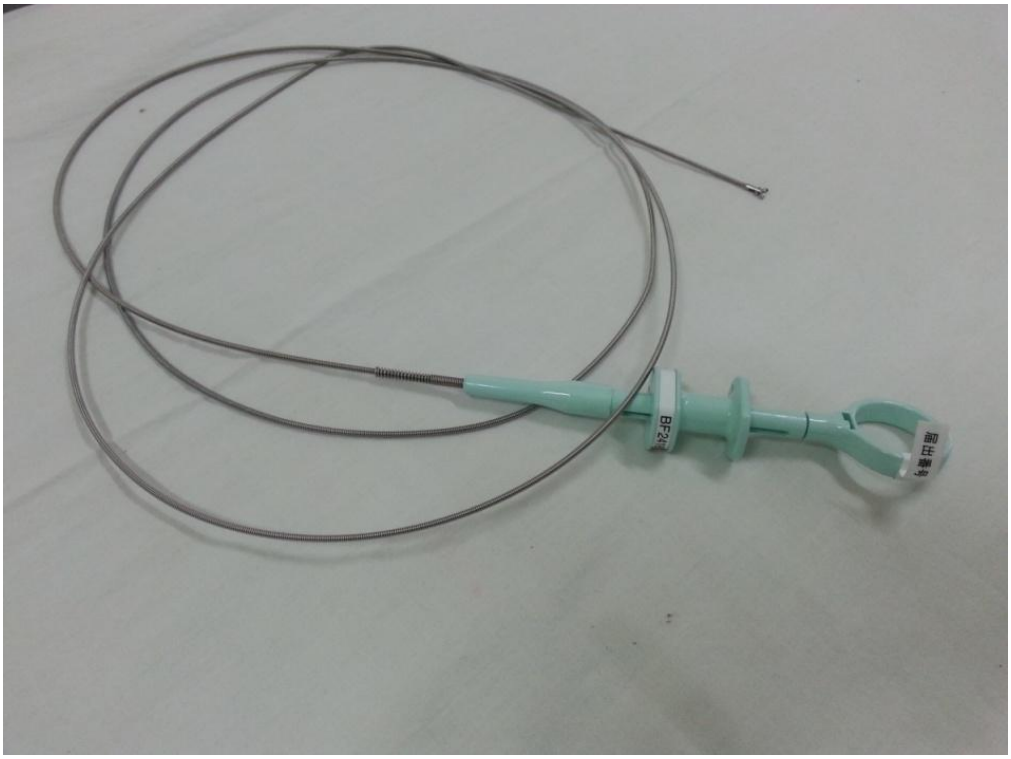

**Fig 3: Endoscopic biopsy forceps used for the study**

Both set of biopsy specimen were subjected to urease test for *H.pylori* separately.

Cholecystectomy was usually performed laparoscopic; the open cholecystectomies were those that were converted from laparoscopic to open; the biopsy from Gallbladder mucosa was subjected to urease test for *H.pylori* in the operation theatre. Rest of the Gallbladder was sent for histopathological examination to the Pathology department.


Ammonia + Phenolic chromogen + hypochlorite → Green coloured complex

Urease if present in the sample hydrolyses urea to ammonia and water. The ammonia formed further reacts with a phenolic chromogen and hypochlorite to form a green coloured complex. (CREST BIOSYSTEMS)


**Fig 4: Positive tube Urease test with gallbladder mucosa on right test tube**

In the control tube we had added urea solution and one drop of urease enzyme present in the kit and chromogen solution to yield the green coloured complex. In the test side we had added the urea solution and the sample tissue (either gallbladder/antral/duodenal mucosa) and then added the chromogen solution. And the test was considered to be positive if it turned into a green coloured solution ,the colour of which was compared to the control test tube as shown in Fig:4. The positive test indicated the presence of urease and hence *H.pylori* in the test sample.

**Statistical analysis**: Data were analysed by using INSTAT software (Graphpad prism software, Inc,. La Zolla, CA. USA). Demographic data were studied using unpaired Student's t-test. The student t-test of significance/chi square test was used wherever deemed necessary. The p-value $\leq 0.05$ was taken as significant.

## III. Results And Observations:

Out of 50 patients in the study, there were 10 male and 40 female patients, age ranged from 18 to 64 years (mean =38.3). Out of these 50 patients, 24 were diagnosed to be having *H. pylori* (46%) either in the stomach, duodenum or gallbladder. It was seen that only 2 out of 10 males and 22 out of 40 females harbored*H.pylori* infection. Out of the *H.pylori* positive cases, gallbladder positivity was found in 13 cases, gastric antral positivity in 24 cases and duodenal positivity in 12 cases. In those 13 cases of gallbladder positive for *H. pylori*infection , all (13/13) cases had *H. pylori* positive antrum and 6/13 cases had duodenum positivity for *H.pylori* and all (13/13) patients had positive finding on upper GI endoscopy.

**Table 1: Organ-wise distribution of *H.pylori* positivity**

| *H.Pylori* | Gallbladder (n=50) | Antrum (n=50) | Duodenum (n=50) |
|---|---|---|---|
| Positive | 13 | 24 | 12 |
| Negative | 37 | 26 | 38 |

**Table 2: Relationship between *H.pylori* status in gallbladder and their antrum and duodenum *H.pylori* status**

| Gallbladder *H.pylori* status (n=50) | Corresponding Antrum | | Corresponding Duodenum | |
|---|---|---|---|---|
| | positive | negative | positive | negative |
| Positive (n=13) | 13 | 0 | 6 | 7 |
| Negative (n= 37) | 11 | 26 | 6 | 31 |

**Table 3: Comparison of endoscopic Findings with *H.pylori* status**

| **Endoscopic Findings** | **No. of pts (n=50)** | ***H.pylori*** | | | |
|---|---|---|---|---|---|
| | | **Antrum** | | **Duodenum** | |
| | | **+ve** | **-ve** | **+ve** | **-ve** |
| Normal | 22 | 0 | 22 | 0 | 22 |
| Antral Gastritis only | 12 | 11 | 1 | 0 | 12 |
| Antral Gastritis with duodenitis | 7 | 6 | 1 | 6 | 1 |
| Healed Duodenal Ulcer | 2 | 2 | 0 | 2 | 0 |
| Duodenitis only | 4 | 3 | 1 | 3 | 1 |
| Healed Antral Ulcer | 2 | 1 | 1 | 0 | 2 |
| Pangastritis with duodenitis | 1 | 1 | 0 | 1 | 0 |

Among the 13 cases of *H. pylori* positive gallbladders subjected to histopathological examination; 10 showed chronic cholecystitis; 2 showed chronic cholecystitis with pyloric metaplasia and 1 showed chronic cholecystitis with foci of cholesterosis. Among the 37 cases of negative *H. pylori* gallbladders subjected to histopathological examination; 27 showed chronic cholecystitis , 7 showed chronic cholecystitis with foci of cholesterosis, 3 showed chronic active cholecystitis, however none showed any evidence of pyloric metaplasia.

**Table 4: Histopathological findings in *H.pylori* positive gallbladder cases**

| | |
|---|---|
| Chronic cholecystitis with pyloric metaplasia | 2 |
| Chronic cholecystitis with foci of cholesterosis | 1 |
| Chronic cholecystitis | 10 |

**STATISTICAL ANALYSIS:**

**Table 5: Correlation between *H.pylori* status in gallbladder and antrum**

| | **Antrum$^{(+)}$** | **Antrum$^{(-)}$** | **No. of cases** | **P value** |
|---|---|---|---|---|
| Gb$^{(+)}$ | 13 | 0 | 13 | <0.0001 |
| Gb$^{(-)}$ | 11 | 26 | 37 | |
| Total | 24 | 26 | 50 | |

The correlation between *H. Pylori* infection in antrum and gallbladder was considered to be statistically significant ($p<0.0001$) .

**Table 6: Correlation between *H.pylori* status in gallbladder and duodenum**

| | Duodenum$^{(+)}$ | Duodenum$^{(-)}$ | No. of cases | P value |
|---|---|---|---|---|
| Gb$^{(+)}$ | 6 | 7 | 13 | 0.0550 |
| Gb$^{(-)}$ | 6 | 31 | 37 | |
| Total | 12 | 38 | 50 | |

However the correlation between *H. Pylori* status in gallbladder and duodenum was also analysed but it was not statistically significant with p value of 0.0550.

**Table 7: Correlation between *H.pylori* status in gallbladder and pyloric metaplasia on histopathology**

| | Chronic cholecystitis with Pyloric metaplasia | Chronic cholecystitis with no Pyloric metaplasia | No. of cases | P value |
|---|---|---|---|---|
| $Gb^{(+)}$ | 2 | 11 | 13 | 0.0637 |
| $Gb^{(-)}$ | 0 | 37 | 37 | |
| Total | 2 | 48 | 50 | |

The correlation between *H. Pylori* status in gallbladder and histopathological finding of pyloric metaplasia was analysed but it was not statistically significant with p value of 0.0637.

**Table 8: Correlation between UGIE findings and *H.pylori* infection**

| | UGIE findings | | | | | | |
|---|---|---|---|---|---|---|---|
| | Antral Gastritis Only (n=12) | Antral Gastritis with Duodenitis (n=7) | Duodenitis Only (n=4) | Healed Ulcers (n=4) | Normal (n=22) | Pangastritis with duodenitis (n=1) | P value |
| *H.pylori*$^{(+)}$ | 11 | 6 | 3 | 3 | 0 | 1 | <0.0001 |
| *H.pylori*$^{(-)}$ | 1 | 1 | 1 | 1 | 22 | 0 | |

The correlation of different UGIE findings and presence or absence of *H.pylori* was also analysed statistically and correlation was found to be significant with p value of <0.0001. All the 22 normal UGIE finding cases were found to be negative for *H.pylori* infection. Among the 28 UGIE cases with positive findings, 24 cases (85.71%) were found to harbour *H.pylori*.

## IV. Discussion:

The presence of *H.pylori* in gallbladder mucosa was first confirmed by Kawaguchi et al[6] in 1996. However, although *H.pylori* has been found 3.5 times more frequently in presence of chronic cholecystitis, whether this agent contributes in the pathogenesis of this biliary disease is still poorly understood. Firstly, it is difficult to verify the potential entry routes of *H.pylori* to the gallbladder including either ascending duodenum infection or the portal system circulation pathway. Secondly, since successful demonstration of *H.pylori* in gallbladder was mostly based on the indirect detection methods such as PCR for *H.pylori* specific components rather than direct bacterial culture, it is possible that *H.pylori* is only a ‘‘stagger’’ but not an ‘‘invader’’ in biliary system. In the present study, we used the rapid urease test on the antral,duodenal and gallbladder mucosa to demonstrate the presence of *H.pylori* because it is rapid, reliable and inexpensive method .

***H.pylori* in gallbladder and Stomach/duodenum**

In the present study, the gallbladder positivity for *H.pylori* was 13 out of 50 cases (26%) of chronic cholecystitis whereas Wafi Attaallah et al [7] demonstrated the presence of *H. pylori* in the gallbladder in 37% of patients (35 cases of 94) with symptomatic gallstones (with either Giemsa, Immunohistochemistry or rapid urease test) and Di Zhou et al [8] found *H. pylori* infection in gallbladder mucosa in 20.55% (67/259) of the cholecystitis patients (either by culture, Warthin- Starry staining or PCR). M. Shirin Sabbaghian et al[9] showed evidence of *Helicobacter* spp. in 12 of 36 patients with benign gallbladder disease, suggesting that *Helicobacter* spp. were involved in 33.3% of patients studied. They used PCR for identification of *Helicobacter spp* in bile and gallbladder tissue of patients with benign gallbladder disease. Arshad Hussain Abro et al[10] had found the presence of *H.pylori* in resected gallbladder by rapid urease test and gram smear and in serum by means of serology. But they hadn’t correlated it with the presence of *H.pylori* in stomach or duodenum. Dong-Feng Chen et al[11]using Warthin-Starry silver stain found that *H.pylori* could be seen in 71 (13.6%) out of 524 specimens of calculous cholecystitis. *H. pylori* can damage human gallbladder epithelial cells and could be the key factor that leads to clinical cholecystitis. A meta-analysis of published work has shown strong association between *H.pylori* and gall bladder disorders.[12]

In the present study we found there were 24 out of 50 cases (48%) with *H.pylori* positivity either in antrum, duodenum or gallbladder. Out of the 50 cases of chronic cholecystitis included in the present study 13 (26%) had gallbladder positive for *H.pylori*. In all these 13 cases of gallbladder positive for *H. pylori*, antrum also was positive for *H.pylori*; this finding was found to be statistically significant with a p-value of <0.0001.

Di Zhou et al [8] and Wafi Attaallah et al[7] hypothesized that *H.pylori* in the gastrointestinal system might be a potential candidate for increasing the risk of chronic inflammation of the gallbladder and that *H. pylori* might reach the biliary system via sphincter of Oddi by the reflux mechanism. Also chronic *H.pylori* infection of the gallbladder may impair gallbladder contractility and leading to increase in the precipitation of bile components to form stones.

Thus the present study, like that of Wafi Attaallah et al[7] and Di Zhou et al[8] indicates that *H.pylori* positivity in gallbladder is definitely related to *H.pylori* positivity in gastric antrum which appears to support the hypothesis.

We found that in 13 of those gallbladders positive for *H.pylori* cases ; 6 cases also were positive for *H.pylori* in the duodenum. We also analysed the correlation between *H.pylori* infection in gallbladder and duodenum alone and it showed a p-value of 0.0550 which was not statistically significant . All the above 6 cases of duodenal positivity were associated with antral positivity for *H.pylori* . We could not find similar published data in English literature to compare the association of gallbladder positivity with duodenal positivity. It is difficult to verify the potential entry routes of *H.pylori* to the gallbladder including either ascending duodenum infection or the portal system circulation pathway.

Di Zhou et al[8]in their study had compared the pathological features between the two groups in gallbladder mucosa (*H.pylori* positive and *H. pylori* negative). Metaplasia was found in 9/67 (13.43%) cases of *H.pylori* positive gallbladder as compared to 14/259 (5.41%) *H.pylori* negative gallbladders and this difference was statistically significant (p= 0.022). The metaplastic lesions were predominantly of pyloric type (21 cases, 84% from the total of metaplastic cases), characterized by structures similar to the pyloric glands in the lamina propria. Intestinal type which was characterized by the presence of goblet cells and enterocyte-like cells, was detected in only 4 cases (16%) of all the metaplastic patients. No difference was found in the distribution of the pyloric and intestinal metaplastic types between the two groups of *H.pylori* positive and negative gallbladders (p=0.602). In the present study even though we have found pyloric metaplasia only in *H.pylori* positive gallbladders it was statistically insignificant (p=0.0637) in contrast to Di Zhou et al[8] which may be attributed to small sample size.

## V. Conclusion:

*H.pylori* in the gallbladder was found to be positive in 26% of cases with chronic cholecystitis. *H.pylori* in gallbladder is always associated with *H.pylori* in the stomach and this association was significant. The source of *H.pylori* in the gallbladder maybe the stomach. Unlike the significant association between gallbladder positivity and antral positivity for *H.pylori* , the same is not true for gallbladder and duodenal positivity. Chronic cholecystitis with pyloric metaplasia was noted in 2/13 cases (15.38%) and was associated with *H.pylori* positive gallbladders only, however the corelation was statistically insignificant.When positive UGIE findings were found, *H.pylori* was present in majority of the cases with a significant association.

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