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Study of association of *H. pylori* infection of the gall bladder and calculous cholecystitis

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Abstract

Background: Helicobacter pylori infection of the gallbladder has been shown to increase the precipitation of cholesterol to form stones. Also it has been shown that urease induced calcium precipitation by Helicobacter species may initiate gallstone formation. This has raised a question about the possible role of Helicobacter species in the aetiology of gallbladder diseases especially cholelithiasis. Owing to conflicting results on the topic in the studies presently available and more so, only a few studies being reported in Northern India regarding this aspect and additionally having discordant findings. Our study was conceptualized in the Southern India scenario.

Methods: During 18 months study period those diagnosed with calculous cholecystitis with USG, patients underwent Laparoscopic Cholecystectomy, the gall bladder specimen sent for histopathological examination for the presence of *H. pylori* and the mucosal changes associated with infection of the gallbladder with Helicobacter pylori.

Results: No statistical significance was seen between *H. pylori* and gender. Among those having *H. pylori* majority were females. ($p=0.23$). No statistical significance was seen between *H. pylori* and type of stone. Among those having *H. pylori* majority were pigmented type. ($p=0.67$) High statistical significance was seen between *H. pylori* and Inflammatory mononuclear infiltrate. $P<0.01$, High statistical significance was seen between *H. pylori* and degree of fibrosis. $P<0.0001$, High statistical significance was seen between *H. pylori* and degree of hyperplasia. $P<0.003$.

Conclusions: There is no significant positive correlation between *H. pylori* infection and Calculous Cholecystitis. *H. pylori* infection is associated with high degree of hyperplasia, degree of fibrosis and Inflammatory mononuclear infiltrate of Gall bladder.

Keywords: *H. pylori*, calculous cholecystitis, histopathology, mucosal changes, degree of fibrosis, inflammatory mononuclear infiltrate

Introduction

The prevalence of Helicobacter infection in the digestive tract varies in different populations, suggesting epidemiological differences in the distribution of the organism in different regions. It infects 50% of the world's population [1, 2] and about 80% of the Indian population [3]. Recently, it has been found that Helicobacter pylori has certain relationship to some diseases in the organs other than stomach and duodenum [4]. Helicobacter pylori (*H. pylori*) infection of the gallbladder has been shown to increase the precipitation of cholesterol to form stones. Also it has been shown that urease induced calcium precipitation by Helicobacter species may initiate gallstone formation [5]. Of late, some studies have revealed the presence of Helicobacter pylori in hepatobiliary system with the prevalence of infection varying from 0% [6] to up to 70% [7].

Cholelithiasis is assumed to have multifactorial aetiology. The prevalence of gallstones is related to many factors including age, gender, and ethnic background with some unidentified genetic and environmental factors involved in the pathogenesis of gallstones [8]. This has raised a question about the possible role of Helicobacter species in the aetiology of gallbladder diseases especially cholelithiasis. Owing to conflicting results on the topic in the studies presently available and more so, only a few studies being reported in Northern India regarding this aspect and additionally having discordant findings [9, 10]. Our study was conceptualized in the Southern India scenario.

Objectives

- Primary objective: To determine the association of calculous cholecystitis and Helicobacter pylori infection of the gallbladder.

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▪ Secondary objective

- To co-relate the type of stone on the basis of its gross appearance with presence of *Helicobacter pylori*.
- To co-relate the mucosal changes due to *Helicobacter Pylori* infection of the gallbladder.

Methods

Study design: The present study type was a prospective Exploratory type of study.

Study area: A Tertiary care hospital.

Study setting: Surgery department of tertiary care hospital.

Study period: November 2019 to May 2021

Registration was done when patient got admitted in Surgery department. On registration the patients having exclusion criteria were not taken for the study. Objective of the study was to determine the association of calculous cholecystitis and *Helicobacter pylori* infection of the gallbladder, to co-relate the type of stone on the basis of its gross appearance with presence of *Helicobacter pylori* and to co-relate the mucosal changes due to *Helicobacter Pylori* infection of the gallbladder.

At registration, the basic information was enrolled especially with respect to clinical findings, sociodemographic factors, and all other investigations. Thus all patients enrolled were followed up in Surgery department till they were discharged. The data collected was analysed to study of association of *H. pylori* infection of the gall bladder and calculous cholecystitis.

Sample size: 250 cases

$$S = Z^2 * p * q / d^2$$

$$= 1.96 * 1.96 * 0.2 * 0.8 / 0.05 * 0.05$$

$$= 245 \sim 250$$

Study participant: Patients with calculous cholecystitis.

Inclusion criteria

- Age group 15 to 75 years
- Both gender
- Patients with clinically and radiologically proven calculous cholecystitis.
- Willing to participate

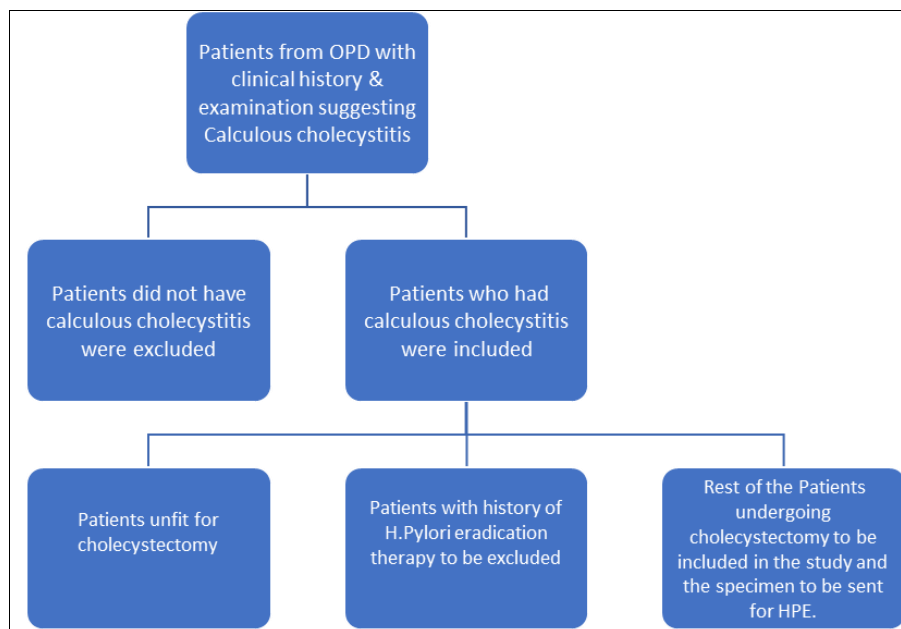
Exclusion criteria

- Patient with history of treatment helicobacter pylori eradication kit.
- Patient unfit for cholecystectomy.
- Not willing to participate

Data collection

Participants were told about the study and written informed consent was taken. Study participants coming to the outpatient department with differential diagnosis of chronic calculous cholecystitis and underwent confirmatory test i.e. USG for the confirmation of chronic calculous cholecystitis. These patients went through several tests for fitness for surgical procedure and the ones fit for laparoscopic cholecystectomy were taken for the procedure were studied.

Data was collected by using a pre designed questionnaire which consisted of standard questions related to clinical condition, socio demographic factors, addiction among family members, and so on, were interviewed. In addition, questions related to past and present medical history and health seeking behaviour were also studied. Clinical examination, diagnosis, investigations details of previous operative procedure was done.



The patients were diagnosed as Calculous Cholecystitis on the basis of clinical examination, laboratory examinations and ultrasonography report, and subjected to surgical treatment in the form of laparoscopic cholecystectomy.

After laparoscopic cholecystectomy Gall bladder was taken out in sterile conditions and put into a sterile container with formalin

and to be sent to the pathology department for the histopathological examination for the presence of *H. pylori* and the mucosal changes associated with infection of the gallbladder with helicobacter pylori.

Mucosal changes were graded with the help of the following table ^[5].

Pathological Changes of Chronic Cholecystitis	Definition
Inflammatory mononuclear infiltrate	
Mild	Diffuse, ≤ 10 inflammatory cells per HPF in any layer
Moderate	Diffuse, between 11 to 30 cells per HPF
Severe	Diffuse, more than 31 cells per HPF or follicular
Degree of fibrosis	
Mild	Uneven collagen deposition in $\leq 20\%$ of material
Moderate	Uneven collagen deposition in 21% to 70% of material
Severe	Uneven collagen or lamellar fibroplasia in $\geq 71\%$ of material
Thickness of the muscular layer	
Mild	Less than one third of the whole thickness
Moderate	One third to two thirds of the wall
Severe	More than two thirds of the wall thickness
Addipose tissue deposition	
Mild	Up to 10% of the material
Moderate	11% to 60% of the material
Severe	More than 60% of the material
Degree of hyperplasia	
Diffuse	$\geq 70\%$ of the whole sections
Focal	$< 70\%$ of the whole sections
Degree of dysplasia	
Low-grade	Resemble tubular adenomas of the colon without intestinal metaplasia
High-grade	Markedly pleomorphic nuclei and/or prominent nucleoli
Metaplasia	
Pyloric type	Structures similar to the pyloric glands in the lamina propria
Intestinal type	Goblet cells and enterocitlike cells
Gastric surface type	Epithelial cells of gallbladder mucosa replaced by tall columnar cells with abundant mucin and basally located nuclei

HPF: high power field.
doi:10.1371/journal.pone.0070265.t001

Statistical analysis

All data was collected and compiled in Microsoft excel. Results of continuous (quantitative data) measurement were presented on Mean +/- SD (min-max) and result on categorical (qualitative data) measurements was presented in percentage and proportions (%). Comparison of qualitative variable was analysed by chi-square test. Wherever necessary between groups, comparison of quantitative variables was analysed by independent student t test according to distribution. A p value of 0.05 was taken as level of significance and was considered statistically significant. Data analysis was done using and open epi version 2.3.1.

Results

Table 1: Age Distribution Among Study Population

Age In Years	Frequency	Percentage
15 To 30	42	16.8%
30 To 45	84	33.6%
45-60	76	30.4%
60-75	48	19.2%
Total	250	100%

Mean Age Was 46.56 ± 14.3 . Ranging from 18 To 75 Years.

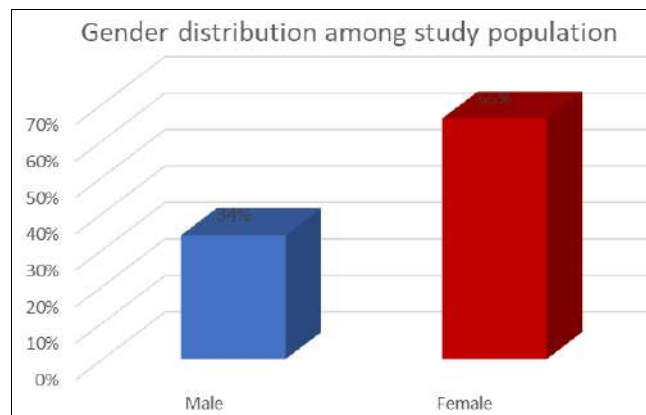


Fig 1: Gender Distribution Among Study Population

Table 2: Presence of H Pylori In Gall Bladder Mucosa

H Pylori	Frequency	Percentage
Present	14	5.6%
Absent	236	94.4%
Total	250	100%

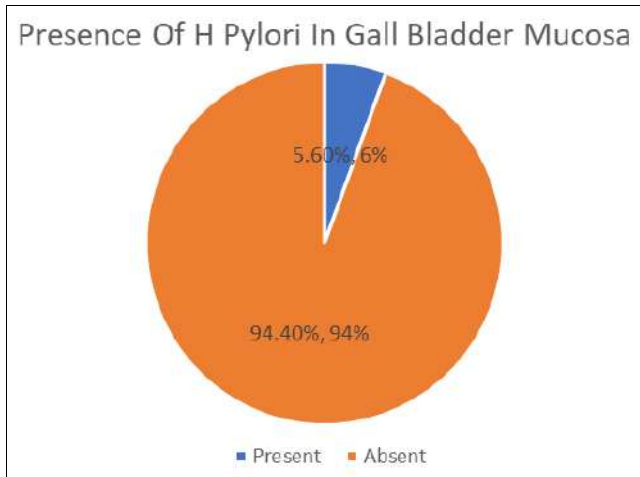


Fig 2: Presence of H Pylori in Gall Bladder Mucosa

Table 3: Stone Type in Gall Bladder

Stone Type	Frequency	Percentage
Cholesterol	68	27.2%
Pigmented	150	60%
Mixed	32	12.8%
Total	250	100%

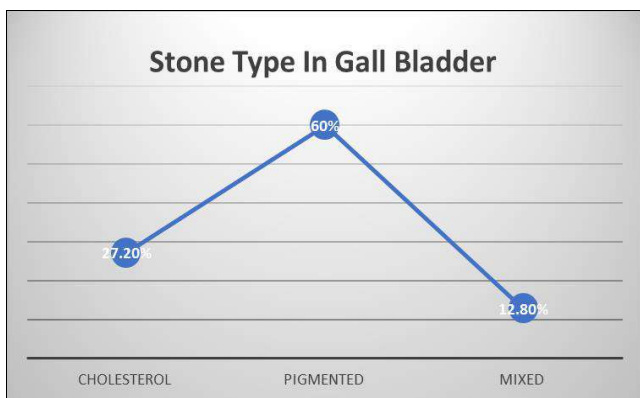


Fig 3: Stone Type in Gall Bladder

Table 4: Inflammatory Mononuclear Infiltrate

Inflammatory Mononuclear Infiltrate	Frequency	Percentage
Absent	178	71.2%
Mild	52	20.8%
Moderate	3	1.2%
Severe	17	6.8%
Total	250	100%

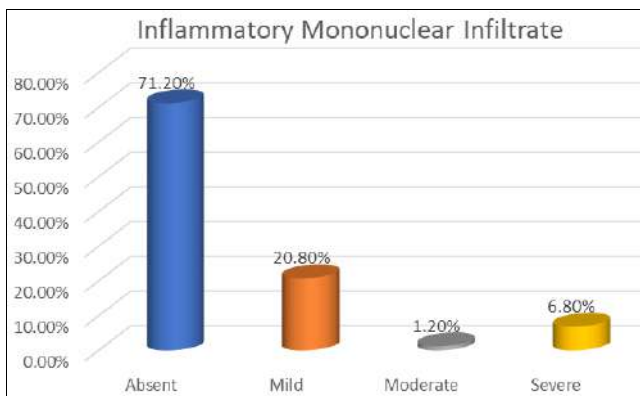


Fig 4: Inflammatory Mononuclear Infiltrate

Table 5: Degree of Fibrosis

Degree of fibrosis	Frequency	Percentage
Absent	247	98.8%
Mild	1	0.4%
Moderate	1	0.4%
Severe	1	0.4%
Total	250	100%

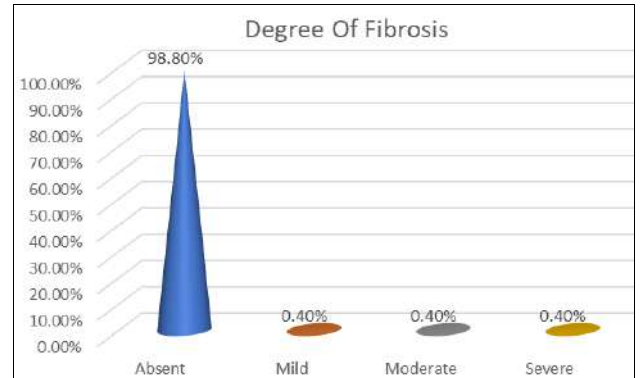


Fig 5: Degree of Fibrosis

Table 6: Thickness of Muscular Layer

Thickness of muscular layer	Frequency	Percentage
Mild	248	99.2%
Moderate	2	0.8%
Severe	0	0%
Total	250	100%

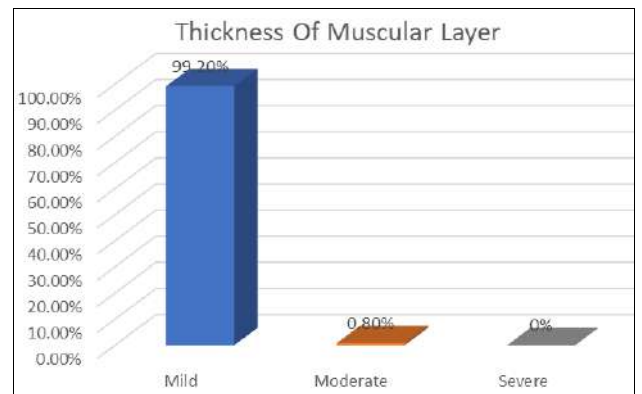


Fig 6: Thickness of Muscular Layer

Table 7: Adipose Tissue Deposition

Adipose tissue deposition	Frequency	Percentage
Absent	250	100%
Present	0	0%
Total	250	100%

Table 8: Degree of hyperplasia

Degree of hyperplasia	Frequency	Percentage
Focal	5	2%
Diffuse	1	0.4%
Absent	244	97.6%
Total	250	100%

Table 9: Degree of Dysplasia

Degree of dysplasia	Frequency	Percentage
Absent	250	100%
Present	0	0%
Total	250	100%

Table 10: Degree of Metaplasia

Degree of metaplasia	Frequency	Percentage
Absent	250	100%
Present	0	0%
Total	250	100%

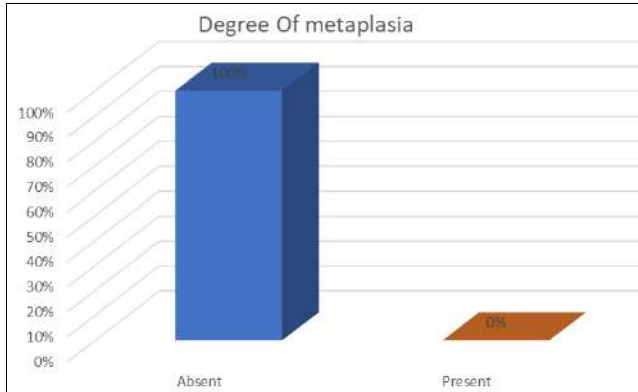


Fig 7: Degree of metaplasia

Association tables

Table 11: Association between H Pylori and age

H Pylori	15-30	30-45	45-60	60-75	Total
Present	1	4	6	3	14
Absent	41	80	70	45	236
Total	42	84	76	48	250

Applying chi square test p value is 0.63, as p value>0.05, shows No statistical significance.

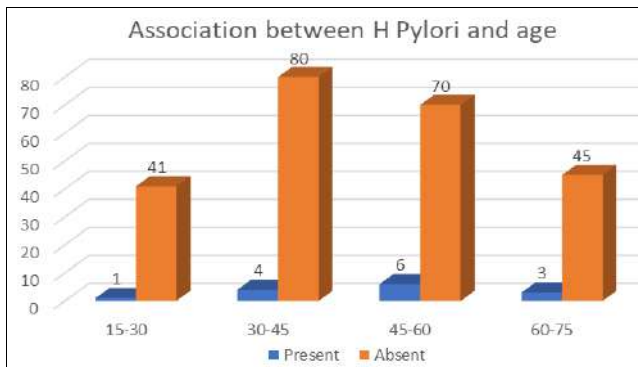


Fig 8: Association between *H. pylori* and age

Table 12: Association between *H. pylori* and gender

<i>H. pylori</i>	Male	Female	Total
Present	6	8	14
Absent	79	157	236
Total	85	165	250

Applying chi square test p value is 0.23, as p value>0.05, shows No statistical significance

Table 16: Association between *H. pylori* and degree of hyperplasia

<i>H. pylori</i>	Focal	Diffuse	Absent	Total	p value (<0.05)
Present	2	0	12	14	
Absent	3	1	232	236	
Total	5	1	244	250	< 0.003

Applying chi square test p value is<0.003, as p value <0.05, shows statistical significance

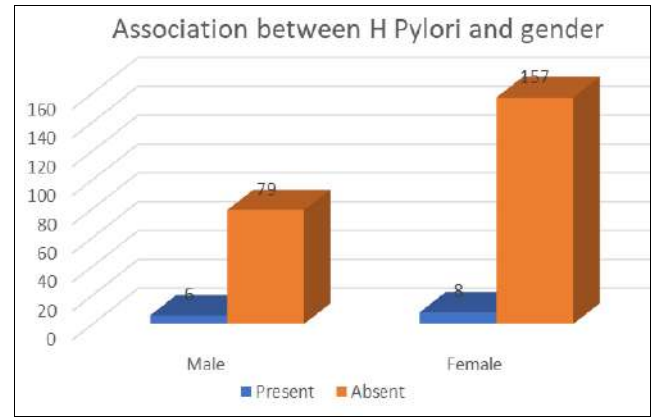


Fig 9: Association between *H. pylori* and gender

Table 13: Association between *H. pylori* and type of stone

<i>H. pylori</i>	Cholesterol	Pigmented	Mixed	Total
Present	5	8	1	14
Absent	63	142	31	236
Total	68	150	32	250

Applying chi square test p value is 0.67, as p value>0.05, shows no statistical significance.

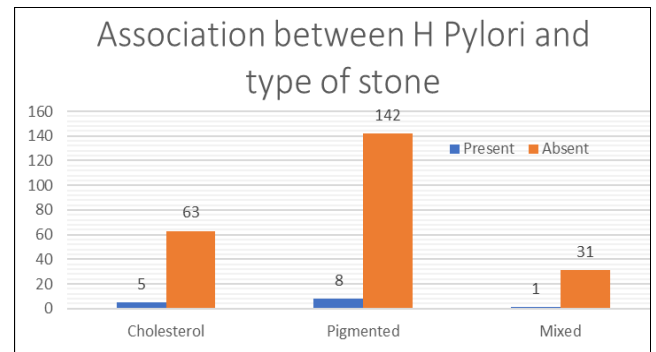


Fig 10: Association between *H. pylori* and type of stone

Table 14: Association between *H. pylori* and Inflammatory mononuclear infiltrate

<i>H. pylori</i>	Mild	Moderate	Severe	Absent	Total	p value (<0.05)
Present	1	1	3	9	14	
Absent	51	2	14	169	236	
Total	52	3	17	178	250	<0.01

Applying chi square test p value is <0.01, as p value<0.05, shows statistical significance

Table 15: Association between *H. pylori* and degree of fibrosis

<i>H. pylori</i>	A	Mild	Moderate	Severe	Total	P value(<0.05)
Present	13	0	1	0	14	
Absent	234	1	0	1	236	
Total	247	1	1	1	250	<0.0001

Applying chi square test p value is <0.0001, as p value <0.05, shows statistical significance

Discussion

Age and Gender distribution

In Our study mean Age Was 46.56 ± 14.3 . Ranging from 18 to 75 Years. Majority 33.6% were in age group of 30 to 45 years. Majority 66% were females and 34% were males. Liang Wang *et al.* [11] showed that risk of gallstone diseases increases with age in all racial groups. Because of female sex hormones and increased cholesterol secretion, females with obesity are more likely to get gallstones. Bohr *et al.* [12] found that the most significant risk factors for gallstone disease were patients with age >65 years and being overweight. Fatemi SM *et al.* [13] showed that mean age of 46.85 ± 14.53 consisting of 58 (67.25%) women and 19 (32.75%) men were included. Doraiswamy S *et al.* [14] showed that mean age was 42.76 years. 71% were females. Similar results were seen in present study.

H. pylori in gall bladder mucosa

In Our study 5.6% *H. pylori* was present in gall bladder mucosa. Liang Wang *et al.* [11] showed that 23.6% had *H. pylori* infection.

Fatemi SM *et al.* [13] showed that 8% had positive *H. pylori* report. Doraiswamy S *et al.* [14] showed that 7 cases were tested positive for *H. pylori* from cholecystectomy specimen. Also in a study by Takahashi *et al.*, there was found to be gallstone prevalence of 6.08% in *H. pylori* positive patients [15].

Gall bladder stones and their types

In Our study Majority 60% had pigmented gall bladder stone, 27.25 had cholesterol and 12.8% had mixed type stones. Doraiswamy S *et al.* [14] showed that 10 patients had calculous cholecystitis and 48 had chronic cholecystitis without calculi.

Mucosal lining

In Our study Inflammatory Mononuclear Infiltrate were present in 28.8% cases. Among them 20.8% were mild, 1.2% were moderate and 6.8% were severe. Degree of fibrosis was seen among 3 cases (1.2%), among them mild, moderate, severe was 0.4% each respectively. Majority 99.25 showed mild Thickness of Muscular Layer, 0.8% had moderate.

No patient had Adipose Tissue Deposition

Only 2.4% had Degree of Hyperplasia, among them 25 had focal and 0.4% had diffuse hyperplasia. No patient had Degree of Dysplasia and No patient had Degree of Metaplasia.

Misra *et al.*, [16] found that *H. pylori* was present in 50 of 111 (45%) sections with metaplasia.

Association results

In Our study No statistical significance was seen between *H. pylori* and age. Among those having *H. pylori* majority were in age group of 45 to 60 years. ($p=0.63$). Bulajic *et al.* [7] noted that the possibility of detecting *H. pylori* in the bile increased steadily with increasing age. In Our study No statistical significance was seen between *H. pylori* and gender. Among those having *H. pylori* majority were females. ($p=0.23$) similar results were found by Liang Wang *et al.* [11] where they showed that female gender was not statistically significant. In Our study No statistical significance was seen between *H. pylori* and type of stone. Among those having *H. pylori* majority were pigmented type. ($p=0.67$).

In Our study High statistical significance was seen between *H. pylori* and Inflammatory mononuclear infiltrate. $P<0.01$. In Our study High statistical significance was seen between *H. pylori* and degree of fibrosis. $P<0.0001$. In Our study High statistical significance was seen between *H. pylori* and degree of hyperplasia. $P<0.003$.

Table 17: COMPARISION with Similar Studies

Reference year	Place	Method used	Disease	Specimen	Organism identified	Helicobacter sp (+)
Misra 2007 [17]	Allahabad	HPE, IHC for GB PCR for GS	Cholecystitis	Gall bladder & stones	<i>H. pylori</i>	50/111(GB) 8/11(stones).
Chaudhary PK 2015 [18]	Mullana	HPE	Cholecystitis, cholelithiasis	Gall bladder	<i>H. pylori</i>	0/50
Bansal 2012 [19]	Delhi	RUT, culture HPE, PCR	Benign biliary disease	Gall bladder and bile	<i>H. pylori</i>	16/49
RR Mishra 2011 [16]	Varanasi	RUT, HPE, PCR, culture ELISA	Gallstone disease, gallbladder carcinoma	Gall bladder serum	<i>H. pylori</i>	18/54 GBC, 15/54 GSD
Present study	Mysore	HPE	Calculous cholecystitis	Gall bladder mucosa and stones	<i>H. pylori</i>	14/250

Conclusions

- Mean Age Was 46.56 ± 14.3 . Ranging From 18 to 75 Years. Majority 33.6% were in age group of 30 to 45 years.
- Majority 66% were females and 34% were males
- Among 5.6% *H. pylori* was present in gall bladder mucosa
- Majority 60% had pigmented gall bladder stone, 27.25 had cholesterol and 12.8% had mixed type stones.
- Inflammatory Mononuclear Infiltrate were present in 28.8% cases. Among them 20.8% were mild, 1.2% were moderate and 6.8% were severe.
- Degree of fibrosis was seen among 3 cases (1.2%), among them mild, moderate, severe was 0.4% each respectively.
- Majority 99.25 showed mild Thickness of Muscular Layer, 0.8% had moderate.
- No patient had Adipose Tissue Deposition
- Only 2.4% had Hyperplasia, among them 25 had focal and

- 0.4% had diffuse hyperplasia.
- No patient had Dysplasia
- No patient had Metaplasia
- No statistical significance was seen between *H. pylori* and age. Among those having *H. pylori* majority were in age group of 45 to 60 years. ($p=0.63$)
- No statistical significance was seen between *H. pylori* and gender. Among those having *H. pylori* majority were females. ($p=0.23$)
- No statistical significance was seen between *H. pylori* and type of stone. Among those having *H. pylori* majority were pigmented type. ($p=0.67$)
- High statistical significance was seen between *H. pylori* and Inflammatory mononuclear infiltrate. $P<0.01$
- High statistical significance was seen between *H. pylori* and degree of fibrosis. $P<0.0001$

- High statistical significance was seen between *H. pylori* and degree of hyperplasia. $P < 0.003$

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Declarations

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Conflict of interest: No conflict of interest declared

Ethical approval: Institution ethical clearance taken

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