Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 12, 2024

Original research article

A study of association of *H. pylori* with gallstone disease

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Abstract

The prevalence of gall bladder stones in our country varies between 10-20% of the population, with approximately 4.3% of individuals experiencing symptoms. The presence of an imbalance in the composition of bile, bile stasis, or gallbladder infections might potentially result in the development of gallstones. Nevertheless, numerous research have examined and documented the association between H. pylori and disorders affecting organs outside the stomach and duodenum. Keywords: Gall bladder, Helicobacter pylori, association.

Keywords: Gallstone disease, *H. pylori*, gallbladder infections, bile composition, prevalence

Introduction

The gallbladder is an oblong organ located on the inferior surface of the liver in the gallbladder fossa. Its primary function is to store bile [1]. Imbalances in the composition of bile can result in the development of gallstones [2]. The gallbladder is susceptible to the development of various conditions such as cancer and inflammation [3]. The prevalence of gall bladder stones in our country varies between 10-20% of the population, with approximately 4.3% of individuals experiencing symptoms. Gallstones can occur when there is an imbalance in the composition of bile, stasis of bile, or infections in the gallbladder [4, 5]. The gallbladder is susceptible to several disorders such as cancer and inflammation [7, 8]. The prevalence of gallstone disease in India is relatively high, ranging from 10-20% [9]. Prevalence is higher among females. Recently, many scientists have become interested in studying the relationship between *H. pylori* infection and different hepatobiliary diseases. However, due to the intricate pathophysiologic mechanisms involved in this infection, the diagnostic procedures for detecting H. pylori in extra gastric specimens have not met the desired standards of accuracy [11, 23]. Nevertheless, the association between *H. pylori* and disorders affecting organs outside the stomach and duodenum has been extensively examined and documented in several studies [24, 26].

Aim and Objective

To find out the incidence of H. pylori infection in the gallbladder of patients with symptomatic gallstone disease.

Materials and Methods

Study Design: Observational study Study setting: Yenepoya Medical College Hospital (YMCH) is a 900 bedded tertiary care teaching hospital situated in Deralakatte, a suburban locality of Mangaluru, Dakshina Kannada. It provides general and specialist healthcare to the coastal and central parts of Karnataka and northern part of Kerala.

Type of Study: Prospective study. Study Population Patients undergoing cholecystectomy for symptomatic gallstone disease in Yenepoya Medical College Hospital.

Study period This was conducted between May 2021 and October 2022.

Inclusion Criteria

- Patients admitted with symptomatic gallstone disease undergoing cholecystectomy at Yenepoya medical college hospital.
- Age of a patients more than 18 years.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 12, 2024

Exclusion criteria
Patients who have undergone any previous hepatobiliary intervention.

Surgeryor
- Patients who received *H. pylori* eradication treatment in the last 1 year.
- Intervention. No intervention other than the surgery needed.

Sampling procedure: Convenient sampling
Randomization: Nil.
Purposive sampling: Nil.
Sample Size: 42.
Sample Size Formula: n = Z2 -α/2 × pq/d² at 5% level of significance and 54.2% of proportion (from related article) with 15% of margin of error, the total sample size is 42.

- Study was conducted in YMHC from May 2021-August 2022.
- After the ethical committee and SRB clearance, the study was started.
- Patients were selected according to the criteria; informed consent was taken from every patient. Method of collection of data.
- All patients who meet the predefined criteria were counseled regarding the study following which those patients who give a written informed consent on their free will without any monetary gain will be chosen for the study. They then underwent complete medical history and clinical evaluation a pre anesthetic checkup. After being cleared from the anesthetists, they underwent surgery.
- Each patient underwent an endoscopy to rule out any stomach or duodenal pathology as the cause for upper abdominal pain. A ultrasound was done to look at the status of the gallbladder. On the day planned for surgery, the patient is kept nil by mouth for at least 8 hours. Following extraction of the gall bladder the following samples are collected Gallbladder mucosa biopsy, for Rapid urease testing, where the principle that H. pylori ferment urea is utilized to identify the presence of H. pylori. This test was done using a RUT dry test kit, A small 2-3 mm size of mucosal tissue is taken and introduced onto the yellow coloured media on the kit. If there is a change in the colour of the media from yellow to pink then it is called as a positive test result.

Another mucosal specimen was collected and stored at -80 degees for DNA analysis.
Protocol: DNA was extracted from the tissue samples using QIAamp DNA mini kit (cat. Nos. 51304 and 51306) by following the manufacturer‘s protocol.
Quantitative analyses of DNA were carried out using a Colibri microvolume spectrophotometer (Berthold Technologies GmbH & Co.KG). Contaminating protein was removed by treating Proteinase-K according to manufacturer‘s protocol and q-RT-PCR assay CFX96 q-PCR instrument (BioRad) was used.

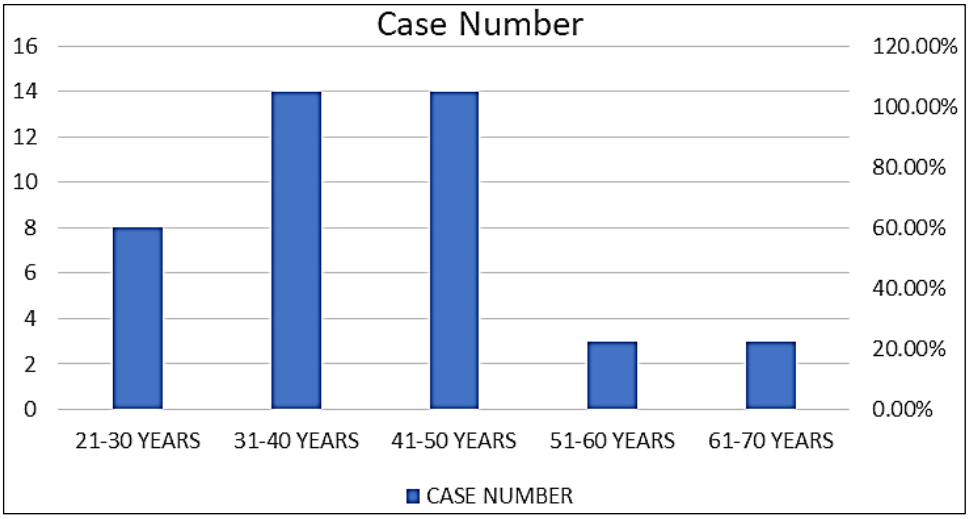
Statistical Analysis
- Descriptive Statistics.
- Mean and Standard deviation for categorical data Frequency and percentage for categorical data.
- Pearson‘s correlation was used to find out correlation between RUT and DNA.

Results
This Prospective study observational study was conducted at Yenepoya Medical College Hospital (YMCH) is a 900 bedded tertiary care teaching hospital situated in Deralakatte, a suburban locality of Mangaluru, Dakshina Kannada with patient population from local, neighboring districts as well as northern part of Kerala. The study period was between May 2021 and October 2022 on Patients undergoing cholecystectomy for symptomatic cholelithiasis in Yenepoya Medical College Hospital.

Table 1: Age

Age in Years	Case Number	Percent of Cases
21-30 Years	08	19.09%
31-40 Years	14	33.33%
41-50 Years	14	33.33%
51-60 Years	03	07.14%
61-70 Years	03	07.14%
Total	42	100.00%

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 12, 2024



Graph 1: Age

The age of the cases in the study ranged between 22 years and 67 years, the mean age of the cases was 41.28 years SD +/- 10.87 years.

Table 2: Gender

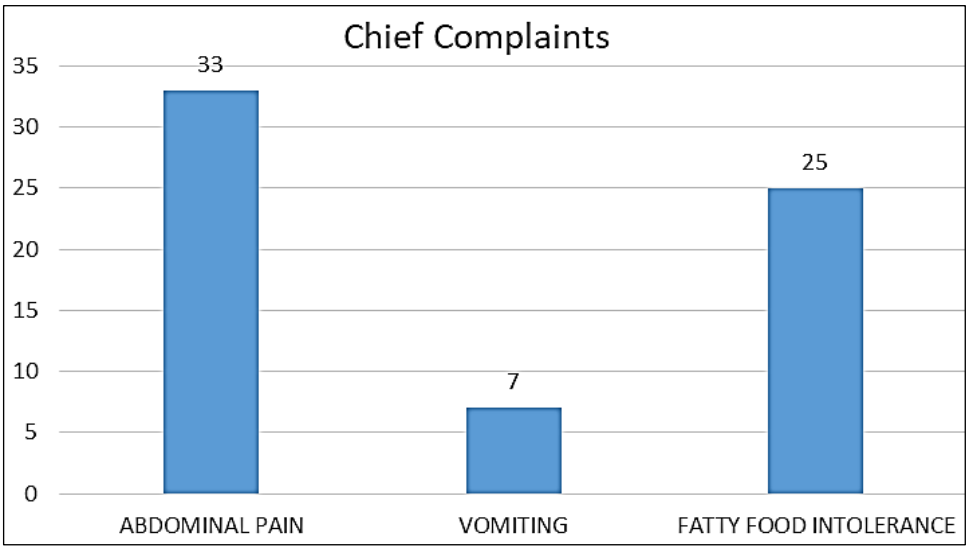
Gender	Case Number	Percent of Cases
Females	33	78.57%
Males	09	21.42%
Total	42	100.00%

Graph 2: Gender

33 (78.57%) cases were females and 09(21.42%) cases were males. Chi square p=0.023 significant, male to female ratio was 0.27:1

Table 3: Presenting Complaints

Presenting Complaints	Case Number	Percent of Cases
Abdominal Pain	33	78.57%
Vomiting	7	16.67%
Fatty Food Intolerance	25	59.52%



Graph 3: Presenting Complaints

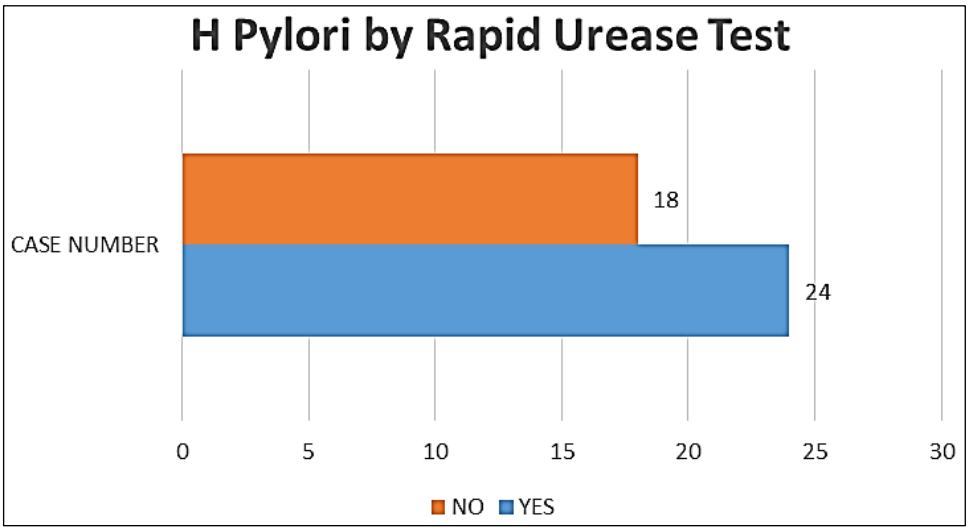
The presenting complaints were as follows

78.57% of the cases had abdominal pain, 16.67% of the cases had vomiting, and 59.52% had complaints of fatty food intolerance.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 12, 2024

Table 6: H Pylori by Rapid Urease Test

Rapid urease test	Case number	Percent of cases
Positive for H Pylori	24	58.54%
Negative for H Pylori	18	43.90%

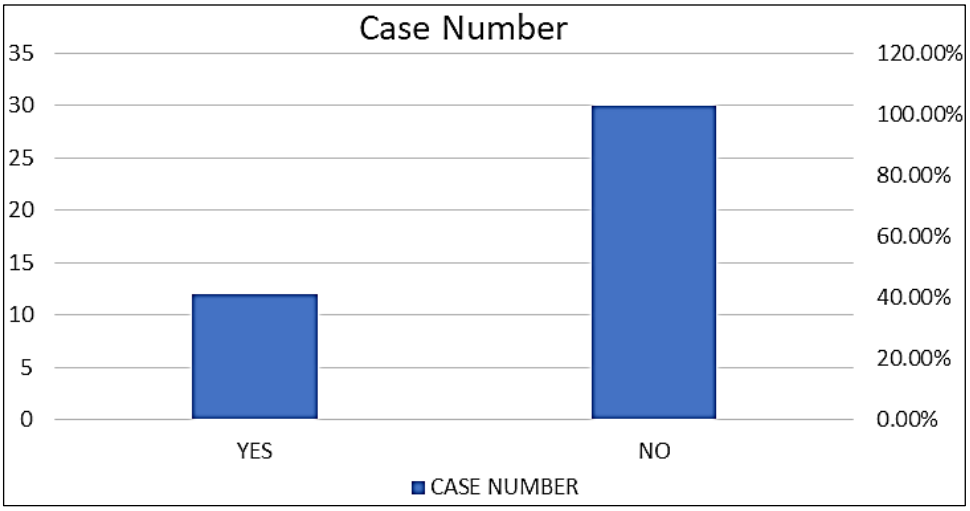


Graph 6: H Pylori by Rapid Urease Test

Rapid urease test results showed that 58.54% (24) of the gallbladder mucosal specimens were positive for H pylori and 43.9% (18) of the gallbladder mucosal specimens were negative for presence of H pylori.

Table 7: Hpylori by DNA

H Pylori DNA Isolation	Case Number	Percent of Cases
Yes	12	28.57%
No	30	71.42%



Graph 7: Hpylori by DNA

H pylori by the method of DNA isolation was seen in 12 cases (28.57%).

Table 8: Results of H Pylori Rapid Urease and DNA Analysis

Sample. No	RUT Result	DNA analysis results (q-RT PCR)	cq value
1	Positive	Positive	27.59
2	Negative	Positive	13.57
3	Positive	Negative	NA
4	Negative	Negative	NA
5	Positive	Negative	NA

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 12, 2024

6	Positive	Negative	NA
7	Positive	Negative	NA
8	Negative	Negative	NA
9	Negative	Negative	NA
10	Positive	Positive	30.39
11	Negative	Negative	NA
12	Positive	Positive	19.59
13	Positive	Negative	NA
14	Positive	Negative	NA
15	Positive	Positive	37.74
16	Positive	Negative	NA
17	Negative	Negative	NA
18	Positive	Negative	NA
19	Negative	Negative	NA
20	Positive	Positive	28.15
21	Positive	Negative	NA
22	Negative	Negative	NA
23	Negative	Positive	28.16
24	Negative	Positive	28.12
25	Positive	Positive	29.4
26	Positive	Positive	29.02
27	Negative	Negative	NA
28	Positive	Negative	NA
29	Negative	Negative	NA
30	Negative	Negative	NA
31	Positive	Positive	12.12
32	Positive	Negative	NA
33	Negative	Negative	NA
34	Negative	Negative	NA
35	Positive	Negative	NA
36	Positive	Negative	NA
37	Positive	Negative	NA
38	Negative	Negative	NA
39	Negative	Negative	NA
40	Positive	Positive	27.29
41	Positive	Negative	NA
42	Negative	Negative	NA

It was found that on DNA analysis a total of 12 samples were found to have the presence of the Helicobacter Pylori DNA, out of which 9 samples also tested positive for the presence of H pylori on Rapid Urease testing and 3 samples were tested negative of Rapid urease testing.
Base on the above results, the sensitivity, specificity, Positive predictive value, negative predictive value and accuracy were calculated as shown in the table below.

Table 9: Sensitivity of H Pylori Detection by Rapid Urease as Compared to DNA Isolation

Statistic	Value	95% CI
Sensitivity	80.00%	51.91% to 95.67%
Specificity	60.00%	22.26% to 98.74%
Positive Likelihood Ratio	2.60	0.39 to 6.55
Negative Likelihood Ratio	1.40	0.07 to 2.23
Disease prevalence (*)	88.24%	63.56% to 98.54%
Positive Predictive Value (*)	92.31%	74.57% to 98.00%
Negative Predictive Value (*)	35.00%	25.65% to 64.97%
Accuracy (*)	76.47%	50.10% to 93.19%

(*) These values are dependent on disease prevalence.

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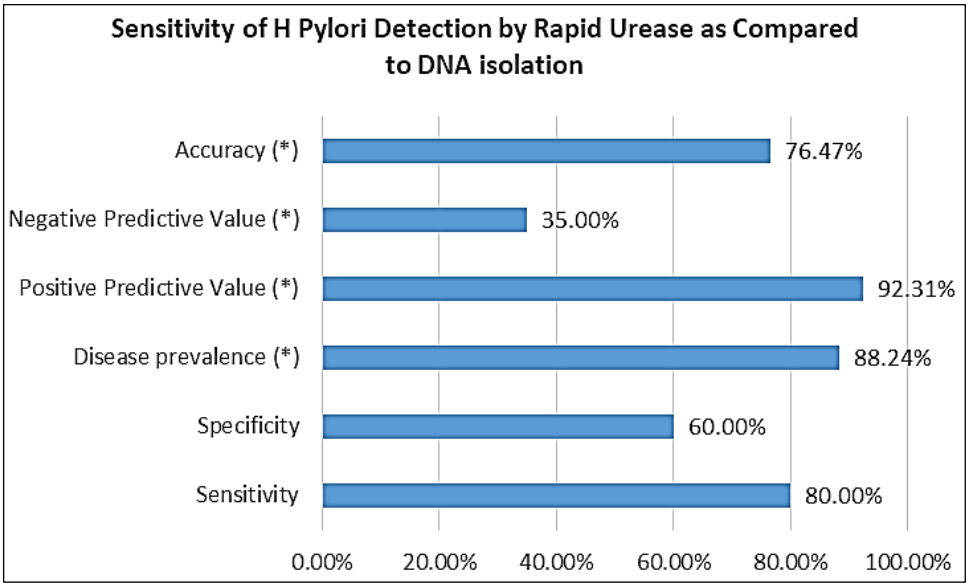

Graph 8: Sensitivity of H Pylori Detection by Rapid Urease as Compared to DNA Isolation

Taking DNA isolation as the gold standard test and comparing the Rapid urease testing with the results showed that:

- H PYLORI Detection by rapid urease testing had a sensitivity of 80%, specificity of 60%, positive predictive value of 92.31%, negative predictive value of 35% accuracy of 76.47%.

Discussion

This prospective observational study was carried out at Yenepoya Medical College Hospital (YMCH) in Deralakatte, Mangaluru, Dakshina Karnataka, from May 2021 to October 2022. The study focused on patients receiving cholecystectomy for symptomatic cholelithiasis. The following is a summary of the results. The age spanned from 22 to 67 years, with a mean age of 41 years and a standard deviation of 10.87 years. 31 instances, accounting for 73.81% of the total, were females. Out of the total number of cases, 11 cases, which accounts for 26.19% of the total, were men. The chi square test yielded a p-value of 0.023, indicating statistical significance. The male to female ratio was 0.33:1. *H. pylori* the quick urease detection method had a sensitivity of 80% and a specificity of 60%. The disease prevalence was 88.24%. The positive predictive value was 92.31%, while the negative predictive value was 35%. The accuracy of the test was 76.47%. The prevalence of gallstones rises with age. The prevalence of this condition is higher in women than in men, with a ratio of 4:1. Additionally, around 50% of patients do not exhibit any symptoms. The development of gallstones is influenced by multiple factors. Alongside alterations in bile composition or bile stasis, the primary cause of gallstone production appears to be the presence of an infectious agent. The phrase coined by Moynihan, which states that a gallstone is a memorial created within the organism, remains accurate in the present time. Laparoscopic cholecystectomy (LC) is a widely performed surgical operation that is considered the most effective treatment for symptomatic gallstones.8 Gallstone disease is prevalent in middle-aged females, with its occurrence rising between the ages of 30 to 50. The prevalence of gall bladder stones in our country varies between 10% and 20% of the population, and it causes symptoms in approximately 4.3% of individual.

Conclusion

After assessing all the results of the conducted study, it is found that there is presence of Helicobacter Pylori infection in gallbladder mucosa of significant amount of patients suffering from cholelithiasis by DNA analysis and Rapid urease testing.

Rapid urease testing is the most commonly used test to detect the presence of H pylori after routine endoscopies. During this study it is found that the sensitivity and Positive predictive value of Rapid urease testing is satisfactory but the Sensitivity, Negative predictive value of the said test is poor when compared to the gold standard DNA isolation.

This shows that further studies have to be conducted to look for a more promising, specific and accurate method to detect the presence of H pylori, which can be used on a daily basis and is both financially and technically easy to perform.

This study also indicates that there is need for further research to look into the association between the pathogenesis of H pylori and the formation of Gallstones and also for probable prevention of gallstone formation by eradication H Pylori infection in the selected population.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 12, 2024

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