International Surgery Journal
Kosma V et al. Int Surg J. 2023 Jun;10(6):1051-1056
http://www.ijsurgery.com
pISSN 2349-3305 | eISSN 2349-2902

DOI: https://dx.doi.org/10.18203/2349-2902.isj20231733

Original Research Article

A study of *H. pylori* infection as risk factor for the gall stone disease

Vijayalaxmi Kosma[1], S. N. Gole[1], S. L. Nirala[1], Rabia Parveen Siddiqui[2], Sujan Narayan Agrawal[3]*

[1]Department of General Surgery, [2]Department of Pathology, Pt. Jawaharlal Nehru Memorial Medical College and Dr. Bhim Rao Ambedkar Memorial Hospital, Raipur, Chhattisgarh, India
[3]Department of Surgery, District Hospital, Jagdalpur (Bastar), Chhattisgarh, India

Received: 25 April 2023
Revised: 17 May 2023
Accepted: 22 May 2023

***Correspondence:**
Dr. Sujan Narayan Agrawal,
E-mail: drsujanagrawal@gmail.com

ABSTRACT

Background: Gallstones are one of the widely occurring digestive/hepatobiliary disorders. Chronic cholecystitis, cholelithiasis or symptomatic gallbladder is a prolonged mechanical or functional disorder of abnormal gallbladder emptying. Nearly eighty percent of the gallstones are cholesterol gallstones, and 20% are pigmented stones consisting of bilirubin and calcium, the two components present in the bile. The diagnosis is established by a variety of diagnostic tools like ultrasonography, CT scan, ERCP, liver function tests and pancreatic enzyme studies. The hypothesis of the presence of H. pylori in the biliary epithelium of the patients with hepatobiliary ailments has been sporadically investigated. *Helicobacter pylori* (*H. pylori*) is a spiral, microaerophilic, gram-negative bacterium. There are many suggestive evidences that the DNA components of H. pylori is found in the bile, gallbladder tissue and/or cholesterol gallstones. The association of gallstones with Helicobacter pylori has been investigated by many authors, but not clearly established.
Methods: The present study is aimed to identify the *Helicobacter pylori* infection as a risk factor for gallstone disease. The histopathological samples of gall bladder mucosa were examined by staining with modified giemsa stain, haematoxylin, and eosin.
Results: Our study showed the presence of *H. pylori* in only one case which is not statistically significant. Moreover, the present study was based on H and E stains only and they should have been substantiated with immunohistochemistry and PCR studies. Thus, further studies are required to establish a causal relationship between the *Helicobactor pylori* infection and gallstone formation.
Conclusions: *Helicobacter pylori* as a risk factor for gallstones is yet to be proven.

Keywords: Biliary sludge, Cholecystectomy, Cholecystitis, Cholelithiasis, *H. Pylori*

INTRODUCTION

Gallstones are one of the widely occurring digestive/hepatobiliary disorders. It is one of the most frequent surgical ailments in clinical practice. Chronic cholecystitis, cholelithiasis or symptomatic gallbladder is a prolonged mechanical or functional disorder of abnormal gallbladder emptying. The symptoms of gallstone disease range from mild, nonspecific symptoms to a severe right quadrant abdominal pain. Most of the patients have recurrent pain attacks (acute biliary colic), but when pain lasts more than 24 hours, it requires urgent surgical intervention and if the pain subsides with conservative management, elective cholecystectomy is

planned.[1-2] Several factors such as chemical composition, structure, morphology, and microbiological findings have been reported as various mechanisms leading to the formation of gallstones.[3-4] Gallstones forms as a result of a settling process in which, solid particulates settle to the bottom of liquid bile and form a sediment. The major organic solutes in the bile are bilirubin, bile salts, phospholipids, and cholesterol.[5] Nearly eighty percent of the gallstones are cholesterol gallstones, and 20% are pigmented stones consisting of bilirubin and calcium, the two components present in the bile.[6-7] Symptoms of gallstone-related clinical conditions vary from nausea, vomiting and fatty dyspepsia to severe right hypochondrial and epigastric pain, jaundice, fever and shock. The diagnosis is not difficult and can be established by a variety of diagnostic tools including ultrasonography, CT scan, ERCP, liver function tests and pancreatic enzymes.[8-9] The hypothesis of the presence of *H. pylori* in the biliary epithelium of the patients with hepatobiliary ailments has been sporadically investigated.[10-11]

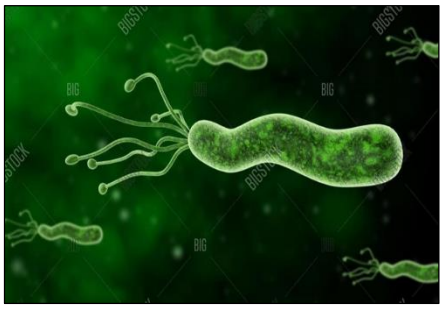

Figure 1: 3D image of *Helicobacter pylori*.

H. pylori is a spiral, microaerophilic, gram-negative bacterium.[4] It lives in the stomach and is related to acute and chronic gastritis, gastric and duodenal ulcer, gastric and pancreatic adenocarcinoma, and lymphoma of gastric mucosa-related lymphoid tissue.[12-13] Literatures is replete with the suggestive evidence of H. pylori DNA components in bile, gallbladder tissue and/or cholesterol gallstones.[14-15] However, Monstein et al reported that detecting bacterial DNA of *H. pylori* in cholesterol gallstone may indicate that *H. pylori* is a normal flora in the gallstone or, alternatively, the formation of cholesterol gallstone might be predisposed by the colonization of *H. pylori* in the biliary tract.[4] Over the last decade, an escalating number of studies have reported the association of *H. pylori* infection with extra-digestive conditions.[16] lately, different authors have described that DNA of bile tolerant *Helicobacter* spp. has been found in the human bile colonizing the biliary tract.[17] Majority of these studies have identified infection caused by *H. pylori* based on immunological assays and urease breath test (UBT), but rarely by using molecular approaches. Interestingly, no one could isolate any *H. pylori* using culture-based methods.[18] The association of gallstones with *Helicobacter pylori* has been investigated but not clearly demonstrated.[19] Pathways of *H. pylori* penetration into the bile have not been completely understood but there are possible routes of *H. pylori* migration and colonization in the biliary tract such as *H. pylori* translocation from the duodenum via sphincter of Oddi and/or its hematogenous spread to the liver and then excretion into the bile.[20] The present study was aimed to identify the *Helicobacter pylori* infection as a risk factor for gallstone disease. *Helicobacter pylori* is curved gram-negative rod that colonizes stomach and is associated with peptic ulcer disease and gastric carcinoma.

The aims and objectives of this study were to find out whether the *Helicobactor pylori* is a risk factor for the patients of gallstone disease, and to find out whether there is any association of *Helicobactor pylori* and gallstone disease.

METHODS

The study conducted at the Department of General surgery Pt. J.N.M. Medical College, Raipur. It is an observational study during the study period from June 2021 to June 2021 (12 months). The inclusion criteria for the study mainly consists of patients with gall stone disease, cholecystitis, biliary sludge, and the diagnosed cases of cholelithiasis. All the patients with gallstone disease are appropriately investigated and those who have been found fit for elective cholecystectomy, are included in the study. The consent of the patient was taken to participate in the study. The approval of the ethical committee is also taken to conduct the proposed study. Those patients were excluded from the study who have not given consent and/or unfit for cholecystectomy. A total of 35 patients are found fit to undergo the proposed study. The specimen of gall bladder after cholecystectomy are submitted for histopathology examination. The histopathology examination consists of staining the gall bladder mucosa with modified giemsa stain, haematoxylin and eosin stain and Warthin starry stains. A total of 35 cases were investigated during this period. After cholecystectomy the specimen are send for histopathology examination. The samples are tested for presence or absence of *Helicobactor pylori* by staining with modified giemsa stain, haematoxylin and eosin, Warthin starry stain. The results are recorded as the presence or absence of *Helicobactor pylori* infection.

RESULTS

The mean age of study subjects was 47.34±14.70 years. Maximum number of cases were between 51 to 60years (25.71%) followed by age group of 31-40 years. (22.86%) Among study subjects 60% were female and 40% were male, showing that it is predominantly a disease found in female.

All the patients presented with pain in abdomen, the duration of which varied from one to eight months. Maximum number of patients had pain since past one to

three months (51.42%). Vomiting was found in 54.29% cases.

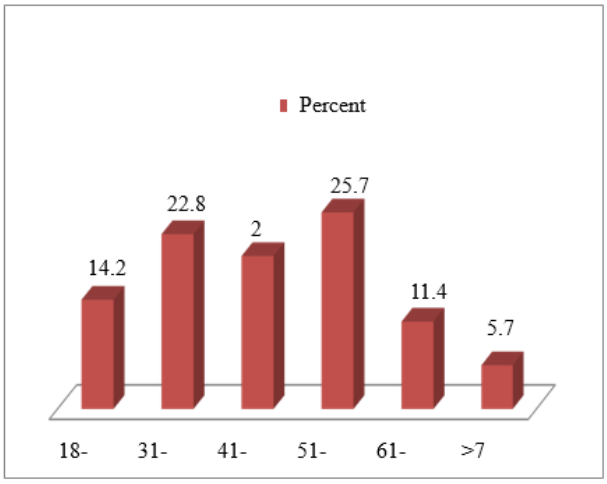

Figure 2: Age distribution among study subjects.

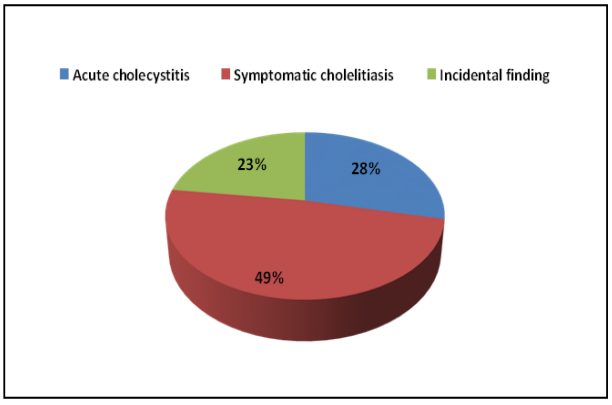

Figure 3: Clinical feature/ presentation.

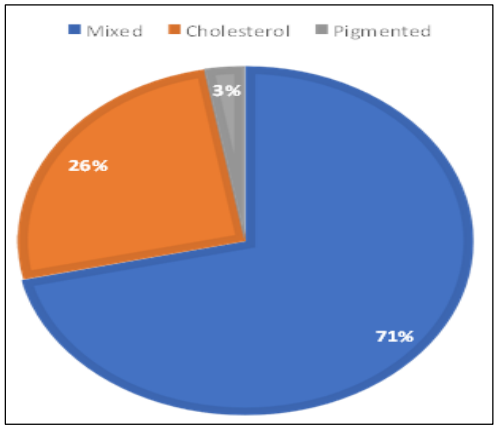

Figure 4: Type of gallstone.

Among the study subject 29 (82.86%) were non diabetic and 27 (77.14%) were non-hypertensive. 71.42% (25 cases) subjects presented with mixed type of gallstones, 25.72% (9 cases) with pure cholesterol type of gallstones and 2.85% (1 case) had pigmented gallstone. 65.71% patients had multiple stones and 34.29% had one stone. The gall bladder wall thickening was around 4mm in 42.86% cases and 40% had 3mm thickness of the wall. It was interesting to note that the *H. pylori* infection was found only in one case.

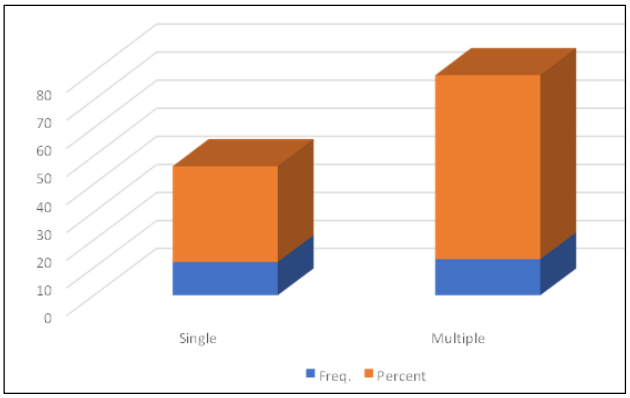

Figure 5: Number of stone among study subjects.

Table 1: *H. pylori* infection among study subjects.

H. Pylori	Frequency	Percent (%)
Absent	34	97.1
Present	1	2.9
Total	35	100

In this case gall bladder wall showed sloughing of mucosa with presence of inflammatory cell infiltrates in the lamina propria with occasional suspicious organism on H and E stain. On modified Giemsa stain and Warthin starry stain, *H. pylori* were seen on the denuded mucosal surface and within the mucinous material.

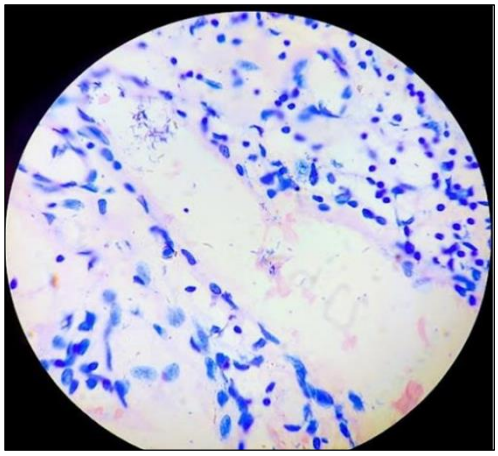

Figure 6: Modified Giemsa stain. *H. pylori* seen on the denuded mucosal surface and within mucus.

DISCUSSION

The present study was conducted with the purpose to study the infection of Helicobacter pylori as a risk factor

for gallstone disease at department of General Surgery at Pt JNM Medical College, Raipur. Abro et al did a hospital-based study on *H. pylori* infection in patients with calculous cholecystitis. The mean age of overall study population was 48.72±8.78 years.[21] Fikry et al did a cross sectional study the *H. pylori* infection in patients with chronic calculous cholecystitis. Of all 140 patients 62.1% were females, mean age of our patients was 39.04±7.3 years.[22] Motie et al study the relationship between cholecystitis and presence of helicobacter pylori in the gallbladder. A total of 84 studied patients with mean age of 45.19±1.78, 27 were male (32.1%) and 57 were female (67.9%).[23] Findings of the present study were comparable with the other studies. In present study all the patients had abdomen pain and the duration of pain was one to two months in 25.71% and 11.43% had pain for six months. 54.29% of cases had complain of vomiting also. Fikry et al found that in the chronic calculus cholecystitis most of the patients presented with abdominal pain (92%) and vomiting (64%). As far as the comorbidities are concerned, the common co-morbidities are peptic ulcer disease (PUD), Diabetes and hypertension as reported by other authors and in the present study also.[24-26] In this and various other studies, it was seen that prediabetic and diabetic status were associated with complicated GSD. In this study, subjects 51.43% had multiple stones and 34.29% had one stone. The size of stone ranges from 5mm to 15mm. Maximum 28.57% had stone size of 8 mm and 25.71% had stone size of 25.71%. In the case control study by Dhamnetiya et al, 31.7% has single stone and 68.3% had multiple stone. 54.2% had stone size of 10mm and 45.8% had stone size of >10mm.[27] Other authors also have the similar findings.[28-29] In present study out of 35 cases only one case showed presence of *H. pylori*. In this case gall bladder wall showed sloughing of mucosa with presence of inflammatory cell infiltrates in the lamina propria with occasional suspicious organism on H and E stain. Abro et al did a hospital-based study on *H. pylori* infection in patients with calculous cholecystitis. Total 100 patients of cholelithiasis underwent laparoscopic cholecystectomy were recruited. *H. pylori* infection was identified in 55% patients with calculous cholecystitis. Helaly et al study the detection of *H. pylori* infection in Egyptian patients with chronic calculous cholecystitis. Gall bladder positivity for *H. pylori* was accompanied by chronic quiescent gastritis (40.9%). In conclusion, *H. pylori* infection may be an etiological factor leading to cholecystitis. Cen et al did a systematic review and meta-analysis on *H. pylori* infection of the gallbladder and the risk of chronic cholecystitis and cholelithiasis. *H. pylori* infection of the gallbladder was significantly associated with an increased risk of chronic cholecystitis and cholecystitis (OR = 3.022).[28] Fatemi et al study the correlation between *H. pylori* and *Enterohepatic helicobacter* species and gallstone cholecystitis. In this study, 77 subjects with acute and chronic cholecystitis DNA of 10 *Helicobacter* spp. were detected in the bile of the patients with cholecystitis of eight *patients*.[18] Khorsheed et al study the prevalence of *H. pylori* among patients with cholelithiasis. In this study was conducted on 95 patients. Out of the 95 patient's 31(33%) were positive for *H. pylori* UreC gene. In the present study the presence of *H. pylori* was found only in one case and is not statistically significant. Tsuchiya Y et al and Ari et al has also reported that there is no significant difference between case and control.[30]

This study also does not show relationship of gallstone and helicobacter pylori in contrast to previous reports. This study also suggests that the helicobacter pylori does not play role in development of gallstones. Similarly, Bashir et al has found out that out of the 150 cases of cholecystectomy specimens, Giemsa stain on 150 cases was negative for *H. pylori*. 35 specimens were subjected to immunohistochemistry (IHC) stains and none detected *H. pylori*. This study also correlates with findings of my study.[31] However, this study was based on H and E stain and special stain only. We did not perform IHC or PCR for the diagnosis of *H. pylori*. Hence case control study with layer groups and ancillary techniques are needed to establish a causal relationship of *H. pylori* with gallstones.

CONCLUSION

Helicobacter pylori as a risk factor for gallstones is yet to be proven. In all the studies previously done, studies with PCR tests for DNA of *H. pylori* have shown some association with its presence in gallbladder. However, it has not been established as the sole factor for gallstones. *H. pylori* mainly inhabits stomach mucosa; its positivity and its role as a causal factor in conditions such as gastritis and stomach cancer have been well established, and extensively documented. To establish helicobacter pylori as a risk factor for gallstones, confounding factors for gallstone formation first have to be eliminated and then special stains, IHC and PCR studies should be done. Our study showed presence of *H. pylori* in only one case which is not statistically significant. Moreover, this study was based on H and E stain and special stain only. We did not perform IHC or PCR for the diagnosis of *H. pylori*. Hence case control study with larger groups and ancillary techniques are needed to establish a causal relationship of *H. pylori* with gallstones.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Jahantab MB, Salehi V, Mehrabi S, Abedini L, Yavari Barhaghtalab MJ. Cholecystomegaly: a case report and review of the literature. Case Rep Gastroint Med. 2020;2020:1-5.
2. Internal Clinical Guidelines Team (UK) Gallstone Disease: diagnosis and management of cholelithiasis, cholecystitis and choledocholithiasis.

London, UK: National Institute for Health and Care Excellence (UK); 2014. Available at: https://www.nice.org.uk/guidance/cg188/resources/gallstone-disease-diagnosis-and-management-pdf-35109819418309. Accessed on 3 March 2023.
3. Ari A, Tatar C, Yarikkaya E. Relationship between *Helicobacter pylori* positivity in the gallbladder and stomach and effect on gallbladder pathologies. J Inter Med Res. 2019;47(10):4904-10.
4. Monstein HJ, Jonsson Y, Zdolsek J, Svanvik J. Identification of *Helicobacter pylori* DNA in human cholesterol gallstones. Scand J Gastroenterol. 2002;37(1):112-19.
5. Haisley R, Hunter JG. Gallbladder and the extrahepatic biliary system. In: Brunicardi FC, Andersen DK, Billiar TR, Dunn DL, eds. Schwartz's Principles of Surgery. 11 ed. New York, NY: McGraw-Hill; 2019.
6. Qiao T, Ma RH, Luo XB, Yang LQ, Luo Z, Zheng PM. The systematic classification of gallbladder stones. PloS One. 2013;8(10):10.
7. Belzer C, Kusters JG, Kuipers EJ, van Vliet AHM. Urease induced calcium precipitation by Helicobacter species may initiate gallstone formation. Gut. 2006;55(11):1678-79.
8. Leong R, Sung J. Helicobacter species and hepatobiliary diseases. Aliment Pharmacol Therap. 2002;16(6):1037-45.
9. Testoni PA. Acute recurrent pancreatitis: Etiopathogenesis, diagnosis and treatment. World J Gastroenterol. 2014;20(45):168-91.
10. Dunn BE, Vakil NB, Schneider BG, Miller MM, Zitzer JB, Peutz T, et al. Localization of *Helicobacter pylori* urease and heat shock protein in human gastric biopsies. Infect Imm. 1997;65(4):1181-8.
11. Silva CP, Pereira-Lima JC, Oliveira AG, Guerra JB, Marques DL, Sarmanho L, et al. Association of the presence of *Helicobacter* in gallbladder tissue with cholelithiasis and cholecystitis. J Clin Microbiol. 2003;41(12):5615-8.
12. Bostanoglu E, Karahan ZC, Bostanoglu A, Savas B, Erden E, Kiyan M. Evaluation of the presence of *Helicobacter* species in the biliary system of Turkish patients with cholelithiasis. Turkish J Gastroenterol. 2010;21(4):421-27.
13. Attaallah W, Yener N, Ugurlu MU, Manukyan M, Asmaz E, Aktan AO. Gallstones and concomitant Gastric *Helicobacter pylori* Infection. Gastroenterol Res Pract. 2013;2013:1-4.
14. Nilsson HO, Stenram U, Ihse I, Wadström T. *Helicobacter* species ribosomal DNA in the pancreas, stomach and duodenum of pancreatic cancer patients. WJG. 2006;12(19):3038.
15. Buck GE. *Campylobacter pylori* and gastroduodenal disease. Clinical Microbiol Revi. 1990;3(1):1-12.
16. Erden E, Kiyan M. Evaluation of the presence of *Helicobacter* species in the biliary system of Turkish patients with cholelithiasis. Turk J Gastroenterol. 2010;21(4):421-7.
17. Abrams DN, Koslowsky I, Matte G. Pharmaceutical interference with the [14C] carbon urea breath test for the detection of *Helicobacter pylori* infection. J Pharm Pharm Sci. 2000;3(2):228-33.
18. Fatemi SM, Doosti A, Shokri D, Ghorbani-Dalini S, Molazadeh M, Tavakoli H, et al. Is There a Correlation between *Helicobacter pylori* and *Enterohepatic Helicobacter* Species and Gallstone Cholecystitis? Middle East J Digest Dis. 2018;10(1):24
19. Attaallah W, Yener N, Ugurlu MU, Manukyan M, Asmaz E, Aktan AO. Gallstones and concomitant gastric *Helicobacter pylori* infection. Gastroenterol Res Pract. 2013;2013:643109.
20. Helaly G, El-Ghazzawi E, Kazeiw A, Dowidar N, Anwar M, Attia N. Detection of *Helicobacter pylori* infection in Egyptian patients with chronic calcular cholecystitis. Brit J Biomed Sci. 2014;71(1):13-8.
21. Abro AH, Haider IZ, Ahmad S. *Helicobacter pylori* infection in patients with calcular cholecystitis: a hospital-based study. J Ayub Med Coll Abbottabad. 2011;23(1):30-3.
22. Fikry AA, Kassem AA, Shahin D, Shabana HA, Mostafa S. *Helicobacter pylori* infection in patients with chronic calcular cholecystitis a cross-sectional study. J Surg. 2014;2(4):58-62.
23. Motie M, Rezapanah A, Abbasi H, Memar B, Arianpoor A. The relationship between cholecystitis and presence of *Helicobacter pylori* in the gallbladder. Zahedan J Res Med Sci. 2017;19(7):e9621.
24. Wang F, Wang J, Li Y, Yuan J, Yao P, Wei S, et al. Gallstone disease and type 2 diabetes risk: a Mendelian randomization study. Hepatol. 2019;70(2):610-20.
25. Lv J, Yu C, Guo Y, Bian Z, Yang L, Chen Y, et al. Gallstone disease and the risk of type 2 diabetes. Sci Repor. 2017;7(1):15853.
26. Khorsheed BA, AL-Hasnawi SMJ, Assi AN. Prevalence of *Helicobacter pylori* among patients with cholelithiasis. IJMSCR. 2019;2(5):93-7.
27. Dhamnetiya D, Goel MK, Dhiman B,Pathania OP. Clinical presentation of gallstone disease: Evidence froma case-control study. Arch Int Surg. 2019;9:1-4.
28. Cen L, Pan J, Zhou B, Yu C, Li Y, Chen W, et al. *Helicobacter pylori* infection of the gallbladder and the risk of chronic cholecystitis and cholelithiasis: A systematic review and meta-analysis. Helicobacter. 2018;23(1).
29. Lodha M, Chauhan AS, Puranik A, Meena SP, Badkur M, Chaudhary R, et al. Clinical profile and evaluation of outcomes of symptomatic gallstone disease in the senior citizen population. Cureus. 2022;14(8).
30. Tsuchiya Y, Mishra K, Kapoor VK, Vishwakarma R, Behari A, Ikoma T, et al. Plasma *Helicobacter pylori* antibody titers and helicobacter pylori infection positivity rates in patients with gallbladder cancer or cholelithiasis: a hospital-based case-

Control Study. Asian Pac J Cancer Prev. 2018;19(7):1911-15.
31. Bashir S, Saldanha P. A study of *Helicobacter pylori* in chronic cholecystitis and gallbladder carcinoma. MGM J Med Sci. 2021;8:95-101.

Cite this article as: Kosma V, Gole SN, Nirala SL, Siddiqui RP, Agrawal SN. A study of *H. pylori* infection as risk factor for the gall stone disease. Int Surg J 2023;10:1051-6.