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Frequency of Helicobacter Pylori Infection in Patients with Gallstone Disease Presented at Chandka Medical College Teaching Hospital, Larkana

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ABSTRACT

OBJECTIVE: To assess the frequency of H. pylori seropositivity among gallstone patients at Chandka Medical College Hospital (CMCH), Larkana.

METHODOLOGY: This descriptive cross-sectional study was conducted in the surgery department of CMCH, Larkana, from March to September 2024. A total of 135 patients (aged 20–60 years) with ultrasound-verified gallstones for ≥2 weeks were enrolled. The sample size was calculated using the WHO formula (95% CI, 7% margin of error, 50% expected frequency). Serological testing (anti-H. pylori IgG) was used to detect H. pylori seropositivity. Data were analyzed using SPSS version 22.

RESULTS: The average age of the patients was 56.83 years, with 63.7% female. H. pylori positivity was found in 62.2% (n=84). Significant associations with H. pylori infection were observed for female sex (p=0.04), urban residence (p=0.02), smoking (p<0.01), obesity (p<0.01), anemia (p<0.01), folate deficiency (p<0.01), hepatitis B/C infection (p<0.01), hypertension (p=0.05), diabetes mellitus (p=0.02), and elevated C-reactive protein (p=0.03). Borderline associations were noted for hypertension (p=0.05). In this cohort, male patients had higher H. pylori seropositivity (73.5%) than females (55.8%), and the difference was statistically significant (p=0.04). For diabetes mellitus, a higher proportion of non-diabetic patients (66.7%) were H. pylori seropositive compared to diabetic patients (53.8%), which was statistically significant (p=0.02).

CONCLUSION: It is concluded that H. pylori seropositivity in gallstone cases at CMCH Larkana is very high (62.2%). H. pylori seropositivity was significantly associated with obesity, smoking, and metabolic comorbidities, indicating a possible pathogenic association.

KEYWORDS: Helicobacter pylori, Gallstones, Cholelithiasis, Pakistan, Larkana, Prevalence

INTRODUCTION

Gallstone disease, also known as cholelithiasis, remains one of the most common surgical disorders worldwide. Because of its high morbidity and medical expenses, it poses a serious threat to public health[1]. In Pakistan, cholelithiasis is a common cause of abdominal surgery, particularly among women of childbearing age, with regional variations in prevalence influenced by dietary habits, genetic predisposition, and socioeconomic factors[2,3]. Gallstone formation is a multifactorial process related to genetic predisposition, insulin resistance, abnormal lipid profiles, excess body weight, sedentary lifestyle, and dietary patterns[4,5]. Usually, the aetiology includes decreased gallbladder motility, nucleation of lipid crystals, and high levels of cholesterol in bile.

Helicobacter pylori, a classic gastric pathogen which has been linked with peptic ulcer disease and gastric cancer, has now been associated with various extra-intestinal diseases including hepatobiliary diseases such as cholecystitis, cholangiocarcinoma, and cholelithiasis in recent years[6]. A recent transcriptomic study also showed that H. pylori was a significant component of the gallbladder epithelial lining of the gallbladder, and that its CagA virulence factor directly affected the permeability of the epithelial permeability, as well as the local inflammatory process that promotes lithogenesis and inhibits gallbladder emptying via neurohormonal pathways[7,8].

The reported seroprevalence of H. pylori in gallstone patients in Pakistan varies from 17% to 72.7%, depending on demographic characteristics, regional variations, and diagnostic techniques used9–11, but specific epidemiological data from Larkana district of Upper Sindh are not available. Considering the high occurrence of gallstone disease in the region and the possible role of H. pylori in the postoperative management and recurrence rate of gallstone patients, the purpose of this study was to determine the frequency of H. pylori seropositivity and related risk factors among gallstone patients visiting the tertiary care facility in Larkana. The discovery of such links may lead to the development of specific screening and eradication programs that will decrease the morbidity associated with gallstones.

METHODOLOGY

This descriptive cross-sectional study was conducted in the Department of Surgery at Chandka Medical College Hospital (CMCH), Larkana, from March to September 2024. CMCH is a major tertiary healthcare referral hospital of Larkana district and surrounding Upper Sindh. The Institutional Review Board (IRB) of Shaheed Mohtarma Benazir Bhutto Medical University, Larkana, granted ethical clearance (CPSP/REU/SGR-2019-221-10269, Date: February 15 2021).

A total of 135 patients (age 20–60 years) with ultrasound-verified gallstones for ≥2 weeks were enrolled. The sample size was calculated using the WHO formula (95% CI, 7% margin of error, 50% expected frequency). The Medical Superintendent of CMCH, Larkana, granted official consent to gather data. Each study participant provided written informed consent after being educated in their mother tongue (Urdu or Sindhi) of the study's goals, methods, possible dangers, and advantages. Throughout the trial, we rigorously upheld patient data confidentiality.

We used a non-probability consecutive sampling approach to enrol 135 patients. Using a 95% confidence interval, a 7% margin of error, and a 50% predicted frequency of H. pylori in gallstone patients (based on limited local data), we determined the sample size using the WHO sample size algorithm. Included patients aged 20 to 60 years, of either gender, with ultrasonographically confirmed gallstones and symptoms attributable to gallstones for at least two weeks. We selected the 20-60 year age range to exclude confounding factors associated with extreme ages, such as age-related physiological changes in gastric mucosa and immune function, and to focus on the working-age population most commonly presenting with gallstone disease at our centre. The exclusion of patients aged 20-34 years reflects the relatively lower incidence of gallstone disease in this younger demographic at our institution during the study period.

Patients were excluded if they were pregnant females, had undergone previous gastric or small bowel resection, had malabsorption syndrome, peritonitis, gastrointestinal malignancy, liver cirrhosis, or were already taking H. pylori eradication therapy or lipid-lowering agents (statins or fibrates) at the time of enrollment.

Data collection was performed using a structured pre-tested proforma, which included the following variables: age, gender, place of residence (urban/rural), duration of gallstone symptoms, smoking habits, body mass index (BMI), obesity, and medical history (hypertension, diabetes mellitus, hepatitis B/C status, anemia, folate deficiency). Hemoglobin levels less than 12 g/dL for females and less than 13 g/dL for males were considered anemia. Serum folate levels less than 4 ng/mL were considered folate deficient.

Under aseptic conditions, 2 cc of venous blood was drawn from each participant. Before analysis, samples were centrifuged, serum separated, and stored at -20°C. Samples from all patients were forwarded to the hospital laboratory and examined by a senior pathologist in a blind clinical setting. Anti-H. pylori IgG enzyme-linked immunosorbent assay (ELISA) kit (Wondfo Biotech, Guangzhou, China) was used to diagnose H. pylori seropositivity. Serology cannot differentiate between active Infection and past exposure; therefore, our findings represent seroprevalence rather than active Infection. A C-reactive protein (CRP) >5 mg/L was used as the definition of elevated.

IBM SPSS Statistics for Windows, version 22.0, was used to gather and analyze the data. Categorical data (gender, residence, smoking, obesity, comorbidities) were represented by frequencies and percentages. Quantitative variables were summarised as mean ± standard deviation (SD). We evaluated the relationships between categorical factors and H. pylori positivity using the chi-square test. A p-value of < 0.05 was considered statistically significant.

RESULTS

This study included all 135 patients with gallstone disease. The mean age of the participants was 56.83 ± 6.74 years (range: 35 to 60 years). The average BMI was 30.77 ± 2.83 kg/m², indicating that the study population was, on average, overweight to obese. The average time to onset of symptoms associated with gallstones was 6.95 ± 3.82 weeks. **(Table I)** The overall seroprevalence rate of H. pylori was 62.2% (84/135) **(Figure 1).** The sex distribution of the 135 patients showed a predominance of female patients (63.7%) over male patients (36.3%), as has been established for gallstone disease. Most of the patients belonged to the 40-49 years age group (33.3%, n=45), followed by the 50-59 years group (31.1%, n=42). Of the 135 respondents, 57.8% (n=78) were from urban areas, and 42.2% (n=57) were from rural areas. Sixty percent of this group (n=82) identified as non-smokers. Obesity was present in 60.7% (n=82), hypertension in 55.6% (n=75), and diabetes mellitus in 57.8% (n=78). **(Table II)** Regarding other parameters, anemia was found in 48.1% (n=65), folate deficiency in 38.5% (n=52), hepatitis B or C seropositivity in 22.2% (n=30), and elevated CRP in 58.5% (n=79).

On inferential analysis using the chi-square test, H. pylori seropositivity revealed a statistically significant relationship with gender; however, contrary to what might be expected from the discussion of female predominance in gallstone disease, we found that males had a higher rate of H. pylori seropositivity: Among males, 73.5% (36/49) were H. pylori seropositive compared to 55.8% (48/86) among females (p=0.04). This indicates that women with gallstones are more likely to harbor H. pylori infection than men. Additionally, living in an urban area was substantially associated with H. pylori infection: 70.5% (55/78) of urban residents tested positive versus 29.5% (23/78) negative, compared to rural residents where 50.9% (29/57) were positive and 49.1% (28/57) negative (p=0.02), suggesting possible environmental or lifestyle-related transmission hazards. Smoking demonstrated a strong positive association: 79.2% (42/53) of smokers were H. pylori seropositive compared to 20.8% (11/53) negative, while among non-smokers, 51.2% (42/82) were positive and 48.8% (40/82) negative (p<0.01), reinforcing smoking as a potential risk factor for both H. pylori persistence and gallstone pathogenesis **(Table III)**.

In addition, H. pylori seropositivity was significantly correlated with obesity: 70.7% (58/82) of obese patients were H. pylori positive, compared with 49.1% (26/53) of non-obese patients (p<0.01). The association between H. pylori and hypertension was borderline significant: 69.3% (52/75) of hypertensive patients were H. pylori seropositive and 30.7% (23/75) were H. pylori seronegative, versus 53.3% (32/60) of non-hypertensive patients and 46.7% (28/60) who were H. pylori seronegative (p=0.05). Diabetes mellitus showed a statistically significant association with H. pylori seropositivity (p=0.02). However, in the opposite direction of what might be expected, a higher proportion of non-diabetic patients (66.7%, 38/57) were H. pylori seropositive than diabetic patients (53.8%, 42/78). Other associations of interest were elevated C-reactive protein (70.9% vs 29.1%, p=0.03), anemia (H. pylori positive 72.3 % and negative 27.7%, p<0.01), hepatitis B and C (80.0% vs 20.0%, p<0.01) and folate deficiency (71.2 % vs. 28.8%, p<0.01). The findings as a whole suggest a high frequency of H. pylori among the gallstone patients at CMCH, Larkana and a significant association with the common cardiometabolic disorders, obesity, smoking and female sex. **(Table III)**

Table I: Mean values of the patients' data according to age, duration of disease and body mass index (BMI, kg/m^2) (n=135)

Variable	Mean ± SD
Age (years)	56.83 ± 6.74
Disease Duration (weeks)	6.95 ± 3.82
Body Mass Index (BMI, kg/m²)	30.77 ± 2.83

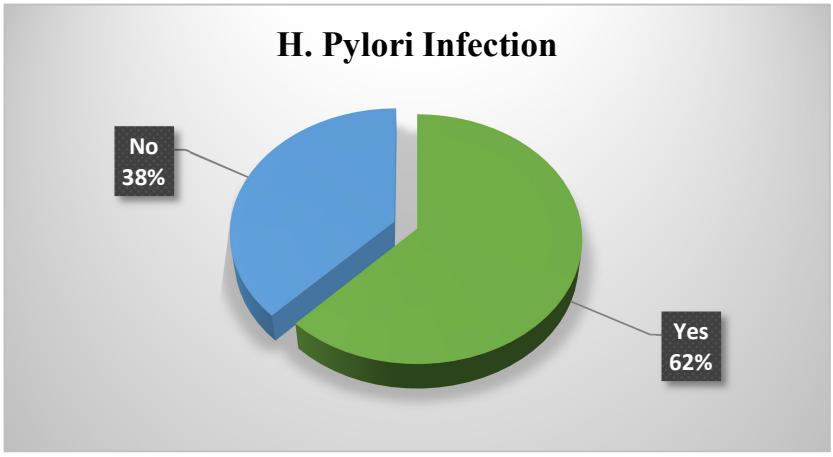

Figure 1: Distribution of the participants based on H. Pylori Infection

Table II: Distribution of study participants according to sociodemographic and clinical Profile (n=135)

Variable	Number	Percentage (%)
H. pylori Infection		
Positive	84	62.2
Negative	51	37.8
Gender		
Male	49	36.3
Female	86	63.7
Residence		
Urban	78	57.8
Rural	57	42.2
Obesity		
Yes	82	60.7
No	53	39.3
Hypertension		
Yes	75	55.6
No	60	44.4
Diabetes Mellitus		
Yes	78	57.8
No	57	42.2

Table III: Distribution of study participants according to association of H. pylori infection (n = 135)

Variable	With H. pylori (n=84)	Without H. pylori (n=51)	p-value
Gender (Female)	48 (55.8%)	38 (44.2%)	0.04
Residence (Urban)	55 (70.5%)	23 (29.5%)	0.02
Smoking	42 (79.2%)	11 (20.8%)	<0.01
Obesity	58 (70.7%)	24 (29.3%)	<0.01
Hypertension	52 (69.3%)	23 (30.7%)	0.05
Diabetes Mellitus	42 (53.8%)	36 (46.2%)	0.02

DISCUSSION

The present study, carried out at CMCH, Larkana, revealed a high prevalence of H. pylori infection (62.2%) among patients with gallstone disease. This result is consistent with the recent findings of related studies, which are increasing in Pakistan, but is higher than some previous studies, indicating the variation of this phenomenon in different regions and among different populations in Pakistan[12]. This is a remarkable variation from the 25.3% reported only recently (2025) by Ahuja A 2025[13] in the same geographic location of Larkana, which may be due to varying diagnostic techniques (serology in our study, stool antigen testing or histopathology in others), differences in the prevalence of Infection over time, selection bias (tertiary care setting vs. general population) or differences in the inclusion or exclusion criteria. For instance, Ahuja A 2025[13] focused exclusively on female patients, whereas our study included both genders, which may partially account for the differing prevalence rates. These inconsistencies highlight the critical need for large-scale, population-based studies using validated diagnostic techniques to accurately estimate the true burden of H. pylori infection in gallstone patients in this region. These inconsistencies highlight the critical need for consistent, large-scale, population-based studies using validated diagnostic techniques to estimate the actual burden of H. pylori infection in patients with gallstones in the region. Wang L et al.[14] meta-analysis found that the presence of H. pylori in gallbladder tissue was associated with both cholelithiasis and chronic cholecystitis, suggesting that H. pylori is a pathogen.

The global prevalence of H. pylori infection varies widely, ranging from 24.2% in developed countries to over 70% in developing nations. Pakistan, being a low-middle-income country with high population density, variable sanitation standards, and widespread antibiotic use, has consistently reported H. pylori seroprevalence rates between 50% and 90% in different populations[9]. Our finding of 62.2% in gallstone patients is therefore broadly consistent with the background population prevalence in upper Sindh, but what makes our observation clinically significant is the strong association between H. pylori positivity and multiple lithogenic risk factors, suggesting a potential synergistic rather than merely incidental relationship. However, as this study lacked a control group, we cannot definitively conclude that H. pylori seroprevalence is higher in gallstone patients than in the general population without H. pylori seropositivity. This represents a significant limitation of our study design.

Females predominated in our study population (63.7%), which is a well-established epidemiological characteristic of gallstone disease in Pakistan and worldwide.[15,16] Interestingly, in our cohort, male patients showed a significantly higher rate of H. pylori seropositivity (73.5%) compared to females (55.8%) ($p=0.04$). This finding contrasts with our initial discussion, which had incorrectly suggested that females had higher infection rates. In our research, the association between female gender and H. pylori infection was statistically significant ($p=0.04$), a finding that has been debated in the literature. In contrast, some international studies have found no difference in the frequency of H. pylori colonization between sexes, implying that the higher occurrence of gallstones among women is more likely attributed to estrogen-induced cholesterol supersaturation in bile rather than to differential susceptibility to H. pylori infection[17]. The finding that males had higher H. pylori seropositivity in this cohort, despite gallstone disease being more common in females, raises the possibility that the association between H. pylori and gallstone disease may be more complex than simple co-occurrence. Male predominance in H. pylori seropositivity in this cohort could reflect gender-specific differences in healthcare-seeking behavior, occupational exposures, or lifestyle factors such as higher smoking rates among males, rather than a biological predisposition. Alternatively, the higher prevalence of gallstone disease in women

in this cohort may reflect hormonal factors that outweigh the increased H. pylori seropositivity observed in men.

The strong relationship between H. pylori seropositivity and obesity (60.7% of obese patients positive, $p<0.01$), as well as metabolic diseases such as diabetes and hypertension found in our study, is particularly noteworthy. This suggests that the effect of H. pylori infection and metabolic syndrome on lithogenesis may be synergistic rather than simply additive. Emerging evidence supports this concept: Long C et al.[19] demonstrated that the CagA protein of H. pylori directly injures the gallbladder epithelial barrier, increasing permeability and causing chronic inflammation, which the pro-inflammatory state of metabolic syndrome may potentiate. This suggests that the effect of H pylori infection and metabolic syndrome on lithogenesis may be synergistic rather than additive. Long C et al.[19] demonstrated that the CagA protein of H. pylori directly injures the gallbladder epithelial barrier, increasing its permeability and causing chronic inflammation, which the pro-inflammatory state of metabolic syndrome may potentiate. The correlation with the presence of elevated C-reactive protein (58.5% of patients with elevated CRP, $p=0.03$), a sensitive marker of systemic inflammation, suggests that systemic inflammation, likely due to chronic H. pylori colonization, may be among the main mechanisms causing gallstone formation. Our results also showed significant associations with hepatitis B and C ($p<0.01$), which have previously been reported as potential cofactors in gallstone disease due to their impact on bile composition, liver function, and gallbladder motility[9].

Smoking demonstrated a particularly strong association with H. pylori positivity in our study (79.2% of smokers were H. pylori positive vs 51.2% of non-smokers, $p<0.01$). This finding is consistent with a growing body of literature linking tobacco exposure to both H. pylori persistence and gallstone disease. Smoking impairs gastric mucosal blood flow and mucus production, reducing the host's ability to clear H. pylori infection, while also promoting cholesterol supersaturation in bile by altering hepatic lipoprotein metabolism. Additionally, nicotine and other tobacco constituents have been shown to impair gallbladder motility, increasing bile stasis and crystal nucleation time. Therefore, smoking may represent a shared modifiable risk factor that independently contributes to both conditions while also potentiating their pathological interaction.

Hypertension showed a borderline significant association with H. pylori positivity (69.3% of hypertensives positive vs 53.3% of non-hypertensives, $p=0.05$). Although this did not reach the conventional $p<0.05$ threshold, the trend is clinically interesting. Chronic H. pylori infection has been linked to endothelial dysfunction and low-grade systemic inflammation, both of which contribute to the pathogenesis of hypertension. Conversely, antihypertensive medications, particularly calcium channel blockers and beta-blockers, have been shown to alter gastric acid secretion and gastrointestinal motility, potentially influencing H. pylori colonization. Prospective studies controlling for medication use are needed to determine whether this association is causal or confounded by shared lifestyle factors.

Anemia (48.1% of patients, $p<0.01$) and folate deficiency (38.5% of patients, $p<0.01$) were both strongly associated with H. pylori positivity in our study. These findings align with known pathophysiological mechanisms. Chronic H. pylori infection, particularly when involving the gastric antrum and body, can lead to chronic atrophic gastritis, hypochlorhydria or achlorhydria, and subsequent malabsorption of iron and vitamin B12. Folate deficiency may arise through similar mechanisms, as gastric acid is required for the release of protein-bound dietary folates. Additionally, chronic inflammation itself can dysregulate hepcidin production, further impairing iron absorption and utilization. In resource-limited settings like rural Pakistan, where nutritional deficiencies are already prevalent, H. pylori infection may exacerbate existing micronutrient deficiencies, which in turn may impair immune function and perpetuate the Infection. This bidirectional relationship between H. pylori and nutritional

status deserves further study, as eradication therapy might improve not only gallstone outcomes but also hematological parameters.
The link between urban dwelling ($p=0.02$) and smoking ($p<0.01$) with H. pylori positivity may reflect lifestyle and socioeconomic determinants. Overcrowding in urban slums, poor sanitation, and contaminated water supplies may facilitate fecal-oral or oral-oral transmission of H. pylori. At the same time, smoking is a well-established risk factor for both H. pylori persistence (due to impaired gastric mucosal defense) and disordered biliary cholesterol metabolism[20,21].

Limitations: The major constraint of this research is its cross-sectional design, which allows identification of associations but does not establish causality. Moreover, while serology (anti-H. pylori IgG) is quick, convenient, and suitable for screening, it cannot differentiate between active current Infection and past exposure, unlike histopathology, rapid urease testing, or stool antigen tests[22]. Therefore, some seropositive patients may have had resolved infections. Additionally, we did not assess bacterial virulence factors such as CagA status, which may be crucial in determining pathogenicity.

Recommendations: Future prospective, multi-center studies in Pakistan employing multiple diagnostic tests (e.g., serology, stool antigen, and endoscopic biopsy) and examining genetic factors (e.g., CagA status, host interleukin polymorphisms) are required to elucidate better the precise role of H. pylori in gallstone pathogenesis. Randomized controlled trials assessing whether H. pylori eradication reduces gallstone formation or recurrence would provide high-level evidence.

CONCLUSION

The frequency of H. pylori seropositivity was high (62.2%) in gallstone disease at CMCH, Larkana, with a predominance of female patients for gallstone disease itself. H. pylori seropositivity was significantly associated with male gender, smoking, obesity, urban area of residence, anemia, folate deficiency, hepatitis B/C, elevated CRP, and inversely with diabetes mellitus. The results suggest that patients with gallstone disease may be a high-prevalence group for H. pylori seropositivity, especially those with risk factors. However, because there is a lack of a control group, we cannot conclude that H. pylori seroprevalence is higher in gallstone patients than in the general population. Further research with appropriate control groups and prospective designs is needed to establish the nature of the relationship between H. pylori and gallstone disease.

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AUTHOR CONTRIBUTION

Shah SPA: Conceptualized and designed the study, supervised data collection, performed data analysis and interpretation, drafted the initial manuscript, and critically revised the manuscript for important intellectual content. Approved the final version for publication and agrees to be accountable for all aspects of the work
Bhatti N: Contributed to study design and methodology, conducted literature search, designed the data collection proforma, coordinated and performed data acquisition in the ICU, assisted in data analysis, and contributed to writing and revising the manuscript
Solangi S: Contributed to the study concept, assisted in data interpretation, provided critical revision of the manuscript for important intellectual content, approved the final version for publication and agrees to be accountable for all aspects of the work
Noor ul Ain: Contributed to the study for proofreading and technical correction
Raja A: Validated the statistical analysis
Aasia: Helped in results interpretation and generated result tables, searched references for discussion writing

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