DEVELOPMENTAL MEDICO-LIFE-SCIENCES

ISSN (P): 3007-2786, (E): 3007-2794 DOI: https://doi.org/10.69750/dmls.02.07.0127

ORIGINAL RESEARCH ARTICLE Open Access

Prevalence of *Helicobacter pylori* Among Dyspeptic Patients in Lahore: A Gender and Age-Based Cross-Sectional Analysis

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ABSTRACT

Background: *Helicobacter pylori* (*H. pylori*) is a major pathogenic factor associated with peptic ulcers, gastric malignancies, and chronic dyspeptic conditions. Its prevalence remains a significant health burden in developing countries such as Pakistan, where epidemiological data stratified by age and gender are limited.

Objective: To determine the prevalence of *H. pylori* among dyspeptic patients in Lahore and to evaluate age- and gender-based differences using manual and automated ELISA methods.

Methods: A descriptive cross-sectional study was conducted from June 2022 to April 2023 in three tertiary care hospitals in Lahore. A total of 455 dyspeptic patients aged 15–85 years were enrolled. Serum samples were collected and tested for *H. pylori*-specific IgG antibodies using both manual and automated ELISA kits. Data were analyzed using SPSS version 21.0, and associations with age and gender were assessed using the Chi-square test, with $p < 0.05$ considered significant.

Results: Out of 455 patients, 102 (22.41%) tested positive for *H. pylori*. A higher prevalence was observed in males (56.86%) compared to females (43.13%). The highest prevalence occurred in the younger age groups: 15–25 years (28.4%) and 26–35 years (23.5%). A significant correlation was found between younger age and *H. pylori* positivity ($p < 0.05$).

Conclusion: *H. pylori* infection remains an important cause of dyspepsia in Lahore, with higher prevalence in males and younger individuals. These findings highlight the need for early screening, timely diagnosis, and targeted treatment strategies in younger populations to prevent long-term complications.

Keywords: *Helicobacter pylori*, dyspepsia, prevalence, ELISA, age distribution, gender differences, Pakistan.

Received: 12/04/2025
Revised: 05/08/2025
Accepted: 10/09/2025
Published: 22/09/2025

INTRODUCTION

Helicobacter pylori (*H. pylori*) is Gram-negative, microaerobic, spiral bacterium, and colonizes gastric mucosa of a human stomach that plays a key role in the pathogenesis of a broad spectrum of gastrointestinal diseases [1]. Barry Marshall and Robin Warren initially described it as a benign colonizer as a gastric commensal organism in 1982 [1]. Later studies however proved its pathogenic role, and is today classified by the World Health Organization (WHO) as Class I carcinogen, highly linked with peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, chronic gastritis, and functional dyspepsia [2,3].

One of the most significant reasons of outpatient visits in the world is dyspepsia or the syndrome of recurrent or persistent pain in the upper abdomen [4]. Clinical signs will consist of bloating, early fullness, nausea, vomiting, epigastric pains or burning [4]. Dyspepsia can be functional (non structural, non-ulcer) as well as dyspepsia has a multifactorial pathogenesis. However, one of the most common contributory factors is the *H. pylori* infection especially in the endemic areas [5].

The world has a population of about half estimated to be harboring *H. pylori* [6]. Its weight is dissimilarly high in less developed nations where congestion, absence of hygiene and lack of access to safe water influence transmission [7]. Reports of prevalence in Pakistan were between 56-60% [8], and the contraction of the infection is usually done during early childhood by interfamilial infection or exposure to the environment [9].

Although the clinical significance of *H. pylori* has been well reported in the global literature, relative lack of epidemiological data that specifically deals with dyspeptic patients with regard to demographical factors including age and gender is observed in the Pakistani locality. Lahore, as a highly populated city, has its individual socioeconomic and environmental risk factors that lead to the prevalence of infections [10]. In addition, diagnostic methods and laboratory infrastructure differences also cause inconsistency in the overall diagnostic rates, highlighting the significance of locally based prevalence study [11].

This gap was aimed to be covered by the current study as it sought to establish the prevalence of the *H. pylori* infection in dyspeptic patients at Lahore who experienced symptoms lasting more than one month. The enzyme-linked immunosorbent assay (ELISA) technique was used to detect *H. pylori*-specific IgG antibodies using both manual and automated techniques. Further, the relationships between infection, age, and gender were logically compared to outline the patterns of distribution of this pathogen among study participants [12-14].

The results of this study will contribute to the establishment of baseline epidemiological information to inform the policy of the Pakistani population on the issue of health promotion, the formulation of a specific diagnostic plan, and the imposition of the therapeutic intervention plan of an individual with dyspeptic. Additionally, its results can be used as a basis of future studies that can include molecular diagnostic and antimicrobial resistance profiling [15].

METHODS AND MATERIALS

This cross-sectional descriptive study was done to identify the prevalence of *Helicobacter pylori* in patients who have dyspeptic symptoms. The researchers conducted the study during a ten-month period, i.e. between June 13, 2022 and April 13, 2023, in three tertiary care hospitals in Lahore, Pakistan, i.e. City Hospital Sharif Medical Complex, Jinnah Hospital and Bahria International Hospital.

These hospitals were selected due to high turnover of patients in such hospitals, provision of diagnostic facilities, and the access to the wide range of urban population.

Both male and female patients of age group 15-85 and with a history of dyspeptic symptoms lasting over one year and gave informed consent were included. The exclusion criteria included those with mixed gastrointestinal infections who reported gastric pain due to other causes, test samples that were improperly labeled or delayed, patients that had taken some antibiotics in less than four weeks, and those who had undergone any antimicrobials in the recent past as their detection of *H. pylori* could be interfered.

Trained phlebotomists used yellow-top vacutainer tubes that had clot activator in collecting venous blood samples of eligible patients. Routine aseptic precautions were followed with the skin being disinfected with 70% ethanol prior to venipuncture. A different patient identification number was used in each of the samples and the samples were sent to the diagnostic laboratories of the respective hospitals. A 10-minute centrifugation running at 3000 rpm were performed and the serum was separated and the result used in the detection of antibodies.

The identification of *H. pylori* was done through manual and automated enzyme-linked immunosorbent assay (ELISA). Anti-*H. pylori* IgG antibodies were identified in the manual ELISA method by the help of a commercial ELISA kit. The aliquot of serum was diluted by 10 µL of the dilution in 200 µL and incubated in the microwells that were impregnated with the *H. pylori* antigens. Following the various washing procedures, enzyme conjugate, substrate, and stop solution were loaded in that order. A microplate reader was used to determine the optical density at 450 nm. The positivity cut-off was set in terms of positive controls (maximum of the average OD of the negative controls and 0.9) values below 0.9 were defined as negative and values equal to or above 1.1 were defined as positive [13].

A smaller subset of samples was also tested with the Cobas e411 automated immunoassay analyzer that also utilizes the chemiluminescense immunoassay technology in order to improve the diagnostic sensitivity and reduce the human error. Under this technique the streptavidin-coated microparticles were incubated with biotinylated *H. pylori* antigens to form sandwich-type immune complexes which were determined using the analyzer. Each of these runs had internal controls to ensure accuracy and this automated method was faster, more sensitive and more reliable trying to process the ELISA than manual ELISA [14].

Strict measures relating to quality control were followed in the course of the study. Both positive and negative control sera were added to each diagnostic batch in order to confirm the test validity. There was consistent use of standardized reagents and procedures and there were internal audits advanced by top laboratory technologist in order to maintain accuracy and reproducibility.

The analysis of obtained data was performed with the help of the SPSS version 21.0 and GraphPad Prism version 9. The findings were reflected in frequencies, percentages and proportions. The Chi-square test was used to determine associations between *H. pylori* seropositivity and demographic variables age and gender and a p-value of less than 0.05 was the considered statistically significant [15].

RESULTS

A total of 455 patients presenting with dyspeptic symptoms were enrolled from three tertiary care hospitals in Lahore. Among them, 102 patients (22.41%) tested positive for *Helicobacter pylori*-specific IgG antibodies, while 353 patients (77.58%) were negative, as

determined by both manual and automated ELISA assays (Table 1). These findings demonstrate that approximately one-fifth of the dyspeptic population in the study cohort was seropositive for *H. pylori*. Table 1 presents the overall prevalence of *H. pylori* infection in the study population, while Figure 1 illustrates the distribution of positive and negative cases by gender.

Table-1: Overall Prevalence of *H. pylori*

Status	n	Percentage
***H. pylori* Positive**	102	22.41%
***H. pylori* Negative**	353	77.58%
Total	**455**	**100%**

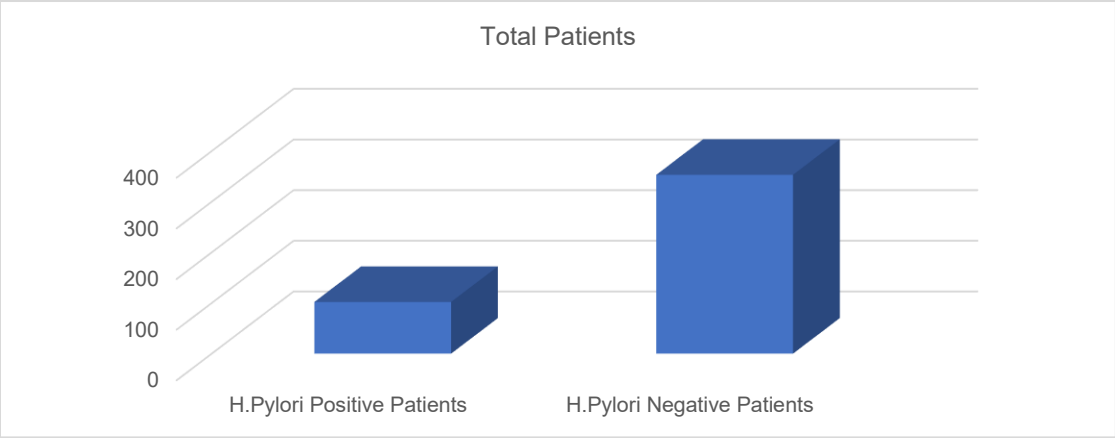

Figure 1: Distribution among genders of patients that tested positive/negative against *H. pylori* presented as a bar graph.

When analyzed by gender, the prevalence of *H. pylori* positivity was found to be higher in males compared to females. Out of the 102 positive cases, 58 were males (56.86%) and 44 were females (43.13%), indicating a male predominance (Table 2). The total study population consisted of 254 males and 201 females, suggesting that men were more frequently affected than women in this cohort. The graphical representation of this distribution is provided in Figure 1, which shows a higher frequency of positive cases among males.

Table-2: Gender Distribution of *H. pylori* Positive Patients

Gender	Positive	Percentage
Male	58	56.86%
Female	44	43.13%

The analysis of prevalence across age groups revealed that the infection was most common among younger patients. The highest proportion of positive cases occurred in the 15–25-year age group, where 29 individuals (28.4%) tested positive. This was followed by the 26–35-year age group with 24 cases (23.5%) and the 36–45-year group with 23 cases (22.5%). The prevalence then progressively decreased with age, with 14 cases

(13.7%) in the 46–55 group, seven cases (6.86%) in the 56–65 group, three cases (2.94%) in the 66–75 group, and only two cases (1.96%) in the 76–85 group (Table 3). These results clearly demonstrate a trend in which younger individuals were more frequently affected compared to older patients. This distribution is visually summarized in Figure 2, which displays the decline in *H. pylori* positivity with advancing age.

Table-3: These results demonstrate that the prevalence of infection decreases with the age.

Age Group	n	Percentage
15–25	29	28.4%
26–35	24	23.5%
36–45	23	22.5%
46–55	14	13.7%
56–65	7	6.86%
66–75	3	2.94%
76–85	2	1.96%

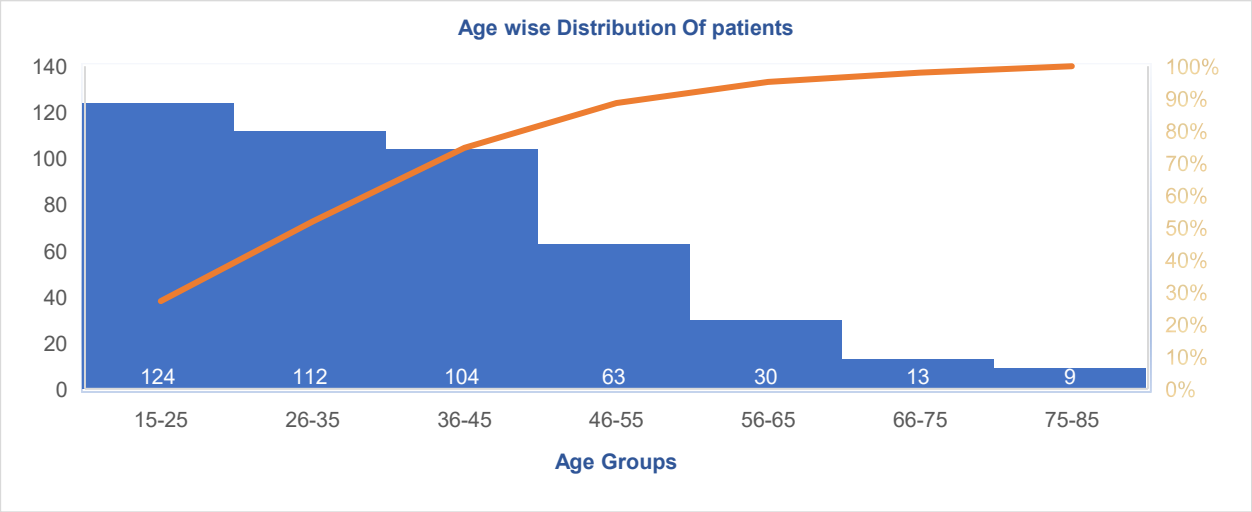

Figure-2: Bar graph to show distribution of *H. pylori*- positive patients according to their age.

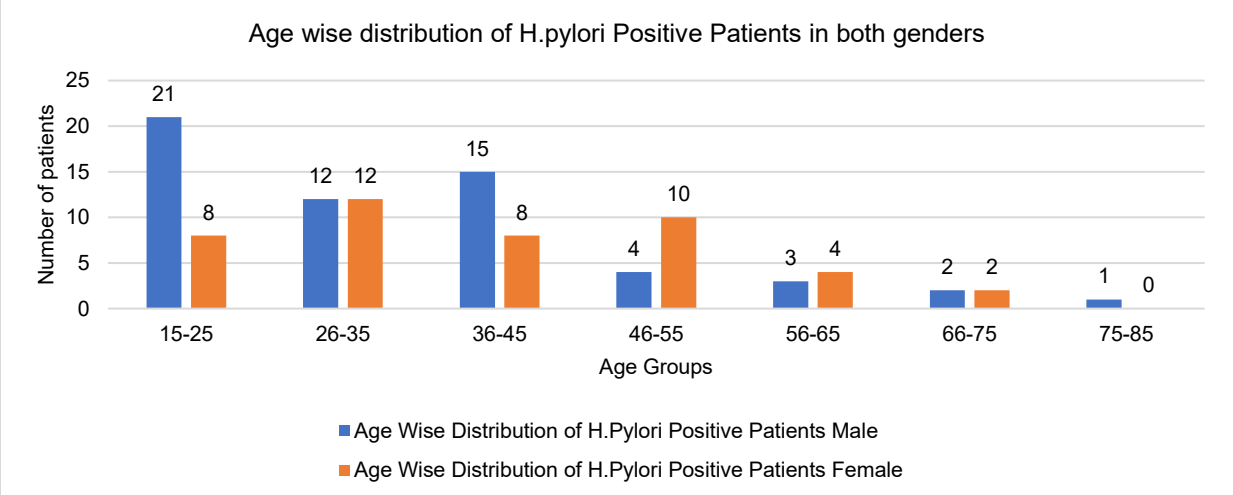

Figure-3: Age-wise distribution of *H. pylori* positive patients by gender (male vs. female) in a sample of 102 seropositive cases.

Further analysis of age stratification by gender revealed interesting differences. In the 15–25-year age group, 21 males and eight females were positive for *H. pylori*. In the 26–35-year group, the number of positive cases was equal for both genders (12 males and 12 females). In the 36–45 group, 15 males were positive compared with eight females, whereas in the 46–55 group the trend was reversed, with 10 females testing positive compared to four males. In the older age groups (56–65, 66–75, and 76–85 years), men remained slightly more affected, although the overall numbers were low (Table 4).

This disaggregated gender-by-age distribution highlights that younger males carried the highest burden of infection, while in middle age the prevalence was more balanced, and in older groups the overall number of positives was low. These findings are presented in Figure 3, which compares age-specific prevalence between males and females, and in Figure 4, which provides a stacked bar chart showing the combined age- and gender-specific positivity trends. Taken together, these results suggest that *H. pylori* infection was significantly more prevalent among young adults, particularly males, with a steady decline in prevalence as age increased. The consistent patterns across both manual and automated ELISA detection reinforce the robustness of these findings.

Table-4: This disaggregation indicates that more men were affected in majority of age groups

Age Group	Male	Female
15–25	21	8
26–35	12	12
36–45	15	8
46–55	4	10
56–65	3	4
66–75	2	2
76–85	1	0

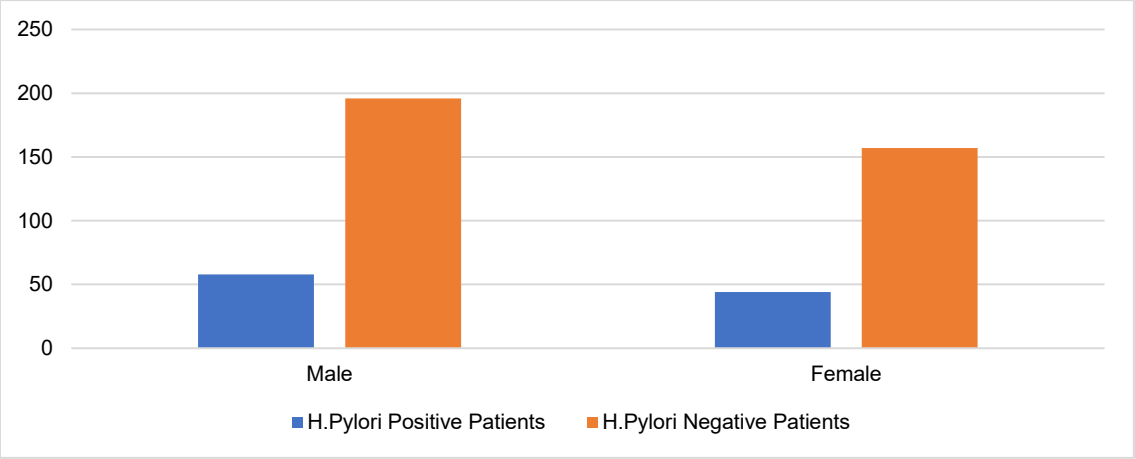

Figure-4: Bar chart of age wise *H. pylori* positivity in both genders (stacked).

DISCUSSION

This cross-sectional study provides important insights into the prevalence and demographic distribution of *Helicobacter pylori* infection among dyspeptic patients in Lahore, Pakistan. Using both manual and automated ELISA methods, we found an overall seroprevalence of 22.41% in a cohort of 455 patients. This result is consistent with studies conducted in eastern Pakistan and neighboring regions, where comparable rates have been observed in populations with similar socioeconomic and environmental conditions [13,14].

A notable observation in our study was the gender difference in prevalence, with 56.86% of positive cases occurring in males and 43.13% in females. Similar male predominance has been documented in other Pakistani studies, as well as in study from Kuwait and Iran, where cultural, behavioral, and occupational factors increase men's exposure to risk [15,16,17]. Possible explanations include greater involvement of men in outdoor work, lower adherence to hygiene standards in public settings, and higher consumption of street-prepared food. In contrast, Japanese data have reported a higher prevalence among women, suggesting that gender-specific dietary practices and health-seeking behaviors can influence infection dynamics differently across populations [18].

Age-based analysis revealed the highest infection rates in the 15–25 year age group (28.4%), followed by 26–35 years (23.5%), with a progressive decline in prevalence among older adults. This pattern contrasts with international studies, which often show an accumulation of infection with advancing age [19]. The discrepancy may be explained by early acquisition of *H. pylori* in Pakistani populations, where household crowding, poor sanitation, and limited access to clean water increase transmission during childhood [20]. Familial clustering of infection and repeated exposure within densely populated urban households have been reported as major determinants of early acquisition [21]. Furthermore, improvements in hygiene, antibiotic availability, and diagnostic access over the past two decades may explain the reduced persistence of infection in older age groups, as clearance or suppression of the bacterium becomes more likely [22].

The diagnostic approach used in this study strengthens the reliability of the results. Manual ELISA remains an inexpensive and widely available method for antibody detection, but it is subject to variability and operator error. The use of the Cobas e411 automated immunoassay system in a subset of samples minimized these limitations by providing higher sensitivity, improved reproducibility, and greater throughput [23]. Despite these advantages, serological assays detect prior exposure rather than active infection, and therefore cannot be used as definitive clinical diagnostic tools. Urea breath testing (UBT) and stool antigen assays remain superior for confirmation of active disease and should complement antibody testing in clinical practice [24].

Comparison with local and regional studies highlights both consistencies and differences. Sarwar et al. reported a positivity rate of 61.8% in a Lahore-based cohort, a much higher prevalence than observed in the present study [15]. The difference may be attributable to differences in study design, as their sample was biased toward middle-aged men and did not exclude recent antibiotic users, whereas our broader age distribution and stricter inclusion criteria likely contributed to a lower prevalence. In Iran, Kianmehr et al. found a prevalence of 23.2% among patients with gastric disorders, which closely mirrors our findings and supports the notion that cultural and socioeconomic

similarities may result in comparable infection patterns [16].

The clinical and public health implications of these findings are significant. Our data indicate that younger adults, particularly males, represent a high-risk group for *H. pylori* infection in Lahore. Since early infection is often asymptomatic yet predisposes to long-term complications such as peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric carcinoma, timely screening and management are critical [25]. Preventive strategies, including improvements in sanitation, safe water provision, and health education, are essential to reducing transmission within families and communities [26]. Early intervention may not only lower the prevalence of infection but also decrease the future burden of severe gastrointestinal diseases in Pakistan.

These findings align with international consensus recommendations. Both the Maastricht Consensus Report and the American College of Gastroenterology guidelines advocate for testing and treating *H. pylori* in patients with unexplained dyspeptic symptoms, given the risk of progression to more serious disease if left untreated [27,28]. Incorporating such recommendations into local clinical practice would allow for earlier diagnosis and targeted treatment in high-risk groups. Additionally, the use of serological assays for preliminary screening, followed by confirmatory UBT or stool antigen tests, could provide a cost-effective diagnostic strategy in resource-limited healthcare settings [29].

Despite the strengths of a large and diverse study population, this study has certain limitations. Being cross-sectional in design, it cannot establish causal relationships. They depend on antibody detection in order to differentiate between previous and present infections and, thus, prevalence could be underestimated or overestimated on certain cases. Moreover, the paper has not examined the patterns of antibiotic resistance; that is, still a major determinant of the treatment of *H. pylori* in Pakistan [30]. Molecular diagnostic equipment and antibiotic susceptibility typing should be incorporated in future investigations to maximize the treatment measures [31,32].

Overall, the present study illustrates that the *H. pylori* infection is a major cause of dyspepsia in Lahore with the prevalence being higher in males and younger people. The results confirm the necessity of special screening and early diagnosis, especially in high risk groups so as to avoid chronic complications. This will involve enhancing the diagnostic capacity, ensuring sanitation and hygiene and aligning the local management practice with the international practice to curb the load of *H. pylori*-associated disease in Pakistan [33–35].

CONCLUSION

This study determined that *Helicobacter pylori* infection was more common in younger patients and males in patients with dyspeptic patients in Lahore. Manual and automated ELISA were both effective in detecting the disease at an early stage, and hence they assist in the early diagnosis. The burden of infection needs to be decreased through improved hygiene, increased diversity in diagnostics, and awareness about health care in the community. Active infection by other means such as molecular should be justified in future studies to determine resistance to antibiotics to best inform treatment regimens.

Conflict of Interest:
The authors report no conflicts of interest.
Funding:
No external funding was received for this study.
Acknowledgments:
We gratefully acknowledge our colleagues and all study participants for their valuable contribution.

Authors' contributions:

Conceptualization: FM, AYM
Data Collection: AU, AK
Laboratory Analysis: FM
Statistical Analysis: AYM
Manuscript Drafting: AU
Critical Review & Editing: AK
Final Approval: All authors

Data Availability Statement:

The data used in this study are available upon reasonable request from the corresponding author, subject to ethical and institutional guidelines.

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This Article May be cited As: *Maqsood F, Mujahid AY, Umar A, Khan A. Prevalence of Helicobacter pylori Among Dyspeptic Patients in Lahore: A Gender and Age-Based Cross-Sectional Analysis: H. pylori Prevalence in Dyspeptic Patients of Lahore. DEVELOPMENTAL MEDICO-LIFE-SCIENCES. 2025;2(7): 23-32. doi: 10.69750/dmls.02.07.0127*