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HELICOBACTER PYLORI PREVALENCE AND ASSOCIATED RISK FACTORS IN INDIVIDUALS WITH RECURRENT EPIGASTRIC PAIN: LAHORE, PAKISTAN

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Abstract

A number of stomach diseases, including gastritis, peptic ulcers, and gastric cancer, have been related to Helicobacter pylori (H. pylori), creating serious public health concerns. The current investigation aimed to determine the frequency of H. pylori infection and related risk factors in individuals having symptoms of recurring epigastric pain.

Methods: *The present study involves 191 participants having recurring epigastric pain. Sociodemographic data, blood, and tissue samples were obtained from 112 patients between September 2020 to August 2021. Biochemical and molecular methods were used to estimate the infection frequency.*

Results: *The findings revealed that the overall infection ratio was 58% (n=65 out of 112). Males (n=41, 63.1%) contracted the infection more frequently than females. The participants belong to the 26-30 age group (n=37, 56.9%) low socioeconomic status (n=45, 69.2%), resided in rural areas (n=54, 83.1%), and consumed unfiltered water (n=57, 87.7%) had observed with significantly higher infection rates. The regression analysis demonstrated greater probability of contracting infection in male gender (OR 5.91, 95%CI: 2.40-12.9), in 26-35 age group (OR 6.16, 95%CI: 1.75-21.6), tap water users (OR 4.42, 95%CI: 1.72-11.3), rural area residents (OR 3.31, 95%CI:1.39-9.76), and low economic status group (OR 5.26, 95%CI:1.84-16.5) as p< 0.05).*

Conclusion: *The findings revealed the incidence of H. pylori infection (58%) and its associated risk factors in individuals having recurring epigastric pain. The results suggest that larger datasets are needed for future studies to accurately reflect infection frequency and risk factors to diagnose and treat the disease.*

Keywords: *Epigastric pain, Frequency, Gastritis, Helicobacter pylori, Risk factors*

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a pathogenic microaerophile linked to several upper gastrointestinal illnesses. It adheres to the stomach epithelial lining and can lead to significant gastric ailments such as peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer (1-3). *H. pylori* infection is highly prevalent on a global scale, affecting approximately 50% of individuals in developing countries and 10-20% in developed nations. The prevalence of this infection exhibits regional disparities, with higher rates in developing regions and lower rates in developed ones. In some areas, the frequency of *H. pylori* infection can reach as high as 80%. An extensive analysis conducted in 2015 reported approximately 4.4 billion cases of *H. pylori* infection worldwide (4-8).

Numerous factors influence the frequency of illnesses, as shown by the wide variations in *H. pylori* prevalence rates between nations. Incidence rates are rising in developed countries due to inadequate sanitation, contaminated water and food, and poor living circumstances. If left untreated, peptic ulcer disease and stomach cancer can develop in 10 to 15 % of infections (6, 9). Studies reported that *H. pylori* infection has been linked to upper gastrointestinal pain. In Turkish patients experiencing dyspepsia and persistent stomach discomfort, 66% were found to have *H. pylori* infections. In another study, *H. pylori* was detected in only 8% of individuals with persistent stomach discomfort (10-12). *H. pylori* colonization might persist throughout a lifetime if not adequately treated. There is substantial debate over the appropriateness

of testing and treating *H. pylori* in those who experience recurrent epigastric discomfort. A rational approach is necessary to shed light on the situation (13, 14).

The conventional method for addressing *H. pylori* infections has historically relied on a trial-and-error strategy, combining several antibiotics with acid-suppressing medications. Unfortunately, this approach has encountered significant challenges, primarily stemming from the growing problem of antibiotic resistance, leading to a high rate of treatment failure (15). Recognizing the escalating prevalence of *H. pylori* infections and the concurrent rise in antibiotic resistance, the World Health Organization (WHO) has designated *H. pylori* as a global priority pathogen (16). Early diagnosis of *H. pylori* in individuals suffering from recurrent epigastric discomfort might affect early treatment options, lowering the risk of developing *H. pylori*-related serious diseases such as gastric cancer.

The current investigation's objective was to estimate *Helicobacter pylori's* prevalence rate as an underlying organic disease in the local community suffering from recurring epigastric discomfort, as well as how different sociodemographic factors influence the risk of disease development. Frequency analysis might help emphasize the importance of early treatment for *H. pylori* infection in individuals suffering from persistent epigastric discomfort in developing nations. Analyzing common risk factors associated with the development of diseases may serve in developing prognostic criteria and selecting the appropriate treatment strategy.

MATERIALS AND METHODS

STUDY DESIGN

The present study adopted a cross-sectional research design. It involved the collection of sociodemographic data, biopsy samples, and blood samples from patients experiencing recurrent epigastric pain at a public hospital in Lahore, Pakistan. Data related to the participants' sociodemographic characteristics were acquired through a concise questionnaire designed for this purpose. The sample and data were gathered using a purposive sampling approach. The data collection period extended from September 2020 to August 2021.

SAMPLE COLLECTION

Infection prevalence was determined through biochemical and molecular analyses of blood and biopsy samples. The study involved a total of 191 adult individuals aged between 20 and 70 years who were experiencing epigastric discomfort. Among these individuals, gastric biopsy samples and blood samples were obtained from 112 patients who were slated for biopsy procedures, which encompassed both corpus and antrum biopsies. However, 79 cases were excluded from the study due to various factors, including the use of antibiotics, non-endoscopic conditions, and pregnancy.

METHODS OF *H. PYLORI* DIAGNOSIS

Four diagnostic methods were employed to determine the frequency status of *H. pylori* in the target population.

WHOLE BLOOD ANALYSIS FOR ANTIBODY DETECTION

According to the manufacturer's instructions, anti-*H. pylori* antibody strips (Chemtrue one-step test cassette, San Diego, USA) were used to analyze the blood samples.

H. PYLORI ISOLATION: BACTERIAL CULTURE

Biopsy specimens were cultured in sterile BHI medium containing 5% calf serum, DENT antibiotics (MP Biomedicals, Inc. France), and urea (0.6 g). Inoculated broths with specimens were cultivated for 7 days under microaerophilic conditions at 37°C in a CO_2 incubator (17).

BIOCHEMICAL ANALYSIS

Following the cultivation and isolation of *H. pylori* on agar plates, the cultures were subjected to two standard biochemical assays, catalase (18) and urease (19).

MOLECULAR CHARACTERIZATION: 16SrRNA PCR ANALYSIS

A bacterial genomic DNA extraction kit was used to extract genomic DNA from biochemically verified isolates (Thermo Scientific).The 500 bp gene product was amplified from isolated genomic DNA using forward and reverse primers previously developed and published by Al Sulami et al. for the 16SrRNA sequence (20). A 25 µL reaction mixture including 2 µL reaction buffer, 1 µL $MgCl_2$, 0.5 µL dNTPs, 1 µL forward and reverse primer, 1 µL DNA samples, 18.0 µL double distilled water, and 0.5 µL Taq polymerase was employed for amplification. Denaturation at 94°C for three minutes, annealing at 56°C for 45 seconds, extension at 72°C for one minute, and final extension for five minutes (35 cycles) are the PCR conditions. Amplified products were electrophoresed in 2% agarose gels and then exposed to UV light.

ETHICAL STATEMENT

The study only included participants with informed consent, having symptoms of recurrent epigastric pain, an endoscopic diagnosis, no active bleeding, and no biopsy-related contraindications. The Helsinki Declaration for the ethical conduct of human research was followed in this study. The ethics review board of the University of Punjab, Lahore approved the study since it meets the standards for fundamentally ethical human subjects research (Letter No./D158/FIMS).

STATISTICAL ANALYSIS

The SPSS 21 (Statistical Package for Social Sciences, Chicago, IL, USA) was used for data entry, demographic analysis, descriptive analysis of diagnostic tests, chi-square, and logistic regression analysis. To analyze the relationships between *H. pylori* infection and related risk variables, the chi-square test was used. The effect size of the association strength between infection and the independent variable was calculated using the phi/V Cramer's value. In order to evaluate possible risk factors, the logistic regression model was employed to obtain odds ratios and 95 % confidence intervals (CI).

RESULTS

DEMOGRAPHIC DETAILS OF PARTICIPENTS

The 112 participants had an average age of 36.15±10.72 (range, 20 – 70). Table I lists the participants' demographic information, including age, gender, location of residence, amount of water consumed, preference for consuming street food, and socioeconomic status. The gender distribution of the participants shows that women contribute 54.0% of the total participants, while men are 46.0% of the total sample. Most (42.9%) of individuals favored eating street food often, whereas more than two-thirds (76.8 %) utilized tap water as their primary source of drinking. According to the residential area, the vast majority of the inhabitants lived in rural regions. Low socioeconomic status was predominated (57.1 %).

DESCRIPTIVE STATISTICS OF DIAGNOSTIC VARIABLES

Four diagnostic methods were employed to identify the presence of *H. pylori* in the samples (Table II). Colonies having clear, greyish appearance on BHI agar medium with diameter of more than 2.0 mm were assumed as *H. pylori* positive and were selected for further biochemical and molecular screening (Fig.1a). The catalase test (Fig.1b) identifies the presence of *H. pylori* infection in 59.8% of the total (n=112) samples, while the urease test (Fig. 1c) confirmed presence infection in 56.3% (n=63) of the total cases. On the other hand, whole blood analysis of the patients with blood anti-*H. pylori* antibody strips, revealed that 57.1 % of the total samples were positive for *H. pylori* (Fig.1d). In order to estimate the total infection prevalence in the target population, the results of the PCR (16SrRNA) study were utilized to validate the results from the four diagnostic procedures. PCR (16SrRNA) analysis verified the infection in 58.0% (n=65) of the total cases (Fig. 1e).

Table I. Descriptive statistics of demographic variables and linked risk factors of *H. pylori*

Variables	Category	Frequency	%age
Gender	Male	52	46.0
	Female	60	54.0
Age	20-25	13	11.6
	26-35	49	43.8
	35-50	35	31.3
	Above 50	15	13.4
Residence location	Urban	30	26.8
	Rural	82	73.2
Drinking Water source	Filtered	26	23.2
	Tap	86	76.8
Street Food	Prefer	48	42.9
	Do not prefer	46	41.1
	Occasionally	18	16.1
Economic Status	Low	64	57.1
	Average	28	25.0
	High	20	17.9
	Total	112	100.0

Table II. Summary of biochemical and molecular examination of samples

Test Variables	Infection Status	Frequency	%age
Catalase	HP +ve	67	59.8
	HP -ve	45	40.2
Urease	HP +ve	63	56.3
	HP -ve	49	43.8
Strip Test	HP +ve	64	57.1
	HP -ve	48	42.9
PCR Analysis	HP +ve	65	58.0
	HP -ve	47	42.0

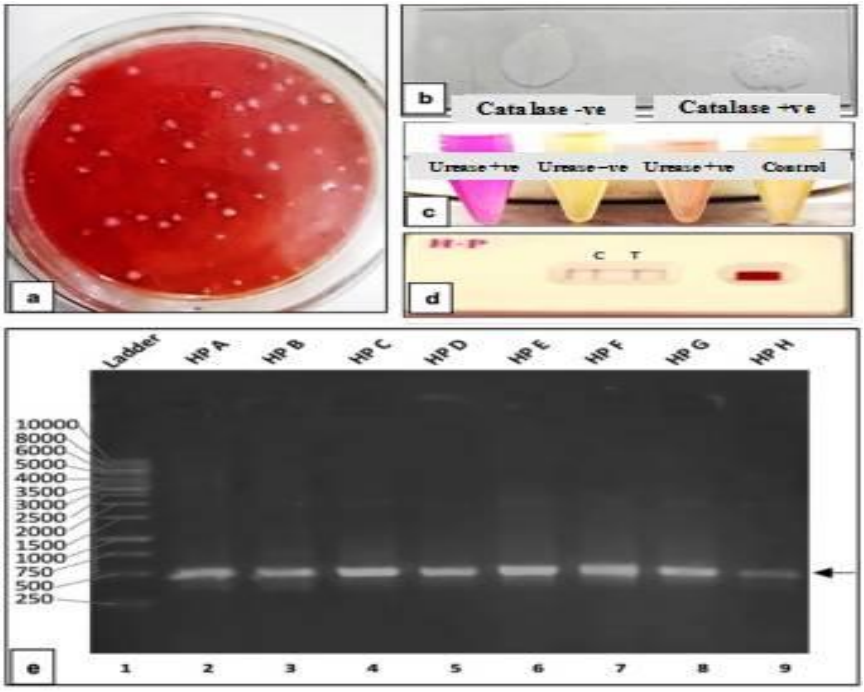

Fig. 1*a*. Grey color *H. pylori* colonies on BHI agar medium; b. Bubble formation in positive isolates; c. A magenta to light pink sample from the urease test indicates urease activity; d. Positive *H. pylori* blood antibody test; e. 16SrRNA PCR findings on 2% agarose gel; lane 1 showed 1 kb gene ruler, lane 2-9 showed 500 bp 16SrRNA product

ANALYSIS OF *HELICOBACTER PYLORI* PREVALENCE

The screening of 112 samples indicated that *H. pylori* infection was present in 65 individuals (58.0%), while 47 individuals (42.0%) tested negative (Fig. 2).

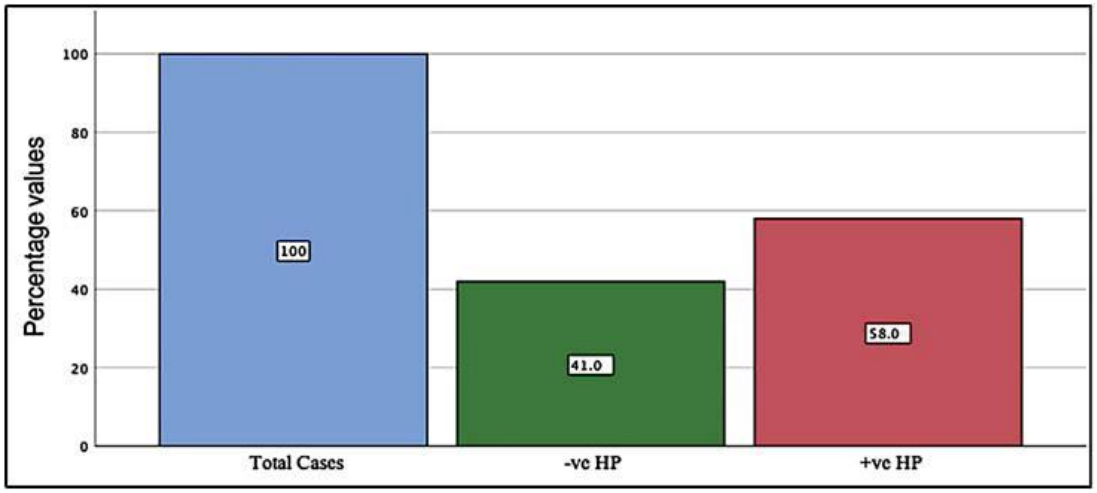

Figure 2. Frequency of *H. pylori* infection in patients having recurring epigastric pain

The probability of *H. pylori* infection among the middle age groups was significantly higher since the majority of individuals with *H. pylori* infection belong to 26 - 35 (56.9%) and 36 – 50 (32.3%) age groups ($\chi 2$ = 19.66, P value < 0.05). On the other hand, Males 54 (63.1%), rural residents 54 (83.1 %), tap water consumers 57 (87.7%), and low socioeconomic status 45 (69.2%) had a substantially greater probability of *H. pylori* infection. The phi or Cramer's V values exhibiting medium effect size for the significant association strength of gender, age, residence area, drinking water source and socioeconomic status with *H. pylori* infection occurrence (Chi square values = 15.702, 19.668, 6.531, 8.930, and 11.158 respectively) as p<0.05. Furthermore, there appears to be no significant strength of relationship between *H. pylori* infection and street food intake, as ($\chi 2$ =.809, p =.667, phi /Cramer's V=.085) P is greater than 0.05 (Table III).

Table III. *Helicobacter pylori* prevalence in epigastric pain patients

Variables	Category	Prevalence				
		Hp +ve (%)	df	Chi square ($\chi 2$)	Effect size (Phi / Cramer's V)	P- value
Gender	Male	41 (63.1)	1	15.702	.393	.000*
	Female	24 (36.9)				
Age	20-25	2 (3.1)	3	19.668	.419	.000*
	25-35	37 (56.9)				
	35-50	21 (32.3)				
	50 Above	5 (7.7)				
Residence Location	Urban	11 (16.9)	1	6.531	.262	.011*
	Rural	54 (83.1)				
Drinking Water Quality	Filtered	8 (12.3)	1	8.930	.304	.003*
	Tap	57 (87.7)				
Street Food	Prefer	28 (43.1)	2	0.809	.085	.667
	Never	25 (38.5)				
	Occasionally	12 (18.5)				
Economic Status	Low	45 (69.2)	2	11.158	.316	.004*
	Average	14 (21.5)				
	High	6 (9.2)				

The Pearson Chi-squared test was performed to look for statistical differences. N stands for number. *Symbol shows significant strength of relationship among the variables.

H. PYLORI INFECTION AND ASSOCIATED RISK FACTORS

The logistic regression model shows that men had significantly higher probability of contracting the infection (OR 5.91, 95 % CI: 2.40-12.9) than women. Risk is also significantly higher in 26-35 age group (OR 6.16, 95%CI: 1.75-21.6), tap water users (OR 4.42, 95%CI: 1.72-11.3), rural area residents (OR 3.31, 95%CI:1.39-9.76), and low economic status group (OR 5.26, 95%CI:1.84-16.5). The risk of infection was non-significantly lower in the 20-25 age range (OR 0.36, 95 % CI: 0.05-2.31) and among consumers who do not prefer street food (OR 0.59, 95 % CI: 0.19-1.85) compared to those over 50 and those who eat street food occasionally (Table IV).

Table IV. Logistic regression analysis of linked risk factors of *H. pylori*

Variables	Category	Odd Ratios (95% CI)	P Value
Gender	Female	1	
	Male	5.91 (2.40-12.9)	0.000
Age	Above 50	1	
	36-50	3.00 (0.84-10.6)	0.090
	26-35	6.16 (1.75-21.6)	0.005
	20-25	0.36 (0.05-2.31)	0.284
Residence location	Urban	1	
	Rural	3.31 (1.39-9.76)	0.007
Drinking Water source	Filtered	1	
	Tap	4.42 (1.72-11.3)	0.002
Street Food	Prefer	1.20 (0.22-2.17)	0.538
	Do not prefer	0.59 (0.19-1.85)	0.372
	Occasionally	1	
Economic Status	Low	5.26 (1.84-16.5)	0.002
	Average	2.33 (0.69-7.82)	0.170
	High	1	

DISCUSSION

H. pylori infections affect nearly 3 billion individuals globally, accounting for more than half of the population, with the majority of cases occurring in developing countries. It is a main predisposing factor for a range of stomach diseases in humans, including atrophic gastritis, gastric lymphoma, gastric adenocarcinoma, intestinal metaplasia, and ulceration disease. Hundreds of million people will suffer peptic ulcers at some time in their lives, and tens of millions will acquire stomach cancer. Most developed countries have lower infection rates than developing countries. Poor hygiene, a low socioeconomic standing, and contaminated water and food all raise the chance of infection by *H. pylori* (21-23). Nigeria, Portugal, Estonia, Kazakhstan, and Pakistan had the greatest *H. pylori* burdens in comparison to the rest of the world, while Switzerland had the lowest (24).

The current study investigated the prevalence of *H. pylori* in recurrent epigastric patients from metropolitan Lahore using four diagnostic methods. These methods were effective, easy, and widely accessible. The results of three biochemical diagnostic methods were confirmed by PCR to estimate the target population's overall infection prevalence (16SrRNA).

In patients with recurrent epigastric pain from metropolitan Lahore, the overall infection rate was 58%, which is greater than past research on *H. pylori* prevalence in Pakistan (25). *H. pylori* infection was identified in 87.7% of individuals experiencing epigastric stomach pain, as reported by Shokrzadeh et al. (26). In another study Daisy et al. (27) observed a prevalence of *H. pylori* infection at 42.4% among adult patients with dyspepsia. Additionally, similar results (28) were reported by wang *et al* and are consistent with the results of the current investigation. Changes in *H. pylori* incidence in current research and other investigations might be attributed to differences in study location, population, socioeconomic conditions, and diagnostic procedures, all of which have been shown to influence illness prevalence rate.

Based on demographic factors, the current analysis found a significant difference in the distribution of illnesses by gender and age groups. In addition to the increased frequency of *H. pylori* shown in men and people in their middle years, patients who resided in rural areas, drank unfiltered water, and had poor

socioeconomic status also had significantly higher ($p<0.05$) infection rates. Additionally, the logistic regression analysis revealed that tap water users, rural area inhabitants, low socioeconomic status individuals, and men are more likely to have an infection. These findings are consistent with earlier studies (12, 29, 30). Socioeconomic stress unhygienic living conditions and low income can have a variety of physiological impacts, such as a decline in immune function (31,32), which can affect the prevalence of *H. pylori* infection.

A substantial body of research demonstrates that immunological responses differ between genders (32-34). Females have a lower infection risk than males, possibly due to a greater protective immunological response to *H. pylori*. Several studies have found that males have weaker immune responses than females due to testosterone's immunosuppressive effects (32, 35, 36). This might be why, according to the present study, males with epigastric discomfort are more prone to get the illness. This research could helpful in raises concerns about the danger of *H. pylori* infection in patients having recurrent epigastric discomfort in specific region as well as globally. More research is needed to better understand the rate of incidence of *H. pylori* infection in people who experience chronic epigastric discomfort. Extending the dataset would allow for a more accurate representation of infection frequency and risk factors association in epigastric pain patients.

CONCLUSION

In conclusion, *H. pylori* infection was observed among 58% of patients having persistent epigastric discomfort in metropolitan Lahore. Study findings revealed that males and middle-aged people are more inclined to get infected with *H. pylori*. Consumption of unfiltered water, residential area status, and low socioeconomic position have all been associated to an increased risk of illness development. Current investigation demonstrated the frequency of *H. pylori* and associated risk factors in individuals with recurring epigastric pain complaints. The study highlights the need for a regional effort for both illness diagnosis and prevention as well as the necessity for additional study to fully understand the epidemiology of *H. pylori* infection in the patients experiencing epigastric discomfort.

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Authors contribution:

Conceptualization; ZQS and Am H; Research performance and Curation of data ; Am H, Ar H, Manuscript writing of original draft, Am H; the writing review and editing, Ar H, Software; Am H, and supervision, ZQS. A final version of the manuscript has been approved by all the authors.

Conflicts of Interest:

The authors have declared no conflicts of interest in the writing of this publication. The authors have declared no relationships with manufacturers of anti- *H. pylori* medication.

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