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Exploring *Helicobacter pylori*'s Impact on Clinical Manifestations and Gastroesophageal Reflux Disease Spectrum

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Key words
C-reactive protein, Gastroesophageal reflux disease, *H. pylori*, Histology, Risk factors, Xerostomia

ABSTRACT

Gastroesophageal reflux disease (GERD), a disorder resulting from the retrograde flow of gastric contents into the esophagus, affects an estimated 10–30% population worldwide. Several pathophysiological factors influence the development and the course of GERD, and *Helicobacter* (*H.*) *pylori* infection might be considered as one of them. Thus, this cross-sectional study was aimed to evaluate the impact and association of *H. pylori* infection with GERD. For this, a total of seventy-five volunteers comprising of sixty patients with GERD and sixteen healthy individuals with similar exclusion and inclusion criteria were recruited. Among them, the uneducated, unemployed, and single showed strong correlations in the disease prevalence. Symptoms like acid reflux, heartburn, halitosis, dyspepsia, dysphagia, and bloating were frequently observed among those with non-erosive reflux disorders with gastritis and those with reflux esophagitis, whereas xerostomia was found to be common in both groups. Routine tissue histological examination revealed dilated intercellular spaces, basal cell hyperplasia, papillary elongation, and elevated eosinophil levels in the reflux esophagitis group. The prevalence of *H. pylori* infection was higher in the non-erosive reflux esophagitis with gastritis group than in the other groups. Esophageal inflammation, high-sensitive (hs-CRP) levels, and potential links to gastritis and *H. pylori* infection warrant further investigation to improve diagnostic and therapeutic approaches.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic digestive disorder caused by stomach acid reflux into the esophagus, leading to symptoms such as heartburn and discomfort. The illness is prevalent even in industrialized countries, impacting around 15-20% of the global population and placing a significant burden on healthcare systems (Maret-Ouda *et al*., 2020). GERD has emerged as the leading cause of influx because of rising workplace stress and dietary changes. Clinical research has recently revealed that the incidence of GERD has been increasing worldwide (Al-Ghadeer *et al*., 2021). The reported prevalence of GERD in Pakistan, based on a few hospital-based studies, ranges from 22.2% to 24.0%. However, there is limited data available on the prevalence of GERD in Pakistan, as a recent global review did not include specific information (Rasool *et al*., 2021).

Typically, GERD appears to be the most prevalent disease affecting the digestive system and commonly denotes a pathological condition resulting from the

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retrograde movement of gastric contents into the esophagus, causing tissue damage. Esophageal inflammation is regarded as the most common complication of GERD. Lower esophageal sphincter dysfunction, an increase in transient lower esophageal sphincter relaxation, hiatus hernia, delayed stomach emptying, inefficient esophageal clearance, and the existence of an acid pocket are some factors which may influence the condition (Simadibrata *et al*., 2023; Zhang *et al*., 2019). Sedentary lifestyle and bacterial infections contribute as an important factor in enhancing disease symptoms.

In clinical terms, GERD predominantly manifests in two distinct clinical forms: non-erosive reflux disease (NERD) and reflux esophagitis (RE). NERD is distinguished as symptomatic acid reflux without apparent mucosal breaks in the esophagus, while RE represents an advanced stage of NERD, characterized by the appearance of erosive esophagitis along with visible mucosal damage (Azer and Reddivari, 2023). Within the spectrum of GERD, NERD represents the milder end, while the condition advances towards RE, Barrett's esophagus (BE), and ultimately esophageal adenocarcinoma (EAC). However, existing clinical evidence to substantiate this progression paradigm is limited. For instance, current data suggest that only a minority (approximately 10 %) of NERD cases progress to reflux esophagitis (Zhou *et al*., 2020).

Helicobacter pylori plays a vital role in the pathogenesis of several gastrointestinal (GI) illnesses, including gastric ulcers, duodenal ulcer and gastric cancer. The association between *H. pylori* and GERD is a multifaceted and intricate topic that requires additional research to fully comprehend (Jonaitis *et al*., 2018; Ranjbar *et al*., 2017; Yucel, 2019). There have been conflicting findings regarding the correlation between *H. pylori* infection and GERD, with some studies indicating a greater incidence of *H. pylori* infection in individuals with GERD, while others suggest the opposite (Ford *et al*., 2005; Yaghoobi *et al*., 2010; Zhang *et al*., 2023). It is challenging to support this association because GERD is influenced by a variety of risk factors, such as obesity, smoking, lifestyle choices, and host characteristics (Chen and Brady, 2019; Dunlap and Patterson, 2019; Jonaitis *et al*., 2018).

The specific pattern of gastritis in patients with dyspepsia who develop GERD remains poorly understood. Additionally, no reported data regarding the prevalence of different GERD phenotypes and associated pathologies in Pakistan are currently available. Therefore, conducting a study in Pakistan to assess the pathology and risk factors associated with GERD would have significant implications in filling this knowledge gap and providing valuable insights into the disease in the local population. In this study, we hypothesized that the GERD phenotype, especially the presence of gastritis, is likely to influence the correlation between GERD and *H. pylori*. We recruited patients with different subtypes of GERD, analyzed the factors influencing GERD, and evaluated the correlation between *H. pylori* infection and different GERD subtypes to identify the influencing factors affecting the development of GERD.

MATERIALS AND METHODS

Patients enrollment and ethical approvals

This cross-sectional study was conducted from 2019-2022 at the Centre for Liver and Digestive Diseases, Holy Family Hospital, Rawalpindi, Pakistan. The Ethical approvals were obtained from the Research Review Forum/Institutional Research Forum, Rawalpindi Medical University, Rawalpindi, Pakistan 1 (Ref: R-74/RMU) and the Ethics Committee for Research at the Dalian Medical University, School of Basic Medical Science, Dalian, China.

A total of 75 probands with NERD (n=23), NERD + gastritis (n = 20), RE (n=17), and healthy controls (n = 15) were enrolled in this research study. Patients who experienced heartburn and regurgitation and were not taking any drugs for treatment were also prospectively enrolled. RE patients followed the endoscopic criteria of the Los Angeles Grade. NERD participants had no endoscopic evidence of esophageal disease (Niknam *et al*., 2015), and normal histology but had experienced reflux symptoms within the last 3 months and more than 8 symptoms as score on 'GerdQ' (Jones *et al*., 2009). We included subjects with reflux symptoms and no intestinal metaplasia or atrophy score > 1 (mild) for histological evaluation in the NERD + gastritis group (NERD+G). Healthy volunteers (controls) were selected using the following criteria: no symptoms of heartburn and regurgitation, GerdQ questionnaire score < 8, normal esophagus under endoscopy, and normal esophageal squamous epithelium based on histological examination. Normal clinical examination and negative ^{13}C urea breath test for *H. pylori*.

Metadata collection and questionnaire

In order to achieve the necessary sample size, a simple and convenient sampling technique was employed to gather data from patients experiencing gastrointestinal symptoms who willingly consented to participate. The data was gathered through a self-administered questionnaire, Initially, participants received a concise study introduction along with guidelines on completing the questionnaire. For those who encountered difficulties in filling questionnaire, were assisted readily. The questionnaire was comprised

three sections. The first part is the information section including patient's demographic data, the second part focused on lifestyle and risk factors related to GERD development, and the third part is the gastroesophageal reflux disease questionnaire (GerdQ), a diagnostic tool used for GERD. The GerdQ has been found to have 65% sensitivity and 71% specificity for diagnosing GERD. Participants with a GerdQ score of ≥ 8 were considered to have the disease.

Endoscopic evaluation

The individual underwent endoscopic evaluation to assess the condition of various disease enrollees. Local oropharyngeal anesthesia was administered with lidocaine 5% after explaining the procedure. Endoscopy involved careful assessment of the distal esophagus and stomach for erosions, erythema, or ulceration, and pinch biopsies were obtained from the distal esophagus, 2 cm above the EGJ, and the gastric antrum for all participants.

Sample collection and analysis

A mucosal biopsy was taken from each participant and was placed in 10% formalin for H and E staining. Blood samples of 4-6 ml were collected in tubes (K3EDTA DIA-VAC Huanan Medical Technology Co., LTD) with and without ethylenediaminetetraacetic acid (EDTA) (Dunbar *et al*., 2016). After centrifugation at 3000 rpm for 5 min, serum samples were obtained and processed immediately for C-reactive protein (CRP) analysis using the Boditech i-CHROMA™ instrument and kit following the company protocol. The established cut-off value of hs CRP for healthy individuals is < 6 mg/L. EDTA samples were analyzed using the Mindray BC-5150 hematology analyzer (Mindray, China) to measure the complete blood count (CBC) (Altun *et al*., 2019).

Diagnosis of H. pylori *infection with Nuclear stable isotopic technique*

The determination of *H. pylori* infection was carried out using a standard non-invasive nuclear isotope ^{13}C UBT technique. In brief, patients were required to fast overnight, and a breath sample was collected before administering a dose containing 75 mg of ^{13}C-enriched urea (provided by Cambridge Isotope Laboratories, United States). After 30 min, a post-dose breath sample was collected. The $^{13}CO_2/^{12}CO_2$ ratio in the breath samples was analyzed using the BreathMAT^{plus} mass spectrometer (Thermo Finnigan, Germany) and the Delta V Plus mass spectrometer (Thermo Scientific, United States). A positive result was determined if there was a change in the δ ^{13}C value over baseline of more than 3‰ (Aziz *et al*., 2023; Rasheed *et al*., 2012).

Histopathological findings for GERD

Esophageal biopsies were fixed in 10% formalin solution and subjected to H and E staining for routine histopathological examinations. Typical histologic findings in RE included dilated intercellular space (DIS), intraepithelial neutrophil and eosinophil infiltration, basal cell hyperplasia (BCH), and papillary elongation (PE). The scoring criteria were used for DIS, intraepithelial neutrophils or eosinophils, BCH, and papillary length. Scores of 1 and 2 were assigned for the presence, and 0 for the absence of five histologic parameters (Grin and Streutker, 2015).

Statistical analysis

Continuous variables with a normal distribution are expressed as mean (standard deviation, SD), while categorical variables are presented as frequencies and percentages. Data analyses were conducted using SPSS statistical software (version 20.0; SPSS Inc., Chicago, IL, USA), and statistical significance was determined at a two-sided p-value < 0.05. Two independent samples t-tests were used for statistical differences in numerical data, and the Fisher exact test was used for categorical data analysis. Pearson's chi-square test was used for calculating mean with standard deviation and analysis of the variance test (ANOVA) were used to evaluate continuous variables, p-values < 0.05 were set as statistically significant (Nobakht *et al*., 2016).

RESULTS

Sociodemographic characteristics

Our study revealed a significant difference with respect to marital status between the control and NERD + G groups. Additionally, there were substantial differences in education between the NERD groups as well as between the control and RE groups. The employment status exhibited a significant association with the RE. group, however there were no differences found in sex and age distribution among the groups (Table I).

Clinical characteristics of enrolled participants

In our study, we observed that acid reflux, heartburn, and halitosis were more prevalent in all the disease groups. Additionally, dyspepsia and dysphagia were relatively higher in the RE group. Furthermore, bloating exhibited a significant increase in both NERD+G and RE groups, while xerostomia was considerably elevated in NERD and R.E. groups. The p-values showed the level of statistical significance for each comparison between groups (Table II).

Table I. Demographic data of enrolled participants.

Variables	Control (n = 15)	NERD (n = 23)	NERD+G (n = 20)	R. E (n = 17)
Gender: No (%)				
Male	9 (60)	11 (47.8)	7 (35.0)	12(70.6)
Female	6 (40)	12 (52.0)	13 (65.0)	5 (29.4)
Age groups(Y)				
18–35	6 (40)	13 (56.5)	9 (45)	5 (29.4)
36–50	4 (26.6)	4 (17.3)	8 (40)	4 (23.5)
51–70	5 (33.3)	6 (26.0)	3 (15)	8 (47.0)
Marital status				
Married	12 (80)	13 (56.5)	13 (65.0)	14(82.4)
Unmarried	3 (20)	10 (43.5)	7 (35.0)	3 (17.6)
Education				
Illiterate	2 (13.3)	4 (17.4)	5 (25.0)	5 (29.4)
School level	5 (33.3)	11 (47.8)	8 (40.0)	7 (41.2)
Intermediate	1 (6.7)	3 (13.3)	3 (15.0)	3 (17.6)
Graduation	6 (40.0)	4 (17.4)	3 (15.0)	2 (11.8)
Master and PhD	1 (6.7)	1 (4.3)	1 (5.0)	0 (0)
Employment status				
Professional	7 (46.7)	8 (34.7)	6 (30.0)	4 (23.5)
Unemployed	4 (26.7)	8 (34.7)	8 (40.0)	8 (47.0)
Student	3 (20.0)	4 (17.4)	4 (20.0)	3 (17.6)
Labor	1 (6.7)	3 (13.0)	2 (10.0)	2 (11.8)

*Baseline characteristics of the study population: values include the patient number (%), NERD, non-erosive reflux disease, NERD+G, non-erosive reflux disease+ gastritis, RE, reflux esophagitis. Statistical differences in numerical data were assessed using two independent samples t-tests, while the Fisher exact test was used for categorical data analysis. *$p < 0.05$.

Table II. Clinical characteristics of enrolled participants.

Clinical symptoms		Control (n = 15)	NERD (n = 23)	NERD+G (n = 20)	R. E (n = 17)
Acid reflux	Yes	1(6.7)	23(100.0)*	19(95.0)*	16(94.1)*
Abdominal pain	Yes	11(73.3)	15(65.2)	18(90.0)	14(82.4)
Heartburns	Yes	1(6.7)	23(100.0)*	19(95.0)*	17(100)*
Vomiting	Yes	8(53.3)	15(65.2)	12(60.0)	11(64.7)
Bloating	Yes	2(13.3)	9(39.1)	11(55.0)*	13(76.5)*
Dyspepsia	Yes	1(67.7)	9(39.1)	5(55.0)	15(88.2)***
Dysphagia	Yes	4(26.7)	6(26.1)	6(30.0)	13(76.5)**
Xerostomias	Yes	2(13.3)	19(82.6)*	13(65.0)	15(88.2)**
Halitosis	Yes	3(20.0)	14(60.9)*	13(65.0)*	13(76.5)*

*Clinical characteristics: values include the patient number (percent), NERD= non-erosive reflux disease, NERD+G, non-erosive reflux disease + gastritis, RE, reflux esophagitis.

Histopathological findings for GERD

Our study results indicated that patients with RE exhibited high levels of DIS, BCH, PE, and intraepithelial eosinophil counts with H-E staining, while there was no statistically significant difference between the NERD, NERD+G, and control groups (Table III, Fig. 1).

Table III. Relationship between histologic findings and GERD phenotypes.

	Control (n= 15)	NERD (n= 23)	NERD+G (n= 20)	R.E (N=17)
H-E staining				
DIS	6(40)	8(34.8)	7(35.0)	14(82.3) **
Eosinophils	4(26.6)	7(30.0)	6 (30.0)	9(52.9) *
Neutrophils	3(20)	5(21.7)	5(25.0)	5(29.4)
BCH	2(13.3)	4 (17.3)	4 (20.0)	13(76.4) ***
PE	1(6.6)	2 (8.6)	2 (10.0)	12 (70.6) ***

Values include the patient number (percent). NERD, non-erosive reflux disease; NERD+G, non-erosive reflux disease + Gastritis; RE, reflux esophagitis; HE, hematoxylin-eosin; DIS, dilated intercellular space; BCH, basal cell hyperplasia; PE, papillary elongation. The data is displayed either as a number (%) or as the mean with standard deviation. * Analyzed p values using the chi-square test and one-way ANOVA to determine differences between the four groups, with statistical significance set at $p < 0.05$.

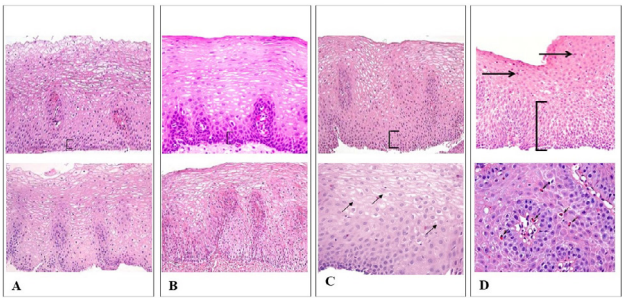

Fig. 1. Routine histological examination: (A) Control: normal esophageal mucosa, no inflammatory cells, epithelium basal layer thickness is often less than 15% of the total thickness. (B) Non-erosive reflux diseases: no inflammatory cells in squamous mucosa, mild basal cell hyperplasia, mild papillae elongation (C) NERD + Gastritis: few neutrophils or eosinophils in squamous mucosa. (D) Reflux esophagitis: numerous neutrophils or eosinophils (5-15/HPF) in squamous mucosa, elongated papillae, more than 30% of epithelial thickness is basal cells. (Magnification: 40x,20x) with hematoxylin-eosin staining.

Risk factors and lifestyle characteristics related to GERD phenotypes

The significant associations were observed between low income, household with animals, and the sewage

system across all diseased groups. In addition, high body mass indices (BMIs) were found significant in the NERD and RE groups while positive associations with the NERD and NERD+G groups were seen in handwashing before meals and after using the restroom. We also found cockroach infestation, smoking, and employment status to be highly associated in the RE group ($p < 0.005$). Likewise, there was a substantial correlation between the NERD+G group and passive smoking (Table IV).

Table IV. Risk factors associated with GERD phenotypes. The values in brackets show percentages.

Parameters	Total participants (n=75)	Control (n = 15)	NERD (n = 23)	NERD+G (n = 20)	R. E (n = 17)
BMI (%)					
Normal	30(40)	14 (93.3)	5(21.7)	7(35.0)	4(23.5)
Underweight	7(9.3)	0(0)	0(0) *	2(10.0)	5(29.4) *
Overweight	38(50.6)	1 (6.7)	18(78.3)	9(45.0)	10(59)
Income					
<25000PKR	35(46.6)	1(6.7)	11(47.8)	12(60.0)	11(64.7)
26000-50000	32(42.6)	10(66.7)	10(43.5) *	6(30.0) *	6(35.3) *
>50000	8(10.7)	4(26.7)	2(8.7)	2(10.0)	0(0)
No of rooms					
up to 3	37(49.3)	5(33.3)	11(47.8)	12(60.0)	9(52.9)
4 to 7	36(48)	10(66.7)	11(47.8)	7(35.0)	8(47.1)
more than 8	2(2.6)	0(0)	1(4.3)	1(5.0)	0(0)
No of persons					
1 to 4	9(12)	0(0)	2(8.7)	4(20.0)	3(17.6)
5 to 8	40(53.3)	7(46.7)	14(60.9)	8(40.0)	11(64.7)
>8	26(34.6)	8(53.3)	7(30.4)	8(40.0)	3(17.6)
Physical activity	No	4(26.7)	8(34.7)	12(60.0)	11(64.7)
	Rarely	3(20.0)	7(30.4)	3(15.0)	4(17.6)
	Frequently	5(33.3)	4(17.3)	1(5.0)	1(5.8)
	Moderately	3(20.0)	4(17.3)	4(20.0)	1(5.8)
Washing hands before a meal	Always	7(46.7)	11(47.8)	10(50.0)	4(23.5)
	Often	8(53.3)	11(47.8) *	9(45.0)	12(70.6)
	Seldom	0(0)	1(4.3)	1(5.0)	0(0)
	Never	0(0)	0(0)	0(0)	1(5.9)
Hand wash after the toilet	Always	7(46.7)	11(47.8)	11(55.0)	4(23.5)
	Often	8(53.3)	11(47.8)	8(40.0)*	10(58.8)
	Seldom	0(0)	1(4.3)	1(5.0)	3(17.6)
	Never	0(0)	0(0)	0(0)	0(0)
Hand wash with soap	Always	4(26.7)	6(26.1)	4(20.0)	1(5.9)
	Often	4(26.7)	8(34.8)	8(40.0)	5(29.4)
	Seldom	7(46.7)	9(39.1)	8(40.0)	11(64.7)
	Never	0(0)	0(0)	0(0)	0(0)
Sewage system	Proper	13(86.6)	10(43.3) *	7(35.0)	5(29.4)
	Damage	2(13.3)	13(56.5)	13(65.0)	12(70.5)

Table continues on next page.....

Parameters	Total participants (n=75)	Control (n = 15)	NERD (n = 23)	NERD+G (n = 20)	R. E (n = 17)
Household animals	Yes	8(53.3)	18(78.2)	13(65.0)	13(76.4)
Cockroaches in house	Yes	8(53.3)	12(52.2)	12(60.0)	12(70.6)*
House flies	Yes	12(80.0)	20(86.9)	17(85.0)	15(88.2)
Smoking	Yes	6(40)	12(52.1)	11(55)	12(70.5)*
Living with someone who smokes	Yes	6(40.0)	10(43.5)	12(60.0) *	9(52.9)
Family history	Yes	1(6.7)	17(73.9)	14(70.0)	6(35.3)
Miswak usage	Always	0(0)	0(0)	0(0)	1(5.9)
	Often	1(6.7)	4(17.4)	6(30.0)	1(5.9)
	Seldom	5(33.3)	10(43.5)	3(15.0)	8(47.1)
	Never	9(60.0)	9(39.1)	11(55.0)	7(41.2)
Toothbrush	Always	2(13.3)	7(30.4)	5(25.0)	2(11.8)
	Often	9(60.0)	8(34.8)	6(30.0)	7(41.2)
	Seldom	1(6.7)	4(17.4)	5(25.0)	5(29.4)
	Never	3(20.0)	4(17.4)	4(20.0)	3(17.6)

*Values include the patient number (percent)., NERD, non-erosive reflux disease; NERD+G, non-erosive reflux disease + Gastritis, and R.E, reflux esophagitis. The Wilcoxon test was used to determine the statistical significance of differences between the four groups, with a significance level of $p < 0.05$ specified. Variables with *p*-values less than 0.05 were considered statistically significant.

Dietary associated risk factors of the study population

There was higher prevalence of all GERD phenotypes associated with frequent consumption of chili/spicy and junk foods. Moreover, in the RE group, the consumption of processed foods showed a significant association with the disease. This table includes the corresponding *p*-values for each variable (Table V).

Table V. Dietary pattern of the study population. The values in brackets show percentages.

		Control (n=15)	NERD (n=23)	NERD+G (n=20)	RE (n = 17)
Salt intake	No	0(0)	0(0)	0(0)	0(0)
	Low	3(20.0)	1(4.3)	1(5.0)	0(0)
	Normal	10(66.7)	17(73.9)	17(85.0)	15(88.2)
	High	2(13.3)	5(21.7)	2(10.0)	2(11.8)
Spicy intake	No	3(20)	2(8.7)	2(10.0)	1(5.8)
	Rarely	5(33.3)	5(21.7) *	3(15.0)	3(17.6)*
	Frequently	2(13.3)	10(43.5)	8(40.0)	9(52.9)
	Moderately	5(33.3)	6(26.1)	7(35.0)	4(26.6)
Black tea	No	1(6.7)	1(4.3)	3(15.0)	2(11.8)
	Rarely	4(26.7)	7(30.4)	1(5.0)	3(17.6)
	Frequently	7(46.7)	8(34.8)	9(45.0)	9(52.9)
	Moderately	3(20.0)	7(30.4)	7(35.0)	3(17.6)
Green tea	No	0(0)	10(43.5)	8(40.0)	7(41.2)
	Rarely	13(86.7)	9(39.1)	12(60.0)	7(41.2)
	Frequently	1(6.7)	1(4.3)	0(0)	1(5.9)
	Moderately	1(6.7)	3(13.3)	0(0)	2(11.8)
Fresh fruits	No	0(0)	0(0)	1(5.0)	0(0)
	Rarely	3(20.0)	9(39.1)	8(40.0)	10(58.8)
	Frequently	2(13.3)	8(34.8)	6(30.0)	4(23.5)
	Moderately	10(66.7)	6(26.1)	5(25.0)	3(17.6)
Eating Rice	No	0(0)	1(4.3)	0(0)	0(0)
	Rarely	7(46.7)	6(26.1)	6(30.0)	8(47.1)
	Frequently	6(40.0)	11(47.8)	10(50.0)	7(41.2)
	Moderately	2(13.3)	5(21.7)	4(20.0)	2(11.8)
Potatoes	No	0(0)	1(4.3)	0(0)	0(0)
	Rarely	8(53.3)	5(21.7)	7(35.0)	9(52.9)
	Frequently	3(20.0)	8(34.8)	4(20.0)	5(29.4)
	Moderately	4(26.7)	9(39.1)	9(45.0)	3(17.6)
Eating meat	No	3(20.0)	0(0)	0(0)	1(5.9)
	Rarely	8(53.3)	13(56.5)	11(55.0)	8(47.1)
	Frequently	1(6.7)	2(8.7)	3(15.0)	3(17.6)
	Moderately	3(20.0)	8(34.8)	6(30.0)	5(29.4)
Pro-cessed food	No	10(66.7)	7(30.4)	8(40.0)	4(23.5)
	Rarely	2(13.3)	5(21.7)	3(15.0)	5(29.4) *
	Frequently	1(6.6)	9(39.1)	7(35.0)	7(41.1)
	Moderately	2(13.3)	2(8.6)	2(10)	1(5.9)
Sweets	No	1(6.7)	2(8.7)	1(5)	2(11.8)
	Rarely	9(60.0)	8(34.8)	10(50.0)	2(11.8)
	Frequently	2(13.3)	8(34.8)	4(20.0)	10(58.8)
	Moderately	3(20.0)	5(21.7)	5(25.0)	3(17.6)
Junk food	No	9(60)	8(34.8)	6(30.0)	6(35.3)
	Rarely	3(20)	6(26.1) *	5(25.0) *	3(17.6) *
	Frequently	1(6.6)	5(21.7)	7(35.0)	7(41.2)
	Moderately	2(13)	4(17.4)	2(10.0)	1(5.9)
Drinking water	Tape	3(20.0)	3(13.0)	4(20.0)	1(5.9)
	wells	7(73.3)	14(60.9)	13(65.0)	16(94.1)
	Boiled	1(6.3)	1(4.3)	3(10.0)	0(0)
	filter	0(0)	5(21.7)	1(5.0)	0(0)

Table continues on next column.....

Values include the patient number (percent), NERD, non-erosive reflux disease; NERD+G, non-erosive reflux disease+Gastritis; RE, reflux esophagitis. The p-value in the table was obtained using the Wilcoxon test, and variables with a p-value less than 0.05 are considered statistically significant.

Hematological investigation and inflammatory factor

In comparison to patients in the control group, patients in the NERD+G and R.E. groups exhibited significantly higher average total white blood cell counts (TWBCs) and elevated levels of CRP. In contrast, HB and platelet counts did not display any significant differences when compared with the control group (Table VI).

Table VI. Hematological and inflammatory data.

Variable	Control (n = 15)	NERD (n = 23)	NERD+G (n = 20)	R. E (n =17)
WBC	1.18±0.4	1.13±0.5	1.08±0.5*	7.42±2.5**
HB(g/dl)	12.7±1.6	12.1±1.3)	11.5±1.7	12±1.5
Platelets	1.91±0.26	1.91±0.3	2.08±0.37	2.2±0.49
CRP	3.8 ±1.4	6.7±4.4	14.4±11.9**	15.0±8.7***

Mean±SD hematological data and Inflammatory marker: NERD, non-erosive reflux diseases; NERD+G, non-erosive reflux diseases with gastritis; R.E, reflux esophagitis; WBC, white blood cells count; HB, hemoglobin; CRP, C reactive protein.

Relationship of H. pylori *with different GERD phenotypes*

The *H. pylori* infection has significant interlinkage with NERD+G group when compared to the NERD ($p = 0.042$) and RE groups ($p = 0.034$), Conversely, the *p*-value of 0.81 suggests non-significant association between *H. pylori* status in the NERD as well as RE groups (Table VII).

Table VII. Relationship of *H. Pylori* infection with different GERD phenotypes. The values in brackets show percentages.

H. pylori status	Diseases status		
	NERD N (%)	NERD+G	R. E
Present	9(39.1) *	14(70) *	6(35.2)
Absent	14(60.9)	6(30)	11(64.7)

H. pylori, *Helicobacter pylori*; NERD, non-erosive reflux diseases; NERD+G, non-erosive reflux diseases with gastritis; R.E, reflux esophagitis. A chi-square test was used to calculate the p-values.

Association between H. pylori *infection with hematological and inflammatory factor*

According to the findings of our study, all gastroesophageal reflux disease (GERD) phenotypes showed a positive correlation between the inflammatory markers C-reactive protein (CRP) and *H. pylori* infection. *H. pylori* also showed a notable correlation in the NERD+G group and the R.E. group. Additionally, there was a connection between *H. pylori* infection and Hb (hemoglobin) levels in the NERD+G group (Fig. 2).

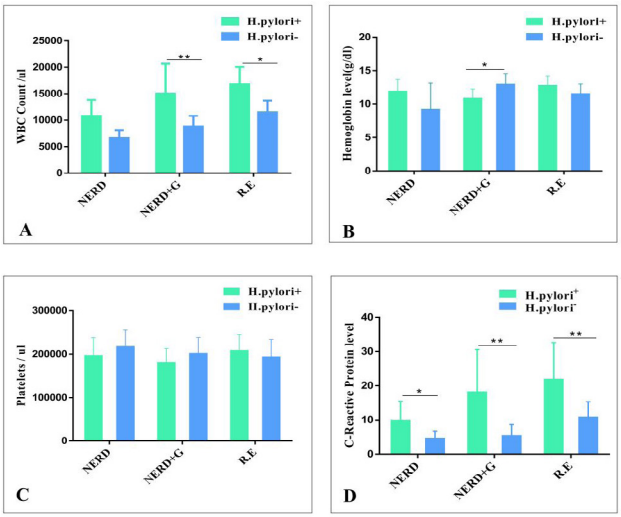

Fig. 2. Association between *H. Pylori* and inflammatory factors: A, *H. pylori* and white blood cells in GERD phenotypes, B, *H. pylori* and hemoglobin in GERD phenotypes. C, *H. pylori* and platelets in GERD phenotypes. D, *H. pylori* and C-Reactive protein.

DISCUSSION

GERD is a prevalent condition which affects a large portion of the general population and can have a substantial impact on daily life. Two subtypes of GERD: NERD and R.E, have distinct clinical presentations and varying impacts on the quality of life of the affected probands (Savarino *et al*., 2013). GERD negatively impacts the lives of an individual owing to physical limitations, social difficulties, sleep disturbances, and reduced work productivity (He *et al*., 2022). The purpose of this study was to identify the risk factors for GERD, explore its association with gastritis patterns in *H. pylori*-infected patients, characterize GERD phenotypes and differentiating factors, and investigate the relationship between hs-CRP and GERD diagnosis, subtypes, and its symptom severity.

This research study revealed significant associations between acid reflux, heartburn, and halitosis in all the disease groups, whereas dyspepsia and dysphagia were significantly higher in the RE group, while bloating was more prevalent among individuals in the NERD+G and R.E. groups, as in line with recent studies (Ha *et al*., 2010; Naik and Vaezi, 2015; Thrift *et al*., 2013). Moreover, a higher incidence of xerostomia had been observed in patients with NERD and RE groups (Kabbir *et al*., 2019). RE groups experience serious clinical symptoms due to prolonged exposure of the esophageal lining to stomach acid lead to further potential complications in the form of

severe tissue damage. Consistent with prior research, our findings reinforce the importance of these symptoms in the respective disease groups, highlighting the impact of chronic exposure and associated complications on severity of symptoms.

Further exploration of histopathological examination in different GERD phenotypes aids in understanding the underlying mechanisms and pathological processes. In our study, patients with RE showed increased DIS, BCH, and PE, with a notable increase in the number of eosinophils compared with controls and patients with NERD. Intraepithelial eosinophil and neutrophil infiltration did not differ significantly between the controls, NERD and NERD+G groups. Based on histopathological examination to differentiate between the GERD types, previous studies found that patients with RE had higher basal cell hyperplasia and papillary elongation, indicating a positive correlation (Chandrasoma *et al*., 2000; El-Serag *et al*., 2014; Hershcovici and Fass, 2010; Kahrilas *et al*., 2008; Yeh *et al*., 2021). In contrary to our findings, studies have reported an elevated presence of neutrophil infiltration in GERD, specifically esophagitis (Kandulski *et al*., 2015; Zand-Irani *et al*., 2021). These conflicting findings may be due to the diverse methodologies used to measure eosinophil and neutrophil infiltration, small sample size, and insufficient evaluation of other histological markers of GERD, such as intraepithelial lymphocytes and mast cells (Rossi *et al*., 2023; Yu *et al*., 2011).

Additionally, our study revealed significant associations between socioeconomic status, sanitation system, household pets, and the occurrence of GERD phenotypes compared with the control group. This implies that these factors may be involved in the onset or worsening of gastrointestinal problems. Our research study found a specific association between higher BMI in NERD and R.E groups compared with the control group. Similar studies were conducted by Mansour-Ghenaei *et al*. (2013), and Wajed *et al*. (2001) which demonstrate that individuals with a high BMI are at a higher risk of experiencing severe cases of GERD. These studies further validate and support our findings, suggesting that BMI may contribute to the development or severity of these gastrointestinal diseases in our study, and no significant association between sex and GERD was found. However, Yamamichi *et al*., (2012) reported female gender, while Kim *et al*. (2008) reported male gender as a significant factor associated with GERD.

Moreover, in our study high percentage of participants in the GERD+G and R.E groups had no physical activity, aligning with a population-based study (Nilsson *et al*., 2004), showing a protective effect of exercise against GERD symptoms. Physically active individuals have a lower risk of developing GERD symptoms (Nocon *et al*., 2006), whereas strenuous exercise may trigger GERD; moderate exercise does not show the same association (Jóźków *et al*., 2007; Ravi *et al*., 2005). The exact mechanism by which exercise induces this reflex remains unclear. However, maintenance of a routine of mild physical activity along with dietary modifications is recommended to prevent reflux symptoms. Additionally, our study revealed a significant association between smoking addiction and the RE group, as reported by Rasool *et al*. (2021). smoking plays a vital role in the development of RE by negatively affecting LES, increasing stomach acid production, and impairing acid clearance from the esophagus, resulting in higher acid reflux, chronic inflammation, and damage to the esophageal lining.

Identifying the most reflexogenic diet is important as certain foods can induce or exacerbate GERD symptoms (DeVault and Castell, 2005). Data of our study revealed a significant association between chili/spicy consumption and junk food with all phenotypes of GERD, implying their potential contribution to the development of GERD symptoms across different subgroups. Studies have shown conflicting results regarding the association between spicy and fast food consumption and the risk of GERD (Eslami *et al*., 2017; López-Colombo *et al*., 2017). While some studies found no significant contribution, others emphasized the link between high-spice food intake and accelerated GERD development. (Alkhathami *et al*., 2017; Asl *et al*., 2015; Kariri *et al*., 2020). In Asian populations, the combination of high-spice food consumption and lying down after eating increases the risk of GERD. Chili consumption may lead to increased acid production and esophageal irritation, while junk food can contribute to gastric distension and relaxation of the lower esophageal sphincter, promoting acid reflux (Wu *et al*., 2013; Asl *et al*., 2015; Heidarzadeh-Esfahani *et al*., 2021). Our study found a significant association between processed foods and reflux esophagitis as it contains preservatives, artificial flavors, and additives that can irritate the esophageal mucosa, causing inflammation and tissue damage and have a significant impact on esophageal health, particularly in cases of RE (Ahmed *et al*., 2020; Eslami *et al*., 2017).

In addition to clinical characteristics, histological parameters, and risk factors, inflammatory and hematological parameters can shed light on GERD's underlying inflammatory processes. Elevated levels of hs-CRP and WBC were seen in patients with RE and *H. pylori* infection. However, no association was found between hs-CRP levels and NERD. These results are consistent with previous research linking increased hs-CRP levels to the severity of *H. pylori* gastritis compared to gastritis without *H. pylori* infection (Rahmani *et al*., 2016; Raut *et al*., 2015). Studies by Raut *et al*. (2015) and Rahmani *et*

al. (2016) support our findings, showing higher hs-CRP levels in patients with *H. pylori* infection and increasing severity of reflux esophagitis, respectively. Higher hs-CRP levels are associated with severe RE, indicating an inflammatory response. Furthermore, our findings suggest a potential link between RE and elevated WBC count. The immune response triggered by esophageal inflammation in RE may contribute to the increased production of WBCs.

To investigate the relationship between *H. pylori* and different GERD phenotypes is important to understand the potential role of this bacterium in disease pathogenesis. *H. pylori* infection was detected in 39.1% of NERD patients, 70% of NERD+G patients, and 29.4% of RE patients, suggesting a possible link between GERD and *H. pylori*-induced gastritis. However, the evidence for a cause and effect connection is lacking. *H. pylori* can affect acid secretion, and its presence in specific areas of the stomach may influence acid production and the development of esophagitis (Ashktorab *et al*., 2012; Kim *et al*., 2014; Saad *et al*., 2012).

H. pylori infection is highly prevalent, making it challenging to exclude its influence when evaluating patients with reflux disease. Many previous studies have examined the association between heart disease and GERD (Miao *et al*., 2014), While this study focused on *H. pylori*-positive patients and investigated the relationship between gastritis and GERD. The findings showed a significant association between antral predominant gastritis and GERD, which differs from previous studies (Niknam *et al*., 2022). Additionally, the study found a lower prevalence of *H. pylori* infection in GERD patients, supporting an inverse correlation between the two conditions. These results align with the findings of He *et al*. (2022) who also observed a lower prevalence of *H. pylori* infection in patients with GERD.

CONCLUSION

Our research study showed that GERD is common in Pakistan and highlights its modifiable risk factors that aids in its development. Sociodemographic factors associated with GERD include marital and employment status. Our study highlights the complex connection between H. *pylori* infection, different types of GERD, and their effects on patients. The observed differences in symptoms, inflammation, and risk factors across various GERD types underscore the diverse nature of the condition. Notably, the potential link between H. *pylori* gastritis and GERD is of significance, prompting the need for more exploration. These findings provide valuable information for customized treatments, enhancing our grasp of GERD and its relationships, ultimately leading to better care for patients. A potential association between GERD and gastritis requires further studies with larger sample size.

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Ethical statement and IRB approval

This study was ethically approved from the Centre for Liver and Digestive Diseases, Holy Family Hospital, Rawalpindi, Pakistan. The Ethical approvals were obtained from the Research Review Forum/Institutional Research Forum, Rawalpindi Medical University, Rawalpindi, Pakistan l (Ref: R-74/RMU) and the Ethics Committee for Research at the Dalian Medical University, School of Basic Medical Science, Dalian, China.

Statement of conflict of interest

The authors have declared no conflict of interest.

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