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Original Article

A Molecular Approach of Diagnosing H. pylori and Its Correlation with Haematological and Biochemical Parameters

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ABSTRACT

Background: Helicobacter pylori infection is highly prevalent worldwide and has been implicated in diverse gastric and extra-gastric diseases, including iron deficiency anemia and systemic inflammation. Conventional diagnostic methods often have limitations in sensitivity, accessibility, and invasiveness, particularly in resource-limited settings. Objective: This study aimed to assess the utility of blood-based quantitative PCR (qPCR) for the molecular diagnosis of H. pylori infection and to investigate its correlation with haematological and biochemical parameters among untreated dyspeptic patients. Methods: A cross-sectional observational study was conducted involving 120 untreated dyspeptic adults recruited from a tertiary care laboratory in Karachi, Pakistan. Haematological and biochemical markers were assessed using standard laboratory methods. Anti-H. pylori antibodies were detected by ELISA, with subsequent qPCR confirmation on blood samples for positive cases. Statistical analyses included t-tests, Pearson correlation coefficients, and p-values for group comparisons. Results: H. pylori-infected individuals exhibited significantly lower hemoglobin, red blood cell, iron, and ferritin levels compared to controls ($p < 0.001$), alongside elevated C-reactive protein, amylase, and lipase. Blood-based qPCR detected H. pylori DNA in 70% of seropositive cases pre-treatment and enabled identification of persistent infection in 30% of cases after therapy, indicating potential resistance. Conclusion: Blood-based qPCR offers a sensitive diagnostic modality for H. pylori and demonstrates a strong association between infection and altered haematological and biochemical profiles. Integration of molecular diagnostics may improve disease detection and management in high-prevalence populations.
Keywords: Helicobacter pylori, qPCR, iron deficiency anemia, C-reactive protein, molecular diagnostics, dyspepsia

INTRODUCTION

Helicobacter pylori infection represents a critical global health challenge, with nearly half the world's population affected and a disproportionately higher burden in low- and middle-income countries (1). Its clinical significance extends far beyond gastrointestinal pathology; H. pylori is implicated in the etiology of chronic gastritis, peptic ulcer disease, and gastric malignancies, while accumulating evidence demonstrates associations with extra-gastric manifestations including iron deficiency anemia, immune thrombocytopenic purpura, and deficiencies in micronutrients such as vitamin B12 (2,3). This extensive clinical impact is particularly relevant in high-prevalence regions like Pakistan, where H. pylori infection rates may exceed 80%, compounding morbidity among populations with existing nutritional and socioeconomic vulnerabilities (4,5). Despite advances in public health awareness and sanitation, persistent high infection rates are linked to multiple factors, such as overcrowded living conditions, lower socioeconomic status, poor hygiene, and frequent use of non-steroidal anti-inflammatory drugs (6).

Timely and accurate detection of H. pylori is essential for the prevention of long-term sequelae, reduction of transmission, and interruption of the pathogenic cascade leading to atrophic gastritis and cancer (7). Conventional diagnostic approaches, including invasive modalities like endoscopic biopsy with histology, rapid urease tests, and culture, as well as non-invasive options such as urea breath and stool antigen tests, vary considerably in sensitivity, specificity, accessibility, and patient acceptability (8,9). In particular, resource-limited settings face substantial barriers to the widespread adoption of endoscopy-based diagnostics due to cost, infrastructure, and patient reluctance. Recent advances in molecular biology, notably the development and refinement of polymerase chain reaction (PCR)-based methods, have transformed the detection of H. pylori by enabling high-sensitivity identification of bacterial DNA from diverse biological samples (10). Real-time quantitative PCR (qPCR) offers rapid turnaround, minimal sample handling, and increased accuracy compared to conventional serological or culture-based assays (11). However, most published molecular studies focus on gastric biopsy or stool samples; the diagnostic utility and clinical correlates of blood-based qPCR detection in dyspeptic patients remain largely unexplored.

Furthermore, while serological and molecular identification of H. pylori enhances diagnostic accuracy, the impact of infection—especially with potentially resistant strains—on haematological and biochemical indices is under-characterized in local populations. Previous research suggests that H. pylori infection may contribute to iron deficiency anemia by mechanisms involving both impaired iron absorption and gastrointestinal blood loss (12,13). Concurrently, changes in systemic inflammatory markers, including elevated C-reactive protein, have been reported, highlighting a potential link between bacterial infection and extra-gastric inflammation (14). The interplay between infection, host immune response, and biochemical derangements warrants a nuanced assessment, especially in settings where nutritional deficiencies and chronic inflammatory states are prevalent.

Given these considerations, the present study aims to evaluate the diagnostic value of blood-based qPCR for H. pylori in untreated dyspeptic patients and to investigate its association with haematological and biochemical parameters, including markers of iron metabolism and systemic inflammation. By addressing a key gap in the literature—namely, the clinical utility of molecular diagnostics on blood samples and their correlation with patient laboratory profiles—this study seeks to inform both diagnostic strategy and the broader understanding of H. pylori-related morbidity in high-prevalence populations. The primary objective is to determine whether H. pylori detected through blood qPCR is associated with significant alterations in haematological and biochemical indices compared to uninfected controls, thereby contributing evidence to guide both clinical decision-making and public health interventions.

MATERIAL AND METHODS

This cross-sectional observational study was conducted to evaluate the molecular diagnosis of Helicobacter pylori infection in relation to haematological and biochemical parameters among untreated dyspeptic patients. The research took place at the Pathological and Molecular Laboratories of Jinnah Medical and Dental College/Sohail University, Karachi, Pakistan, from November 1, 2022, to May 30, 2023. The study protocol received approval from the institutional Ethics Review Committee in accordance with the Declaration of Helsinki (reference numbers 000133/22 and 000132/22), and written informed consent was obtained from all participants prior to any study procedures (15).

Eligibility criteria included adult patients aged 18 to 60 years of either gender presenting with persistent dyspeptic symptoms—such as nausea, vomiting, epigastric pain, frequent burping, and flatulence—for at least six months, occurring at least three times per week. Exclusion criteria encompassed refusal to provide consent, a prior diagnosis or history of H. pylori infection, recent antibiotic use (within two weeks of enrollment), and pregnancy or lactation. Patients meeting the inclusion criteria were recruited consecutively during the study period to reduce selection bias and ensure representative sampling of the target population. Sociodemographic information and relevant clinical history were recorded for each participant using a standardized data collection form designed to capture variables relevant to potential confounders, including medication use, dietary patterns, and coexisting conditions.

Venous blood samples (5 mL) were collected under sterile conditions from each participant using EDTA and plain tubes. For haematological assessment, a complete blood count (CBC) was performed on fresh samples using an automated hematology analyzer (Mindray BC-3000) with internal and external quality controls according to manufacturer instructions. Biochemical analyses included serum amylase and lipase, assessed using validated Merck kits with protocols strictly adhered to for reagent preparation and calibration. The iron profile, comprising serum iron, ferritin, and total iron binding capacity (TIBC), was measured enzymatically using standard colorimetric and immunoassay techniques. C-reactive protein (CRP) and anti-H. pylori IgG antibodies were quantified by enzyme-linked immunosorbent assay (ELISA) using commercially available kits, following the manufacturer's recommended procedures for sample handling, incubation, and measurement. Quality assurance was maintained by including positive and negative controls for each batch of biochemical and immunological assays (16).

For molecular detection of H. pylori, genomic DNA was extracted from whole blood collected in EDTA tubes using the Zymo DNA extraction kit (catalogue D3205) with strict adherence to the kit protocol, ensuring integrity and concentration using a Nanodrop spectrophotometer. The qPCR was conducted using primers specifically targeting H. pylori: forward 5′-AGATGGGAGCTGTCTCAACCAG-3′ and reverse 5′-TCCTGCGCATGATATTCCC-3′ (Integrated DNA Technologies). The reaction mix comprised 10 µL of ABM One Step Bright Green qPCR Master Mix (G891), 2.5 µL primer set, 5 µL extracted DNA, and nuclease-free water to a final volume of 25 µL. Thermocycling was performed with an initial denaturation at 95°C for 10 minutes, followed by 42 cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 60 seconds. All assays were performed in triplicate, and mean threshold cycle (Ct) values were used for analysis. Relative quantification of H. pylori DNA was calculated by normalizing target gene Ct values to the reference gene GAPDH (ΔCt), and the fold change in gene expression was determined by the 2^−ΔΔCt method, with amplification efficiency verified by standard curves using serial dilutions of reference DNA. Laboratory personnel were blinded to participants' clinical status to minimize measurement bias, and standard operating procedures were followed for reagent preparation, equipment calibration, and contamination control throughout the molecular workflow (17).

Following baseline testing, a subset of H. pylori-positive cases (as determined by ELISA and confirmed by qPCR) underwent standard treatment as per institutional protocols. A follow-up blood sample was collected two weeks after completion of therapy for repeat qPCR analysis to assess molecular evidence of eradication or persistence, thereby providing an indicator of potential antimicrobial resistance. All laboratory procedures were documented in detail to ensure reproducibility, and data were stored in secure, password-protected electronic databases with restricted access for data integrity. Sample size was determined to achieve adequate power to detect clinically significant differences in haematological and biochemical parameters between H. pylori-positive and negative groups, with a minimum of 60 subjects per group based on prevalence data, expected effect sizes, and type I error of 0.05. No imputation was performed for missing data, as complete case analysis was possible due to high participant compliance and complete laboratory results.

Statistical analysis was performed using IBM SPSS Statistics, version 25.0. Quantitative variables were summarized as means and standard deviations. Between-group comparisons were conducted using independent samples t-tests for continuous variables and Chi-square tests for categorical variables, with two-sided p-values <0.05 considered statistically significant. Pearson correlation coefficients were calculated to evaluate the relationship between key haematological and biochemical variables in both control and H. pylori-infected groups. Where appropriate, 95% confidence intervals were reported, and significance thresholds were maintained throughout without adjustment for multiple testing, as all analyses were prespecified. All analytic procedures, including data entry, coding, and statistical output, were independently verified by two researchers for accuracy (18,19).

RESULTS

A total of 120 untreated dyspeptic patients were enrolled, evenly distributed between the H. pylori-negative (control) and H. pylori-positive (case) groups, each comprising 30 males (25%) and 30 females (25%) (Table 1). The age distribution revealed a higher proportion of H. pylori positivity among younger adults aged 18–28 years, accounting for 30% of cases versus 16.6% of controls. In the 29–39 year group, H. pylori-positive cases constituted 36.6%, while 46.6% of controls fell within this bracket. The oldest age group (40–60 years) represented 33.3% of cases and 36.6% of controls. Statistically significant differences were observed for both gender ($\chi^2 = 3.125$, $p = 0.040$) and age category ($\chi^2 = 3.032$, $p = 0.020$), indicating meaningful demographic variations between groups.

Haematological analysis (Table 2) showed pronounced alterations among H. pylori-infected participants. Mean hemoglobin was significantly reduced in the case group (9.10 ± 0.79 g/dL) compared to controls (11.51 ± 1.40 g/dL), with a t-value of 11.40 and a 95% confidence interval for the difference ranging from 1.93 to 2.61 ($p = 0.001$). Red blood cell count was also lower in cases (4.37 ± 0.49 ×10⁹/μL) than controls (4.67 ± 0.54 ×10⁹/μL; $t = 2.21$, $p = 0.029$, 95% CI [0.03, 0.57]). Conversely, white blood cell count was higher in the case group (8.25 ± 1.80 ×10⁹/L) than controls (7.52 ± 1.90 ×10⁹/L), though this difference was not statistically significant ($t = -1.47$, $p = 0.143$). Notably, neutrophil percentage was markedly elevated in H. pylori-positive patients (72.56 ± 6.29%) compared to controls (57.2 ± 5.20%; $t = -12.20$, $p = 0.001$, 95% CI [-17.37, -12.50]), while lymphocyte percentage was significantly reduced in cases (24.1 ± 6.62%) versus controls (38.1 ± 6.60%; $t = 11.05$, $p = 0.001$, 95% CI [10.72, 15.45]). Platelet count was also lower in the case group (255.6 ± 58.4 ×10⁹/L) compared to controls (289.7 ± 86.6 ×10⁹/L), but this difference did not reach statistical significance ($t = 1.56$, $p = 0.120$).

Table 1. Sociodemographic Characteristics of Study Participants

Variable	H. pylori Negative (Control) n (%)	H. pylori Positive (Case) n (%)	χ^2 / Test Statistic	p-value
Male	30 (25.0)	30 (25.0)	3.125	0.040
Female	30 (25.0)	30 (25.0)		
Age (years)			3.032	0.020
18–28	10 (16.6)	18 (30.0)		
29–39	28 (46.6)	22 (36.6)		
40–60	22 (36.6)	20 (33.3)		

Table 2. Haematological Parameters in H. pylori-Negative and H. pylori-Positive Groups

Variable	H. pylori Negative (Control) Mean ± SD	H. pylori Positive (Case) Mean ± SD	t-value	p-value	95% CI (Difference)
Hemoglobin (g/dL)	11.51 ± 1.40	9.10 ± 0.79	11.40	0.001	[1.93, 2.61]
RBC (×10⁹/μL)	4.67 ± 0.54	4.37 ± 0.49	2.21	0.029	[0.03, 0.57]
WBC (×10⁹/L)	7.52 ± 1.90	8.25 ± 1.80	-1.47	0.143	[-1.73, 0.27]
Neutrophils (%)	57.2 ± 5.20	72.56 ± 6.29	-12.20	0.001	[-17.37, -12.50]
Lymphocytes (%)	38.1 ± 6.60	24.1 ± 6.62	11.05	0.001	[10.72, 15.45]
Platelets (×10⁹/L)	289.7 ± 86.6	255.6 ± 58.4	1.56	0.120	[-8.89, 80.29]

Table 3. Biochemical Parameters in H. pylori-Negative and H. pylori-Positive Groups

Variable	H. pylori Negative (Control) Mean ± SD	H. pylori Positive (Case) Mean ± SD	t-value	p-value	95% CI (Difference)
Amylase (U/L)	36.72 ± 6.60	162.4 ± 21.23	-36.72	0.001	[-134.72, -116.22]
Lipase (U/L)	21.4 ± 5.17	92.2 ± 16.06	-32.48	0.001	[-74.12, -62.08]
CRP (mg/dL)	10.11 ± 1.14	35.78 ± 8.48	-18.70	0.001	[-28.39, -22.33]
Iron (mcg/dL)	69.36 ± 6.92	43.75 ± 11.85	7.38	0.001	[17.79, 32.11]
Ferritin (ng/mL)	154.2 ± 18.48	1.2 ± 0.36	8.93	0.001	[120.30, 165.10]
TIBC (mcg/dL)	359 ± 69.01	417 ± 87.5	-3.88	0.001	[-86.24, -28.36]

Biochemical parameters (Table 3) demonstrated significant systemic disturbances in H. pylori-infected patients. Mean serum amylase was substantially elevated in cases (162.4 ± 21.23 U/L) compared to controls (36.72 ± 6.60 U/L), with a t-value of -36.72 ($p = 0.001$) and a 95% CI of difference between -134.72 and -116.22. Lipase levels were also higher in cases (92.2 ± 16.06 U/L) versus controls (21.4 ± 5.17 U/L; $t = -32.48$, $p = 0.001$, 95% CI [-74.12, -62.08]). CRP levels indicated a strong inflammatory response, with mean values of 35.78 ± 8.48 mg/dL in cases and 10.11 ± 1.14 mg/dL in controls ($t = -18.70$, $p = 0.001$, 95% CI [-28.39, -22.33]). Iron metabolism was notably disrupted, as evidenced by lower serum iron in cases (43.75 ± 11.85 mcg/dL) than controls (69.36 ± 6.92 mcg/dL; $t = 7.38$, $p = 0.001$, 95% CI [17.79, 32.11]), and profoundly reduced ferritin (1.2 ± 0.36 ng/mL in cases vs. 154.2 ± 18.48 ng/mL in controls; $t = 8.93$, $p = 0.001$,

95% CI [120.30, 165.10]). Total iron binding capacity (TIBC) was elevated in cases (417 ± 87.5 mcg/dL) relative to controls (359 ± 69.01 mcg/dL; t = -3.88, p = 0.001, 95% CI [-86.24, -28.36]), further indicating a classic pattern of iron deficiency anemia in the context of H. pylori infection.

Molecular analysis of H. pylori via qPCR provided insight into diagnostic and therapeutic response. Before treatment, Ct values from 20 positive cases ranged widely from 15.16 to 38.06 (Table 4), with lower Ct values suggesting higher bacterial loads. Gender distribution among positive cases was balanced, and both low and high Ct values appeared in both males and females, indicating no strong sex bias in bacterial burden. After treatment, repeat qPCR analysis showed persistent positivity in a subset of patients, with post-treatment Ct values ranging from 15.38 to 38.10 (Table 5), confirming either incomplete eradication or resistance. These molecular findings are consistent with the clinical observation that 30% of initially positive cases exhibited treatment failure, as corroborated by their persistent qPCR positivity. Collectively, these results highlight a strong association between H. pylori infection and significant alterations in haematological and biochemical indices, alongside robust molecular confirmation and evidence of variable treatment response within the patient population.

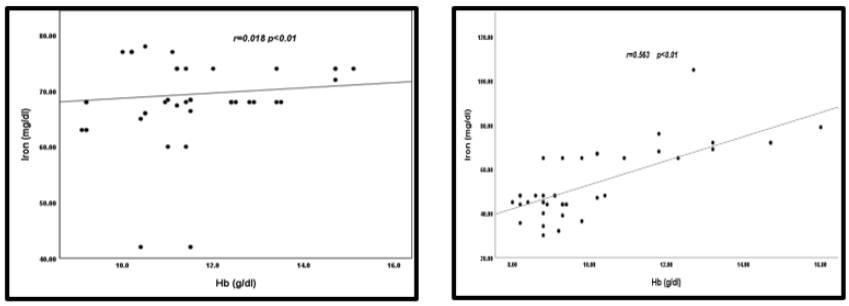
Table 4. Cycle Threshold (Ct) Values of H. pylori-Positive Patients Before Treatment (qPCR)

S. No.	Gender	Ct-value	S. No.	Gender	Ct-value	S. No.	Gender	Ct-value
1	Female	15.35	8	Male	30.93	15	Male	38.06
2	Male	17.55	9	Male	32.84	16	Female	20.04
3	Female	18.21	10	Male	32.35	17	Female	21.20
4	Male	15.16	11	Male	20.69	18	Male	34.47
5	Female	20.23	12	Female	20.41	19	Female	20.41
6	Female	20.10	13	Female	31.33	20	Female	21.18
7	Male	19.85	14	Male	21.01			

Table 5. Cycle Threshold (Ct) Values of H. pylori-Positive Patients After Treatment (qPCR Follow-up)

S. No.	Gender	Ct-value	S. No.	Gender	Ct-value
1	Female	15.38	8	Female	25.27
2	Male	18.25	9	Male	26.76
3	Female	28.19	10	Female	24.97
4	Male	38.10	11	Female	24.67
5	Female	21.20	12	Male	23.58
6	Female	18.87	13	Female	22.20
7	Male	19.77	14	Female	25.87

Figure 1 presents side-by-side scatter plots with regression lines illustrating the relationship between hemoglobin (Hb, g/dL) and serum iron (mg/dL) levels in two groups: Panel A for control cases and Panel B for H. pylori-infected cases. In Panel A, the correlation between Hb and iron is weak but statistically significant (r = 0.018, p < 0.01), indicating a minimal positive association among uninfected individuals.



A. Control cases B *H. pylori* infected cases

Figure 1 Pearson correlation coefficient analysis between Haemoglobin and Iron levels

Serum iron values cluster primarily between 60 and 80 mg/dL as Hb rises from roughly 10 to 16 g/dL, showing limited dispersion. In contrast, Panel B demonstrates a much stronger, positive association (r = 0.563, p < 0.01) between Hb and iron among H. pylori-infected cases. Here, iron values vary widely, ranging from 20 to over 100 mg/dL, with a steeper regression slope as Hb increases, suggesting that reductions in hemoglobin are closely paralleled by decreases in iron. This heightened correlation in infected individuals highlights the clinically significant disruption of iron metabolism associated with H. pylori infection, in contrast to the relatively stable relationship seen in controls.

Figure 2 displays amplification curves from quantitative PCR (qPCR) analysis for H. pylori detection prior to treatment, with each curve representing a patient sample. The X-axis shows cycle numbers (1–39), while the Y-axis (Rn) quantifies fluorescence as a proxy for the amount of amplified DNA. Most curves begin to rise between cycles 20 and 35, with a range of steepness and plateau heights, reflecting varied cycle threshold (Ct) values among positive samples. The majority of positive reactions display fluorescence crossing the detection

threshold between cycles 17 and 38, consistent with Table 4 data. These amplification profiles confirm H. pylori DNA presence in multiple patients prior to therapy and demonstrate inter-individual differences in bacterial load, as indicated by the spread of Ct values.

Figure 3 depicts the follow-up qPCR amplification curves two weeks after treatment, again with the X-axis for cycles and the Y-axis for relative fluorescence. Post-treatment, fewer curves show early amplification, with several only reaching significant fluorescence at higher cycle numbers (closer to cycle 35–39) and some remaining flat or with delayed rise, indicating either low bacterial DNA or negativity. Compared to Figure 2, this visual shift represents a marked reduction in H. pylori DNA detection among treated patients, although persistent amplification in a subset of samples highlights cases of possible antibiotic resistance or treatment failure.

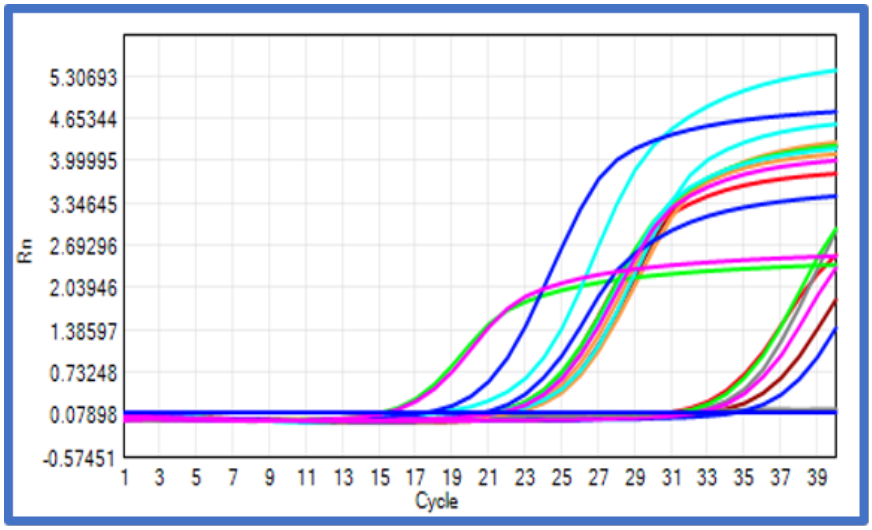


Figure 2 Ct (cycle threshold) value of the patients with H. pylori-positive cases before treatment.

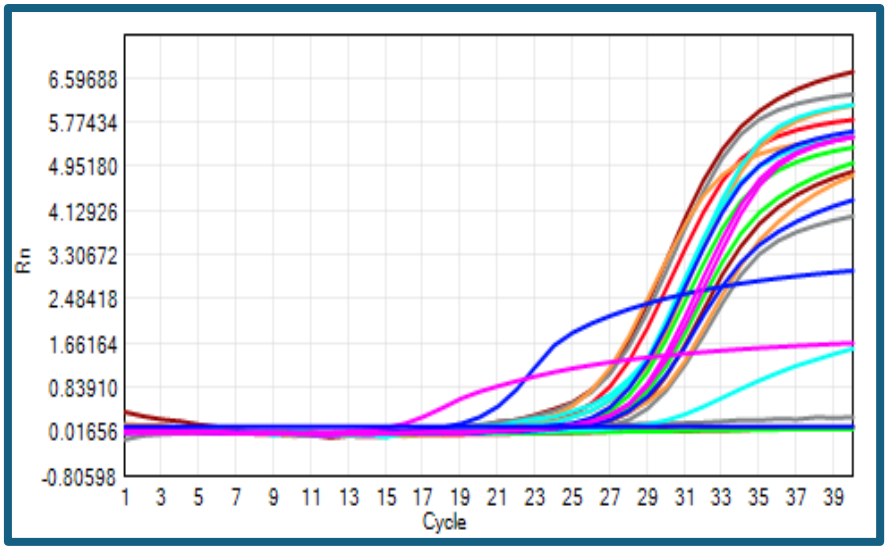


Figure 3 Ct (cycle threshold) value of the patients with H. pylori-positive cases after treatment.

The overall rightward shift and increased variability in amplification onset visually corroborate the reduction in molecular positivity following therapy, in line with Table 5 data. These integrated visualizations (Figures 1–3) collectively reinforce the study's central findings: the strong disruption of iron metabolism and hemoglobin levels in H. pylori infection, and the dynamic molecular response to therapy as quantified by qPCR, including identification of both successful eradication and persistent, potentially resistant infections.

DISCUSSION

The present study provides compelling evidence for the complex interplay between Helicobacter pylori infection and both hematological and biochemical disturbances in a cohort of untreated dyspeptic patients from a high-prevalence region. Consistent with global data, approximately half of our study population tested positive for H. pylori, and the infection was equally distributed among males and females, reinforcing the notion that gender is not a major determinant in acquisition or persistence of infection (1,2). While recent meta-analyses have documented a gradual decline in global prevalence—particularly in developed countries—H. pylori remains highly endemic in low- and middle-income countries, including Pakistan, owing to socioeconomic, environmental, and hygienic factors that sustain transmission (3,4).

The hematological impact of H. pylori observed in our study, including significantly lower hemoglobin, red blood cell counts, and markedly reduced serum iron and ferritin levels, underscores the organism's role in iron deficiency anemia (5,6). The robust positive correlation

between hemoglobin and serum iron among infected individuals ($r = 0.563$, $p < 0.01$) is particularly notable, indicating that H. pylori-related anemia is not merely coincidental but reflects a pathogenic disruption of iron metabolism. Previous research has attributed this phenomenon to both direct and indirect mechanisms: chronic gastritis induced by the bacterium impairs gastric acid secretion, which is essential for dietary iron absorption, and chronic inflammation or microbleeding from the gastric mucosa further exacerbates iron loss (7,8). Additionally, proinflammatory cytokines and hepcidin upregulation in response to infection can reduce iron bioavailability and contribute to functional iron deficiency (9). The reversal of iron deficiency anemia following H. pylori eradication, as reported in other clinical series, lends further weight to the causal relationship (10).

Beyond hematologic effects, our findings of significantly elevated amylase, lipase, and C-reactive protein (CRP) levels in H. pylori-positive patients point toward a broader systemic inflammatory response that may involve extra-gastric organs such as the pancreas. Growing evidence suggests that H. pylori infection may act as a co-factor in the pathogenesis of pancreatitis and possibly pancreatic cancer, through mechanisms including molecular mimicry, chronic systemic inflammation, and the potential for direct bacterial DNA presence in pancreatic tissue (11–13). These results highlight the necessity of viewing H. pylori not solely as a gastrointestinal pathogen but as a contributor to multi-system morbidity, particularly in populations with concurrent nutritional deficiencies or inflammatory conditions. A key strength of this investigation lies in the application of blood-based qPCR for molecular detection of H. pylori, which offers several advantages over traditional diagnostics. Unlike serology or stool antigen tests, qPCR provides high sensitivity and specificity and allows for the quantification of bacterial DNA, facilitating both diagnosis and treatment monitoring (14,15). In our cohort, qPCR detected H. pylori DNA in 70% of seropositive patients before treatment and was instrumental in identifying those with persistent infection after therapy, likely reflecting antimicrobial resistance. This molecular approach is especially valuable in resource-constrained environments where endoscopy and gastric biopsy may not be readily available or acceptable to patients (16). Furthermore, repeat qPCR after treatment enables early identification of eradication failure, supporting prompt modification of therapeutic regimens and potentially reducing the risk of complications related to chronic infection (17).

Nevertheless, several limitations warrant consideration. The cross-sectional design precludes inference of temporal causality between H. pylori infection and hematological or biochemical changes, and unmeasured confounders—such as underlying nutritional status, occult gastrointestinal bleeding from other causes, or concurrent infections—may have influenced the observed associations. The sample size, although adequate for primary analyses, may limit subgroup exploration and generalizability. Importantly, while the use of qPCR on blood samples demonstrates clear clinical utility, future research should address standardization of target genes, optimal sample types, and cost-effectiveness relative to existing diagnostic strategies (18,19).

Despite these limitations, the present study advances the understanding of H. pylori's systemic effects and supports the integration of molecular diagnostics into routine practice. The strong correlation between infection and disruption of iron homeostasis in our population echoes global findings and underscores the potential for targeted interventions, such as iron supplementation combined with eradication therapy, to improve patient outcomes. Furthermore, the identification of biochemical markers of inflammation and pancreatic involvement expands the clinical horizon for H. pylori-related disease and invites further longitudinal research to clarify the direction and magnitude of these associations (20,21). In summary, our findings reinforce the clinical value of blood-based qPCR for H. pylori detection and monitoring, highlight the organism's impact on iron metabolism and systemic inflammation, and advocate for a multidisciplinary approach to diagnosis and management in endemic settings. Ongoing surveillance of antimicrobial resistance and integration of molecular methods into broader clinical workflows will be crucial as the epidemiology and therapeutic landscape of H. pylori continue to evolve (22–25).

CONCLUSION

This study demonstrates a significant association between Helicobacter pylori infection and alterations in haematological and biochemical parameters among untreated dyspeptic patients, with notable reductions in hemoglobin, iron, and ferritin, as well as elevated inflammatory and pancreatic markers. The application of blood-based quantitative PCR provided high diagnostic accuracy for detecting H. pylori and proved useful in monitoring treatment response, including identification of persistent or resistant cases. These findings underscore the clinical utility of molecular diagnostics in high-prevalence, resource-limited settings, and highlight the need for integrated management strategies targeting both infection and its systemic sequelae. Further large-scale, prospective studies are warranted to clarify causal pathways and optimize diagnostic and therapeutic protocols for H. pylori-associated morbidity.

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