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ORIGINAL ARTICLE

MOLECULAR IDENTIFICATION OF *Helicobacter pylori* AND THEIR INFLUENCE ON HEMATOLOGICAL PARAMETERS IN PATIENTS WITH IRON-DEFICIENCY ANEMIA

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ABSTRACT

Iron-deficiency anemia (IDA) is a global health concern that may be related to *Helicobacter pylori* infection. Precise molecular identification of *H. pylori* and its virulence factors is essential for understanding the pathogenic mechanisms fundamental to IDA. The current study aims to correlate molecular detection of *H. pylori* and its virulence factor genes and hematological parameters in patients with IDA compared to non-diseased controls. This study included 120 participants (80 patients with IDA and 40 healthy controls). Gastric biopsies were obtained from patients admitted to Al-Nasiriyah Teaching Hospital, Thi-Qar, Iraq. DNA was extracted for polymerase chain reaction (PCR)-based detection of the *H. pylori*-16S rRNA and virulence factor genes (*cagA* and *babA2*). Hematological parameters, such as hemoglobin, serum ferritin, and iron levels, were measured from blood samples. The infection with *H. pylori* was detected in 62.5% of IDA patients, compared with 25% in controls. The *cagA* gene was detected in 76% of *H. pylori*-positive IDA patients versus 40% in positive controls. The *babA2* gene was detected in 68% of *H. pylori*-positive IDA patients compared with 30% in positive controls. Patients with both virulence factors showed significantly lower hemoglobin and ferritin levels. This study concluded that there is a significant association between *H. pylori* infection and IDA, highlighting that virulent strains carrying *cagA* and *babA2* may contribute to iron deficiency.

KEY WORDS: Gastritis; iron absorption impairment; Gram-negative; virulence factors.

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INTRODUCTION

Iron-deficiency anemia (IDA) affects approximately 1.62 billion individuals annually and is one of the most widespread nutritional diseases worldwide (Xia, 2017). Although nutritional and bleeding are the traditional causes of IDA, recent research indicates that *Helicobacter pylori* infection may contribute significantly to the beginning of iron deficiency and chronic blood loss due to gastric inflammation (Fernandez-Caso et al., 2022). *Helicobacter pylori* is a Gram-negative, microaerophilic bacterium that colonizes the human stomach and affects more than 50% of the global population (Ali & AlHussaini, 2024). It is identified as an important etiological factor in gastritis, gastric ulcer, and gastric cancer (Mendoza et al., 2019). Previous studies identified an association between infection by *H. pylori* and IDA manifestations, such as reduced iron absorption and chronic blood loss due to gastric inflammation (Li et al., 2017). The pathogenesis of *H. pylori* is primarily affected by the appearance of some virulence factors (Wang et al., 2025). The *cagA* gene encodes a cytotoxin-associated protein that is translocated into host epithelial cells via a type IV secretion channel, resulting in cellular malfunction and increased inflammation (Hatakeyama, 2017). Blood group antigen-binding adhesin A2 (*babA2*) promotes bacterial adhesion to abdominal epithelial cells that express Lewis B blood group antigens, thereby extending colonization and the duration of infection (Chey et al., 2017).

Traditional techniques for detecting *H. pylori*, such as serology, stool antigen screening, and urea breath test, can have limitations in testing, e.g., reduced sensitivity, especially in patients with atrophic gastritis or those treated with proton pump inhibitors (Wang et al., 2025). Molecular methods, especially the polymerase chain reaction (PCR), provide increased accuracy for the detection of virulence genes that may affect the severity and clinical consequences of the disease (Doohan et al., 2021). The correlation between *H. pylori* and IDA is not entirely clear. This study aimed to identify the virulence factors of *H. pylori* and to assess hematological parameters in IDA patients compared with healthy controls in Thi-Qar province/Iraq.

MATERIAL AND METHODS

Study Design and Participants

This cross-sectional study was conducted at Al-Nasiriyah Teaching Hospital from December 2024 to May 2025. The study protocol was approved by the Institutional Ethics Committee (Ethics approval number: IEC/2024/45), and written informed consent was obtained from all participants. A total of 120 participants were recruited and divided into two groups: 80 patients diagnosed

with IDA and 40 healthy volunteers without anemia. Different criteria were included in this investigation for both groups, such as age (18-65 years) and normal values for hemoglobin, serum ferritin, and transferrin saturation, which were <12 g/dL (females) or <13 g/dL (males), <15 ng/mL, and <16%, respectively. Excluded criteria were pregnancy, lactation, history of gastric surgery, recent use of antibiotics, bismuth, or proton pump inhibitors (within four weeks), presence of other chronic diseases (liver disease, chronic kidney disease, malignancy), and blood transfusion within the past three months.

Sample Size

A total of 120 blood samples were collected from both IDA patients and healthy controls. The sample size was calculated a priori using G Power software (version 3.1.9.7). Based on an anticipated medium effect size (Cohen's d = 0.5), an alpha level (α) of 0.05, and a statistical power of 80%, a minimum of 64 participants per group was required. To compensate for potential attrition, the total sample size was increased to 120 individuals.

Laboratory Investigations

Complete blood count (CBC) (BC-700®, Mindray, China), Serum iron, ferritin, and total iron-binding capacity (TIBC), transferrin saturation, and vitamin B12 and folate levels (AGD 4400®, India) were measured. Eighty biopsies were taken by physicians from patients with IDA and used for molecular identification of *H. pylori* and to investigate the prevalence of *H. pylori* virulence genes.

DNA Extraction

DNA extraction from gastric biopsy samples was performed using the QIAamp DNA Mini Kit (Qiagen®, Germany) according to the manufacturer's instructions. The extracted DNA was quantified using a NanoDrop spectrophotometer (Avans®, China) and stored at -20 °C until PCR analysis.

PCR Amplification

The 16S rRNA gene serves as the primary target for *H. pylori* identification due to its species-specific sequences and high sensitivity. Commonly used primer sets include: [HP-1 Forward: 5'-CTGGAGAGACTAAGCCCTCC-3'; HP-2 Reverse: 5'-ATTACTGACGCTGATTGTGC-3'; expected amplicon size: 294 bp]. Initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 7 minutes

these were the main conditions that were used in the 16S rRNA gene detection by PCR (Bioneer®, Korea) (Ho et al., 1991). The *cagA* gene amplification was performed using: [cagA Forward: 5'-GATAACAGGCAAGCTTTTGAGG-3'; *cagA* Reverse: 5'-CTGCAAAAGATTGTTTGGCAGA-3'; expected amplicon size: 349 bp]. Initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 1 minute, 58 °C for 1 minute, and 72 °C for 1 minute, with final extension at 72 °C for 10 minutes (Yamaoka et al., 1999). Likewise, *babA2* gene amplification was performed using a specific primer [*babA2* Forward: 5'-AATCCAAAAAGGAGAAAAAGTATGAAA-3'; *babA2* Reverse: 5'-TGTTAGTGATTTCGGTGTAGGACA-3'; expected amplicon size: 832 bp]. Initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 61 °C for 30 seconds, and 72 °C for 1 minute, with final extension at 72 °C for 7 minutes (Kadhim, et al., 2018, Abdulhusein, et al., 2023). PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized under UV light after ethidium bromide staining.

Statistical Analysis

Data were analyzed using SPSS version 26 (IBM®, Chicago, IL, USA). The Chi-square test was used to identify significant differences. Continuous variables were assessed using Student's t-test or the Mann-Whitney U test, as appropriate. A *p*-value lower than 0.05 was considered statistically significant.

RESULTS

The healthy control and IDA patient groups were equivalently matched across all significant demographic factors. The average age of individuals in the IDA patient group was 42.5 ± 14.2 years, whereas in the control group it was 38.7 ± 12.8 years, with no statistically significant difference noted (p= 0.142). The gender distribution was relatively similar across the groups; females represented 65% (52/80) of the IDA cohort and 55% (22/40) of the control group. Whereas males represented 35% (28/80) of the IDA patients and 45% (18/40) of the control group, and there was no significant difference (p= 0.301). The IDA patient group had a reduced mean Body Mass Index (BMI) of 23.4 ± 4.1 kg/m² compared with the control group, which had a BMI of 24.8 ± 3.6 kg/m²; however, this difference was not statistically significant (p= 0.067). No significant differences were observed between the groups for smoking status (p= 0.382) or socioeconomic stratification (p= 0.234) (Table 1).

Table 1. Demographic characteristics of the study participants.

Parameter		IDA Patients *n*= 80 (mean±SD*)	Controls *n*= 40 (mean±SD*)	*p*-value
Age (years)	Range	18-65	20-62	
		(42.5 ± 14.2)	(38.7 ± 12.8)	0.142
Gender	Male	28 (35%)	18 (45.0)	0.301
	Female	52 (65%)	22 (55.0)	
BMI (kg/m²)		23.4 ± 4.1	24.8 ± 3.6	0.067
Smoking status	Smokers	22 (27.5)	8 (20.0)	0.382
	Non-smokers	58 (72.5)	32 (80.0)	
Socioeconomic status	Low	34 (42.5)	12 (30.0)	0.234
	Middle	38 (47.5)	22 (55.0)	
	High	8 (10.0)	6 (15.0)	

*SD= standard deviation

According to Table 2, there was a substantial elevation in *H. pylori* infection in IDA patients compared with healthy controls. Of the 80 IDA patients, 50 (62.5%) were positive for *H. pylori*, while only 10 individuals (25%) in the control group of 40 tested positive. The difference was statistically significant (p= 0.002), demonstrating a robust link between *H. pylori* infection and IDA.

Table 2. Prevalence of *Helicobacter pylori* infection.

Parameter		IDA Patients (*n*=80)	Controls (*n*=40)	*p*-value
H. pylori status	Positive	50	10	<0.002*
	Negative	30	30	

*Statistically significant (p< 0.05)

The findings in Table 3 revealed an association between *H. pylori* infection and hematological parameters. All hematological parameters associated with iron status were statistically significant in the IDA and control groups (p<0.05). Nonetheless, a more significant iron shortage was noted in *H. pylori*-positive IDA patients compared with *H. pylori*-negative patients. *H. pylori*-positive IDA patients demonstrated more pronounced hematological abnormalities, characterized by reduced mean hemoglobin levels (8.2 ± 1.4 g/dL compared to 9.1 ± 1.2 g/dL), diminished serum ferritin (8.3 ± 3.2 ng/mL *vs* 10.5 ± 4.1 ng/mL), and decreased serum iron (42.1 ± 15.3 µg/dL relative to 48.7

± 18.2 µg/dL). Moreover, the pattern of iron deficiency, heightened TIBC, and diminished transferrin saturation was more evident in the *H. pylori*-positive group (TIBC: 478.6 ± 65.2 µg/dL; transferrin saturation: 8.8 ± 3.1%) than in the *H. pylori*-negative IDA patients (TIBC: 445.3 ± 58.7 µg/dL; transferrin saturation: 10.9 ± 4.2%).

Table 3. Association between *Helicobacter pylori* infection and hematological parameters.

	Parameter	IDA Patients (*n*=80)	Controls (*n*=40)	*p*-value
H. pylori Positive	Hemoglobin (g/dL)	8.2 ± 1.4	14.1 ± 1.2	<0.001*
	Serum ferritin (ng/mL)	8.3 ± 3.2	45.2 ± 18.7	<0.003*
	Serum iron (µg/dL)	42.1 ± 15.3	89.4 ± 22.1	<0.001*
	TIBC (µg/dL)	478.6 ± 65.2	312.5 ± 48.9	<0.001*
	Transferrin saturation (%)	8.8 ± 3.1	28.6 ± 6.2	<0.001*
H. pylori Negative	Hemoglobin (g/dL)	9.1 ± 1.2	13.9 ± 1.1	<0.005*
	Serum ferritin (ng/mL)	10.5 ± 4.1	48.3 ± 16.4	<0.001*
	Serum iron (µg/dL)	48.7 ± 18.2	92.1 ± 19.8	<0.001*
	TIBC (µg/dL)	445.3 ± 58.7	318.7 ± 45.2	<0.002*
	Transferrin saturation (%)	10.9 ± 4.2	29.1 ± 5.8	<0.003*

*Statistically significant ($p<0.05$)

Molecular detection of H. pylori

The outcomes of the molecular diagnosis utilizing 16S rRNA and PCR performance metrics are delineated in Table 4. The experiment revealed a considerably elevated detection rate of *H. pylori* in the IDA group (62.5%, 50/80) compared to the control group (25.0%, 10/40), a statistically significant difference ($p<0.001$). The diagnostic efficacy of the 16S rRNA was outstanding (Figure 1). Using a preset reference standard (possibly a combination of other tests), the assay exhibited a high overall sensitivity of 93.8% (60/64) and specificity of 92.9% (52/56). The positive predictive value (PPV) was 93.8%, signifying that a positive PCR result was very indicative of a true infection, while the negative predictive value (NPV) was 92.9%, indicating that a negative result was highly indicative of a true absence of infection. The performance was consistently strong in both the IDA and control categories. The analysis of demographic distribution among PCR-positive patients

indicated no significant age-related differences in infection rates (p=0.821). The infection was uniformly distributed among 18-30, 31-45, and 46-65 (years) age groups. Likewise, there was no statistically significant difference in the gender distribution of *H. pylori*-positive cases between the IDA and control groups (p=0.279), but more infected individuals in the IDA group were female (68.0%), while males accounted for 32.0%.

Table 4. Molecular diagnosis of *Helicobacter pylori* in the 16S rRNA gene.

Parameter		IDA Patients (n=80)	Controls (n=40)	Total (n=120)	p-value
16S rRNA Results, n (%)	Positive	50 (62.5)	10 (25.0)	60 (50.0)	<0.001*
	Negative	30 (37.5)	30 (75.0)	60 (50.0)	
PCR Sensitivity and Specificity	Sensitivity	94.3% (50/53)	90.9% (10/11)	93.8% (60/64)	
	Specificity	88.9% (24/27)	96.6% (28/29)	92.9% (52/56)	
	PPV	94.3% (50/53)	90.9% (10/11)	93.8% (60/64)	
	NPV	88.9% (24/27)	96.6% (28/29)	92.9% (52/56)	
Age (years) Distribution of *H. pylori* Positive Cases	18-30	12 (24.0)	2 (20.0)	14 (23.3)	
	31-45	19 (38.0)	4 (40.0)	23 (38.3)	0.821
	46-65	19 (38.0)	4 (40.0)	23 (38.3)	
Gender Distribution of *H. pylori* Positive Cases	Male	16 (32.0)	5 (50.0)	21 (35.0)	0.279
	Female	34 (68.0)	5 (50.0)	39 (65.0)	

*Statistically significant (p<0.05)

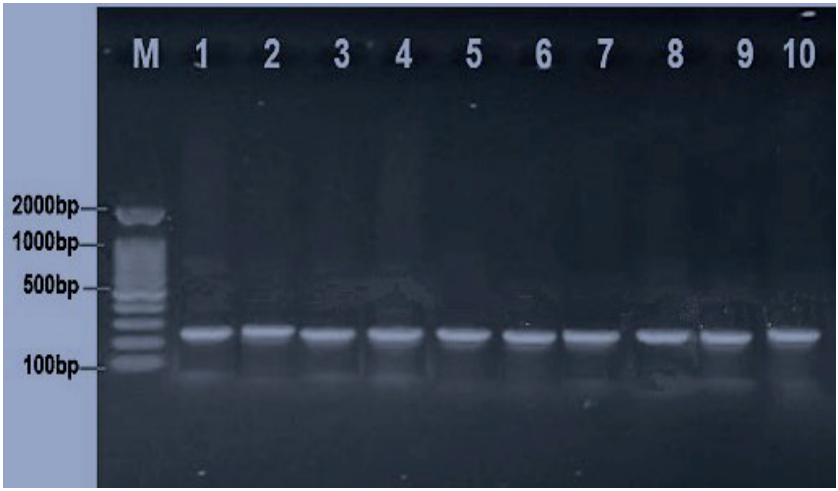

Figure 1. An agarose gel electrophoresis image shows the PCR product analysis of the 16S rRNA gene of *Helicobacter pylori* with a 294 bp PCR product size.

Virulence factor genes of H. pylori

A considerably raised incidence of *cagA* and *babA2* was noted in isolates obtained from IDA patients in comparison to those from healthy controls. While the *cagA* gene was observed in 76.0% (38/50) of isolates from IDA patients compared to 40.0% (4/10) of control isolates (p=0.037), as depicted in Figure 2; the *babA2* gene was detected in 68.0% (34/50) of IDA isolates, in contrast to 30.0% (3/10) of control isolates (p=0.043), as highlighted in Figure 3.

The most notable distinction was noted in the co-occurrence of these virulence factors. The co-occurrence of *cagA* and *babA2* genes was noticeably greater in the IDA group (56.0%, 28/50) in relation to the control group (20.0%, 2/10) (p=0.031). In contrast, isolates lacking genes were primarily identified in the control group (50.0%, 5/10) as opposed to the IDA group (12.0%, 6/50). The existence of these virulence genes was directly correlated with the intensity of iron shortage. Patients with co-occurrence of *cagA* and *babA2* strains demonstrated the most severe hematological deficiency, characterized by the lowest mean hemoglobin (7.8 ± 1.2 g/dL) and the lowest serum ferritin levels (6.9 ± 2.8 ng/mL). A distinct gradient of severity was noted: the patients with co-occurrence of *cagA* and *babA2* strains had the most pronounced effects, followed by individuals possessing a single virulence factor, whereas those with strains devoid of both genes demonstrated the least severe iron deficiency among the infected cohort (Hemoglobin: 9.2 ± 1.1 g/dL; Ferritin: 11.8 ± 4.2 ng/mL). All groups had considerably inferior metrics compared to the health controls (p<0.001) (Table 5).

Table 5. Prevalence of *Helicobacter pylori* virulence genes (*cagA* and *babA2*).

Parameter		IDA Patients (n=50)	Controls (*n*=10)	Total (*n*=60)	*p*-value
Virulence Factor					
cagA Gene, *n*(%)	Positive	38 (76.0)	4 (40.0)	42 (70.0)	0.037*
	Negative	12 (24.0)	6 (60.0)	18 (30.0)	
babA2 Gene, n(%)	Positive	34 (68.0)	3 (30.0)	37 (61.7)	0.043*
	Negative	16 (32.0)	7 (70.0)	23 (38.3)	
Combined Virulence Pattern, *n*(%)	cagA+ babA2+	28 (56.0)	2 (20.0)	30 (50.0)	0.031*
	cagA+ babA2-	10 (20.0)	2 (20.0)	12 (20.0)	
	cagA- babA2+	6 (12.0)	1 (10.0)	7 (11.7)	
	cagA- babA2-	6 (12.0)	5 (50.0)	11 (18.3)	
Hematological Parameters by Virulence Status					
cagA+ *babA2*+ (*n*=30)	Hemoglobin (g/dL)	7.8 ± 1.2	13.8 ± 0.9	8.4 ± 2.1	<0.001*
	Serum ferritin (ng/mL)	6.9 ± 2.8	42.1 ± 15.3	9.8 ± 12.4	<0.001*
cagA+ or *babA2*+ only (*n*=19)	Hemoglobin (g/dL)	8.4 ± 1.3	14.2 ± 1.1	9.1 ± 2.0	<0.001*
	Serum ferritin (ng/mL)	9.1 ± 3.4	46.8 ± 17.2	13.2 ± 15.8	<0.001*
cagA- *babA2*- (*n*=11)	Hemoglobin (g/dL)	9.2 ± 1.1	14.5 ± 1.3	10.8 ± 2.2	<0.001*
	Serum ferritin (ng/mL)	11.8 ± 4.2	48.9 ± 19.1	21.4 ± 18.7	<0.001*

*Statistically significant ($p<0.05$)

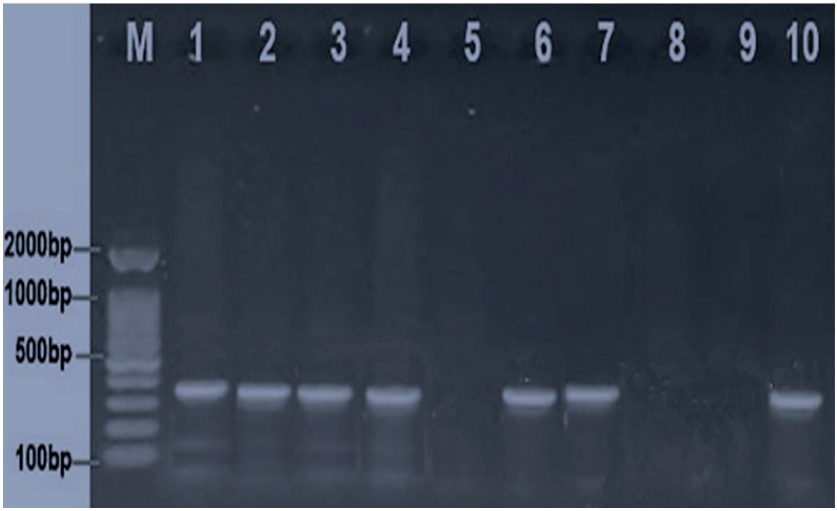

Figure 2. An agarose gel electrophoresis image that shows the PCR product analysis of the *Helicobacter pylori cagA* gene with 349 bp PCR product size.

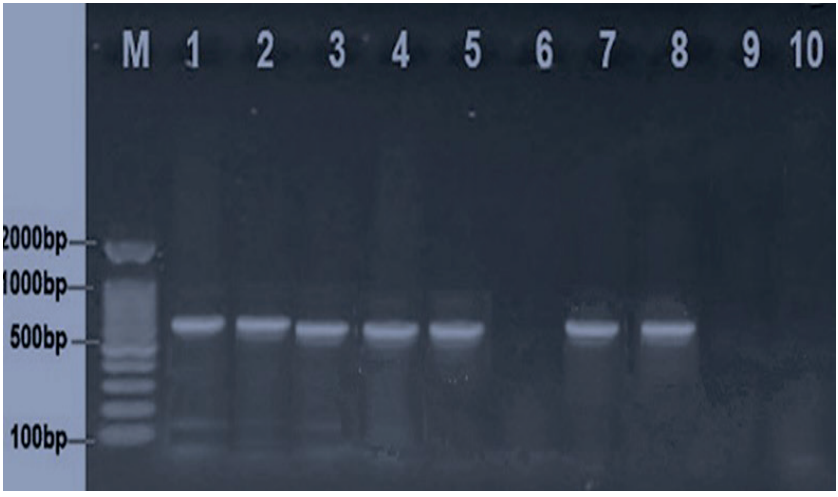

Figure 3. An agarose gel electrophoresis image that shows the PCR product analysis of the *Helicobacter pylori babA2* gene with 832 bp PCR product size.

DISCUSSION

This study presents extensive molecular evidence linking *H. pylori* infection to IDA, revealing significantly elevated infection rates and determinants of pathogenicity in IDA patients relative to the healthy group. The results provide noteworthy insights into the pathogenic mechanisms of *H. pylori*-related iron shortage and endorse the use of PCR-based molecular diagnostics in clinical settings. The findings of this study indicate a considerable correlation between *H. pylori* infection and IDA, with 62.5% of IDA patients testing positive for *H. pylori*, in contrast to 25% of healthy controls. This prevalence aligns with prior research indicating *H. pylori* infection rates between 40-80% in IDA patients (Kato et al., 2022; Motupalli & Terry, 2024). The elevated infection rate in this study of patients with IDA indicates that *H. pylori* may significantly contribute to the onset of iron insufficiency, even when standard etiologies have been ruled out (Pu et al., 2025). The molecular identification using 16S rRNA showed high sensitivity (93.8%) and specificity (92.9%), affirming the reliability of this diagnostic method. PCR-based detection provides numerous advantages compared to traditional approaches, including enhanced sensitivity and the capacity to identify living but non-culturable bacteria, along with less interference from concomitant drugs such as proton pump inhibitors (Chey et al., 2017).

The analysis of virulence factors demonstrated a markedly elevated prevalence of both *cagA* and *babA2* genes in *H. pylori*-positive IDA patients compared with healthy controls. Patients possessing both virulence factors exhibited the most pronounced iron deficiency characteristics, characterized by reduced hemoglobin levels and ferritin concentrations in comparison to individuals with either single or absent virulence factors. The *cagA* gene encodes a cytotoxin-associated protein that is introduced into gastric epithelial cells, resulting in heightened inflammatory responses and epithelial injury (Gluckman, 2020). The *babA2* adhesion enhances robust bacterial attachment to stomach epithelial cells that express Lewis b antigens, hence fostering sustained colonization (Szymczak et al., 2020). This increased adherence may result in more severe and longer stomach irritation, contributing to persistent iron deficiency (Chen et al., 2013). The interplay of these virulence factors seems to establish a notably pathogenic milieu that significantly affects iron homeostasis, with certain investigations indicating a synergistic effect leading to more severe gastroduodenal damage (Ansari & Yamaoka, 2019). *H. pylori* infection enhances chronic stomach inflammation, resulting in heightened synthesis of inflammatory cytokines, including interleukin-6 and tumor necrosis factor-α (Aspholm-Hurtig et al., 2004). These cytokines induce hepatic hepcidin synthesis, which inhibits duodenal iron absorption and macrophage iron release, leading to functional iron insufficiency (Hatakeyama, 2017). Furthermore, *H. pylori* infection may directly contend with the host for

accessible iron (Shahi et al., 2015). The bacteria have iron acquisition systems and may sequester iron for their metabolic requirements, especially in the iron-deficient stomach environment (Moalla et al., 2024). The persistent stomach inflammation linked to virulent *H. pylori* strains may result in microscopic hemorrhage and iron depletion via occult blood loss (Doohan et al., 2021). Moreover, *H. pylori* infection, especially with *cagA*-positive strains, might precipitate gastric atrophy and diminish acid secretion (Flores et al., 2017; Chey et al., 2024). Decreased stomach acid production may directly prevent iron absorption by preventing the conversion of ferric iron to the absorbable ferrous form, even in the absence of inflammation (Wang et al., 2025). The results have significant clinical implications for the management of patients with unexplained IDA. The incidence of *H. pylori* infection in patients with IDA advocates for routine screening for *H. pylori* in individuals with unexplained iron deficiency, especially after conventional explanations have been ruled out, a proposal endorsed by clinical recommendations (Szymczak et al., 2020; Abdelkader et al., 2022). The correlation between virulent *H. pylori* strains and heightened iron shortage implies that molecular characterization of *H. pylori* isolates could aid in predicting treatment efficacy and informing therapeutic choices (Chen et al., 2013; Chey et al., 2024).

Patients infected with *cagA* and *babA2* possessing strains may necessitate more intensive iron supplements and vigilant monitoring after *H. pylori* eradication. The elimination of *H. pylori* infection has demonstrated enhancement of iron status in numerous patients with IDA (Aspholm-Hurtig et al., 2004; Ansari & Yamaoka, 2019). Patients with virulent strains may require more effective eradication protocols or prolonged therapy durations. Other important virulence factors, such as *vacA*, *iceA*, and *oipA* genes, also play a role in iron deficiency (Stein et al., 2017; Motupalli & Terry, 2024). The long-term studies monitoring iron status post-*H. pylori* eradication in patients categorized by virulence factor status would yield significant insights into treatment success (Hooi et al., 2017; Saleem & Howden, 2020).

The examination of host-pathogen interactions, encompassing genetic susceptibility factors and immunological responses, may assist in identifying patients at the greatest risk for *H. pylori*-related iron insufficiency (Shahi et al., 2015; Malfertheiner et al., 2022). The advancement of point-of-care molecular diagnostic technologies for concurrent detection of *H. pylori* and virulence factor profiling will enhance prompt clinical decision-making and individualized treatment strategies (Wang et al., 2025). The investigation of innovative therapeutic targets grounded on virulence factor functionality may yield more efficacious treatment strategies for individuals afflicted with virulent *H. pylori* strains (Hirschl & Makristathis, 2007; Souod et al., 2013).

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CONFLICT OF INTEREST

The authors declares no conflicts of interest.

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