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Original Article

Expression and clinical significance of IL-8 and Wnt2 in *Helicobacter pylori*-infected gastric cancer patients

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Abstract

Introduction: We assessed the expression levels of interleukin-8 (IL-8) and Wnt2 in *Helicobacter pylori*-infected gastric cancer (GC) patients, and investigated the association of these proteins with prognosis.

Methodology: Seventy-two GC patients, including 40 cases with *H. pylori* infection and 32 cases without *H. pylori* infection, were enrolled. Tumor and para-tumor normal tissues were collected. *H. pylori* infection was determined using the rapid urease test and Warthin-Starry silver staining. The expressions of IL-8 and Wnt2 were assessed using immunohistochemistry. The association between *H. pylori* infection, IL-8, Wnt2, and pathological and clinical data was analyzed. The relationship between *H. pylori*, IL-8, Wnt2, and prognosis was assessed using survival curves and regression analyses.

Results: The expression levels of IL-8 and Wnt2 in GC tissue significantly exceeded that in normal mucosa. The expression levels of IL-8 and Wnt2 were significantly higher in patients with *H. pylori* infection, than in those without *H. pylori* infection; and a positive correlation was observed between IL-8 and Wnt2 expressions. The positivity rate of IL-8 and Wnt2 in early-stage GC without lymph node metastasis markedly differed from that in advanced-stage GC with lymph node metastasis. Additionally, the survival rate of GC patients with positive IL-8 and Wnt2 expressions was significantly lower than in patients without such expression patterns. Clinical stage and Wnt2 were identified as independent prognostic factors of GC.

Conclusions: IL-8 and Wnt2 were prognostic indicators for GC. *H. pylori* and IL-8 were not independent prognostic factors, whereas Wnt2 could independently serve as a prognostic factor for GC.

Key words: gastric cancer; *Helicobacter pylori*; interleukin-8; Wnt2; prognosis.

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Introduction

Gastric cancer (GC) is a significant global health issue. According to the GLOBOCAN 2020 report by the International Agency for Research on Cancer, GC accounted for over 1 million new cases and an estimated 769,000 deaths in 2020, ranking fifth globally in incidence and fourth in mortality [1]. GC incidence varies across regions, with higher rates observed in central and eastern Asia, eastern Europe, and Latin America [2,3]. Two-thirds of GC patients were in developing countries, with 44% of them in China, where the incidence rate was higher in northwest China and the southeast coastal areas [3].

Persistent inflammatory responses caused by pathogenic bacterial infections are closely associated with malignant tumor progression. Approximately 25% of malignant tumors are related to infections because chronic inflammation can lead to abnormal immune responses, DNA damage, and potentially cancer [4,5]. *Helicobacter pylori* (*H. pylori*), is a primary carcinogenic factor, and contributes to the development and progression of GC by continuously inducing inflammatory responses in host and peripheral immune cells [6,7]. This persistent inflammatory response can activate the NF-κB pathway, promote interleukin-8 (IL-8) release, and thereby activate the Wnt/β-catenin pathway. IL-8 likely serves as a crucial mediator linking these two signal pathways [8]. Limited studies have explored the association among *H. pylori* infection, IL-8 expression, Wnt2, and the activation of the Wnt/β-catenin pathway in the prognosis of GC.

IL-8, also known as pro-inflammatory CXC chemokine or CXCL-8, is produced by various immune-related cell types including lymphocytes,

macrophages, peripheral blood mononuclear cells, and epithelial cells [9,10]. It is involved not only in the response to inflammation but also in epithelial-mesenchymal transition (EMT) and the remodeling of the tumor microenvironment [11,12]. The *Wnt2* gene is located on human chromosome 7q31 [13]. The canonical Wnt signaling pathway typically regulates proliferation, stem cell maintenance, and homeostasis in normal gastric mucosa, in addition to its role in early embryogenesis [14]. Previous studies have shown frequent up-regulation of *WNT2* mRNA in human GC [15]. Therefore, Wnt2 is considered a tumor marker and the *WNT2* gene is a target for pharmacogenomics in the field of oncology [13]. High expression of Wnt2 and aberrant β-catenin-dependent transcriptional activation drive human carcinogenesis by inducing cancer stem cell (CSC) features, bulk tumor proliferation, and EMT [16]. Our previous animal and cytological experiments [8,17] found that persistent *H. pylori* infection induced Wnt2 overexpression and increased IL-8 release in the gastric epithelial cell line (GES-1) and mouse gastric epithelium. Consequently, the activation of the Wnt-β-catenin/NF-κB/IL-8 pathway is involved in abnormal proliferation, EMT, and CSC properties of the gastric epithelium.

This study aimed to identify the differential expression of IL-8 and Wnt2 in GC patients with *H. pylori* infection and those without infection. Additionally, we aimed to assess whether they can predict the prognosis of GC patients. Our findings may provide a strong theoretical foundation for identifying novel prognostic markers and therapeutic targets for GC.

Methodology

Study participants

Seventy-two patients who were diagnosed with GC and underwent radical gastrectomy between December 2016 and October 2019 at the Gansu Provincial Cancer Hospital were included in the study. Pathologically confirmed GC tissue samples were collected from each patient. Forty cases of normal gastric mucosa adjacent to the cancer (para-tumor normal tissues), located more than 5 cm away, were randomly selected as control. All 72 patients had complete clinical data and were followed up for a period ranging from 3 months to 3 years. The inclusion criteria were (1) patients with a confirmed diagnosis of GC; (2) patients who received subtotal or total gastrectomy with standard lymph node dissection; (3) patients without comorbidities such as diabetes, hepatitis B, cirrhosis, or tuberculosis; (4) patients with a preoperative Karnofsky score of 80 points and normal liver, heart, and kidney function. The exclusion criteria were: (1) patients with a prior history of receiving chemotherapy or radiation therapy for the current disease; (2) patients with brain metastases, mental disorders, substance abuse, or chronic alcoholism, which could hinder postoperative chemotherapy or lead to allergic reactions to chemotherapy drugs. The study received ethical approval from the Ethics Committee at Gansu Provincial Cancer Hospital. The requirement for written informed consent was waived due to the retrospective nature of this study and the anonymity of the patient data.

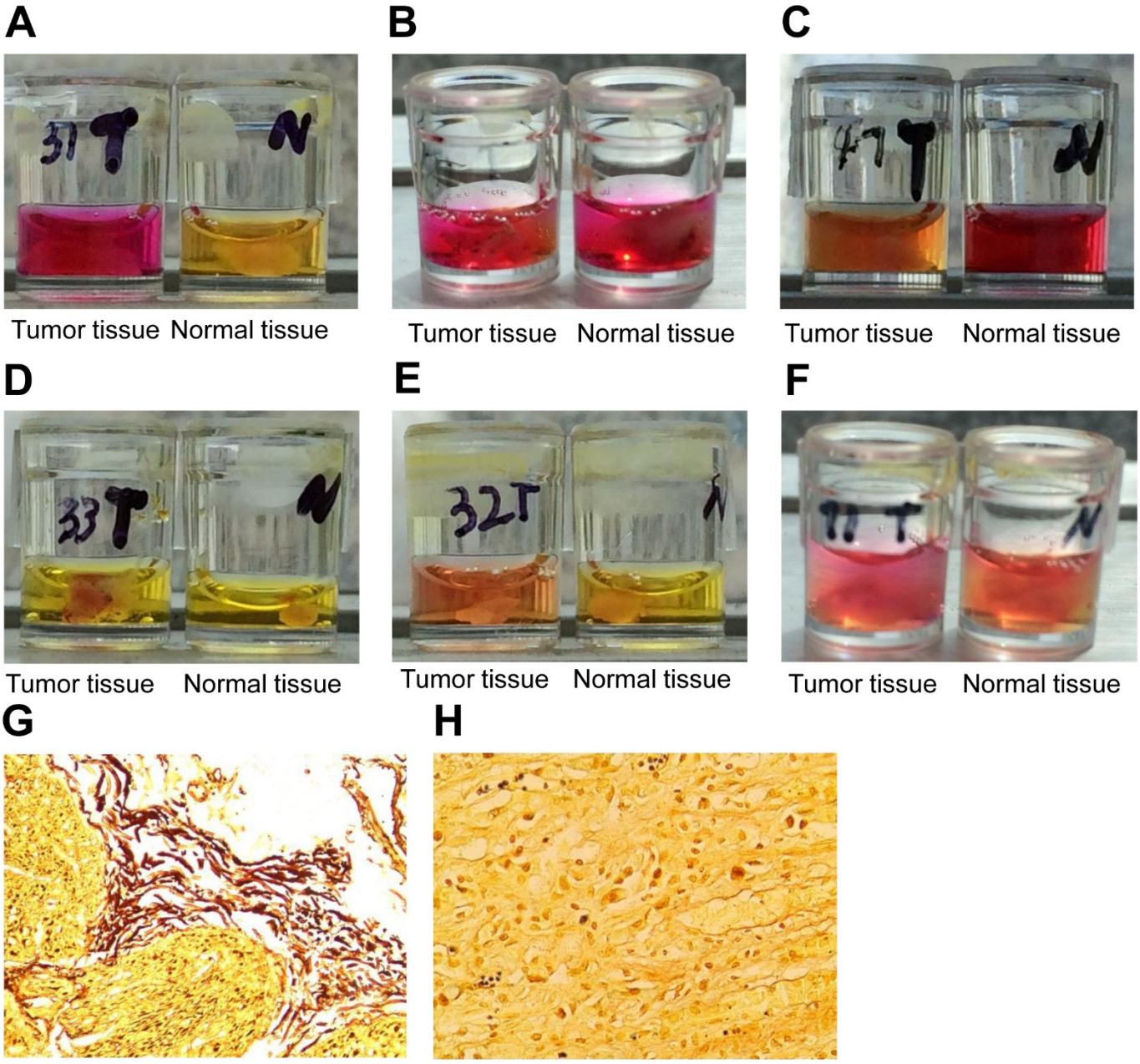
Immunohistochemistry

The immunohistochemistry procedure was conducted as previously described [18]. The paraffin-embedded tissues were sectioned to approximately 3 μm thickness, dewaxed, and hydrated. Subsequently, antigen retrieval was carried out using a pressure cooker, and incubating the slides in EDTA buffer (pH 8.0) for 3 min. Then, the sections were incubated with 10% goat serum for 15 min to prevent nonspecific binding. The samples were then incubated at 37 °C for 1 h following the addition of anti-mouse IL-8 antibody (1:2000 dilution; ImmunoWay, Plano, TX, USA) and anti-rabbit Wnt2 antibody (1:600 dilution; Bioss, Beijing, China). Subsequently, after rinsing, incubation with HRP-polymer anti-mouse and rabbit antibody (Zhongshan Jinqiao Biotechnology Co., Ltd, Beijing, China) was conducted for 1 h. Two pathologists evaluated the staining intensity and percentage of positive staining in a blinded manner. The immunostaining intensity was assessed on a scale of 0 to 3 (0, non-staining; 1, weak; 2, moderate; and 3, strong. The percentage of positive cells was scored as follows: 0 (< 5% positive cells), 1 (6–25% positive cells), 2 (26–50% positive cells), and 3 (51–100% positive cells). According to the product of these two scores, the tumor tissues were classified into four grades: 0 (negative, -), 1–2 (weakly positive, +), 3–5 (moderately positive, ++), and 6–9 (strongly positive, +++) [19].

Detection of H. pylori infection

The rapid urease test was performed to confirm *H. pylori* infection in patients using the gastric *H. pylori* detection kit (Beizhen Biological Technology Co., Ltd, Fujian, China) as per the manufacturer's instructions. Additionally, gastric tissues were stained with the Warthin-Starry silver staining kit (Solarbio, Beijing, China), following standard protocols.

Figure 1. Detection of *Helicobacter pylori* infection in gastric cancer (GC) and para-carcinoma by rapid urease test and Warthin-Starry silver staining (×100).



A–F: rapid urease test results: A. tumor tissue (+) and para-tumor normal tissue (-); B. tumor tissue (+) and para-tumor normal tissue (+); C. tumor tissue (±) and para-tumor normal tissue (+); D. tumor tissue (-) and para-tumor normal tissue (-); E. tumor tissue (±) and para-tumor normal tissue (±). Positive results are indicated by dark red or purplish red coloration, negative results are indicated by faint yellow coloration, and suspicious positive results by pink coloration. G–H: G. positive gastric mucosa with brownish-black rod-shaped bacterial bodies around the periphery; H. negative gastric mucosa.

Statistical analysis

The statistical analysis was conducted using the IBM SPSS Statistics 22.0 software package (IBM Corp., Armonk, NY, USA). Categorical data were compared using the χ^2 test. Measurement data were presented as mean ± SD and analyzed with a t-test. Correlation analysis was performed using Kendall's rank correlation. The Kaplan-Meier method was used for survival analysis. The log-rank method was used for comparative analysis of survival. The Cox proportional hazards regression model was utilized to conduct univariate and multivariate analyses. A significance level of $p < 0.05$ was deemed statistically significant.

Results

Baseline clinical data

There were 31 patients aged ≥ 60 years and 41 patients aged < 60 years. The cohort comprised of 56 males and 16 females. The pathological stages were determined according to the 8th Edition TNM staging of the American Joint Committee on Cancer (AJCC) [20]. Based on this, 35 cases were categorized as early-stage GC (IA–IIB) and 37 cases as advanced-stage GC (IIIA–IIIV). According to the pathological differentiation, 39 cases were of high and moderate differentiation, while 33 cases were of low differentiation and undifferentiation. Regarding the depth of cancer cell invasion, 39 cases had invasion depth not reaching the serosa layer, and 33 cases had invasion depth reaching the entire gastric wall. In terms of lymph node metastasis, 26 cases showed no lymph node involvement.

H. pylori infection rate in GC and para-tumor normal tissues

The tumor (n = 72) and para-tumor normal tissues (n = 40) from GC patients were subjected to the rapid urease assay. Positive reactions in both tumor and para-tumor normal tissues were considered indicative of *H. pylori* infection (Figure 1A-F). Suspicious positive reactions in tumor and para-tumor normal tissues were confirmed using Warthin-Starry silver staining. The urease assay results indicated that there were 36 patients with positive staining, 26 with negative staining, and 10 with suspicious positive staining. Among the 36 patients with positive staining, 15 exhibited positive reactions in tumor tissue but not in para-tumor normal tissue (Figure 1A); 12 showed positive reactions in both carcinoma and para-tumor normal tissues (Figure 1B); and 9 had no positive reactions in tumor tissue but did in para-tumor normal tissue (Figure 1C). The 10 patients with suspicious positive staining (Figure 1E–1F) were subjected to Warthin-Starry silver staining, which revealed that 4 cases were indeed infected with *H. pylori*, while 6 cases were excluded (Figure 1G–H). Based on the results of the two detection methods, 40 out of the 72 GC patients were *H. pylori* positive, yielding an infection rate of 56%.

Differential expression of IL-8 in normal gastric mucosa and GC tissue

The IL-8 staining results were categorized based on staining intensity and positive cell rate as absent (-), low (+), moderate (++), and high (+++) (Figure 2A). The expression of IL-8 was significantly elevated in the GC tissue, whereas it was minimal or absent in normal gastric mucosa (Figure 2C). A comparison of the number of positive cases (+/++/+++) with negative cases (-) and moderately/strongly positive cases (+/++) with negative/weakly positive cases (-/+) (Supplementary Table 1) revealed a significantly higher positivity rate and intensity of IL-8 expression in GC tissue compared to para-carcinoma normal tissues.

Correlation between IL-8 expression and H. pylori infection in GC tissue

The rate of IL-8 positivity in the 40 GC tissues infected with *H. pylori* (92.5%) was significantly higher than in the 32 cases without infection (71.9%). Moreover, 80.0% of the infected cases showed moderate to strong IL-8 positivity, while only 37.5% of the uninfected cases did so. Additionally, the expression level of IL-8 in the infected group was higher than in the uninfected group. As shown in Supplementary Table 2, there was a positive correlation between the expression of IL-8 and *H. pylori* infection, indicating that in GC patients infected with *H. pylori*, the release of IL-8 was significantly higher than in uninfected patients.

Differential expression of Wnt2 in normal gastric mucosa and GC tissue

The Wnt2 staining results were classified as absent (-), low (+), moderate (++), and high (+++) (Figure 2B). Wnt2 exhibited high expression in GC tissue, whereas it showed no or low expression in normal gastric mucosa (Figure 2D). Comparison of positive cases (+/++/+++) and negative cases (-), as well as moderately/strongly positive cases (+/++) with negative/weakly positive cases (-/+) (Supplementary Table 3), revealed a significantly higher positive rate and intensity of Wnt2 expression in GC tissue compared to para-carcinoma normal tissues.

Correlation between Wnt2 expression and H. pylori infection in GC tissue

The positive rate of expression of Wnt2 in GC tissues with *H. pylori* infection (95%) was significantly higher than in those without infection (81.3%). Of the 40 cases with *H. pylori* infection, 34 (85%) showed moderate/strong positive expression of Wnt2, whereas among the 32 uninfected cases, only 17 (53.1%) exhibited such expression. As shown in Supplementary Table 4, the expression level of Wnt2 was significantly higher in the *H. pylori*-infected group than in the uninfected group. There was a positive correlation between the expression of Wnt2 and *H. pylori* infection ($p = 0.01$). This suggests that the expression of Wnt2 was elevated in GC patients infected with *H. pylori*.

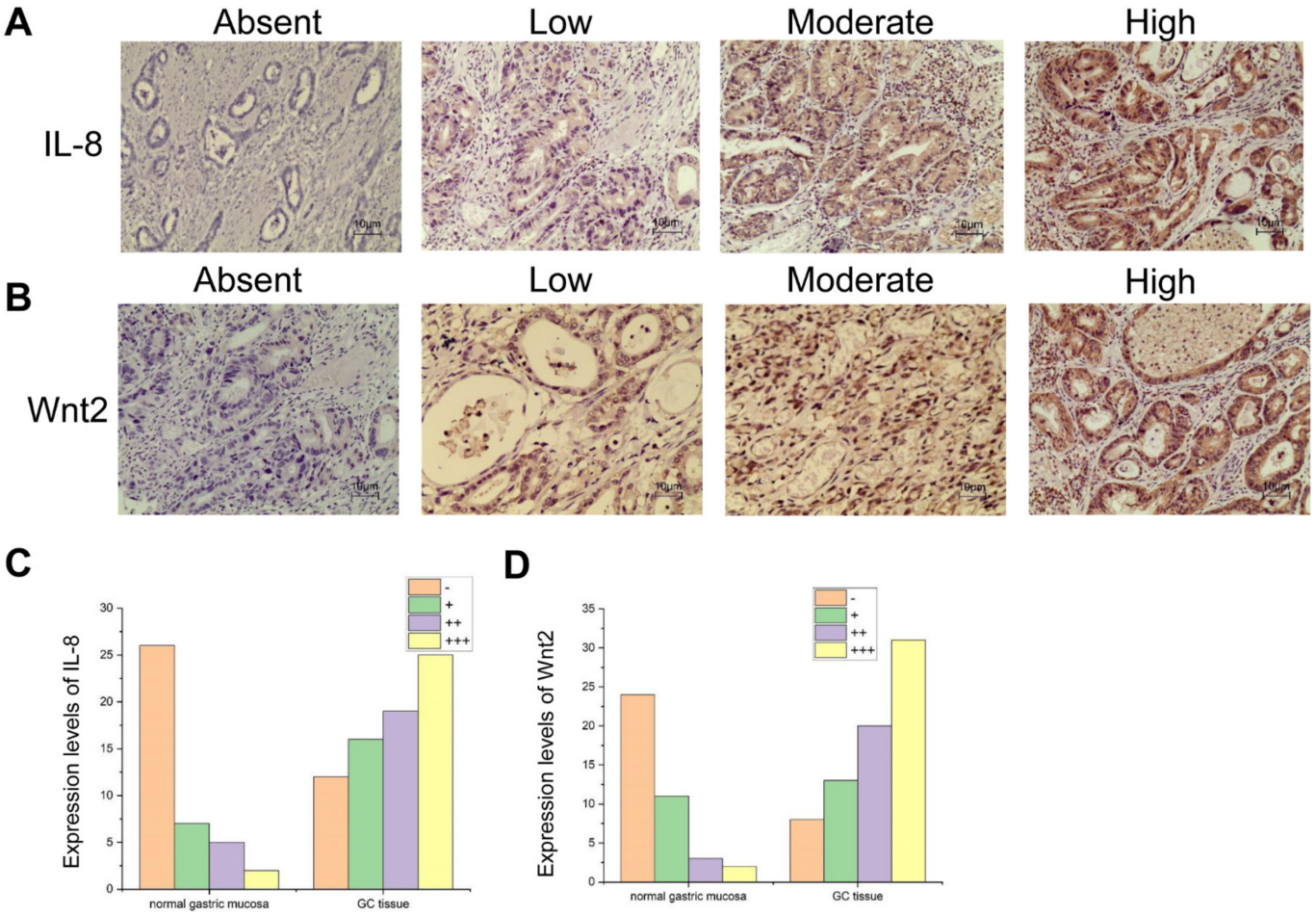
Correlation between IL-8 and Wnt2 expressions in GC tissue

Based on the staining grades, negative and weakly positive expressions were categorized as the negative group, and moderate and strongly positive expressions were categorized as the positive group. Among the 44 IL-8 positive GC tissues, 40 cases (90.9%) expressed Wnt2, while only 11 cases (39.3%) of the 28 IL-8 negative GC tissues expressed Wnt2. As shown in Supplementary Table 5, there was a positive correlation between the expression of IL-8 and Wnt2, implying that the increased IL-8 release in GC patients may increase the Wnt2 positivity rate. Thus, IL-8 and Wnt2 may have a synergistic role in the progression of GC.

Relationship between H. pylori infection, IL-8, and Wnt2 with clinicopathological features in patients with GC

To determine the roles of *H. pylori*, IL-8, and Wnt2 in the development and progression of GC, we analyzed the associations among them. The results showed that

Figure 2. Different expression levels of IL-8 and Wnt2 in gastric cancer (GC) tissue (× 200).



Immunohistochemical staining of IL-8 (A) and Wnt2 (B). Absent, negative (-); low, weakly positive (+); moderate, moderate positive (++); high, strong positive (+++). C. positive rate of IL-8 expression in GC and para-tumor normal tissue. D. positive rate of Wnt2 expression in GC and para-tumor normal tissue.

Table 1. Association between IL-8/Wnt2 co-expression and clinical clinicopathological features in GC patients with IL-8/Wnt2 co-expression.

Clinical pathological features	n	*H. pylori* (+) IL-8/Wnt2 (++/+++)	H. pylori (-) IL-8/Wnt2 (++/+++)	χ^2	*p*
Gender					
Female	10	6	4	1.67	0.299
Male	26	21	5		
Age (years)					
< 60	18	15	3	1.33	0.248
≥ 60	18	12	6		
Differentiation grade					
High/moderate	17	15	2	3.01	0.083
Poorly/undifferentiated	19	12	7		
Clinical stage					
I/II	9	8	1	1.24	0.267
III/IV	27	19	8		
LNM					
No	9	8	1	1.24	0.267
Yes	27	19	8		
Invasion depth					
Invasion depth not reaching the serosa layer	19	16	3	1.82	0.177
Invasion of the entire gastric wall	17	11	6		

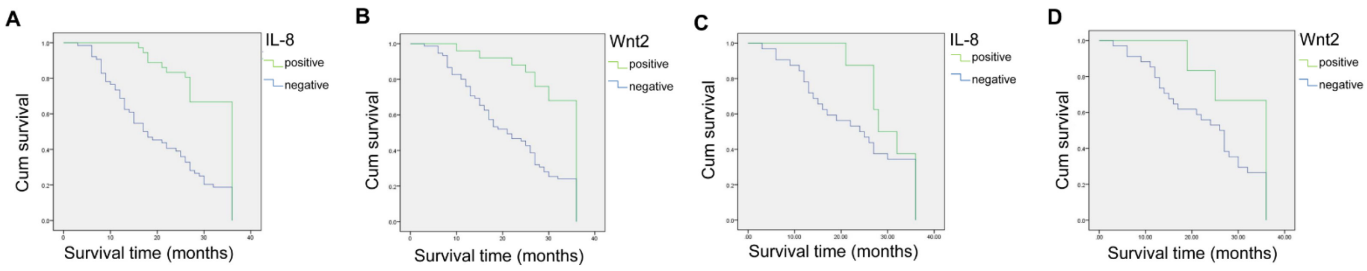
H. pylori: Helicobacter pylori; LNM: lymph node metastasis. Staining intensity and positive cell rate: moderate (++), high (+++).

there was no significant association between *H. pylori* infection and gender, age, differentiation grade, clinical stage, lymph node metastasis (LNM), and invasion depth in 72 GC patients ($p > 0.05$) (Supplementary Table 6). The expressions of IL-8 and Wnt2 were not significantly associated with gender, age, differentiation grade, and invasion depth ($p > 0.05$); however, both were significantly related to clinical stage and LNM ($p < 0.05$). Specifically, the expression of IL-8 in advanced GC (III/IV stage, 29 cases, 40.3%) was significantly higher than that in the early stage (II stage, 15 cases, 20.8%; $p = 0.002$). Similarly, the expression of Wnt2 in advanced GC (III/IV stage, 36 cases, 50.0%) was significantly higher than that in the early stage (II stage, 15 cases, 20.8%; $p < 0.001$). The number of cases with positive expression of IL-8 and Wnt2 in patients with LNM was 32 (44.4%) and 39 (54.2%), respectively; significantly higher than those without LNM (both 12, 16.7%; $p = 0.005–0.001$). All these results indicate that the expressions of IL-8 and Wnt2 were significantly increased in advanced GC and those with LNM, suggesting a potential role for IL-8 and Wnt2 in the progression and metastasis of GC.

Association between IL-8/Wnt2 co-expression and clinical clinicopathological features in GC patients with IL-8/Wnt2 co-expression

We assessed whether the IL-8/Wnt2 co-expression is associated with prognosis in GC patients infected with *H. pylori*. There were 36 patients with IL-8/Wnt2 co-expression. Among these patients, there was no significant association between the IL-8/Wnt2 co-expression and gender, age, differentiation grade, and invasion depth in *H. pylori*-infected GC patients (Table 1; $p > 0.05$). Furthermore, while the IL-8/Wnt2 co-expression in *H. pylori*-infected patients with advanced stage and LNM appeared to be higher than that in uninfected patients, there was no statistical difference ($p > 0.05$). This may be attributed to the limited number of cases.

Figure 3. Analysis of survival curves. The Kaplan-Meier method was used for survival analysis.



A. relationship of IL-8 expression and survival time in gastric cancer (GC); B. relationship of Wnt2 expression and survival time in GC; C. relationship of IL-8 expression and survival time in Helicobacter pylori-infected GC; D. relationship of Wnt2 expression and survival time in H. pylori-infected GC.

H. pylori infection, IL-8, and Wnt2 expressions and prognosis analysis of GC

Kaplan-Meier survival analysis revealed a significant difference in survival time among all patient groups (Figure 3A–B). The survival curves of patients testing positive for IL-8 and Wnt2 (++/+++) exhibited notably lower survival rates compared to those testing negative (-/+), with a p value < 0.001. Specifically, the average survival time for patients in the IL-8 positive group was 21.7 months, in contrast to 31.6 months for those in the negative group (Figure 3A). Similarly, the average survival time for patients in the Wnt2 positive group was 21.8 months, compared to 31.6 months in the negative group (Figure 3B).

The survival curves for 40 *H. pylori*-infected patients are presented in Figure 3C and 3D. Among them, 32 cases were in the IL-8 positive group, with an average survival time of 23.2 months, and 12 cases were in the negative group, with an average survival time of 30.4 months. No significant difference was observed between the two groups (p = 0.362). There were 34 cases in the Wnt2 positive group, with an average survival time of 23.3 months, and 6 cases in the negative group, with an average survival time of 31.3 months, showing no significant difference (p = 0.102). These results indicate that the expression of IL-8 and Wnt2 in *H. pylori*-infected GC patients did not significantly affect their survival, possibly due to the small sample size.

Cox regression analysis of independent prognostic factors

COX regression analysis found that clinical stage and Wnt2 were independent prognostic factors (p < 0.05) (Table 2). Specifically, the B-value of the clinical stage was 1.121 (B > 0), indicating that the risk increased by 3.069 times with the increase in the clinical stages of patients. On the other hand, the B-value of Wnt2 was 0.633, signifying that the risk of a positive Wnt2 was 1.884 times higher than a negative result. However, *H. pylori* differentiation grade, invasion depth, LNM, and IL-8 were not found to be independent prognostic factors (p > 0.05).

Discussion

H. pylori, as a primary carcinogenic factor, can lead to persistent inflammation when gastric mucosa is infected over a long-term [21,22]. One of the key pro-inflammatory chemokines, IL-8, not only contributes to inflammatory response but is also highly expressed in numerous tumor cells. IL-8 influences the tumor microenvironment through tumor cell autocrine and paracrine mechanisms, promoting tumor cell growth and angiogenesis [23,24], and even participating in EMT and CSC-like changes [25,26]. *H. pylori* infection in the gastric mucosa activates the NF-κB signaling pathway, resulting in the release of IL-8 [27]. Additionally, apart from cytotoxin-associated gene A, the outer membrane protein A can also enhance the release of IL-8 by activating NF-κB [28]. Therefore, IL-8 plays a crucial role in the incidence of gastritis and *H. pylori*-infected GC [29].

The gastric epithelial cells do not exhibit overexpression of Wnt protein. Several studies have shown that abnormal activation of the Wnt signaling pathway leads to the initiation and progression of GC [30,31], as well as contributing to the migration and invasion of GC cells [15]. This occurs due to the inhibition of the formation of the complex Axin/APC/GSK3β by the activated Wnt protein, especially Wnt2, resulting in the inability to phosphorylate β-catenin, thus allowing it to enter the nucleus as a transcription factor [32]. Furthermore, *H. pylori* can elevate nuclear β-catenin levels and activate downstream target genes, which play a pivotal role in inducing both CSC-like changes and EMT, thereby promoting the initiation and progression of GC [33].

Some studies have indicated that the NF-κB and Wnt/β-catenin signaling pathways are both implicated in the malignant transformation of gastric cells associated with *H. pylori* infection [34,35], suggesting a close relationship between inflammation and tumors. Our previous studies [8,17] confirmed the association

Table 2. Cox regression analysis of prognostic factors.

Items	B	SE	Wald	p	Relative Risk Exp (B)	Exp (B) 95.0% confidence interval	
						Upper limit	Lower limit
H. pylori	-0.024	0.300	0.006	0.937	0.976	0.542	1.759
Age	0.395	0.264	2.236	0.135	1.484	0.885	2.489
Differentiation grade	0.333	0.266	1.574	0.210	1.396	0.829	2.349
Invasion depth	-0.201	0.264	0.576	0.448	0.818	0.487	1.373
LNM	-0.245	0.181	1.845	0.174	0.782	0.549	1.115
Clinical Stage	1.121	0.312	12.917	< 0.001	3.069	1.665	5.657
IL-8	0.380	0.301	1.590	0.207	1.462	0.810	2.640
Wnt2	0.633	0.311	4.149	0.042	1.884	1.024	3.465

H. pylori: Helicobacter pylori; LNM: lymph node metastasis.

between IL-8 and Wnt2 through cellular and murine experiments. It was also preliminarily demonstrated that IL-8 was involved in the activation of Wnt2. As the upstream molecule of the Wnt/β-catenin signaling pathway, Wnt2 regulates the expression of downstream target genes by activating Wnt/β-catenin, ultimately promoting EMT and the development of CSC-like characteristics in gastric epithelial cells [13,36]. This raises the question of whether there is a differential expression of these two proteins related to *H. pylori* infection in human GC tissue as compared to normal stomach tissue, and whether they can be used to predict the prognosis of GC patients.

As one of the virulence factors of *H. pylori*, cytotoxin-associated gene A is delivered into gastric mucosal cells by the type IV secretion system [37]. This process can activate the AKT/NF κB signaling pathway, contributing to inflammatory responses [34]. Moreover, another virulence factor, vacuolating cytotoxin A, also plays a role in immunoregulation by activating the NF-κB signaling pathway [38]. A study from the 1990s revealed that upon infection of gastric epithelial cells with *H. pylori*, the NF-κB signaling pathway was rapidly activated within 30 min [39]. This activation led to an increase in downstream target genes, such as IL-8 mRNA at 1 h and protein levels at 4 h. The sustained release of IL-8 contributed to the persistence of gastritis. Furthermore, chronic infection with cytotoxin-associated gene A-positive *H. pylori* activates the Wnt/β-catenin/NF-κB/IL-8 regulatory pathway in gastric mucosal cells, inducing abnormal proliferation of gastric mucosal epithelial cells with characteristics resembling cancer stem cells and mesenchymal cells [17]. This process promotes inflammatory responses in the gastric mucosa and facilitates the progression to precancerous transformations [17]. The correlation between IL-8 and Wnt2 expression in GC cells suggests that IL-8 may facilitate GC development by upregulating Wnt2 expression [8,40].

In addition to IL-8, many other pro-inflammatory chemokines are also involved in the regulation of the GC microenvironment. For example, IL-10, which is regulated by highly expressed B lymphocytes, especially B regulatory cells, plays a role in the immune regulation of GC [41]. In patients with GC, IL-10 was the anti-inflammatory cytokine showing the highest correlation with the co-inhibitory molecule PD-L1 and IL-12A; whereas in the patients without cancer, IFN-γ and IL-17A exhibited the highest correlations with IL-10 [42].

In this study, we divided 72 GC patients into *H. pylori* infection and non-infection groups. Our findings revealed a positive correlation between *H. pylori* infection and the expression of IL-8 and Wnt2. Additionally, there was a certain degree of positive correlation between the expression of IL-8 and Wnt2. These results suggest that *H. pylori* infection not only affects the expression of IL-8 and Wnt2 in GC tissue but also implies that IL-8 may play a role in regulating the expression of Wnt2. Furthermore, we found that the expression of both IL-8 and Wnt2 in GC tissue was significantly higher than in normal tissue; and, in advanced GC with LNM, the expression was significantly higher than in early GC without LNM. The survival curves of GC patients in the IL-8 and Wnt2 positive groups all shifted to the left compared with the negative groups, indicating a significantly lower 3-year survival rate in IL-8 and Wnt2 positive GC patients than in negative GC patients. Notably, IL-8 and Wnt2 did not significantly affect the survival of *H. pylori*-infected GC patients. These results indicate that IL-8 and Wnt2 may serve as prognostic indicators for GC. Regression analysis revealed that *H. pylori* and IL-8 were not independent prognostic factors, while Wnt2 was an independent prognostic factor for GC. Nevertheless, GC patient survival is affected by multiple factors beyond just IL-8 and Wnt2 levels, including disease stage, treatment modalities, patient characteristics, comorbidities, etc. Therefore, the relationship between IL-8 and Wnt2 levels and survival time should be considered as a reference point in the broader context of GC management. Further research incorporating a comprehensive assessment of various clinical variables is warranted to validate the significance of IL-8 and Wnt2 in predicting patient outcomes.

This study has some limitations. First, this study is a retrospective study with a small overall sample size and a small number of normal tissue samples, which may introduce bias and limit the ability to establish causal relationships. Second, the diagnostic criteria for *H. pylori* infection lack rigor, which may affect the accuracy of infection diagnosis. Third, this study did not include a control group of individuals without GC. Fourth, this study did not account for potential confounding factors such as other comorbidities, lifestyle factors, or treatment modalities that could influence the expression of IL-8 and Wnt2, as well as the prognosis of GC patients. Further studies are warranted.

Conclusions

In summary, the expressions of IL-8 and Wnt2 were upregulated in GC tissues, and their expressions were more significant in GC patients infected with *H. pylori*. Moreover, there was a strong correlation between IL-8 and Wnt2. In addition, GC patients with high expression of IL-8 and Wnt2 had a lower survival rate. These two proteins also served as prognostic factors for GC. Our findings may provide a theoretical basis for the development of GC treatment targets.

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Authors' contributions

LL: study design, literature search, statistical analysis, data interpretation, manuscript preparation, funds collection; RT: data collection, statistical analysis, data interpretation, funds collection; YC: literature search, statistical analysis, data interpretation, manuscript preparation; QZ: statistical analysis, data interpretation; KY: literature search; statistical analysis; WY: data collection, manuscript preparation; FG: literature search, data collection.

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Annex – Supplementary Items

Supplementary Table 1. Comparison of IL-8 expression in gastric cancer (GC) and para-carcinoma tissues.

IL-8	n	-	+/++/+++	*p*	-/+	++/+++	*p*
Normal gastric mucosa	40	26 (65%)	14 (35%)	< 0.001	33(82.5%)	7 (17.8%)	< 0.001
GC tissue	72	12 (16.7%)	60 (83.3%)		28 (38.9%)	44 (61.1%)	

Pearson Chi-square test was used for the analysis. When comparing the positive group (+/++/+++) with the negative group (-), $\chi^2 = 67.391$, $p < 0.01$; When compared with the negative/weakly positive group (-/+), $\chi^2 = 43.006$, $p < 0.01$.

Supplementary Table 2. Correlation between IL-8 expression and *H. pylori* infection in gastric cancer (GC) tissue.

Helicobacter pylori	IL-8				Total	R	*p*
	-	+	++	+++			
Negative	9	11	5	7	32		
Positive	3	5	14	18	40	0.351	0.001
Total	12	16	19	25	72		

Kendall's rank correlation demonstrated τb = 0.351 and $p < 0.01$, indicating a positive correlation between *H. pylori* and IL-8.

Supplementary Table 3. Comparison of Wnt2 expression in gastric cancer (GC) and para-carcinoma tissues.

Wnt2	n	-	+/++/+++	*p*	-/+	++/+++	*p*
Normal gastric mucosa	40	24 (60%)	16 (40%)	< 0.001	35 (87.5%)	5 (12.5%)	< 0.001
GC tissue	72	8 (11.1%)	64 (88.9%)		21 (29.2%)	51 (70.8%)	

Pearson Chi-square test was used. When comparing the positive group (+/++/+++) with the negative group (-), $\chi^2 = 77.156$, $p < 0.01$; when compared with the negative/weakly positive group (-/+), $\chi^2 = 52.5$, $p < 0.01$.

Supplementary Table 4. Correlation between Wnt2 expression and *Helicobacter. pylori* infection in gastric cancer (GC) tissue.

H. pylori	Wnt2				Total	R	*p*
	-	+	++	+++			
Negative	6	9	7	10	32		
Positive	2	4	13	21	40	0.284	0.01
Total	8	13	20	31	72		

Kendall's rank correlation revealed τb = 0.284 and $p = 0.01$, indicating a positive correlation between *H. pylori* and Wnt2.

Supplementary Table 5. Correlation between IL-8 and Wnt2 expressions in gastric cancer (GC) tissue.

IL-8	Wnt2		Total	R	*p*
	Negative group (-/+)	Positive group (++/+++)			
Negative group (-/+)	17	11	28		
Positive group (++/+++)	4	40	44	0.554	< 0.001
Total	21	51			

Kendall's rank correlation revealed τb = 0.554 and $p < 0.01$, suggesting a positive correlation between IL-8 and Wnt2.

Supplementary Table 6. Association between *Helicobacter pylori* infection, IL-8, and Wnt2 with clinicopathological features in patients with gastric cancer (GC).

Clinical pathological feature	n	*H. pylori*		χ^2	*p*	IL-8		χ^2	*p*	Wnt2		χ^2	*p*
		Positive	Negative			Positive	Negative			Positive	Negative		
Gender													
Female	16	7	9	1.161	0.281	12	4	1.67	0.196	13	3	1.08	0.299
Male	56	33	23			32	24			38	18		
Age (years)													
< 60	41	19	22	3.274	0.07	24	17	0.266	0.606	30	11	0.252	0.616
≥ 60	31	21	10			20	11			21	10		
Differentiation grade													
High/moderate	39	25	14	2.517	0.113	33	22	0.169	0.681	38	17	1.866	0.172
Poorly/undifferentiated	33	15	18			31	14			37	8		
Clinical stage													
I/II	35	17	18	1.345	0.246	15	20	9.549	0.002	15	20	25.802	< 0.001
III/IV	37	23	14			29	8			36	1		
LNM													
No	26	15	11	0.075	0.784	12	14	3.831	0.005	12	14	11.998	0.001
Yes	46	25	21			32	14			39	7		
Invasion depth													
Invasion depth not reaching the serosa layer	39	20	19	0.629	0.428	21	18	1.89	0.169	24	15	3.558	0.059
Invasion of the entire gastric wall	33	20	13			23	10			27	6		

LNM, lymph node metastasis.