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Helicobacter pylori Eradication Is Associated With a Reduced Risk of Metachronous Gastric Neoplasia by Restoring Immune Function in the Gastric Mucosa

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Keywords: endoscopic submucosal dissection | gastric cancer | *Helicobacter* infections | immune system

ABSTRACT

Background: *Helicobacter pylori* infection is a significant contributing factor of gastric cancer. Metachronous neoplasms also pose a risk. The mechanism underlying the impact of *H. pylori* eradication on preventing metachronous gastric cancer is unclear. This study aimed to investigate immunity changes in gastric mucosa after *H. pylori* eradication and to identify mechanisms preventing metachronous recurrence.

Materials and Methods: Patients diagnosed with gastric neoplasm and *H. pylori* infection, who underwent endoscopic resection, were included. Thirty-six cases of metachronous neoplasms occurring after eradication (metachronous group) were compared to 36 controls matched for age, sex, atrophy, and metaplasia (control group). Histological features and immunohistochemical staining for T-cell (CD3, CD4, and CD8) and immune exhaustion (forkhead/winged helix transcription factor and programmed cell death-ligand 1) markers in the non-tumor-bearing mucosa were evaluated.

Results: In histologic features, glandular atrophy and intestinal metaplasia in the gastric mucosa significantly improved following *H. pylori* eradication in the control group ($p < 0.001$, 0.008), whereas they did not improve in the metachronous group ($p = 0.449$, 0.609). CD8 and CD8/CD3 ratios increased in the control group ($p < 0.001$, 0.04), but did not show differences in the metachronous group ($p = 0.057$, 0.245). The CD4/CD3 ratio and programmed cell death-ligand 1/CD4 expression significantly decreased after *H. pylori* eradication in the control group ($p = 0.003$, 0.042), but not in the metachronous group ($p = 0.54$, 0.55).

Conclusions: This observational study suggests that *H. pylori* eradication may prevent the recurrence of gastric neoplasia by improving histological inflammation and overcoming immune exhaustion.

Abbreviations: Foxp3, forkhead/winged helix transcription factor; *H. pylori*, *Helicobacter pylori*; PD-L1, programmed cell death-ligand 1.

The first two authors contributed equally to this article.

The last two authors contributed equally to this article.

1 | Introduction

Gastric cancer is the third leading cause of cancer-related death globally [1]. It is a multifactorial disease, with *Helicobacter pylori* (*H. pylori*) infection identified as the strongest risk factor [2]. *H. pylori* infects approximately half of the world's population and is considered an initial trigger in the Correa cascade, a model of gastric carcinogenesis that progresses from superficial gastritis through atrophic gastritis, intestinal metaplasia, and dysplasia, leading to adenocarcinoma [2–4]. Chronic *H. pylori* infection can also cause genetic instability in the gastric mucosa through a persistent active immune response that lasts throughout the lifespan of the host if the infection is not eradicated [5–7].

Advances in endoscopic techniques have facilitated the treatment of many cases of early gastric cancer with endoscopic resection; however, in contrast to surgery, it leaves most of the stomach intact, raising concerns regarding metachronous recurrence [8]. *H. pylori* eradication after endoscopic resection for early gastric cancer is recommended to reduce the risk of incidence of gastric neoplasms [9–11]. However, the mechanism by which *H. pylori* eradication prevents metachronous recurrence remains unclear.

Prolonged antigen exposure because of chronic infection leads to the progressive loss of T-cell proliferation, cytokine production, and cytotoxic activity [12]. The immune exhaustion of T cells, marked by the upregulation of inhibitory receptors such as programmed cell death 1 (PD-1) and the increased presence of regulatory T cells (Tregs), plays a crucial role in the immune evasion mechanisms of various cancers [13–15]. This exhausted state of T cells impairs the body's ability to control infections and facilitates the development and progression of tumors by allowing cancer cells to evade immune surveillance. Thus, understanding and reversing T-cell exhaustion is critical for improving immune responses in chronic infections and cancer therapy.

PD-1 is a protein expressed on the surface of T cells, B cells, and natural killer cells. PD-1 binds to programmed cell death-ligand 1 (PD-L1), inducing T-cell exhaustion and promoting the differentiation of regulatory T cells, which affects the prognosis of gastric cancer [16–18]. Additionally, among the immunological factors involved in gastric cancer development, Tregs, characterized by the expression of the forkhead/winged helix transcription factor (Foxp3), contribute to carcinogenesis by inhibiting CD8+ cytotoxic T-cells [19, 20].

Therefore, we hypothesized that *H. pylori* eradication after endoscopic resection of early gastric cancer may help prevent metachronous recurrence by recovering immune exhaustion in the gastric mucosa, which could eliminate potential tumor antigens. By comparing the expression levels of exhausted T-cells and Tregs in the surrounding gastric mucosa before and after *H. pylori* eradication, we aimed to investigate the mechanism by which *H. pylori* eradication reduces the risk of metachronous recurrence.

2 | Methods

2.1 | Patients

This 1:1 matched case–control study included patients who underwent endoscopic resection because of a gastric neoplasm, including dysplasia and adenocarcinoma, and with identified *H. pylori* infection at a single center, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, between January 2010 and December 2019. The inclusion criteria were patients with confirmed *H. pylori* infection at the time of the first endoscopic resection who subsequently received eradication treatment. Exclusion criteria included patients with no confirmed *H. pylori* infection ever, prior eradication therapy before the first endoscopic resection, failed eradication, or successful eradication that is not confirmed via rapid urease test or urea breath test, inadequate tissue sample preservation, or follow-up periods shorter than 3 months after eradication.

During this period, a total of 2590 patients underwent gastric endoscopic resection for gastric neoplasms, including low- and high-grade epithelial dysplasia and adenocarcinoma. After reviewing records that followed up these patients through December 2023, we found that 236 patients underwent endoscopic resection for recurrence of gastric neoplasm. From these patients, we selected those who had confirmed *H. pylori* infection during the first endoscopic resection and received eradication treatment. After *H. pylori* eradication and confirmation with a negative result on rapid urease test or urea breath test, 41 patients showed development of metachronous gastric neoplasm (dysplasia or adenocarcinoma) after the first endoscopic resection for a previous gastric neoplasm and were treated by a second endoscopic resection for a new lesion. We categorized them as the metachronous group. Forty-one patients matched by age, sex, and the grade of atrophy and intestinal metaplasia were enrolled and categorized as the control group. The control group comprised patients who underwent endoscopic resection for gastric neoplasm but did not show any metachronous gastric neoplasm during the follow-up period.

We defined metachronous lesions as those appearing at a different site at least 6 months after the previous endoscopic resection. The interval between *H. pylori* eradication and the development of metachronous gastric neoplasms was at least 6 months, allowing for an accurate evaluation of the effect of *H. pylori* eradication on the incidence of metachronous lesions after the previous endoscopic resection. Figure 1 provides a brief flowchart of this study.

This study was approved by the Yonsei University College of Medicine Institutional Review Board (IRB No. 3-2023-0325). The requirement for individual informed consent was waived because of the retrospective design of the study.

2.2 | Endoscopic Procedure

Endoscopic resections were performed using standard techniques, including endoscopic submucosal dissection, by

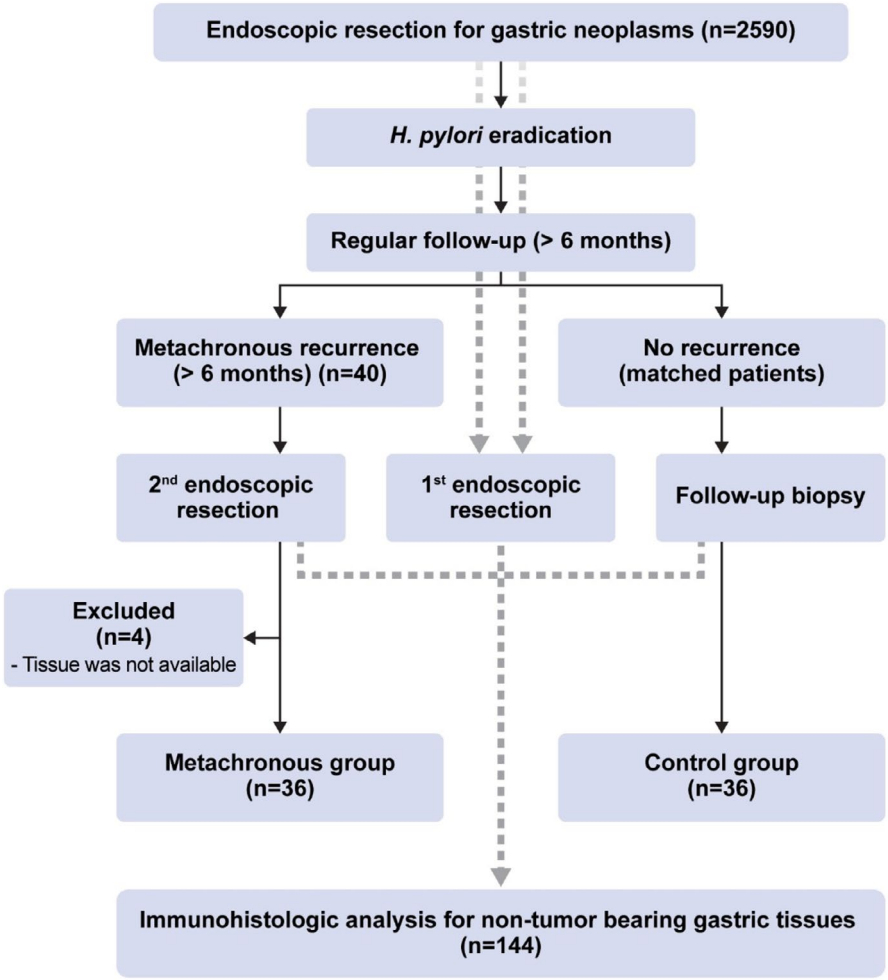

FIGURE 1 | Flowchart of the study. *H. pylori*, *Helicobacter pylori*.

experienced gastroenterologists. The procedures were performed using high-definition endoscopes equipped with narrow-band imaging capabilities. The equipment used included the Olympus GIF-H260, GIF-2TQ260M, and GIF-H290 series, Olympus corporation, Tokyo, Japan, which provide enhanced visualization of the mucosal and vascular patterns.

Endoscopic submucosal dissections were conducted using a dual knife with a 1.5-mm cutting knife (KD-650Q; Olympus, Tokyo, Japan). Injection solution, which consisted of a mixture of glycerol, indigo carmine, and epinephrine, was used to elevate the mucosa. Endoscopic submucosal dissection procedures aimed to achieve en bloc resection to ensure complete removal of lesions with a margin of healthy tissue.

Patients underwent conscious sedation with midazolam, propofol, and pethidine, and all procedures were monitored by an assistant doctor. The resected specimens were pinned on a cork board and fixed in 10% buffered formalin for histopathological examination.

2.3 | Histological/Immunological Evaluation

Representative paraffin-embedded sections from endoscopic submucosal dissection or biopsy tissues were selected for histological evaluation and immunohistochemical staining. The histological features of the gastric mucosa were assessed using 4-μm-thick hematoxylin and eosin-stained slides and graded according to the updated Sydney system scores. One expert pathologist evaluated the degree (0: none, 1: mild, 2: moderate, and 3: severe) of chronic inflammation, neutrophil infiltration, atrophy, and intestinal metaplasia infiltration on each pathology slide [21, 22].

Immunohistochemical staining was conducted to detect T-cell markers (CD3, CD4, and CD8) and immune exhaustion markers (Foxp3 and PD-L1; Figure 2). The antibodies used are detailed in Table S1. All immunohistochemical analyses were performed on 4-μm-thick formalin-fixed paraffin-embedded tissue sections using an automated immunohistochemistry staining device (BenchMark XT; Ventana Medical Systems, Tucson, AZ, USA).

Expression levels were quantified using 3DHistech software (Budapest, Hungary)—NuclearQuant. It is a color- and pattern-based image analysis solution designed to identify different tissue elements in stained samples. Rather than relying on time-consuming manual annotation, the software allows for automated detection and annotation of regions of interest, following a brief training on relevant tissue samples. To avoid misquantification, only areas within the lamina propria, excluding epithelial glands, were annotated, ensuring that the annotated

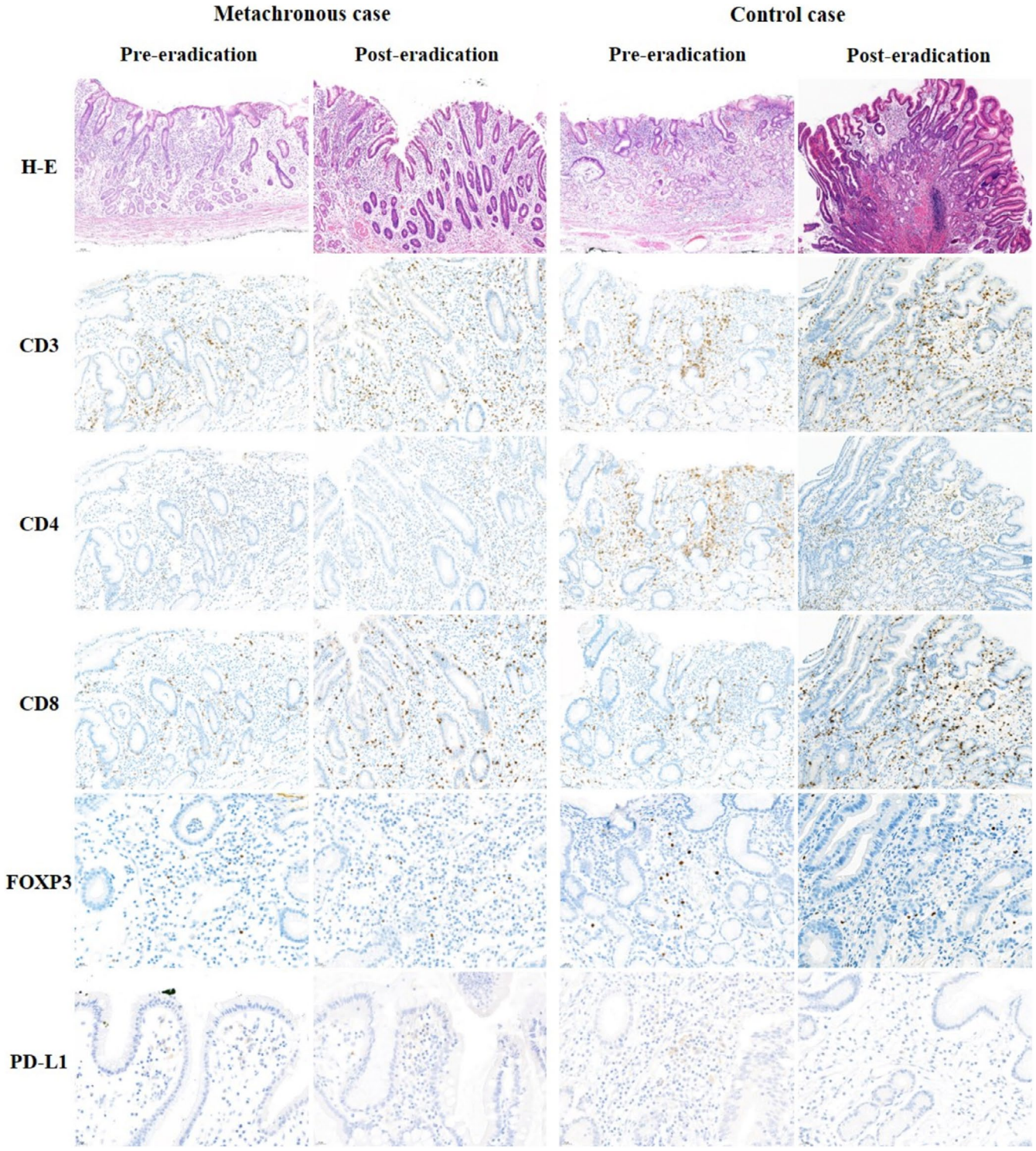

FIGURE 2 | Representative images of metachronous recurrent and control cases and immunohistochemical expression slides (hematoxylin and eosin, CD3, CD4, and CD8, 100× magnification). In the recurrent cases, minimal changes were observed in immune cell populations, aside from an increase in CD3 cells. In contrast, the control cases showed a significant increase in immune cells following eradication.

regions consisted solely of inflammatory cells. The annotated areas included regions of the lamina propria that are larger than a specified size: on average, 0.35 mm^2 for endoscopic submucosal dissection samples and 0.2 mm^2 for biopsy specimens. We used the proportion obtained by dividing the counted cells by the annotated area (Figure 3).

2.4 | Statistical Analysis

The baseline characteristics, except age, lesion size, and follow-up interval, were categorical variables and are presented as mean and standard deviation. All variables of histologic analysis were categorical and those of immunologic expression level

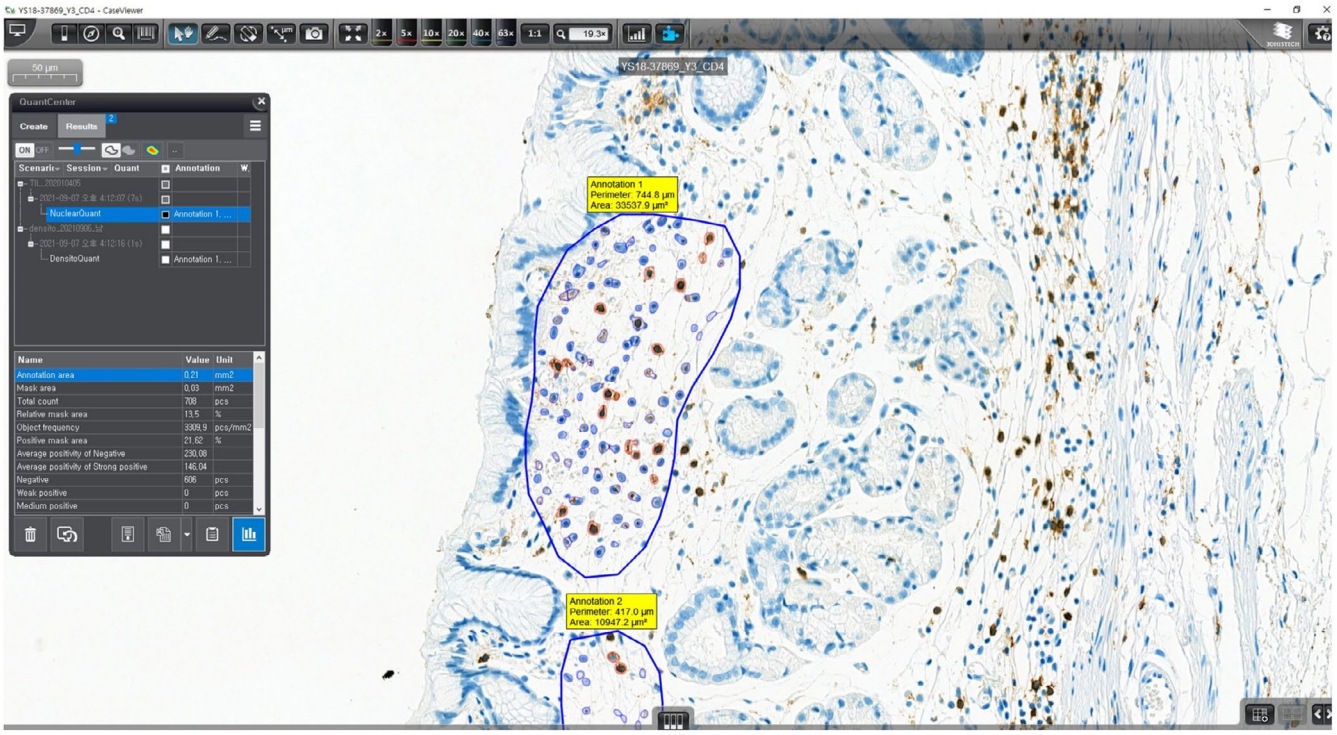

FIGURE 3 | Methods for quantification of immunologic markers. Immune cells are automatically counted by NuclearQuant in the annotated areas of the lamina propria. Red circles indicate the positive immunostained (brown color) CD4 cells, whereas blue circles indicate the negative cells.

were continuous. Chi-squared test and Fisher's exact test were employed to compare categorical variables of clinicopathological factors between groups based on the development of metachronous gastric neoplasms. For non-categorical variables in intergroup comparisons of clinicopathological characteristics, the t-test was used. To evaluate immunohistochemical expression levels, the Wilcoxon signed-rank test and Mann–Whitney U test were applied. Sub-group analysis was conducted by classifying the study participants into various sub-groups based on criteria such as baseline characteristics (age and sex), whether the pathology of the primary lesion was dysplasia or adenocarcinoma, and whether the pathology of the metachronous recurrent lesion was dysplasia or adenocarcinoma. When comparing immunologic expression levels, we attempted both pre- and post-eradication comparisons within the same patient as well as comparisons between different groups. For sub-group analysis based on recurrent lesions, only the matched control groups in a 1:1 ratio were extracted and analyzed.

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant.

3 | Results

3.1 | Baseline Characteristics

Of the 40 patients in the metachronous group, four were excluded because of tissue-sample-preservation abnormalities that precluded analysis, thus including a total of 36 patients in the study. Matched patients in the control group were also excluded, resulting in a total of 72 patients included in the two groups.

Table 1 shows the comparison of various clinical characteristics between the two groups. Age and sex showed no significant differences between the groups because we matched them when enrolling patients. The average lesion size, lesion location, gross appearance, and dysplasia/adenocarcinoma rates were not significantly different ($p = 0.883$, 0.06, and 0.116, respectively). The recurrence interval in the metachronous group was 1055.3 days, and the pathology follow-up interval in the control group was 579.4 days.

3.2 | Histologic Change After *H. pylori* Eradication

On comparison of the pre- and post-eradication histological features between the metachronous and control groups, both groups showed significant improvement in chronic inflammation and neutrophil infiltration post-eradication ($p = 0.006$ and 0.031 in the metachronous group, and $p < 0.001$ for both measures in the control group). In contrast, no significant change was observed in glandular atrophy and intestinal metaplasia scores post-eradication in the metachronous group ($p = 0.449$ and 0.609, respectively). However, the control group showed significant improvement in these scores ($p < 0.001$ and $p = 0.008$, respectively; Table 2).

3.3 | Immunologic Change After *H. pylori* Eradication

We compared T-cell- (CD3, CD4, and CD8) and immune exhaustion- (Foxp3 and PD-L1) marker-expression levels and their ratio before and after eradication in metachronous and control groups (Table 3). In both groups, CD3 showed a

TABLE 1 | Baseline clinicopathological characteristics of patients in two groups.

Variables	Metachronous group ($n=36$)	Control group ($n=36$)	p
Male (n, %)	25 (69.4)	25 (69.4)	—
Age (years, mean ± standard deviation)	68.0 ± 7.8	64.6 ± 10.5	0.199
Size (mm)	18.9 ± 12.8	19.1 ± 12.9	0.883
Location (n, %)			0.06
Upper 1/3	4 (11.1)	1 (2.8)	
Mid 1/3	10 (27.8)	6 (16.6)	
Lower 1/3	22 (61.1)	29 (80.6)	
Gross appearance (n, %)			0.116
Elevated	13 (36.1)	6 (16.6)	
Flat	13 (36.1)	19 (52.8)	
Depressed	10 (27.8)	11 (30.6)	
Dysplasia/adenocarcinoma (n, %)	7 (19.4)/29 (80.6)	6 (16.6)/30 (83.4)	
Recurrence interval (days)	1055.3 ± 809.7		
Pathology follow-up interval (days)		579.4 ± 314.5	

statistically significant increase after eradication ($p=0.017$ and 0.022, respectively). However, in the metachronous group, other T-cell- and immune exhaustion-marker-expression levels and their ratio showed no significant change between before and after eradication. On the other hand, in the control group, CD3 and CD8 expressions increased after eradication ($p=0.022$ and <0.001), and CD4/CD3, CD8/CD4, PD-L1/CD4 ratios changed significantly after eradication ($p=0.003$, 0.04, and 0.042, respectively).

3.4 | Metachronous Cancer-Group Analysis

In the sub-group analysis, we separated only cases in which the pathology of the recurrent lesion was adenocarcinoma and analyzed the expression levels between the two groups before and after eradication. After eradication, we found that the expression of CD8 was statistically higher in the control group, and the PD-L1 and PD-L1/CD4 ratio were significantly lower (Table 4; $p=0.033$, 0.015, and 0.039, respectively).

4 | Discussion

H. pylori eradication in patients who have undergone endoscopic resection for gastric cancer and dysplasia reduces the risk of metachronous recurrence, but the mechanism remains unclear [23]. This study is the first to demonstrate that the immune system, exhausted by *H. pylori* infection and tumors, can recover after eradication, thereby preventing metachronous recurrence. Our study provides additional insights into the immunological mechanisms underlying the beneficial effects of eradication. In this 1:1 age, sex, grade of atrophy, and metaplasia matched case–control study, we observed significant immunohistological changes in the gastric mucosa following *H. pylori* eradication, particularly noting that metachronous recurrence of gastric neoplasm after endoscopic resection was associated with a failure to recover from immune exhaustion. The increase in CD8+ T-cell expression in the control group, which did not exhibit recurrence during the follow-up period after eradication, suggests that restoring T-cell immunity from an exhausted state due to chronic infection may be linked to preventing metachronous recurrence.

CD8+ T-cells are cytotoxic T lymphocytes that produce and express αβ-T-cell receptors with CD8 in the thymus [24]. They are pivotal in the immune system for eliminating tumor antigens. CD8+ T-cells combat viruses, tumors, and other pathogens by recognizing and destroying cells presenting major histocompatibility complex class-I molecules [25]. Chronic inflammation resulting from persistent infection, such as *H. pylori* infection, leads to a reduction in T-cell proliferation and their ability to capture pathogens, ultimately causing CD8+ T-cell exhaustion [14, 24]. This exhaustion is a known factor in the development and poor prognosis of gastrointestinal tract cancers, including gastric cancer, because of the decreased anti-tumor immune function [14]. While the functional impairment of antigen-specific T-cells is a defining characteristic of many chronic infections, the underlying mechanisms of T-cell dysfunction and repair after eradication are still unclear [26]. In our study, the patients who showed increased CD8 expression in the gastric tissue significantly after *H. pylori* eradication did not show metachronous recurrence of gastric neoplasm after previous endoscopic resection. This result suggests that restoring T-cell immunity from an exhausted state because of chronic infection may be linked to preventing metachronous recurrence of gastric neoplasm after *H. pylori* eradication.

Several studies have focused on reversing T-cell exhaustion during chronic infection and cancer. A major area of interest

TABLE 2 | Changes in histologic features following *Helicobacter pylori* eradication.

Variables	Metachronous ($n=36$)			Control ($n=36$)		
	Pre-eradication (n, %)	Post-eradication (n, %)	p	Pre-eradication (n, %)	Post-eradication (n, %)	p
Chronic inflammation			**0.006**			**<0.001**
No	0 (0)	0 (0)		1 (2.8)	6 (16.7)	
Mild	8 (22.2)	19 (52.8)		5 (13.9)	26 (72.2)	
Moderate	19 (52.8)	14 (38.9)		17 (47.2)	4 (11.1)	
Severe	9 (25)	3 (8.3)		13 (36.1)	0 (0)	
Neutrophil infiltration			**0.031**			**<0.001**
No	10 (27.8)	19 (52.8)		10 (27.8)	26 (72.2)	
Mild	20 (55.6)	15 (41.6)		16 (44.4)	9 (25)	
Moderate	6 (16.6)	2 (5.6)		9 (25)	0 (0)	
Severe	0 (0)	0 (0)		1 (2.8)	1 (2.8)	
Glandular atrophy			0.449			**<0.001**
No	3 (8.3)	4 (11.1)		3 (8.3)	20 (55.6)	
Mild	19 (52.8)	21 (58.3)		23 (63.9)	16 (44.4)	
Moderate	14 (38.9)	10 (27.8)		10 (27.8)	0 (0)	
Severe	0 (0)	1 (2.8)		0 (0)	0 (0)	
Intestinal metaplasia			0.609			**0.008**
No	0 (0)	0 (0)		6 (16.6)	11 (30.6)	
Mild	12 (33.4)	16 (44.4)		10 (27.8)	16 (44.4)	
Moderate	16 (44.4)	11 (30.6)		15 (41.6)	8 (22.2)	
Severe	8 (22.2)	9 (25)		5 (14.0)	1 (2.8)	

Note: significance: $p < 0.05$.

TABLE 3 | Comparison of positive cell proportions of CD3, CD4, CD8, Foxp3, and PD-L1 and their ratios before and after *Helicobacter pylori* eradication.

Variables	Metachronous ($n=36$)			Control ($n=36$)		
	Pre-eradication	Post-eradication	p	Pre-eradication	Post-eradication	p
CD3	21.33 ± 9.78	26.71 ± 11.19	**0.017**	21.00 ± 12.07	27.59 ± 10.43	**0.022**
CD4	16.82 ± 14.12	20.51 ± 10.90	0.109	17.65 ± 12.21	15.50 ± 8.81	0.194
CD8	12.91 ± 7.06	14.94 ± 6.93	0.057	11.71 ± 5.52	19.44 ± 8.89	**<0.001**
Foxp3	2.41 ± 2.42	2.71 ± 2.28	0.338	2.40 ± 2.60	2.06 ± 1.45	0.936
PD-L1	0.57 ± 1.21	0.31 ± 0.48	0.799	0.67 ± 1.36	0.28 ± 0.71	0.092
CD4/CD3	0.79 ± 0.56	0.79 ± 0.35	0.54	0.79 ± 0.39	0.57 ± 0.26	**0.003**
CD8/CD3	0.65 ± 0.31	0.59 ± 0.21	0.245	0.65 ± 0.35	0.76 ± 0.37	**0.04**
PD-L1/CD4	0.05 ± 0.14	0.02 ± 0.04	0.55	0.04 ± 0.07	0.02 ± 0.03	**0.042**

Abbreviations: Foxp3, forkhead/winged helix transcription factor; PD-L1, programmed cell death-ligand 1.
Note: significance: $p < 0.05$.

TABLE 4 | Sub-group analysis of immunologic markers in recurrent cases before and after *Helicobacter pylori* eradication.

	Pre-eradication ($n = 21$, each)			Post-eradication ($n = 21$, each)		
Variables	**Recurrence**	**Control**	***p***	**Recurrence**	**Control**	***p***
CD3	18.47 ± 10.29	20.14 ± 12.40	0.394	24.20 ± 10.85	29.15 ± 10.94	0.217
CD4	18.12 ± 14.30	17.71 ± 10.78	0.986	17.00 ± 9.68	17.24 ± 9.82	0.848
CD8	11.08 ± 6.84	11.88 ± 6.26	0.639	13.72 ± 6.16	19.55 ± 9.09	**0.033**
Foxp3	2.42 ± 2.79	2.36 ± 1.85	0.986	2.14 ± 2.22	2.15 ± 1.68	0.903
PD-L1	0.86 ± 1.52	0.76 ± 1.32	0.557	0.44 ± 0.58	0.26 ± 0.82	**0.015**
CD4/CD3	0.98 ± 0.60	0.88 ± 0.30	0.59	0.74 ± 0.37	0.59 ± 0.28	0.149
CD8/CD3	0.69 ± 0.38	0.69 ± 0.42	0.931	0.59 ± 0.20	0.72 ± 0.35	0.192
PD-L1/CD4	0.07 ± 0.18	0.06 ± 0.09	0.913	0.03 ± 0.04	0.01 ± 0.03	**0.039**

Abbreviations: Foxp3, forkhead/winged helix transcription factor; PD-L1, programmed cell death-ligand 1.
Note: significance: $p < 0.05$.

is the PD-1/PD-L1 pathway, which is selectively upregulated in exhausted T-cells [12, 26]. Research has shown that blocking this inhibitory pathway can restore the functions of exhausted CD8+ T-cells [27–29]. PD-L1, a well-known immune checkpoint protein, is expressed on the surface of cancer and stromal cells such as antigen-presenting cells and activated T-cells [30, 31]. PD-L1 expression on memory CD4+ T-cells activates regulatory T-cells, which suppress the activity of naive T-cells, reducing the immune response [13]. This mechanism promotes self-tolerance and suppresses neighboring macrophages and effector T-cells in cancer. A previous study has found that PD-L1 expression in gastric cancer is stronger in the immune stroma than in the tumor, suggesting that PD-L1 may affect the surrounding microenvironment and contribute to cancer development [32]. Based on this mechanism and previous findings, we analyzed the expression level of PD-L1 and the PD-L1/CD4 ratio in our study. While no statistically significant difference was observed, PD-L1 expression decreased more in the control group after eradication, and the PD-L1/CD4 ratio significantly decreased only in the control group after eradication. This suggests that the decrease in PD-L1 expression on CD4+ T-cells may contribute to the reactivation of exhausted CD8+ T-cells.

In this study, we observed significant changes in the CD4/CD3 and CD8/CD3 ratios following *H. pylori* eradication, which provide critical insights into the immune dynamics within the gastric mucosa. We chose the ratio of CD4/CD3 because we considered that it effectively reflects the balance of helper T-cells within the total T-cell population, serving as an indicator of immune regulatory functions. A decrease in this ratio after eradication, observed in the control group, may signify a reduction in Tregs activity, potentially shifting the immune environment towards enhanced cytotoxicity and tumor suppression [33].

Conversely, the CD8/CD3 ratio represents the proportion of cytotoxic T-cells, which is essential for directly targeting and eliminating cancerous cells. The significant increase in the CD8/CD3 ratio in the control group after eradication suggests a restoration of cytotoxic T-cell function, highlighting the critical role of these cells in maintaining effective immune surveillance and preventing metachronous recurrence [33]. These findings underscore the importance of restoring immune balance and enhancing cytotoxic responses through *H. pylori* eradication, contributing to the overall reduction in the recurrence of gastric cancer.

Despite initial expectations that Foxp3 expression would differ significantly between the metachronous recurrence and control groups, our findings did not reveal any significant differences. Foxp3, a key marker for Tregs, plays a crucial role in maintaining immune tolerance and suppressing anti-tumor immune responses [15]. Previous studies have highlighted the association between increased Foxp3+ Treg infiltration and poorer prognosis in various cancers, including gastric cancer [20, 34, 35]. Further, Foxp3-positive cells may have an important role in severe *H. pylori*-associated gastritis [36]. However, in our study, the Foxp3 levels remained relatively unchanged between the two groups, both before and after *H. pylori* eradication. The lack of statistically significant results may not be because of the minimal role of Treg cells, but rather owing to the insufficient number of Foxp3-positive cells, making sensitive analysis by immunohistochemistry challenging. Accurate assessment of Treg cells requires fluorescence activated cell sorter analysis, and further studies using this method may be necessary to elucidate the role of Tregs clearly.

The results of the sub-group analysis, which focused on cases where the recurrent lesion was confirmed to be adenocarcinoma, supported the previous conclusions more strongly. We aimed to determine whether a direct comparison of immune marker expression before and after eradication between the control and recurrent adenocarcinoma sub-groups would reveal any differences in cases that developed more severe metachronous lesions during the same follow-up period. The analysis showed that immune markers did not differ between the recurrent and control groups before eradication. However, after eradication, CD8 expression was significantly higher in the control group, while PD-L1 expression and the PD-L1/CD4 ratio were significantly lower. We speculate that the activation of the immune system by reducing PD-L1 expression and increasing CD8+ T-cells has potential in preventing recurrence, indicating a potential pathway for enhancing treatment outcomes.

The two groups also showed significant differences in the recovery of glandular atrophy and intestinal metaplasia after eradication. Chronic inflammation and neutrophil infiltration were significantly reversed in both groups, but atrophy and intestinal metaplasia were reversed in the control group and not in the metachronous recurrence group. Several studies have explored whether *H. pylori* eradication can reverse atrophic gastritis and intestinal metaplasia, although the results have been controversial [37–39]. The findings of this study not only support previous research findings indicating that reversible changes in atrophic gastritis and intestinal metaplasia are associated with the suppression of gastric neoplasm recurrence after *H. pylori* eradication but also suggest that restoration of T-cell immunity may be linked to these reversible changes. However, the causal relationship remains unclear.

The limitations of this study include its retrospective nature and relatively small sample size, which may affect the generalizability of the findings. Although we matched patients for key variables such as age, sex, and the grade of atrophy and intestinal metaplasia, other confounding factors might have influenced the outcomes. Moreover, the specific factors and mechanisms involved in the recovery of exhausted immunity after eradication remain unclear. Given the retrospective, observational design of our study, we could not definitively establish causality. Future prospective studies including tracking immune changes following *H pylori* infection and eradication are critical to validate the mechanistic pathways suggested by our clinical observations. Additionally, the role of PD-L1 reduction in normal gastric immunity is still unclear. Future studies are needed to determine which specific cell populations express PD-L1 within the gastric mucosa and to use functional assays evaluating whether PD-L1 downregulation directly enhances CD8+ T-cell activity in order to clarify these mechanisms.

Despite these limitations, our study has several important strengths that contribute to its value in the field of gastric neoplasms. To our knowledge, this is the first study to identify an association between T-cell exhaustion and the occurrence of metachronous recurrence after *H. pylori* eradication of gastric neoplasms. Focusing on this mechanism may offer new insights into developing therapies to prevent gastric neoplasm recurrence after eradication. Currently, *H. pylori* eradication is the only treatment option for preventing metachronous recurrence of gastric cancer; however, our study suggests that patients who do not achieve immune recovery following eradication remain at risk for recurrence. These patients may benefit from adjunctive therapies aimed at restoring immune function, such as immune checkpoint inhibitors, which have shown potential in reactivating exhausted T-cells and enhancing anti-tumor immunity. Future studies with larger cohorts and prospective designs are needed to validate these findings and further elucidate the underlying mechanisms.

In conclusion, our study suggests that *H. pylori* eradication contributes to preventing metachronous recurrence in adenocarcinoma by improving histologic inflammation and enhancing immune system recovery. Specifically, the increase in CD8+ T-cells and reduction in PD-L1 expression post-eradication could be critical factors in mitigating recurrence risk. These insights underscore the importance of immune modulation in cancer prevention and may guide future therapeutic strategies aimed at enhancing immune responses in patients with gastric cancer.

Author Contributions

Min-Jae Kim: conceptualization: equal; data curation: equal; formal analysis: lead; investigation: equal; methodology: supporting; resources: lead; visualization: lead; writing – original draft: lead. **Ji Hae Nahm:** conceptualization: equal; data curation: equal; formal analysis: equal; investigation: lead; methodology: equal; visualization: equal; supervision: equal; writing – review and editing: equal. **Yeonjin Je:** conceptualization: equal; data curation: lead; formal analysis: supporting; funding acquisition: equal; investigation: equal; methodology: equal; resources: equal. **Jaeyoung Chun:** investigation: supporting; resources: supporting. **Young Hoon Youn:** investigation: supporting; resources: supporting. **Hyojin Park:** investigation: supporting; resources: supporting. **Jie-Hyun Kim:** conceptualization: lead; funding acquisition: lead; methodology: lead; supervision: lead; writing – review and editing: lead.

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Disclosure

The datasets generated and/or analyzed during the current study are not publicly available due to the policy of human-derived materials research regulations.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Yonsei University College of Medicine (IRB No. 3-2023-0325). The requirement for individual informed consent was waived due to the retrospective design of the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.