Original Article

Cytokine Expression in *Helicobacter pylori*-Associated Gastrointestinal Diseases: Insights from Ulcerative Colitis and Colon Cancer

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) is one of the most common chronic bacterial infections worldwide, associated with gastritis, peptic ulcer, and stomach cancer. Both ulcerative colitis (UC) and colon cancer (CC) are considered gastrointestinal diseases linked with chronic inflammation. **Objective:** the current study aimed to investigate the prevalence of *H. pylori* infection with cytotoxic-associated gene A (CagA) status in CC and UC patients and evaluate the cytokine expression of interleukin-21 (IL-21) and interleukin-23 (IL-23) in both groups to explore their potential role in *H. pylori*-associated pathogenesis. **Materials and Methods:** This is a cross-sectional study of 88 Iraqi patients, including 57 males and 31 females, aged between 45 and 70 years, who were recruited for this study; the data were collected from three different groups: 30 patients with CC, 58 patients with UC, and 30 healthy controls (HCs) aged between 41 and 73 years, with normal mucosa and no *H. pylori* infection. *H pylori* detection was confirmed, and a biopsy was taken from all participants for immunohistochemistry staining to assess IL-21 and IL-23 expression. A blood sample for serum extraction was also performed for *H. pylori*-CagA immunoglobulin G antibodies analysis using an enzyme-linked immunosorbent assay test. **Results:** The prevalence of *H. pylori* was more than half (60.34%) in UC and also high (70%) in CC patients, with no infection in HC. The prevalence of positive serum CagA level revealed that 100% *H. pylori*-positive CC cases and approximately 51.43% *H. pylori*-positive UC patients. The mean IL-21 and IL-23 expression was significantly different ($P = 0.0001$) between all studied groups. The expression level of these cytokines was shown to be higher in *H. pylori*-positive CC followed by *H. pylori*-positive UC compared to other groups. The *H. pylori*-negative UC also showed increased expression of IL-21 and IL-23, as compared to HC. A significant positive correlation between the two cytokines was observed in *H. pylori*-infected patients and *H. pylori*-CagA-positive patients. **Conclusion:** An influential role in modulating immune responses was followed by the pro-inflammatory cytokines during *H. pylori* and *H. pylori*-CagA-positive infection, giving the importance of focusing on CagA status in the pathogenesis of post-CC and post-colon UC.

Keywords: Colon cancer, *Helicobacter pylori*-CagA, IL-21, IL-23, ulcerative colitis

Introduction

Ulcerative colitis (UC) is one of the most common types of inflammatory bowel disease (IBD) that increases the risk of colorectal cancer involving various abnormalities in the immune-modulating mechanisms such as and biased toward T helper 1 (TH1) cells[1] and T cytotoxic cells and impairs T regulatory cell function and cytokine production, leading to damaged colonic epithelial cells.[2]

Helicobacter pylori (*H. pylori*) is an important pathogen that colonizes the human mucosal layer and epithelial mucus of the stomach in approximately 50%–90% of cases.[3] It is the most common chronic bacterial infection worldwide. Specific virulence factors of certain *H. pylori* strains, such as cytotoxin-associated gene A (CagA) and

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specific alleles of vacuolating cytotoxin, have been linked to an increased risk of gastric cancer. However, they may also exert beneficial effects by modulating host immune defenses and other mechanisms.[4]

The expression of the CagA is involved in persistent colonization of some *H. pylori* strains, leading to chronic atrophic gastritis and, ultimately, hypergastrinemia, which is a possible risk factor for development of colorectal cancer.[5] Notably, *H. pylori* infection is associated with increased serum gastrin levels, stimulating rectal cell proliferation and promoting the development of colorectal cancer.[6]

H. pylori infection also leads to alterations in gastric microbiota, contributing to deleterious events that may eventually lead to gastric cancer.[7]

Other epidemiological studies suggest a possible association between *H. pylori* infection and extragastric manifestations, linking the risk of colon cancer (CC) with persistent infection of *H. pylori*.[8] *H. pylori* is well known for its association with gastric disorders. Still, its role in the pathogenesis of other gastrointestinal diseases, such as CC and UC, remains an area of interest.

The abnormal immune response to various factors such as microbiota that disrupt the intestinal balance is linked to the widespread inflammation of the colonic mucosa, potentially leading to chronic inflammation in individuals with UC.[9] It is essential to determine the expression level of some crucial cytokines like interleukin-21 (IL-21) and interleukin-23 (IL-23) to better understand the immune-modulatory action that may cause inflammation in the mucosal lining, which may result in UC and ultimately end in CC.

Cytokine IL-21 is produced by a subset of CD4+ T cells called T Helper 17 (Th 17) cells in response to diverse effects and is involved in both innate and adaptive immune responses.[10] IL-21 can have both pro-tumorigenic and anti-tumorigenic effects.[11] On one hand, IL-21 enhances the function of specific immune cells, such as natural killer cells and cytotoxic T cells, which can help in the antitumor immune response.[12] In contrast, this cytokine has a pro-tumorigenic effect, promoting the growth and survival of certain types of cancer cells, highlighting the role of IL-21 in CC, indicating its pro-and anti-tumorigenic function.[13,14]

Cytokine IL-23 is part of the IL-12 family that is, primarily produced from activated antigen-presenting cells such as dendritic cells and macrophages, promoting the proliferation and maintenance of Th17, leading to the production of a pro-inflammatory cytokine IL-17, and creating the environment for cancer cell progression and growth.[15] Some studies believe that IL-23 is associated with the pathogenesis of IBD.[16,17] Th17 cells produce pro-inflammatory cytokines such as IL-17 and IL-21, contributing to chronic inflammation and tissue damage in the colon, which is characteristic of UC.[18]

This study aims to investigate the prevalence of *H. pylori* infection and its cytotoxic-associated gene A (CagA) status in UC and CC patients, examine the expression levels of IL-21 and IL-23, and to explore their potential roles in *H. pylori*-associated pathogenesis.

Materials and Methods

This is a cross-sectional study which recruited 88 Iraqi patients, including 57 males and 31 females, aged between 45 and 70 years; the data were collected from three different groups: CC patients: the study includes 30 individuals with CC. UC patients: the study comprises 58 individuals with UC. The patients were selected from Al-Yarmouk Teaching Hospital and Gastroenterology and Hepatology Teaching Hospital from January 2022 to August 2022. Additionally, 30 healthy controls (HCs), aged between 41 and 73 years, with normal mucosa and no *H. pylori* infection, were included as controls, who were referred for colonoscopy, and their colons were found to be normal. All subjects underwent colonoscopy, and biopsy samples were obtained using forceps of the same size. The biopsy sample has been fixed in an appropriate solution and cut into 4-µm-thick sections to be prepared for immunohistochemical (IHC) analysis. The histological and bacteriological examinations were performed. For identification of *H. pylori*, rapid urease test, urea breath test, and histological examination were performed.

To identify *H. pylori* from the tissue specimens of the colon, the specimens were placed on a gel with urea as an indicator, leading to a color change during the first hour with *H. pylori* presence. The rapid urease test involved the inoculation of a single biopsy on urea agar slants, which was then followed by an incubation period ranging from 15 min to 1 h at 37 degrees Celsius. The color variations from yellow to pink on the urea slant agar show the portion of urea and hydrolase that converts urea to ammonia, and a change in pH causes color change. Also, biopsy samples are embedded in paraffin and stained with hematoxylin and eosin (H&E) and the Giemsa stain to determine the presence of *H. pylori*.

Blood samples were obtained from all individuals to acquire serum to quantify CagA immunoglobulin G (IgG), employing the sandwich enzyme-linked immunosorbent assay method. The commercial kit of CagA IgG antibodies against *H. pylori* was obtained from Genesis Diagnostics, Cambridge, UK. Immediately after the colonoscopy, blood was collected from the patient and centrifuged at 1500 rpm for 15 min to separate the serum. After that, the serum was frozen to -20°C and maintained until further analysis.

The cutoff value for positivity was calculated and compared with the value provided by the manufacturer. Serum levels higher than the cutoff value were considered

CagA-positive (above 0.5% U/mL indicates significant CagA IgG levels).

Immunohistochemical staining

Each paraffin block was cut into 4-µm sections. From each block, three sections were taken, one for hematoxylin and eosin (H&E stain) for histopathology revision, and two were used on positively charged microscopic slides (Fisher Brand superfrost/plus Fisher scientific, USA) for IHC staining with IL-21 and IL-23 monoclonal antibodies.

IL-21 rabbit polyclonal (ab 154767, Abcam Ltd., Cambridge, UK).

These antibodies were bound to nuclear target cytoplasmic proteins. The bound primary antibodies were detected by the conjugation of secondary antibodies with a specific chromogen (3, 3-diaminobenzidine [DAB]) with a brown-colored precipitate at the antigen location in the tissue, which indicates a positive reaction.

The appearance of brown products at the site of the target antigen is associated with a positive reaction in the peroxidase secondary detection system. A counterstain was then used to stain the cell blue.

Immunohistochemical scoring

Two independent and blinded readers, both experienced histopathologists, conducted the assessment of the IHC staining. In the case of IL-21, the evaluation involved tallying the number of cells displaying brown nuclear staining under light microscopy at 400× magnification. The percentages of stained cells were then calculated for each section. Positive protein expression was identified by the presence of brown nuclear staining.[19,20] Similarly, for IL-23, the same approach as mentioned above was employed to ascertain the percentages of cells exhibiting brown cytoplasmic staining under light microscopy at 400× magnification.[21] Any occurrence of brown cytoplasmic or nuclear staining within cells was considered for determining the proportion score.

The positively stained cells were counted by at least 10 random fields (200× magnification) to assess the frequency of IL-23 in each group. The percentage of positive cells relative to the total cell count was used to obtain the results.

Statistical analysis

For analyzing the correlation between the variables and the research parameters, the statistical analysis system (SAS Institute Inc., Cary, North Carolina, USA) software package was used. A *t* test was carried out to compare the two means. However, the chi-square test was used to analyze the differences in results between groups. An Analysis of Variance was carried out so that the differences in mean scores across a number of different groups could be investigated. After that, either Pearson's or the Spearman's correlation test was used to calculate the correlation coefficient (*r*). A *P* value of less than 0.05 is considered to be statistically significant.

Results

In this study, the age and gender distribution of the HC group consisted of 17 males and 13 females, with a mean age of 55.2 ± 1.3 years. The UC patient group consisted of 36 males and 22 females, with a mean age of 56.3 ± 2.2 years. The CC patient group included 21 males and nine females, with a mean age was 61.2 ± 3.5 years. Regarding *H. pylori* infection, the prevalence was more than half (the 35 individuals, 60.34%) in UC, while 23 individuals (39.66%) tested negative. In the case of CC, the prevalence of *H. pylori* was also high, with 21 cases (70%) testing positive and only nine cases (30%) showing a negative result [Table 1]. Notably, none of the HC subjects showed any positive infection of *H. pylori*.

The prevalence of CagA was examined only for the *H. pylori*-positive cases in both CC and UC patient groups. The results revealed that all *H. pylori*-positive cases with CC (21 cases, 100%) and approximately half of the UC cases (18 cases, 51.43%) had a positive serum level for this gene product, as illustrated in Table 2.

The results in Table 3 revealed substantial variations in IL-21 expression by immunohistochemistry among the study groups. In *H. pylori*-positive CC patients, the mean IL-21 expression was significantly elevated at 69.4 ± 17.7 compared to 51.20 ± 24.1 in *H. pylori*-negative CC patients ($P = 0.0001$). Likewise, *H. pylori*-positive UC patients exhibited higher IL-21 expression (30.86 ± 22.7) than *H. pylori*-negative UC patients (17.63 ± 12.7), with a statistically significant difference ($P = 0.037$) [Figure 1].

Table 1: Prevalence of *Helicobacter pylori* infection in colon cancer and ulcerative colitis patients compared to healthy controls

Variables	CC (*N* = 30)	UC (*N* = 58)	HC (*N* = 30)
HP (*Helicobacter pylori*)			
Positive	21 (70%)	35 (60.34%)	—
Negative	9 (30%)	23 (39.66%)	30 (100%)

CC: colon cancer; UC: ulcerative colitis; HC: healthy controls

Table 2: Prevalence of *H. pylori*-CagA in *H. pylori*-positive cases of colon cancer and ulcerative colitis patients

Variables	Colon cancer (*N* = 21)	Ulcerative colitis (*N* = 35)
HP CagA		
Positive	21 (100%)	18 (51.43%)
Negative	-	17 (48.57%)

CagA: cytotoxic-associated gene A, HP: *Helicobacter pylori*

Table 3: Expression of IL-21 by immunohistochemistry in the studied groups

IL-21		HP-positive CC (*N* = 21)	HP-negative CC (*N* = 9)	HP-positive UC (*N* = 35)	HP-negative UC (*N* = 23)	HC (*N* = 30)
Mean ± SD		69.4 ± 17.7	51.20 ± 24.1	30.86 ± 22.7	17.63 ± 12.7	9.63 ± 11.7
P value compared to	HC	0.0001#	0.0001#	0.018#	0.675	—
	HP-negative UC	0.0001#	0.0001#	0.037#	—	—
	HP-positive UC	0.0001#	0.0001#	—	—	—
	HP-negative CC	0.0001#	—	—	—	—
	All groups			0.0001^		

HP: *Helicobacter pylori*, CC: colon cancer, UC: ulcerative colitis, HC: healthy controls

Value < 0.05 (independent samples *t* test)

^*P* value < 0.05 (ANOVA test)

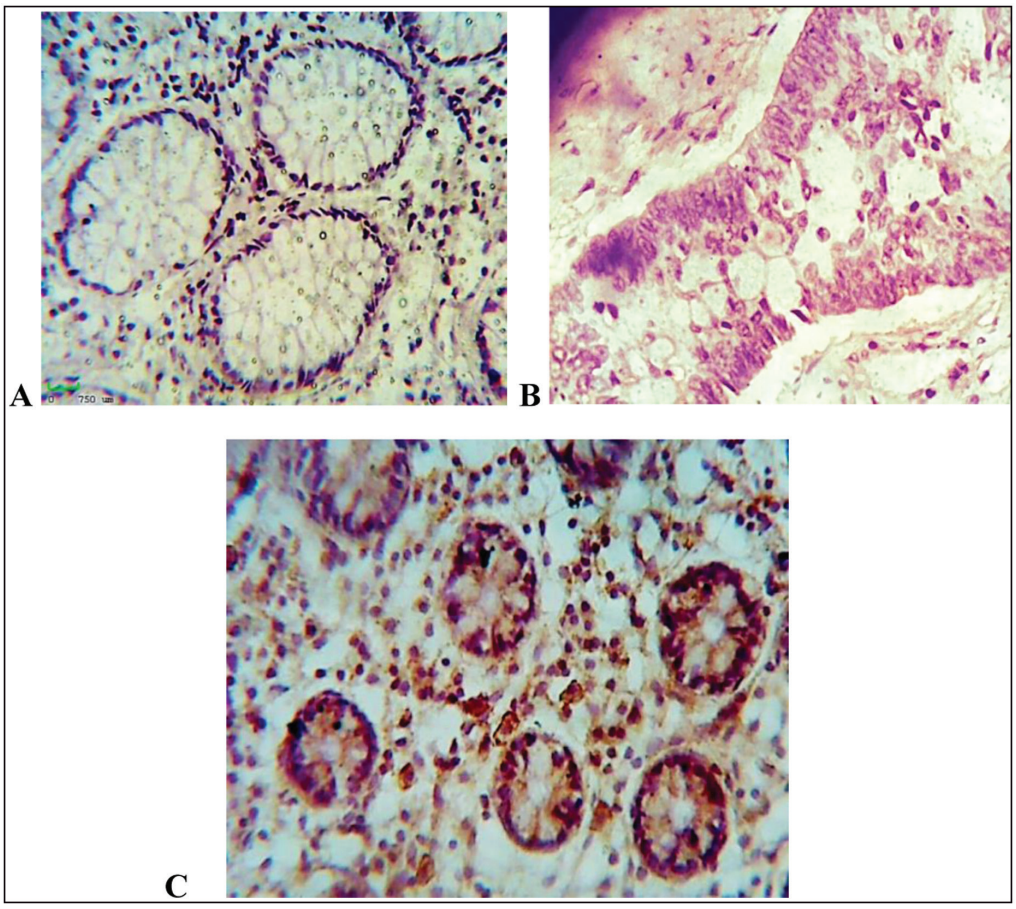

Figure 1: Immunohistochemical staining (IHC) of IL-21 proteins in the tissue of the colon. Staining by DAB chromogen (dark brown) counterstained with hematoxylin. (A) Normal tissue. (B) Positive immunostaining of ulcerative colitis. (C) Positive immunostaining of colon cancer (400×)

When compared to HCs, IL-21 expression in *H. pylori*-positive CC patients showed a significant increase ($P = 0.0001$). Similarly, *H. pylori*-positive UC patients displayed a significantly higher IL-21 expression than HCs ($P = 0.018$) [Table 3].

Table 4 shows the expression of IL-21 in patients infected with *H. pylori* and having CagA. The mean IL-21 expression was significantly different ($P = 0.0001$) between all studied groups: HP CagA-positive CC (69.4 ± 17.7), HP CagA-positive UC (38.7 ± 20.12), HP CagA-negative UC (26.86 ± 13.8), and HC (9.63 ± 11.7).

The findings in Table 5 indicated significant variations in IL-23 expression among the studied groups. The mean expression of HP-positive CC patients (58.4 ± 15.7) was significantly higher than that of HP-negative CC patients (40.7 ± 21.12), and the mean expressions of the two groups were also notably higher than those in the other studied groups (HP-positive UC 22.6 ± 11.2, HP-negative UC 16.63 ± 11.7, and HC 7.23 ± 9.3; $P = 0.0001$). However, no significant difference in IL-23 expression was found between HP-positive UC with HP-negative UC patient and HC ($P = 0.91$ and 0.17, respectively) and HP-negative UC patient and HC ($P = 0.18$) [Figure 2].

Table 4: Expression of IL-21 in patients infected with *H. pylori* and its association with CagA status

IL-21		HP CagA Post. CC (*N* = 21)	HP CagA Post. UC (*N* = 18)	HP CagA Neg. UC (*N* = 17)	HC (*No* = 30)
Mean ± SD		69.4 ± 17.7	38.7 ± 20.12	26.86 ± 13.8	9.63 ± 11.7
P value compared to	HC	0.0001#	0.0001#	0.001#	—
	HP CagA Neg. UC	0.0001#	0.0001#	—	—
	HP CagA Post UC	0.0001#	—	—	—
	All groups		0.0001^		

HC: healthy controls, HP CagA: *Helicobacter pylori* have cytotoxic-associated gene A, HP Neg. UC: *Helicobacter pylori*-negative ulcerative colitis, HP Post. UC: *Helicobacter pylori*-positive ulcerative colitis, HP Post. CC: *Helicobacter pylori*-positive colon cancer

#*P* value <0.05 independent samples *t* test

^*P* value < 0.05 ANOVA test

Table 5: Comparative expression of IL-23 by immunohistochemistry in colon cancer, ulcerative colitis, and healthy controls

IL-23		HP-positive CC (*N* = 21)	HP-negative CC (*N* = 9)	HP-positive UC (*N* = 35)	HP-negative UC (*N* = 23)	HC (*N* = 30)
Mean ± SD		58.4 ± 15.7	40.7 ± 21.12	22.6 ± 11.2	16.63 ± 11.7	7.23 ± 9.3
P value compared to	HC	0.0001#	0.0001#	0.17	0.18	—
	HP Neg. UC	0.0001#	0.0001#	0.91	—	—
	HP Post. UC	0.0001#	0.0001#	—	—	—
	HP Neg. CC	0.0001#	—	—	—	—
	All groups			0.0001^		

HP: *Helicobacter pylori*, HC: healthy controls, HP Neg.: HP-negative ulcerative colitis, HP Post.: HP-positive ulcerative colitis, CC: colon cancer

#P value <0.05 independent samples-*t* test

^*P* value < 0.05 ANOVA test

Table 6: Expression of IL-23 in patients infected with *H. pylori* and its association with CagA status

IL-23		HP CagA Post. CC (*N* = 21)	HP CagA Post. UC (*N* = 18)	HP CagA Neg. UC (*N* = 17)	HC (*N* = 30)
Mean ± SD		58.4 ± 15.7	32.7 ± 20.12	20.86 ± 13.8	7.23 ± 9.3
P value compared to	HC	0.0001#	0.0001#	0.0001#	—
	HP CagA Neg. UC	0.0001#	0.0001#	—	—
	HP CagA Post UC	0.0001#	—	—	—
	All groups		0.0001^		

HC: healthy controls, HP CagA: *Helicobacter pylori* have cytotoxic-associated gene A, HP Neg. UC: *Helicobacter pylori*-negative ulcerative colitis, HP Post. UC: *Helicobacter pylori*-positive ulcerative colitis, HP Post. CC: *Helicobacter pylori*-positive colon cancer

#*P* value<0.05 independent samples-*t* test

^*P* value < 0.05 ANOVA test

Table 6 illustrates the expression of IL-23 in patients infected with *H. pylori* and having CagA. The mean IL-23 expression was significantly different ($P = 0.0001$) between all studied groups: HP CagA-positive CC (58.4 ± 15.7), HP CagA-positive UC (32.7 ± 20.12), HP CagA-negative UC (20.86 ± 13.8), and HC (7.23 ± 9.3).

Table 7 displays the results of the correlation analysis between IL-21 and IL-23 production in the *H. pylori*-infected patients, in both CC and UC. The data revealed a significant positive correlation between the two cytokines ($r = 0.705$, $P < 0.01$). The data for the correlation analysis between IL-21 and IL-23 production in *H. pylori*-infected patients who also tested positive for the CagA gene revealed a significant positive correlation between IL-21 and IL-23 ($r = 0.560$, $P < 0.01$).

Discussion

The abnormal immune response to various factors, such as microbiota which disrupt the intestinal balance, is associated with widespread inflammation of the colonic mucosa, potentially leading to chronic inflammation in individuals with UC.[9] Furthermore, other studies have focused on the role of *H. pylori* in extragastric manifestations.[22] This bacterium, with its virulent component such as CagA, along with other factors contributing to asymptomatic persistence, leads to chronic infection and alters the environment to create a pro-tumor effect controlled by inflammatory mediators.[23] An early-stage investigation may be crucial in preventing disease progression and extending patient survival. CC ranks as the third-most common cancer worldwide, and its incidence is increasing,

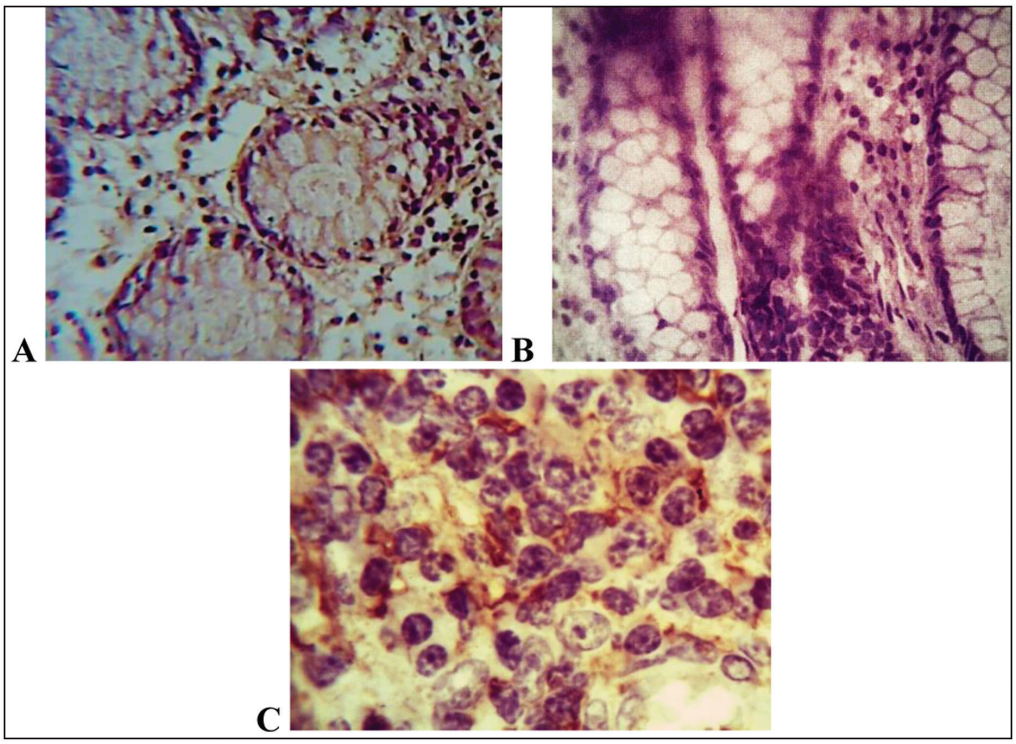

Figure 2: Immunohistochemical (IHC) staining of IL-23 proteins in the tissue of the colon. Staining by DAB chromogen (dark brown) counterstained with hematoxylin. (A) Normal tissue. (B) Positive immunostaining of ulcerative colitis. (C) Positive immunostaining of colon cancer (400×)

Table 7: Correlation between IL-21 and IL-23 production in patients with *H. pylori*

Variable	No.	Correlation coefficient = *r*	*P* value
IL-21 and IL-23 patients with *H. pylori*	56	0.705	<0.01*
IL-21 and IL-23 patients with *H. pylori* and CagA	39	0.560	<0.01*

*At the 0.05 level, the correlation is significant

particularly in developing countries.[24] In this research, exploring the potential coexistence of *H. pylori* along with its virulent component CagA and the assessment of IL-21 and IL-23 expression proved to be particularly intriguing.

The present study has demonstrated a higher prevalence of *H. pylori* infection in UC and CC patients. Regarding UC, there have been controversial published reports about the correlation between *H. pylori* and the development of IBD; some of the studies focused on the inverse correlation between *H. pylori* and IBD, suggesting that hygiene (*H. pylori* eradication) conditions induce intestinal autoimmunity such as IBD.[25] Others agreed with this study and concluded that *H. pylori* infection significantly increased in UC patients, which may induce long-standing chronic inflammation with dysregulated cytokines, leading to IBD.[26] The role of this bacterium exerts a notable virulent impact in the pathogenesis of several extragastric manifestations affecting the gut microbiome, which could play a pivotal role in colorectal cancer, promoting changes in gut microbial signatures that could contribute to tumor development.[27]

The observation in this study that all CC patients were *H. pylori*-positive subjects were CagA-positive while half of the UC patients were positive is in agreement with that of Tepler *et al*.[4] who found evidence supporting the influence and effect of CagA-positive cases in UC and IBD, and this level was even higher in CC than in the control group. Furthermore, an Iraqi study investigated the prevalence of CagA genes among *H. pylori*-infected Iraqi patients with different gastric pathologies and found that CagA is highly prevalent.[28] Another study conducted by Strofilas *et al*.[5] proposed that cytotoxic-associated genes modulate the environment and trigger inflammation in the colonized gastric mucosa, leading to chronic atrophic lining, eventually playing a role in cancer progression and postulating the direct correlation between CagA level and cytokine production, implying the effect of CagA-producing strains of bacteria on chronic inflammation acting in suppressing apoptosis of transformed cells, which is a crucial factor in the pro-tumor microenvironment and immunoediting in humans.[11] This effect could explain that CagA produced by *H. pylori* has a major impact on the pathogenesis of this bacterium by triggering inflammatory cytokines, leading to chronic inflammation and experiencing cellular response by T h1 followed by Th 2, and this shifting in the immune response is thought to create a microenvironment that is, conducive to tumor progression.[29] Another study by Wang

et al.[30] and He *et al*.[31] pointed to the association between CagA and CC in *H. pylori*-CagA-positive patients and explained that this protein induces systemic inflammation and alters gut microbiota, which may contribute to the altered pro-tumor microenvironments, leading to the development of CC.

This study investigated the expression level of some essential cytokines (IL-21 and IL-23) using IHC techniques to better understand the induced immune-modulatory effect. The results showed a significantly elevated expression of IL-21 and IL-23 in *H. pylori*-positive patients with CC and UC compared with HCs. The important immune cell player in *H. pylori* is TH1 and T helper 17.[32] IL-21 is the cytokine produced by the Th17 lineage and plays an important role in the activation and recruitment of neutrophils, an important contributor to inflammatory lesions caused by *H pylori*.[33] The current study examined the significantly elevated expression of this cytokine between the *H. pylori*-positive CC as compared to *H. pylori*-negative CC and *H. pylori*-positive UC, highlighting the persistent chronic inflammation in *H. pylori*-positive CC, which is also explained by Dewayani *et al*.[34] who investigated the role of IL-21 in maintaining Th17 activation and its inflammatory impact on epithelial lining for comprehending the pathophysiology of this bacteria.[35] Patients with UC have excessive IL-21 production in mucosa as compared to control[36] and eventually predisposing to colonic epithelial cells to produce chemokines and recruit macrophages, leading to Th17 and Th1 signaling and activation and displaying extra-vascularization, inflammation, and colitis.[37]

Cytokines of Th17 cells in patients with IBD, such as intestinal and serum IL-23, have been found to be significantly elevated in a study by Bank *et al*.[38] who found that the presence of polymorphism in IL-23 and IL-17 pathway is linked to higher susceptibility to development of IBD. The present study is in agreement with the findings of Datta and Sarvetnick,[39] as it clearly demonstrates a marked increase in the levels of both IL-21 and IL-23 in patients with CC and UC. This supports the notion that IL-21 plays a crucial role in maintaining the function and homeostasis of Treg cells, which are responsible for regulating both the immune response and homeostasis.[39] The cytokine IL-23 is closely related to the pathogenesis of UC which maintains the function and persistence of Th17 cells, which play a crucial role in the inflammatory response in UC through the production of pro-inflammatory cytokines like IL-17 and IL-21, contributes to chronic inflammation and tissue damage in the colon, which is a characteristic of UC.[40] IL-23 is believed to have a pro-tumorigenic role in CC. It promotes the growth and maintenance of Th17 cells, which produce pro-inflammatory cytokines like IL-17. These cytokines can enhance tumor growth and create a pro-inflammatory environment that supports cancer progression.[34] This is particularly true in *H. pylori* infection, which, in certain persons who are unable to tolerate it, may progress to UC and cancer.[41] Additionally, IL-21 may be involved in increasing the risk of mucosal inflammation into inflammatory carcinogenesis.[35] There are other roles that IL-21 may play, but one of its primary functions is to activate Th17 cells, which can then lead to proliferation and differentiation,[42] as well as accumulation in the tissue, which can put a person at risk for inflammatory bowel illnesses. Controlling chronic inflammation may reduce the risk of developing cancer and tumors.[35]

This research, along with others,[41] concludes that there is a strong positive association between IL-21 and IL-23 in *H. pylori*-positive participants. It was not quite evident whether or not these two cytokines were correlated in this investigation of control participants, which suggests that the expression of the cytokine profile in a normal condition is different from its expression in an inflammatory state. Together with IL-21, IL-23 may boost inflammatory responses that affect the intestinal mucosa.[34] Although every cytokine has multiple effects and responsibilities, it needs to be investigated thoroughly to have a deeper comprehension of the clinical results of UC and CC. Together with IL-21, IL-23 plays an essential part in inflammation and has been shown to have either direct or indirect correlations with UC and CC.

Conclusion

The findings suggest potential connections between these cytokines, indicating an influential role as pro-inflammatory cytokines in modulating the immune response during *H. pylori* and *H. pylori*-CagA-positive infection and highlighting the importance of CagA status and cytokine expression in the pathogenesis of post-CC and post-UC.

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Ethical approval

The ethical approval of this study was obtained according to the principles of the Declaration of Helsinki and after obtaining verbal and informed consent from the patients before the taking of samples. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee of the Microbiology Department at the Medical College of Mustansiriyah University Numbered 53 on November 8, 2021, to get this approval.

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Conflicts of interest
There are no conflicts of interest.

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