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## Research Article

### Expression of NF-κB mRNA in *Helicobacter pylori* Positive Iraqi Patients with Inflammatory Bowel Diseases and Colorectal Cancer

Sinan Subhi Farhan 

Department of Anesthesia, College of Medical Science Techniques, The University of Mashreq, Baghdad, Iraq

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#### Abstract

**Background:** Inflammatory bowel diseases (IBD) and colorectal cancer (CRC) are caused by a combination of variables, including environmental, host-related, and nutritional factors. *Helicobacter pylori* (*H. pylori*) is an environmental risk factor for many GIT disorders and is designated as a class I carcinogen. **Aim:** To investigate the prevalence of *H. pylori* in Iraqi patients diagnosed with IBD and CRC and the expression of NF-κB mRNA in those patients. **Methods:** Patients with GIT symptoms were tested for the existence of IBD and CRC in a cross-sectional observational study. In biopsies taken from GIT lesions, biochemical and histochemical approaches are employed to determine the presence of *H. pylori* and the expression of NF-κB mRNA. **Results:** *H. pylori* tests were positive in 33.3% of CRC patients, although this was not statistically significant compared to those who had negative testing. Only 63.3% of IBD patients had *H. pylori* infection. In the CRC group, 53.3% of patients had negative NF-κB expression, whereas all of the patients in the IBD group had a negative test. **Conclusion:** Patients with CRC had a high prevalence of *H. pylori*, whereas IBD patients had a low frequency. Patients with CRC had high levels of NF-κB mRNA expression, whereas patients with IBS had none.

**Keywords:** *Helicobacter pylori*, NF-κB mRNA expression, colorectal cancer, inflammatory bowel disease

التعبير عن NF-κB mRNA في المرضى العراقيين الموجبين لفحص هيليكوبكتر بيلوري المصاب لأمراض الأمعاء الالتهابية وسرطان القولون والمستقيم

#### الخلاصة

**الخلفية:** تحدث أمراض الأمعاء الالتهابية (IBD) وسرطان القولون والمستقيم (CRC) بسبب مجموعة من المتغيرات، بما في ذلك العوامل البيئية والعوامل المتعلقة بالمضيف والعوامل الغذائية. بكتيريا هيليكوبكتر بيلوري هي عامل خطر بيئي للعديد من اضطرابات GIT ويتم تعيينه كمسبب من الفئة الأولى. **الهدف:** التحقيق في انتشار هيليكوبكتر بيلوري في المرضى العراقيين الذين تم تشخيص إصابتهم بمرض ال IBD و CRC والتعبير عن الحمض النووي الريبي NF-κB لدى هؤلاء المرضى. **الطرق:** تم اختبار المرضى الذين يعانون من الإصابة بأمراض IBD و CRC في دراسة رصدية مقطعية. في الخزعات المأخوذة من آفات GIT ، يتم استخدام النهج الكيميائية الحيوية والهيستوكيميائية لتحديد وجود *H. pylori* والتعبير عن NF-κB mRNA. **النتائج:** كانت اختبارات *H. pylori* إيجابية في 33.3% من مرضى CRC ، على الرغم من أن هذا لم يكن مهما إحصائيا مقارنة بأولئك الذين تعرضوا لاختبارات سلبية. فقط 63.3% من مرضى IBD كان لديهم عدوى *H. pylori* في مجموعة CRC ، كان 53.3% من المرضى ظهر لديهم فحص سلبية لمعيار NF-κB ، في حين أن جميع المرضى في مجموعة IBD أظهرت نتيجة سلبية لهذا الفحص. **الاستنتاج:** كان لدى المرضى الذين يعانون من CRC معدل انتشار مرتفع لـ *H. pylori* ، في حين كان المعدل أقل لدى مرضى IBD . وكان المرضى الذين يعانون من CRC مستويات عالية من الحمض النووي الريبي للعامل NF-κB ، في حين أن المرضى الذين يعانون من IBS لم يكن لديهم أي مقدار منه.

\* **Corresponding author:** Sinan S. Farhan, College of Medical Science Techniques, The University of Mashreq, Baghdad, Iraq; E-mail: [sinan.subhi@uom.edu.iq](mailto:sinan.subhi@uom.edu.iq)

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#### INTRODUCTION

Inflammatory bowel diseases (IBD) and colorectal cancer (CRC) are caused by a combination of variables, including environmental, host-related, and nutritional factors [1].

*Helicobacter pylori* (*H. pylori*), gram-negative bacteria, is a known environmental risk factor for many GIT disorders and is designated as a class I carcinogen by the World Health Organization [2]. This bacterium is expected to infect half of the world's population [3,4]. The cag

Pathogenicity Island (cag-PAI) is a virulence determinant area found in *H. pylori* strains, and CagA (cytotoxin-associated gene) is one of many genes encoded by cag-PAI [5]. CagA is translocated from bacteria into human epithelial cells via a type IV secretion system and regulates certain signal transduction pathways to modulate cellular effects [6]. The "Correa's cascade," which involves progression from chronic gastritis to chronic atrophic gastritis, intestinal metaplasia, atypical hyperplasia, and eventually gastric cancer, is triggered by *H. pylori* [7]. Chronic *H. pylori* infection is linked to the activation of many pro-inflammatory signaling pathways [8]. Two of the most prominent pro-inflammatory pathways that are active during carcinogenesis are NF- $\kappa$ B and STAT3 [9]. NF- $\kappa$ B is a transcriptional factor that is activated by *H. pylori* infection and is constitutively activated in many GIT disorders including various cancers [10]. NF- $\kappa$ B activation causes the production of a number of pro-inflammatory cytokines and chemokines implicated in proliferation, angiogenesis, invasion, and apoptosis blocking [11]. Chronic inflammatory conditions are involved in the promotion of carcinogenesis by creating an inflammatory microenvironment [12]. NF- $\kappa$ B is one of the most important inflammatory pathways in the development and progression of numerous malignancies, including gastric and colorectal tumors [13]. The transcriptional activities of NF- $\kappa$ B and STAT3 interact to optimize cytokine production and activation, according to a number of recent studies [14]. In the present study, we investigated the prevalence of *H. pylori* in Iraqi patients diagnosed with IBD and CRC and the expression of NF- $\kappa$ B mRNA in those patients using biochemical and histological methods.

## METHODS

Patients included 90 patients with normal colon (NC), 30 patients with IBD, and 30 patients with colorectal cancer (CRC) who attended the endoscopic unit at Baghdad Teaching Hospital. Biopsies were histopathologically examined to confirm the patients' diagnoses. For the double blind evaluation, two examiners looked at each patient's slide. The patients ranged in age from 15 to 62 years old. The patients were separated into three groups based on their ages: <20 years, 20–40 years, and > 40 years. For at least four weeks, no antibiotics, proton pump inhibitors (PPIs), or H2 blockers should be used by the individuals who have been chosen. They should also have stopped using bismuth-based therapy and NSAIDs for at least three weeks. The patients were put on a particular eating regimen and given laxatives the day before the colonoscopy to help them clear the colon. During colonoscopy, each patient's cecum, ascending, transverse, and descending colons (sigmoid) were sampled for intestinal biopsy. Two to four mucosal punch biopsy specimens were collected after endoscopic diagnosis. A biopsy urease test (BUT) was performed on one biopsy specimen to detect *H. pylori* in

the tissue sample [15]. In Rapid Urease Medium, the urea reaction produced by *H. pylori* is faster than that produced by other urea-splitting organisms. As a result, the formation of a pink-red or red-violet hue is an efficient test for the diagnosis of *H. pylori*. Negative results were stored for up to 20 hours. For the creation of paraffin embedded tissue blocks, other biopsy tissues were preserved with 10% buffered neutral formalin. Sections of 5  $\mu$ m thickness were put on conventional slides for H&E staining and on charged slides for in situ hybridization using a biotinylated DNA probe and an in situ hybridization detection kit (Maxim Biotech/USA) to detect NF- $\kappa$ B mRNA [16].

## Statistical analysis

Statistical analysis was done using software SPSS 16 for Windows. Differences among groups were evaluated by using Chi-square test and Fisher's exact test (2 x 2 Table). Differences were considered to be statistically significant at P value less than 0.05.

## RESULTS

Table 1 illustrates the incidence of IBD and CRC in the patients who were chosen based on their age. The highest prevalence of both illnesses were seen in those over the age of 40 (46.6% and 50%, respectively), while the lowest rates were found in people under the age of 20. (6.8% for each group).

**Table 1:** Distribution of patients diagnosed with CRC disease and IBD according to age groups compared with those with normal colon (Total n=240)

Age ranges (year)	NC (%)	CRC (%)	IBD (%)
<20	21(23.3)	2(6.8)	2(6.8)
20-40	23(25.6)	14(46.6)	13(43.2)
>40	46(51.1)	14(46.6)	15(50)
Total	90(100)	30(100)	30(100)

The prevalence of *H. pylori* in the selected patients with GIT complaints is shown in Table 2. *H. pylori* was found in 66.7% of patients with normal colons, which is statistically significant when compared to patients who tested negative for *H. pylori* ( $P<0.5$ ). Meanwhile, 33.3% of CRC patients had a positive *H. pylori* test, which was not statistically significant when compared to those who had a negative test. Only 63.3% of IBD patients tested positive for *H. pylori*, which was shown to be non-significant when compared to those who tested negative. The majority of patients in the NC group (58.33%) had low *H. pylori* density, whereas 25% had notable *H. pylori* density, according to the H&E staining test of tissue sections. The majority of patients in the CRC group (44.5%) had moderate *H. pylori* density, while 22.2% had significant density.

**Table 2:** *H. pylori* prevalence among different age groups of patients diagnosed with CRT disease and IBD based on BUT results.

Age (year)	NC (n=90)		CRC (n=30)		IBD (n=30)	
	+ve(%)	-ve(%)	+ve(%)	-ve(%)	+ve(%)	-ve(%)
<20	6(28.6)	15(71.4)	1(50)	1(50)	1(50)	1(50)
20-40	9(39.1)	14(60.9)	8(57.1)	6(42.9)	5(38.5)	8(61.5)
<40	15(32.6)	31(67.4)	11(78.6)	3(21.4)	5(33.3)	10(66.7)
Total	30(33.3)	60(66.7)	20(66.7)	10(33.3)	11(36.7)	19(63.3)
P	0.001		>0.05		>0.05	

In the IBD group, only 44% of patients had moderate *H. pylori* density and 11% had significant *H. pylori* density, indicating a similar pattern. Significant differences were not found in statistical analysis of the data (Table 3).

**Table 3:** *H. pylori* density in different groups based on H&E staining method.

Patient group	Mild (%)	Moderate (%)	Marked (%)	Total
NC	14(58.3)	4(16.7)	6(25)	24
CRC	6(33.3)	8(44.5)	4(22.2)	18 <sup>NS</sup>
IBD	4(44.5)	4(44.5)	1(11)	9 <sup>NS</sup>
Total	36(38.7)	35(37.6)	22(23.7)	93

Table 4 shows that 75% of NC patients had negative NF- $\kappa$ B expression, which was not statistically significant when compared to those who had positive NF- $\kappa$ B expression (25%). However, 53.3% of patients in the CRC group had negative NF- $\kappa$ B expression, while all patients in the IBD group had negative NF- $\kappa$ B expression testing. Figure 1 shows the different levels of NF- $\kappa$ B expression in tissue sections taken from the patients compared to sections with no expression.

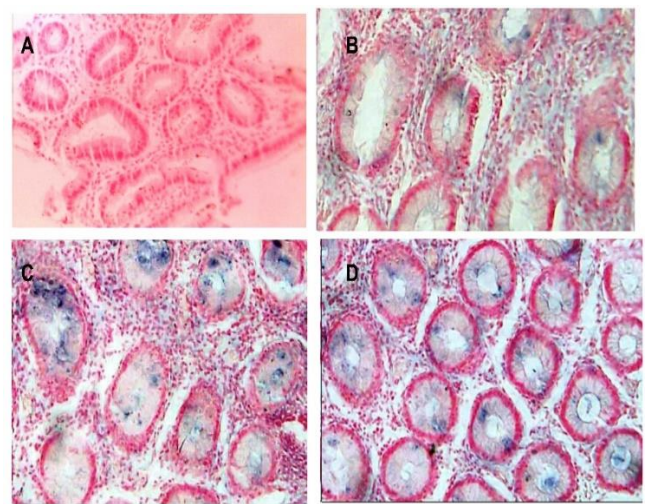
**Table 4:** Expression of NF- $\kappa$ B mRNA in different age groups of patients with CRC and IBD based on in situ hybridization results.

Age (year)	NC (n=24)		CRC (n=30)		IBD (n=30)	
	+ve(%)	-ve(%)	+ve(%)	-ve(%)	+ve(%)	-ve (%)
<20	2(40)	3(60)	0	2(6.7)	0	2(6.7)
20-40	3(42.9)	4(57.1)	8(57.2)	6(42.8)	0	13 (43.3)
<40	1(8.3)	11(91.7)	6(42.8)	8(57.2)	0	15(50)
Total	6(25)	18(75)	14(46.)	16(53.)	0	30(100)
	P>0.05		P>0.05		----	

## DISCUSSION

Infection with *H. pylori* may be the most common human infection and in many populations, and infection rates of 80-90% are not unusual [17]. The prevalence of *H. pylori* infection also varies widely by geographic area, age, race, and socioeconomic status; however, only few of those colonized people developed diseases related to *H. pylori* [18]. Regarding IBD, there are many evidences supporting a multifactorial genesis comprising a combination of genetic predisposition, immune response, and environment, most notably the bacterial gut microbes or

luminal antigens [19]. Certain studies detected *H. pylori* infection among IBD patients, so they suggested a possible role of *H. pylori* in IBD group, although other studies suggested no possible role of *H. pylori* in IBD indicating that the role of *H. pylori* in IBD is still controversial [20]. The present study disclosed presence of *H. pylori* in 66.7% of CRC patients and 36.7% of IBD patients compared to 33.3% in the NC group, and analysis of data reflects non-significant differences. A recent meta-analysis study demonstrates that colorectal adenoma, advanced adenoma, and cancer were all associated with *H. pylori* infection [21], a result that support our primary finding in this regard. The role of inflammation and *H. pylori*-mediated activation of NF- $\kappa$ B in transcriptional regulation in cancer was recently clarified [22].

**Figure 1:** Expression of NF- $\kappa$ B mRNA. A: negative expression, B: Mild expression, C: moderate expression; D: Marked expression [BCIP/NBT stain (dark-blue) by *In Situ* hybridization staining counterstained with NFR].

The present study highlighted the influence of *H. pylori*-induced NF- $\kappa$ B mRNA expression of high rate in CRC patients, while none of the IBD patients demonstrates positive results. However, NF- $\kappa$ B is involved as an important inflammatory signaling pathway in the pathogenesis of IBD [23]. Early studies have reported that constitutive NF- $\kappa$ B activation was found in the inflamed intestinal tissues of IBD patients [24], which was in conflicts with the reported outcomes in the present study. Previous studies also suggest that apoptosis and DNA damage are increased in GIT cells during infection with *H. pylori* [25]. Chronic *H. pylori* infection results in generation of a subpopulation of epithelial cells with high levels of DNA damage that are resistant to apoptosis [26]. The accumulation and survival of cells with damaged DNA heightens the risk of development of GIT cancers [27]. Moreover, the effectiveness of GIT epithelium cytoprotection has been found closely related to the virulence of *H. pylori* strains, particularly CagA subtypes



[28]. The reason for the inconsistent findings reported to date is unclear, but it might be at least partly explained by the methodological issue. These include selection bias, small sample sizes and an inadequate consideration of potential confounding variables in the data analysis. One of the limitations of the present study is the small sample size which do not enable achieving significant differences in the obtained outcomes.

### Conclusion

A relatively high *H. pylori* prevalence was detected only at the site of lesion of patients with colorectal cancers, and low prevalence in IBD patients. Patients with CRC showed high levels of NF- $\kappa$ B mRNA expression, while completely missed in IBS group.

### Acknowledgement

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### Conflict of interests

The author declared no conflicting interests.

### Data sharing statement

The datasets analyzed during the current study will be available from the corresponding author on a reasonable request.

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