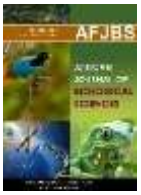
https://doi.org/10.48047/AFJBS.6.13.2024. 2652-2672

African Journal of Biological Sciences



Impact *of Helicobacter Pylori* Infection on Extracellular Matrix Deposition and Tumor Progression in Patients with Colorectal Cancer

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Article History
Volume 6, Issue 13, 2024
Received: 18June 2024
Accepted: 02July 2024
doi:*10.48047/AFJBS.6.13.2024.* 2652-2672

Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection is not limited only to gastrointestinal tract but also associated with various extra-gastric diseases including colorectal cancer (CRC). This work aimed to estimate *H. pylori* impact on extracellular-matrix deposition in CRC-patients. **Methods:** A total of 285 CRC patients and 120 healthy individuals were included. *H. pylori*-antigen and fibronectin were quantified in patients' sera by ELISA. **Results:** Our findings showed that *H. pylori*-infected patients were more susceptible for developing severe tumour features than those without. Patients with late stages (III–IV) and poor tumor differentiation (G3-G4) displayed 7.3 and 11.5-fold increase in *H. pylori*-antigen than with early stages and low grades, respectively. The results showed that patients infected with *H. pylori* were accompanied by significant increase in fibronectin compared to those without. Indeed, the risk of elevated fibronectin for developing severe tumor features (late stages and high tumor grades) was 2.3 and 3.4, respectively, in cases of low *H. pylori*-antigen concentration. Surprisingly, this risk was increased yielding an estimated odds ratio of 5.1 and 15.4 in case of high *H. pylori*-antigen concentration. **Conclusions:** Our findings could indicate the influence of *H. pylori* on enhancing extracellular-matrix deposition subsequently increases patients' susceptibility for developing severe tumor features.

Key words: Colorectal cancer, Extracellular matrix, Fibronectin, *Helicobacter pylori*, Tumor markers.

Introduction

Globally, the prevalence of colorectal cancer (CRC) has been dramatically growing at an alarming rate in recent years. More than 1.9 million new CRC cases and 935,000 deaths were estimated to occur in 2020, representing about one in 10 cancer cases and deaths (Sung et al., 2021). In fact, the development of CRC is associated with multiple genetic and environmental factors. Overall, lifestyle factors including overweight and obesity, sedentary lifestyle, heavy alcohol intake, tobacco smoking, and inappropriate dietary patterns have important roles in CRC etiology. Also, individuals with diabetes mellitus or a personal or family history of colorectal adenoma or CRC are at increased risk for CRC (Ahmed, 2020). As well, gut microbiome, gender, age, and socioeconomical status are known to influence CRC risk (Sawicki et al., 2021).

On the other hand, *Helicobacter pylori* (*H. pylori*) infection is a prevalent bacterial infection that affects the gastric mucosa of humans, with a prevalence ranging from 30% to 90%, depending on the geographical area (Aljaberi et al., 2023). Conclusive evidence has proven that *H. pylori* infection is associated with various health concerns including chronic gastritis, gastric ulcers, and gastric cancer. Interestingly, it is the only bacterium recognized as a type 1 carcinogenic agent (Iannone et al., 2018). Indeed, *H. pylori* infection is not only limited to the gastrointestinal tract and also has been associated with various extra-gastric diseases including CRC (Liu and Zheng, 2016). Unfortunately, most studies have analyzed mainly the presence of serum antibodies to *H. pylori*, which is unable to discriminate between current and past infections and only the current infection of *H. pylori* would elicit both humoral and cellular immune responses that initiate or sustain chronic inflammatory processes in the gastrointestinal tract with potential oncogenic consequences such as metastasis and mortality (Coelho and Coelho, 2021). Therefore, this work shed light on the association between the active infection of *H. pylori* by detecting 58-kDa *H. pylori* antigen and CRC development and progression. Then evaluating if there is an impact for *H. pylori* infection on extracellular-matrix deposition and if so, to what extent would that pathogen affect.

Methods

Patients

This cross sectional study was conducted on two-hundred and eighty-five consecutive Egyptian patients with clinically and laboratory-confirmed CRC recruited from Tropical Medicine department, Mansoura University hospitals, Mansoura, Egypt. All CRC patients were categorized according to TNM staging system and tumor differentiation degree (Sobin et al., 2011). Regarding cancer stages, cancerous patients included 32 cases with stage I, 57 with IIA, 38 with IIIB, 33 with IIIA, 22 with IIIB, 37 with IIIC, and 66 with IV stages. For clinically useful prognostic information, cancerous patients can be classified into two categories: 127 cases (44.6%) with early stages (I-II) and 158 (55.4%) with late stages (III-IV). Furthermore, according to the degree of tumor differentiation, only 57 cases of cancerous patients had well- differentiated tumors (G1), whereas 113 cases had moderately differentiated tumors (G2), 75 cases had poorly differentiated tumors (G3), and 40 cases had undifferentiated tumors (G4). Overall, the majority of cancerous patients had well-moderately differentiated tumors (low grade) [G1-G2; 170 cases], while 115 cases had poorly differentiated tumor (high grade) [G3-G4]. Informed consent was acquired from all participants after providing them with comprehensive information regarding the diagnostic procedures and the nature of the disease. The 1975 Helsinki Declaration's ethical principles were followed by the study protocol. This study was authorized by the Mansoura University Hospitals' ethical and scientific committees, Mansoura University, Egypt (Code Number: MS.21.07.1595). The participants provided verbal consent to participate, which was authorized by the Ethics Committee. Additionally, the study was controlled by 120 healthy individuals.

Laboratory tests

All the study participants fulfilled the following criteria: a negative test for hepatitis B surface antigen (Biomedica, Sorin, Italy); a negative test for anti-HCV antibodies (ETI-AB-HCVK-3 kit, Sorin Biomedica, Diagnostic, Vercelli, Italy). In addition, all patients were subjected to laboratory investigations including, complete blood count on an automated hematology analyzer (Sysmex Corporation, Kobe, Japan), and tumor markers including carcinoembryonic antigen (CEA) and cancer antigen 19.9 (CA19.9) were also detected according to the manufacturer’s instructions of the commercial ELISA kit (CanAg, Diagnostics AB, Gothenburg, Sweden).

Detection of 58-kDa *H. pylori* antigen and fibronectin using ELISA

In this study, the quantification of 58-kDa *H. pylori* antigen and fibronectin (FN) levels was assessed using the enzyme ELISA technique using their mono-specific antibodies (ABC Diagnostics, New Damietta, Egypt) as described by Attallah et al. (Attallah et al., 2013; Attallah et al., 2004). The intensity of color was directly proportional to the amount of bound conjugate and thus correlated with the concentration of *H. pylori* antigen and FN present in the serum sample.

Statistical analysis

Data processing and analyses were performed using the GraphPad Prism package, version 5.0 (GraphPad Software, San Diego, CA), and SPSS software, version 20.0 (SPSS Inc., Chicago, IL). Normally distributed data were expressed as mean± standard deviation (SD), while non-normally distributed data were shown as median (interquartile range). Significant difference was assessed by the student *t*-test or Mann–Whitney *U* test as appropriate. A value of $P<0.05$ was considered statistically significant. Odds ratio (with 95% confidence intervals (CI)) was calculated from a 2×2 contingency table to determine the likelihood risk of advanced tumor stage and grade with *H. pylori* existence.

Results

Patients' characteristics

The patient-related clinical and hematological parameters are summarized in Table 1. The mean (±SD) age of CRC was 50.5 (±11.8) years. Interestingly, CRC patients had a higher median of inflammatory indexes, including neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR). Additionally, CRC patients were associated with significantly higher levels of tumor markers (CEA and CA19.9).

The influence of H. pylori existence on different laboratory parameters

First of all, CRC patients were categorized into two main groups according to *H. pylori*-infection: the *H. pylori*-infected group included 189 patients, and the *H. pylori*-non infected group included 96 patients. The levels of different laboratory parameters were assessed in *H. pylori*-infected patients against those without *H. pylori* in order to evaluate the impact of *H. pylori* existence on these parameters, as shown in Table 2. First, there was no significant difference in the mean age of *H. pylori*–infected patients and those without *H. pylori*, and the prevalence of *H. pylori* infection was

64.6% in males and 68.5% in females. Whereas there was a significant increase in WBC and neutrophil count between the two groups (P<0.0001), while there was a significant decrease in lymphocyte count in *H. pylori*-infected individuals (P=0.019). Also, in regard to inflammatory indexes, *H. pylori*-infected patients had a significant increase in NLR levels (P<0.0001) in comparison to those without infection. Additionally, *H. pylori*-infected patients had a 10.3 and 8.8-fold increase in the level of tumor markers CEA and CA19.9 than those without *H. pylori*, respectively.

The impact of H. pylori existence and colorectal cancer progression

Interestingly, H. pylori-infection was found to be associated with CRC progression. As a result, 128 cases (81.0%) of CRC patients who had late cancer stages were infected with H. pylori in comparison to only 61 cases (48.0%) of CRC patients who had early cancer stages. Additionally, 85.2% (98/115) of CRC patients with high grades (G3-G4) were *H. pylori* infected, while 53.5% (91/170) of patients with low grades (G1-G2). Indeed, *H. pylori*-infected patients were more likely to be susceptible to severe tumor features than those without infection, yielding an estimated odd ratio (OR) of 4.6 and 5.0 for late cancer stages (III-IV), and poorly differentiated tumor (high grade) [G3-G4], respectively.

One of the objectives of this investigation is to explore not only the aspect of *H. pylori* existence, but also the impact of infection levels. Given that, 58-KDa *H. pylori* antigen was quantified using the ELISA technique in our CRC cohort (Figure 1). As a result, *H. pylori* antigen levels were significantly increased (P=0.0001) with CRC stage progression. Patients with late stages (III-IV) displayed a 7.3-fold increase in *H. pylori* antigen levels than those with early stages (I-II). Also, patients with poor tumor differentiation (G3-G4) displayed a 11.5-fold increase in *H. pylori* antigen than patients with low grade (G1-G2) as shown in Figure 1.

Influence of *H. pylori* on fibronectin, a crucial element of the extracellular matrix

Fibronectin (FN) is a vital component of the extracellular matrix that participates in ECM formation and plays roles in a variety of pathological processes such as malignancy and infection; interestingly, there have been a growing number of studies that demonstrate the role of FN in CRC progression. Additionally, it has been reported that its expression in urine and serum can serve as a valuable indicator of the extent of disease advancement. Indeed, our findings are consistent with these observations. Irrespective of the existence of *H. pylori*, CRC patients with severe

tumor features (late cancer stages (III-IV) and high tumor grades (G3-G4)) were accompanied by a significant (P=0.0001) high FN concentration compared to those with early cancer stages (I-II) or low grades (G1-G2) as depicted in Figure 2.

FN levels were then assessed with respect to *H. pylori* infection to examine its influence on this pivotal protein. Overall, CRC patients with *H. pylori* infection was accompanied by risen FN level with a 1.3–fold increase than those without *H. pylori,* as illustrated in Figure 2. Also, in terms of cancer stage, our results demonstrated that at the same stage group, *H. pylori*-infected individuals were accompanied by a significant increase in FN levels compared to those without *H. pylori*-infection. Additionally, late-stage CRC patients who were infected with *H. pylori* had the highest FN levels. The same results were found in terms of tumor histological grade, as shown in Figure 3. Interestingly, these elevated FN levels in *H. pylori* infection patients may indicate their increased susceptibility to tumor progressing faster than those without *H. pylori*.

The impact of *H. pylori* antigen concentration on fibronectin levels

Then, by studying not only the existence of *H. pylori* but also the impact of *H. pylori* antigen concentrations on FN levels, our findings indicated that CRC patients with high *H. pylori* antigen levels were associated with elevated FN levels compared to those with low *H. pylori* antigen levels, as shown in Figure 3. Interestingly, our findings also indicated that in the case of *H. pylori*-infected patients with *H. pylori* antigen levels ≤ 5.0 μg/mL the risk of elevated FN for developing sever tumor features (late cancer stages (III-IV) and high tumor grades (G3-G4)) was found to yielding an estimated odds ratio of and 2.3 and 3.4, respectively. This risk was elevated in the case of high *H. pylori* antigen levels > 5.0 μg/mL with an estimated odds ratio of 5.1 and 15.4, respectively, as showed in Table 3. In fact, the aforementioned findings can indicate the role of active *H. pylori*–infection on one of ECM proteins, FN, that in turn can increase and fasting CRC patients' susceptibility for progressing severe tumor features.

Discussion

Although *H. pylori* infection is limited to the stomach, accumulating epidemiological data proved an association between *H. pylori* infection and different extra-gastric diseases (Gravina et al., 2018). Among those, a higher risk CRC has been reported to be associated with *H. pylori* infection (Zuo et al., 2020). In this study, *H. pylori* infection was found to be associated with CRC progression and *H. pylori*-

infected patients were more susceptible for developing severe tumor features than those without yielding an estimated odds ratio of 4.6 and 5.0 for late cancer stages (III- IV), and poorly differentiated tumor (high grade) [G3-G4], respectively. Moreover, not only the aspect of *H. pylori*-existence, but also bacterial load was associated with CRC development. The results showed *H. pylori* antigen levels were significantly increase (*P*=0.0001) with CRC progression. Patients with late stages (III-IV) or poor tumor differentiation (G3-G4) displayed a 7.3 or 11.5-fold increase in *H. pylori* antigen levels than those with early stages (I-II) or low grade (G1-G2), respectively.

These findings were reproduced by Sonnenberg and Genta's study who showed that *H. pylori*-positive gastritis and intestinal metaplasia, a more easily recognizable pre-malignant lesion, increased the risk for colonic neoplasms while *H. pylori*-negative gastritis did not (Sonnenberg and Genta, 2013). As well, a large recent study also investigated the association of gastric *H. pylori* presence with the risk of colorectal polyps and CRC (Wang et al., 2020). The results confirmed that patients with *H. pylori* infection were 2.19 and 3.05 times more likely to develop colorectal polyps and CRC, respectively, than those without *H. pylori* active infection.

Regarding direct effects, studies evaluating the presence of *H. pylori* in the colorectal neoplastic epithelium are still scarce. Studies using immunohistochemical methods and a PCR technique identified *H. pylori* between 22-27% in samples of polyps or CRC fragments (Soylu et al., 2008). Bacterial adherence to a cell can trigger a cascade of events where adhesins can act as biological effector molecules (Clyne and Drumm, 1993). One study showed that some components of the cell wall of *H. pylori* itself can be carcinogenic to the colorectal epithelial cell lining (Soylu et al., 2008).

Interestingly, it has been hypothesized that *H. pylori* can be directly involved in CRC carcinogenesis; the increased level of serum gastrin promoting epithelial cell growth and proliferation, and hypochlorhydria that might lead to bacterial overgrowth in the gastrointestinal tract and alterations in the colonic microenvironment of the bacterial flora (Coelho et al., 2018; Kanno et al., 2009). Additionally, it had been suggested that *H. pylori* virulence factors including vacuolating cytotoxin A (vacA), Helicobacter cysteine rich protein C and cagA, were significantly associated with increased risk of developing CRC. All these factors may contribute to colonic carcinogenesis . It has been speculated that vacA toxin able to exert, outside the stomach, its effects on cellular vacuolation, cellular permeability, interference with

cellular pathways, in addition to immunomodulatory and pro-inflammatory properties (Chauhan et al., 2019; McClain et al., 2017). One recent suggestion is that vacA forms chloride channels that become inserted into the cell and mitochondrial membranes thereby reducing the membrane potential and mitochondrial energy production, interfering on cell proliferation control. Therefore, it would be biologically plausible that the vacA toxin of *H. pylori* could increase the risk of colon cancer, by chronically altering ionic equilibrium enterocytes exposed to the toxin (Ponzetto and Figura, 2019). Regarding the virulent factor cagA, which requires direct contact between bacteria and host cells, a Japanese study suggested that exosomes containing cagA were detectable in the blood of cagA-positive *H. pylori*-infected individuals and could facilitate the development of multiple extra-gastric diseases. Because cagA is a bacterial oncoprotein, exosome-mediated cagA delivery may also be involved in the development of neoplasias outside the stomach (Shimoda et al., 2016).

Definitive evidence has indicated a strong contribution of *H. pylori*-induced changes in microbiota to the tumor phenotype, and suggests, that *H. pylori*-induced carcinogenesis in the small intestine and colon is a multifactorial process involving the interplay of the pro-inflammatory immune response, alterations in microbiota of the lower gastrointestinal tract, favors mucus-degrading microbiota and induce pro- carcinogenic signaling. Experimental studies with atrophic gastritis patients demonstrate that acid secretion reduction induces colorectal microbiota changes, intestinal bacterial overgrowth, and may favor carcinogenesis (Dash et al., 2019).

On the other hand, it is also well known that the major pro-inflammatory response towards *H. pylori* consists of a mixed T helper (Th)1 and Th17 response (Shi et al., 2010), and is to a large extent leads to chronic inflammation and results in the activation of pro-inflammatory signaling pathways which are major drivers of *H. pylori*-induced gastric carcinogenesis (Mejías-Luque et al., 2017). Interestingly, in the intestinal and colonic epithelium, *H. pylori* induced pro-carcinogenic STAT3 signaling and a loss of goblet cells, changes that have been shown to contribute to combination with pro-inflammatory and mucus degrading microbial signature to tumor development. Similar immune and epithelial alterations were found in human colon biopsies from *H. pylori*-infected patients (Ralser et al., 2023)

Another part of this work was dedicated to investigating the impact of *H. pylori* infection on the circulating levels of Fibronectin in patients with different CRC stages. Of note, fibronectin is a large glycoprotein found in body fluids, on the surfaces of

cells and in the ECM. Interestingly, there is a growing number of studies that demonstrate the role of FN in CRC progression.

Irrespective to the presence of *H. pylori*, CRC patients with severe tumor features had significantly higher FN concentration compared to those with early stages or low grades. Subsequently, in respect to *H. pylori* infection, CRC patients with *H. pylori* infection had a 1.3–fold increase in FN level than those without *H. pylori*. Additionally, late-stage CRC patients who infected with *H. pylori* had the highest FN levels. Interestingly, these elevated FN levels in *H. pylori* infection patients may indicate their increased susceptibility for tumor progressing faster than those without

H. pylori. Moreover, not only the existence of *H. pylori* but also our findings indicated that high *H. pylori* antigen levels were associated with elevated FN levels. Furthermore, the risk of elevated FN for developing sever tumor features (late cancer stages (III-IV) and high tumor grades (G3-G4)) was increased with elevated *H. pylori* antigen yielding an estimated odds ratio of 5.1 and 15.4.

It is generally accepted that ECM plays an important role in cell adhesion, migration, differentiation, and proliferation. In healthy tissue, the ECM is not exposed. However, after tissue trauma it becomes exposed and accessible for interaction with bacteria (Hennig et al., 2005). It is also recognized FN to be the target for many bacterial proteins, which are generally considered to function as bacterial adhesins (Henderson et al., 2011). It is widely accepted that *H. pylori* adheres to receptors in the gastric epithelium by means of specific adhesins. VacA may interact with FN and influence integrin receptor-induced cell signaling and cytoskeleton-dependent cell functions (Hennig et al., 2005). Microbial adhesion to ECM components may retard bacterial clearance by peristaltic flow or other physical forces. Second, it may help organisms to penetrate the intercellular junctions of epithelial cells (Dubreuil et al., 2002). Third, binding of pathogens to ECM components, particularly FN, may facilitate host cell invasion and persistent infection (Schwarz-Linek et al., 2003).

Conclusion

The aforementioned findings could indicate the role of *H. pylori* infection on enhancing extracellular-matrix deposition, which subsequently could increase the susceptibility of CRC patients for developing severe tumor features.

Acknowledgments

This work has been completely carried out at the Biotechnology Research Center, New Damietta, Egypt.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author's contributions

All authors have made a substantial, direct, and intellectual contribution to the work. Mohamed El-Far, Abdelfattah M. Attallah, Khaled Farid and Mohamed S. Albannan were involved in the supervision of the study. All authors contributed to the final manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

The 1975 Helsinki Declaration's ethical principles were followed by the study protocol. This study was authorized by the Mansoura University Hospitals' ethical and scientific committees (Code Number: MS.21.07.1595).

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Table 1: Clinical characteristics of participants (n=285)

Control Variables	**(no= 75)**	**Colorectal cancer (no=285)**	***P* value**
Age (Years)	46.7 ±4.4	50.7±11.8	0.129
Gender (male/female)	38/37	161/124	0.434
Haematology data			
† Hb (g/dL)	12.4±1.6	11.5±0.6	0.007
† RBC ($\times 10^{12}$/L)	4.4±0.6	4.3±0.6	0.661
† WBC ($\times 10^{9}$/L)	4.6 (4.3-6.7)	8.1 (5.5-9.5)	0.00

Neutrophil ($\times 10^9$/L)	3.5 (2.5-5.5)	5.5 (3.6-7.2)	0.032
Lymphocyte ($\times 10^9$/L)	2.1 (1.6-3.6)	1.6 (1.2-2.1)	0.004
Platelet count ($\times 10^9$/L)	294.5 (289-358.0)	276.0 (176.0-324.0)	0.133
Inflammatory markers			
† NLR	1.6 (1.2-4.5)	3.4 (1.8-5.7)	0.004
† PLR	159.0 (97.8-231.0)	152.6 (100.1-229.6)	0.389
Tumor markers			
† CEA (U/L)	0.9 (0.5-1.7)	11.8 (2.9-88.0)	<0.001
† CA19.9 (U/L)	6.1 (1.6-20.3)	31.8 (9.4-163.0)	<0.001

Normally distributed data were expressed as mean± standard deviation (SD) while non-normally distributed data were shown as median (interquartile range).

†Abbreviation: Hb: hemoglobin; RBC: red blood cell; WBC: white blood cell; NLR:

neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; CEA: carcinoembryonic antigen; CA19.9: cancer antigen 19.9. $^{*}P$ >0.05 is considered non-significant while P <0.05 is considered no significant.

Table 2: Influence of *H. pylori* existence on different laboratory parameters and disease progression

Variables	*H. pylori* infection		*P* value*
	Non-infected (n=96)	Infected (n=189)	
Age (Years)	50.9±12.3	48.3±10.3	0.086
Gender (male/female)	57/39	104/85	0.490
Hematology data			
† Hb (g/dL)	12.6±1.7	12.4±1.8	0.822
† RBC ($\times10^{12}$/L)	4.2±0.7	4.3±0.6	0.774
† WBC ($\times10^{9}$/L)	5.1 (4.6-8.2)	8.3(7.0-9.9)	< 0.0001
Neutrophil ($\times10^{9}$/L)	3.1 (2.4-4.8)	6.1 (5.2-8.2)	< 0.0001
Lymphocyte ($\times10^{9}$/L)	1.9 (1.5-2.6)	1.3 (1.0-2.0)	0.019
Platelet count ($\times10^{9}$/L)	241.0 (185.0-318.0)	280.0 (180.0-358.0)	0.789
Inflammatory markers			
† NLR	1.5 (1.2-3.2)	4.4 (3.2-6.7)	< 0.0001
† PLR	146.6 (90.5-200.8)	162.6 (103.8-287.1)	0.058
Tumor markers			
† CEA (U/L)	5.9 (1.1-22.3)	60.5 (4.4-137.5)	0.003
† CA19.9 (U/L)	15.4 (11.0-48.9)	132.0 (6.4-255.5)	0.031
Histological disease progression			
Early stages (I-II)/ late stages (III-IV)	66/30	61/128	< 0.0001
Low grades (G1-G2)/ high grades (G3-G4)	79/17	91/98	< 0.0001

Normally distributed data were expressed as mean± standard deviation (SD) while non-normally distributed data were shown as median (interquartile range). Significant difference was assessed by student *t*-test or Mann–Whitney *U* test as appropriate for continuous variables and X^2 test for categorical variables. †Abbreviation: Hb: hemoglobin; RBC: red blood cell; WBC: white blood cell; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; CEA: carcinoembryonic antigen; CA19.9: cancer antigen 19.9. *P >0.05 is considered non-significant while P <0.05 is considered no significant.

Table 3: Impact of elevated fibronectin level on the colorectal cancer progression in relation to *H. Pylori*-antigen concentration

Categories	Low level of *H. Pylori*		High level of *H. Pylori*	
	Odd ratio	95% CI	Odd ratio	95% CI
Advancement of CRC stages				
III-IV/I-II	2.3	1.1-4.8	5.1	2.2-11.6
Poor CRC grade development				
G3-G4/G1-G2	3.4	2.2-21.5	15.4	5.4-43.8

The adjusted odds ratio (with 95% confidence intervals (CI)) was calculated from a 2×2 contingency table to determine the likelihood risk of advanced tumor stage and grade. The cutoff points of both fibronectin and *H.Pylori* antigen levels were chosen based on receiver-operating characteristic curve.

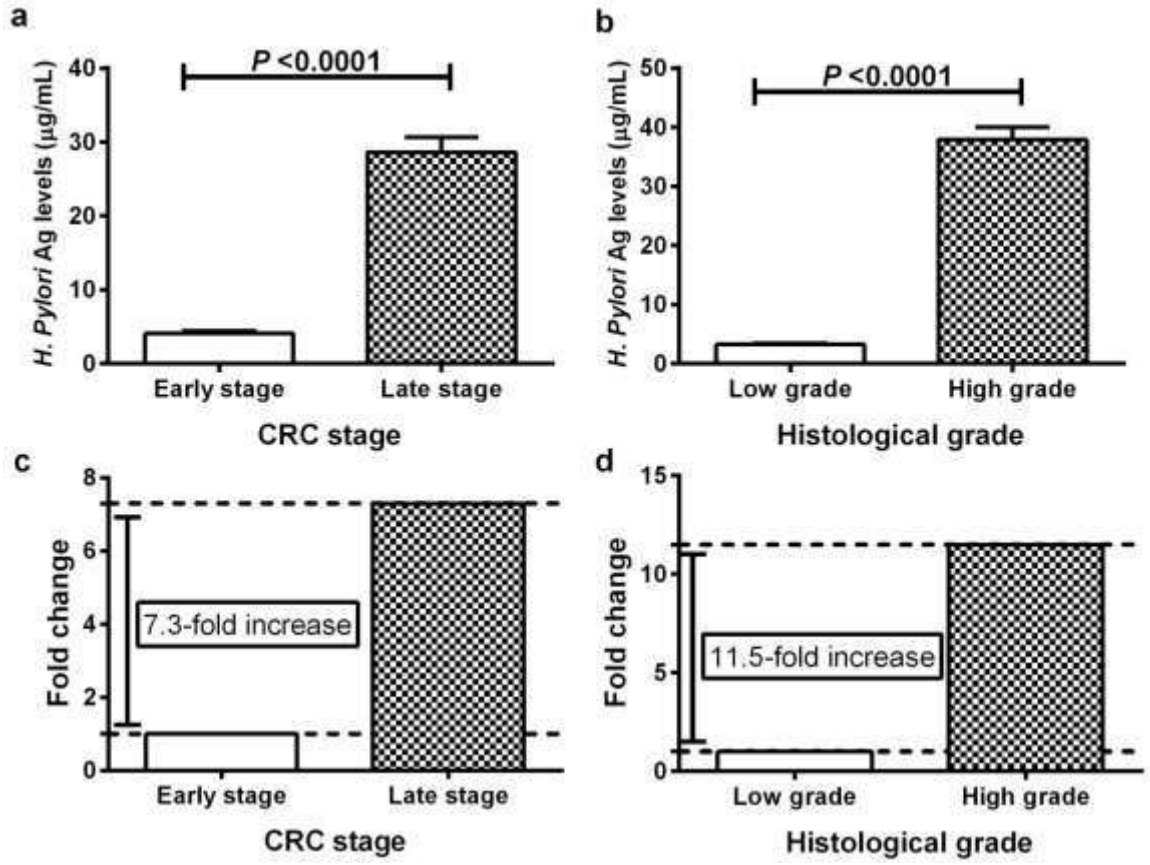

Figure 1. The association between *H. pylori* antigen level and colorectal cancer progression. (**A**): Distribution of *H. pylori* antigen level in accordance to cancer stage and **(B):** histological tumor grade. Observed fold changes of *H. pylori* antigen between

(C) : patients with early cancer stages (I-II) versus those with late stages (III-IV) and

(D) : patients with low tumor grades (G1-G2) versus those with high grades (G3-G4).

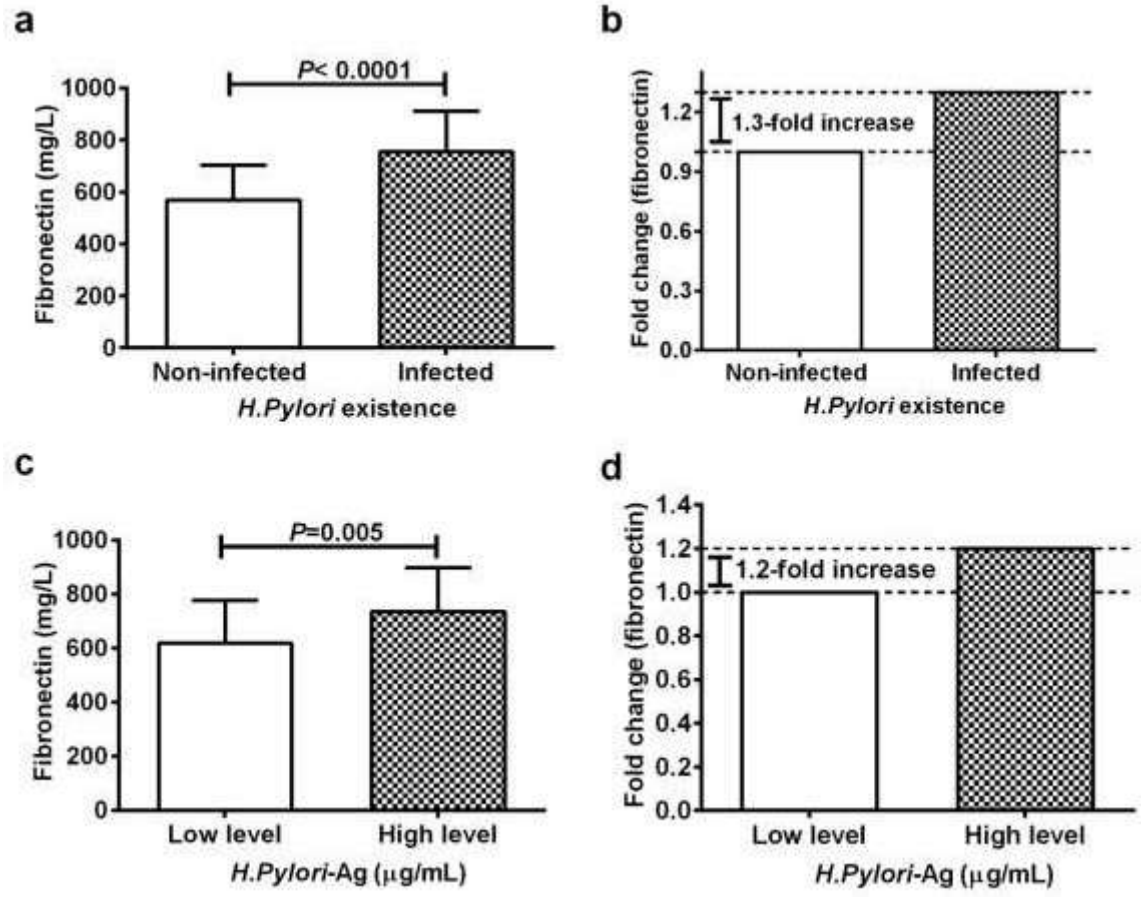

Figure 2. The impact of *H. pylori* existence on fibronectin. Distribution of (**A**): fibronectin level and (**B**): its observed fold change in *H. pylori*-infected patients compared to those non-infected. (**C**): The distribution of fibronectin levels and (**D**): its observed fold change among *H. pylori*-infected patients in relation to *H. pylori* antigen concentration. Low *H. pylori* antigen (≤ 5.0 μg/mL), high *H. pylori* antigen level (> 5.0 μg/mL).

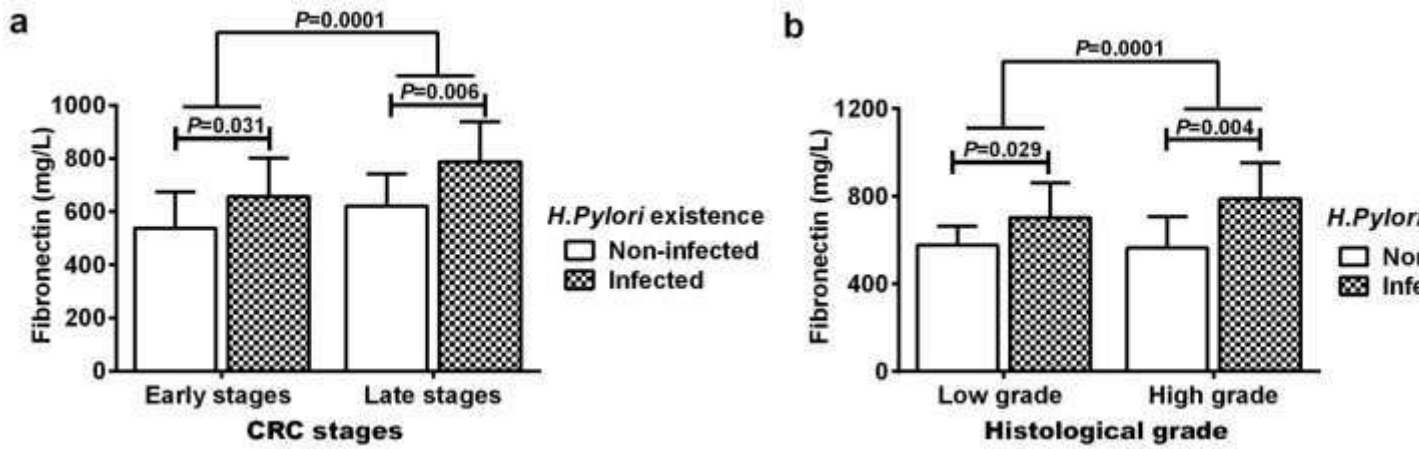

Figure 3. Distribution of fibronectin level in colorectal cancer patients with and without *H. pylori* infection according (**A**): cancer stages and (**B**): tumor histological grades.