



## IMPACT OF INFECTION WITH *HELICOBACTER PYLORI* ON INTERLEUKIN-10 MRNA EXPRESSION IN STOMACH MUCOSA

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*The immuno-regulatory cytokine interleukin 10 (IL-10) is important in reducing the inflammatory response during H. pylori infection. The goal of our study was to explain how H.pylori infection affects mucosal IL-10 mRNA expression. This study included seventy-three patients suffering from gastroduodenal disorders admitted to Assiut University Hospitals, (35 (47.95%) were males, and 38 (52.05%) were females). Three antral biopsies were obtained from each patient during endoscopic examination. Two invasive tests (Rapid urease test (RUT) and polymerase chain reaction (PCR)) were performed for the detection of H. pylori infection in all participants. Real time PCR (RT-PCR) was performed for determination of IL-10 mRNA expression. Results of RUT and PCR indicated that 60.27% of patients were H. pylori infected. Our results showed that patients positive for H. pylori had significantly higher IL10 mRNA expression than H. pylori-negative patients. Finally, overexpression of IL-10 during H. Pylori infection may be implicated in the downregulation of excessive immune response.*

**Keywords:** *H. pylori, interleukin-10, rapid urease test, and real time PCR*

### INTRODUCTION

The spiral, gram-negative, microaerophilic bacterium *Helicobacter pylori* (*H. pylori*) colonises the human gastroduodenal mucosa. The global prevalence of *H. pylori* infection is about 50%<sup>1-3</sup>. *H. pylori* colonization in the gastric environment is the major etiological factor in the prognosis of gastroduodenal disorders, particularly gastritis and peptic ulcer disease (PUD), and is the risk factor for gastric malignancies<sup>4</sup>. Nevertheless, not all colonised patients suffer from severe gastroduodenal disorders, suggesting that the clinical prognosis of *H. pylori* infection is influenced by a number of factors, such as *H. pylori* strain diversity, host genetic factors, and environmental factors<sup>5</sup>. From within the host genetic factors,

interleukins (ILs) are of special significance. Ils are small peptide molecules that communicate the cellular components of the immune system with each other and with other systems, including the endocrine system<sup>6</sup>. The main function of ILs is the coordination of immune response<sup>7</sup>. Several studies have revealed the enhanced secretion of cytokines such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin-1, interleukin-6, and interleukin-8 during *H. pylori* infection<sup>7-10</sup>.

IL-10 is an anti-inflammatory cytokine that regulates the activation and functions of various innate and adaptive immune cells, whereby it diminishes inflammatory cytokines eruption and prevents damage to the host tissue<sup>11-13</sup>. IL-10 is excreted by many cell types of the immune system, like T and B

lymphocytes, dendritic cells, macrophages, mast cells, natural killer cells, eosinophils, neutrophils, in addition to some innate lymphoid cells.<sup>14</sup> *H. pylori* establishes various strategies to escape the immune system in gastric mucosa<sup>15</sup>. One of these strategies is the increased production of regulatory cytokines like IL-10, which plays an important role in the downregulation of inflammatory responses and thus enhances *H. pylori* persistence in the gastric environment<sup>16</sup>. It has been proven that IL-10 has the ability to dampen down the various reactions of the immune system like antigen presentation in antigen presenting cells, inflammatory cytokine production and T-cell activation<sup>17</sup>. The reduced IL-10 production is associated with enhanced intensity of gastric inflammation, which increases the risk of gastric malignancy development during *H. pylori* infection<sup>18&19</sup>.

In this context, our study aimed to determine the IL-10 mRNA expression levels in gastric biopsy specimens from patients with gastroduodenal disorders in Assiut University Hospitals and to explore the effect of *H. pylori* infection on the mucosal IL-10 mRNA expression.

## PATIENTS AND METHODS

### Patients

This study was carried out on patients who had gastroduodenal disorders undergoing upper gastric endoscopy at the endoscopy unit of Assiut University Hospitals. There was no specific age or sex limitation for patients. A full history of each patient was taken, including: name, age, sex, and previous administration of antibiotics. Inclusion criteria included all patients having gastrointestinal complaints, mainly dyspeptic symptoms (as nausea, vomiting, and epigastric pain)<sup>20</sup>. Exclusion criteria included patients who had undergone partial or complete gastrectomy, those with a previous administration of *H. pylori* treatment<sup>21</sup>, and those with a previous administration of antimicrobials within 4 weeks or stomach acid-reducing drugs within the last 2 weeks<sup>22</sup>.

### Collection of Gastric biopsies

During endoscopic examination, three gastric biopsy specimens from the antrum

region were obtained from each participant. The first biopsy specimen was used for RUT, the second was put in normal saline and stored at -70 °C for PCR<sup>23</sup>, and the third was kept in an eppendorf tube containing RNA-later solution and preserved at -70 °C for RT-PCR<sup>24</sup>.

### Rapid urease test

*H. pylori* urease activity in gastric biopsy specimens was detected immediately by using AMA RUT (AMA Co Ltd, Russia)<sup>25</sup>. According to the protocol supplied by the manufacturer, a change in colour around the biopsy on the slide within 3 minutes from yellow to red indicates a positive test.

### DNA extraction and PCR

According to the manufacturer's instructions, DNA was extracted using the Genejet DNA purification kit (Thermo Fisher Scientific Inc, USA)<sup>26</sup>. PCR detection of *H. pylori* was performed by amplification of the glmM gene using the glmM F primer, (5'-AAGCTTTTAGGGGTGT TAGGGGTTT-3') and glmM R primer, (5'-AA GCTTA CTTTCTA AACTAA CGC-3')<sup>27</sup>. The PCR product was 294 bp. PCR amplification reaction was done in a volume of 25 µl containing 1µl forward and reverse primers, 5µl template DNA, and 10 µl of 2X of Master Mix (Bioline, UK). A thermal cycler (Biometra, Germany) was used for performing the reaction under the following conditions; 94°C for 5 minutes initial denaturation, 40 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute and 72°C for 10 minutes final extension<sup>27</sup>. A patient was considered as *H. pylori* infected if the bacteria were detected by RUT and/or PCR and considered as uninfected when the bacteria were negative in two diagnostic tests.

### Extraction of total RNA and semiquantitative RT-PCR

Total RNA was extracted from preserved samples using the Genejet RNA purification kit (Thermo Fisher Scientific Inc., USA), as directed by the manufacturer<sup>28</sup>. A NanoDrop® ND1000 spectrophotometer was used to quantify RNA concentration<sup>29</sup>. After RNA extraction, 1 µg of extracted material was used for cDNA synthesis by random hexamers and the Revert Aid First Strand cDNA Synthesis kit

(Thermo Fisher Scientific Inc., USA) according to the protocol supplied by the manufacturer<sup>30</sup>.

The relative expression level of IL-10 mRNA was measured by RT-PCR utilising an ABI Prism® 7500 system (Applied Biosystems, Foster City, CA, USA). As a control gene, the B-actin gene was employed. The B-actin primer sequences were: B-actin R-5' CGGATGTCCACGTCACAC and F-5' TGCCCTGAGGCACTCTTC 3'<sup>31</sup>. IL-10 F -5' ACGGCGCTGTCATCGATT 3' and IL-10 R -5' GGCATTCTTCACCTGCTCCA 3' were the primer sequences for IL-10<sup>32</sup>. The RT-PCR assays were done in a volume of 20 µl containing 0.5 µl from each primers, 50 ng cDNA, and 10 µl of 2X HERA SYBR Qpcr master mix (Willowfort,UK). The following were the reaction conditions: Initial denaturation at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute, followed by a dissociation step at 95°C for 15 seconds, 60°C for 30 seconds, and 95°C for 15 seconds<sup>31</sup>.

### Statistical analysis

For entering and analysing data, we used SPSS version 23. Demographic and clinical data of patients were expressed as numbers and percentages. Chi-square test was used to compare *H.pylori* infection in different groups. The  $2^{-\Delta\Delta Ct}$  relative quantification method was used to quantify the relative IL-10 mRNA expression normalized to  $\beta$ -actin. Data of IL-10 mRNA expression were reported as mean  $\pm$  standard error of the mean (SEM) and analyzed using the Graph Pad Prism 7. The expression of IL-10 mRNA in different groups was compared using the Student t-test. When the p-value was  $\leq 0.05$ , it was considered significant.

## RESULTS AND DISCUSSION

### Results

#### *H. pylori* infection and patients' distribution according to age, sex, and endoscopy finding

A total of 73 patients examined by upper endoscopy were studied in this work. There were 35 men (47.95%) and 38 women (52.05%) among the patients. The average age of the studied patients was  $42.26 \pm 13.45$  years and the age range was 18.0–74.0 years. Based on endoscopy findings, the patient group was divided into 3 subgroups depending on the type of endoscopic lesion: normal, gastritis, and PUD. The most prevalent endoscopic finding in the studied patients was gastritis, detected in 32 patients (43.8%), followed by normal in 24 patients (32.9%), and peptic ulcer in 17 patients (23.3%). According to results of *H. pylori* detection by PCR (Figure. 1) and RUT, *H. pylori* infection was positive in 60.27% of patients in our study.

#### Association of *H. pylori* infection with age, sex, and endoscopic findings of patients.

Table (1) demonstrates the prevalence of *H. pylori* infection by patient gender and demonstrates that there is no significant association between *H. pylori* infection and patient gender.

Table (2) represents the prevalence of *H. pylori* infection in different age groups. The results in the table show that the age group 18–35 years was the most infected group (65.21%). The results revealed that *H. pylori* infection and age have no statistically significant relationship.

Table (3) illustrates the frequency of *H. pylori* infection among gastroduodenal diseases and shows a marked association of *H. pylori* infection with gastritis and PUD ( $p = 0.0002$  and  $0.0003$ , respectively).



**Fig. 1:** A photomicrograph of 1.5% agarose gel showing the product of a representative PCR experiment for detection of ure C gene of *H. pylori*. lane 1=100 bp DNA ladder, lanes (2, 3,4,6,7,9,11and 12) show the PCR product of ureC gene (297 bp). Lanes (8 and 10) show no PCR product of ureC gene and represent the. Lane 5= water negative control.

**Table 1 :** *H. pylori* infection prevalence by patient gender.

Gender	<i>H. pylori</i>						P. value
	Positive		Ngative		Total		
	No.	%	No.	%	No.	%	
Female	25	65.78	13	34.22	38	100	<b>0.31</b>
Male	19	54.28	16	45.72	35	100	
Total	44	60.27	29	39.73	73	100	

*p* value was determined by Chi-square test to compare between *H.pylori* infection rate according to gender of patients.

**Table 2:** Prevalence of *H. pylori* infection in different age group of patients.

Age group	<i>H. pylori</i> infection						P-value
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	
18-35	15	65.21	8	34.79	23	100	<b>0.813</b>
36-50	19	59.37	13	40.63	32	100	
>50	10	55.55	8	44.45	18	100	
Total	44	60.27	29	39.73	73	100	

*P*.value was determined by Chi-square test to compare between *H.pylori* infection rate according to age group of patients.

**Table 3:** Frequency of *H. pylori* infection among gastroduodenal diseases .

Endoscopy finding	<i>H. pylori</i> infection						P-value
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	
Normal	6	13.60	18	62.10	24	32.90	<b>REF</b>
Gastritis	24	54.50	8	26.60	32	43.80	<b>0.0002</b>
Ulcer	14	31.80	3	10.30	17	23.30	<b>0.0003</b>
Total	44	100	29	100	73	100	

*p*.value was determined by Chi-square test to compare between *H.pylori* infection rate in gastroduodenal diseases.

#### Association between IL10 mRNA expression and *H. pylori* infection

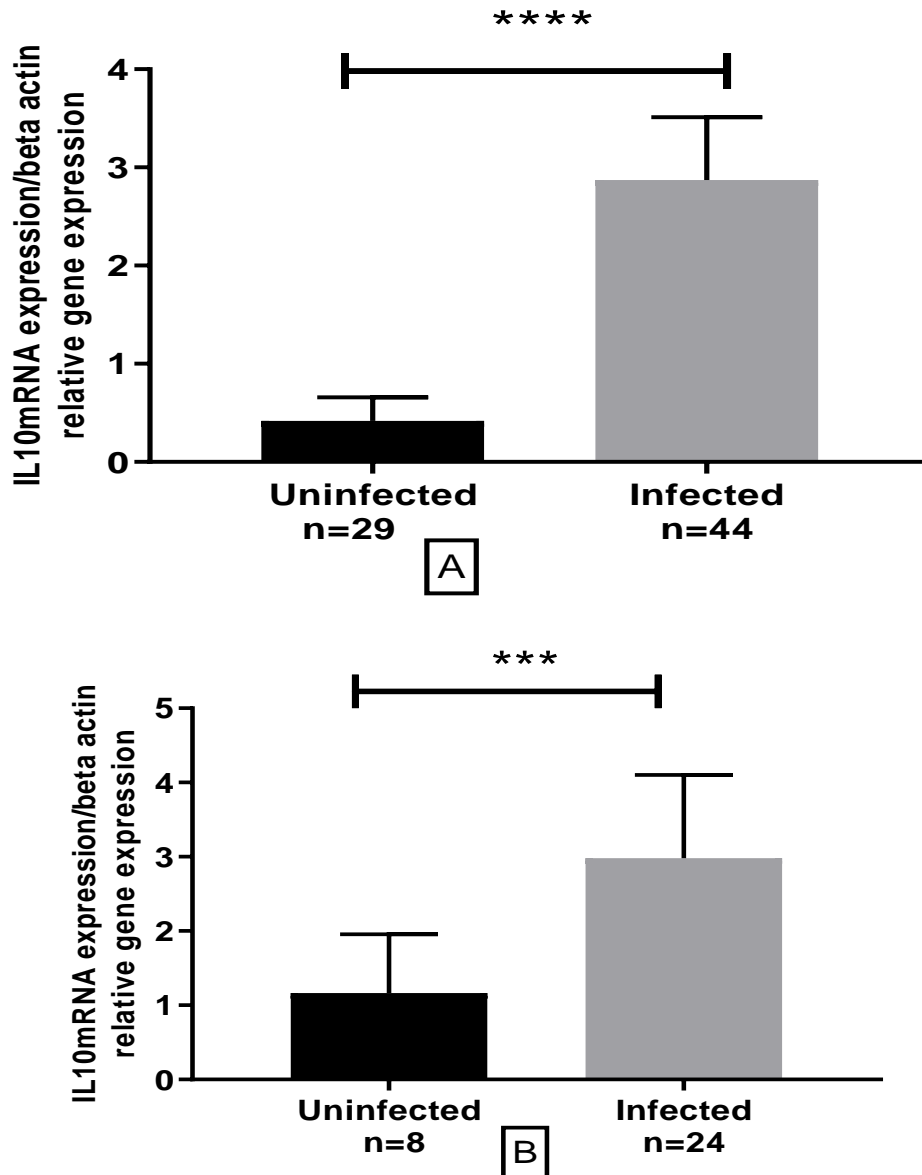
The expression of IL-10 mRNA was markedly increased in patients with *H. pylori*-infection than in patients without *H. pylori* infection (Figure 2 (A)). The change in IL-10 mRNA expression was 7-fold increased in patients with *H. pylori* infection when compared to patients without infection.

#### Association between IL10 mRNA expression and *H. pylori* infection in different endoscopic findings

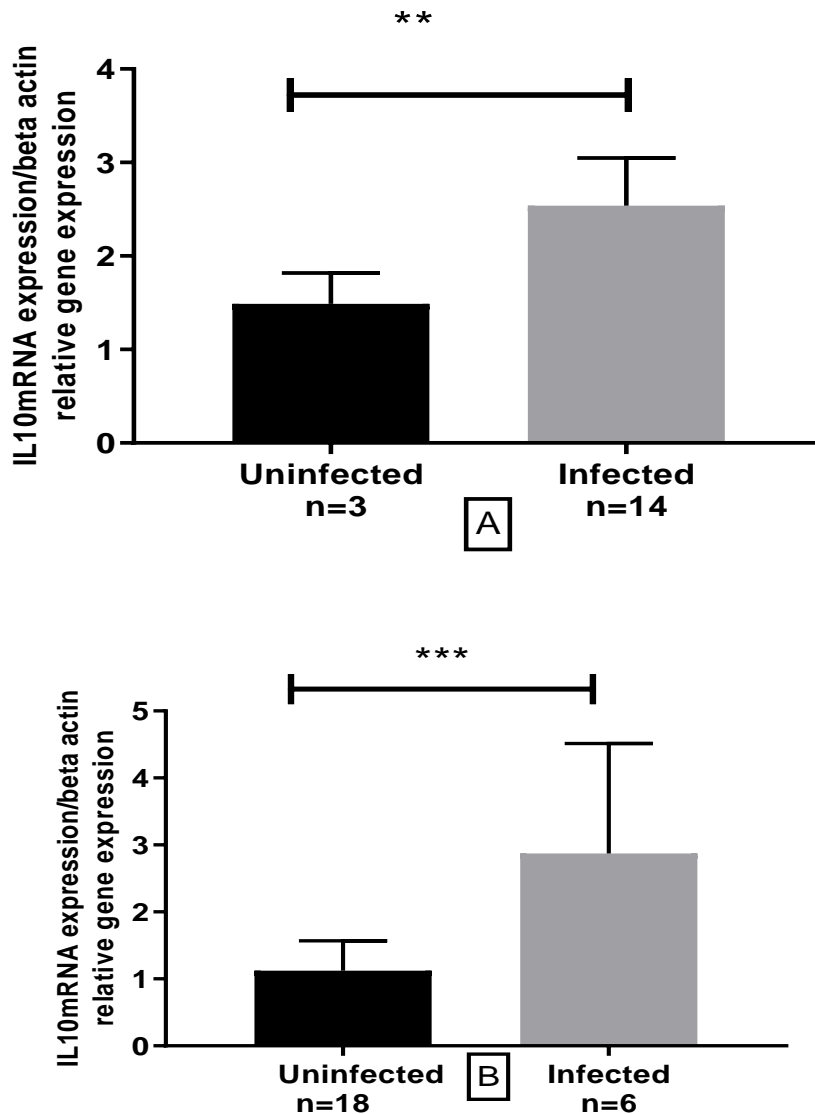
In gastritis and PUD patients, IL10 mRNA expression levels were markedly higher in antral biopsy specimens of patients with *H. pylori* infection than in patients without *H. pylori* infection (Figure 2 (B) & 3 (A)), respectively. In patients with gastritis, the

change in IL-10 mRNA expression was 2.56-fold increased in patients with *H. pylori* infection when compared to patients without infection. In PUD patients, the change in IL-10 mRNA expression was 1.78-fold increased in patients with *H. pylori* infection when compared to patients without infection. Also, in patients with normal mucosa, the expression of

IL10 mRNA was markedly increased in patients with *H. pylori* infection than in patients without *H. pylori* infection (Figure 3 (B)). In patients with normal mucosa, the change in IL-10 mRNA expression was 2.56-fold increased in patients with *H. pylori* infection when compared to patients without infection.



**Fig. 2:** (A) Mucosal IL10 mRNA expression according to *H. pylori* infection. (B) Mucosal IL10 mRNA expression of gastritis patients according to *H. pylori* infection. When the p-value was  $\leq 0.05$ , it was considered significant. A student's t-test was used to compare the IL-10 expression levels among groups. The mean  $\pm$  SEM is used to express the results. \*\*\*\* P < 0.0001, \*\*\*P < 0.001.



**Fig. 3:** (A) Mucosal IL10 mRNA expression of ulcer patients according to *H. pylori* infection. (B) Mucosal IL10 mRNA expression of normal patients according to *H. pylori* infection. When the p-value was  $\leq 0.05$ , it was considered significant. A student's t-test was used to compare the IL-10 expression levels among groups. The mean  $\pm$  SEM is used to express the results. \*\*\*\* P < 0.0001, \*\*\*P < 0.001.

### Discussion

IL-10 is an anti-inflammatory cytokine produced by the gastric epithelium during *H. pylori* infection<sup>24</sup>. IL-10 has negative effect on the production of inflammatory mediated cytokines secreted by Th1 cells such as TNF- $\alpha$  and IFN- $\gamma$ , while enhancing antibody production by B-lymphocytes. However, the primary biological effect of IL-10 is to restrict and eliminate excessive inflammatory responses<sup>33</sup>. Our work showed significantly higher expression of IL10 mRNA in the gastric

mucosa of patients with *H. pylori* infection than in patients without *H. pylori* infection. This result is consistent with the results obtained by Razavi *et al.*,<sup>34</sup> who found considerably higher levels of Foxp3+ cells, IL10, and TGF1 cytokines during *H. pylori* infection. Also, Goll *et al.*,<sup>35</sup> carried out a study on 91 patients and found IL-10 mRNA overexpression in the gastric mucosa of *H. pylori* infected patients. On the other hand, in Belgium, a study carried out on adults and children found a considerably higher level of IL-10 mRNA in children with *H. pylori*

infection than those without *H. pylori* infection, while in adult patients, there was no difference in IL-10 mRNA expression between the *H. pylori* infected group and the *H. pylori* non-infected group<sup>36</sup>. This inconsistency in IL-10 mRNA expression results during *H. pylori* infection could be attributed to the ethnic diversity of patients in different studies or to genotypic differences in *H. pylori* that affect the expression of IL-10. Additionally, single nucleotide polymorphisms in the IL-10 cytokine gene have been linked to varying amounts of IL-10 production at locations -1082, -819, and -592<sup>37</sup>.

Regarding the endoscopic findings of patients, our findings revealed a significant association between *H. pylori* infection and elevated IL10 mRNA expression in patients suffering from gastritis. Karttunen *et al.*,<sup>38</sup> found higher expression of IL-10 mRNA in *H. pylori* infected gastritis patients than in gastritis patients who received successful *H. pylori* treatment, which is consistent with our findings. Similar results were also obtained by Bodger *et al.*,<sup>39</sup> who found that IL-10 concentration was elevated significantly in *H. pylori* infected patients with chronic gastritis than in *H. pylori* negative patients with chronic gastritis.

The present work revealed that the IL-10 mRNA expression level was markedly increased in PUD patients with *H. pylori* infection when compared to PUD patients without *H. pylori* infection. Our findings coincide with those obtained by Holck *et al.*,<sup>40</sup>. In contrast, Milic *et al.*,<sup>41</sup> reported that *H. pylori* infection has no association with the mucosal IL-10 mRNA expression in bleeding duodenal ulcer patients.

The present study revealed a statistically significant association between IL-10 mRNA expression and *H. pylori* infection in patients with normal mucosa. This finding is in accordance with previous studies<sup>42-44</sup>.

### Conclusions

*H. pylori* is a common pathogen associated with many gastric diseases. *H. pylori* infection is independent of "Age and sex" of patients in our study. *H. pylori* infection is strongly associated with duodenal ulcer and gastritis. The improved expression of IL-10 mRNA in stomach mucosa during *H.*

*pylori* infection may be implicated in down-regulation of the inflammatory responses produced by pro-inflammatory cytokines and thus favour infection and *H. pylori* survival.

### Recommendations

Extended large-scale studies are required to determine the prevalence of *H. pylori* infection in the Egyptian population and to assess the role of different cytokines during *H. pylori* infection. Future studies should focus on the evaluation of the association between IL-10 expression and *H. pylori* virulence factors

### Ethical statement

The Local Ethical Committee of the Faculty of Medicine, Assiut University, gave approval to the protocol of this study (Approval No: 17200718). Informed consent was obtained from all participants before the collection of samples.

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## نشرة العلوم الصيدلانية جامعة أسيوط



### تأثير العدوى بالبكتيريا الملوية البوابية على التعبير الجيني للإنترلوكين ١٠ في الغشاء المخاطي للمعدة

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الملوية البوابية هي بكتيريا حلزونية الشكل سلبية الجرام مرتبطة بحدوث العديد من أمراض المعدة والإثني عشر. نتائج الإصابة بهذه البكتيريا تتوقف على عدة عوامل مثل العوامل البيئية، عوامل مرتبطة بالبكتيريا وعوامل مرتبطة بالشخص المصاب. هناك العديد من الأبحاث أوضحت أن العوامل الجينية التي تحكم التعبير الجيني للسيتوكينات والتي تؤثر على احتمالية الإصابة بالبكتيريا الملوية البوابية تلعب دورا هاما في آلية حدوث الأمراض المرتبطة بعدوى البكتيريا الملوية البوابية مثل التهاب المعدة وقرحة المعدة والإثني عشر. الإنترلوكين ١٠ هو إنترلوكين مضاد للإلتهاب ويؤثر على خلايا عديده في الجهاز المناعي ليقوم بعملية تنظيم عمل الجهاز المناعي .

هذه الدراسة هدفت إلى توضيح مدى تأثير العدوى بالبكتيريا الملوية البوابية على مستوى التعبير الجيني للإنترلوكين ١٠ في الغشاء المخاطي للمعدة .

اشتملت تلك الدراسة على ٧٣ مريض ممن يعانون من اضطرابات المعدة والإثني عشر وخضعوا لمنظار المعدة لأخذ عينات بذل من الغشاء المخاطي المبطن للمعدة لفحصها باستخدام اختبار اليوريز السريع وتفاعل البلمرة التسلسلي للكشف عن البكتيريا. أيضا تم إجراء تفاعل البلمرة التسلسلي الكمي لقياس التعبير الجيني للإنترلوكين ١٠ .

هذه الدراسة التي شملت ٧٣ مريض نسبة النساء كانت ٥٠,٠٥% ونسبة الرجال كانت ٤٧,٩٥% وأعمار المرضى تتراوح من ١٨-٧٤ سنة ومعدل الأعمار ٤٢,٢٦ سنة . نسبة الإصابة بالبكتيريا الملوية البوابية كانت ٦٠,٣%. لم يكن هناك أى علاقة مهمة ما بين الإصابة بالبكتيريا والعمر أو الجنس. الإصابة بالبكتيريا الملوية البوابية في هذه الدراسة مرتبطة بحدوث التهابات المعدة وقرحة المعدة .

نتائج الدراسة أوضحت ان الإصابة بالبكتيريا الملوية البوابية تزيد من التعبير الجيني للإنترلوكين ١٠ في الغشاء المخاطي المبطن للمعدة.

نستنتج من هذه الدراسة أن الإنترلوكين ١٠ تزداد مستوياته في حالة الإصابة بالبكتيريا الملوية البوابية لتهدئة ردة الفعل المناعية التي ينتجها الجسم لمحاولة التخلص من البكتيريا الملوية البوابية.