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# Helicobacter Pylori-Oncogenic Protein Cytotoxin-Associated Gene A and Assessment of CD14 and CD163 in Duodenal Ulcer and Gastric Cancer Patients

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**Abstract**--Nearly half of the world's population is infected with *Helicobacter pylori* (*H.pylori*). A duodenal ulcer or stomach cancer can be caused by the infection by this bacterium. The aim of this work is to assess the levels of CD14 and CD163 in *H.Pylori*-positive patients infected with duodenal ulcer (DU) and gastric cancer (GC) and determine the prevalence of *Helicobacter Pylori*-oncogenic protein cytotoxin-associated gene A strains (*H.pylori*-CagA). This study included 89 individuals distributed as follows: 20 healthy individuals as controls and 69 patients infected with *H. Pylori* have been divided as follows: 27 patients infected with *H.pylori* only (*H.Pylori*+), 22 *H.pylori*+DU and 20 *H.pylori*+GC. *H. Pylori*-oncogenic protein cytotoxin-associated gene A strains (*H-pylori*-CagA) were diagnosed based on a qualitative reverse-phase Enzyme Immunoassay Technique. CD163 and CD14 were measured in all individuals' serum using the Enzyme-Linked Immunoassay (ELISA) test. Out of 69 patients infected with *H.pylori*, there was one CagA strain in *H.pylori*+; two and five strains were recorded in *H.pylori*+DU and *H.pylori*+GC, respectively. CD14 and CD163 serum concentrations were significantly higher ( $P \leq 0.05$ ) in *H. pylori*+, *H. pylori*+DU and *H. pylori*+GC than in controls. **Conclusions:** Patients with CagA strains infection are at risk of developing a duodenal ulcer and stomach cancer. CD14 and CD163 may serve as potential biomarkers for assessing the severity of *H.pylori* infection. The presence of elevated levels of CD14 and CD163 might be used as an early indicator of duodenal ulcer and gastric cancer in patients infected with *H.pyloti*.

**Keywords**---CD14, CD163, CagA, DU, GC, *H.pylori*.

## **Introduction**

*Helicobacter pylori* is a spiral, motile, gram-negative bacteria that is considered one of the most common types of bacteria that infect humans at all ages due to their resistance to stomach acidity and causes many post-infection complications such as duodenal ulcers, peptic ulcer, and stomach cancer (Sun and Zhang, 2019; Jolaiya et al., 2020; Rashad and Aljanaby, 2021). Several studies have indicated that this pathogen affects about half of the population, especially in developing countries (Alhasnawi and Aljanaby, 2022a). When *H. pylori* invades the host, it causes a robust immunological response, resulting in chronic inflammation of the stomach mucosa (Wang et al., 2014; Hou et al., 2019). Innate and adaptive immunity play a pivotal role against this pathogen due to producing many kinds of immune markers such as IL-10, IL-2, CD14+, and CD163+. CD14+ is a human protein produced mainly by macrophages and monocytes, and is considered an essential part of the innate immune system (Michalkiewicz et al., 2015; Lehours and Robinson, 2020). The primary role of this CD marker is to bind with bacterial lipopolysaccharides (LPS) and pathogen-associated molecular patterns (PAMP); therefore, it has a recognizing and defense function in the host. The elevated production of CD163 (a macrophage-specific protein) is one of the primary modifications in the macrophage flip to alternate activated phenotypes in inflammation, a result, macrophages with high CD163 expression are associated with tissues reacting to inflammation (Fehlings et al., 2012; Etzerodt et al., 2013; Zhu et al., 2020). The cytotoxin-associated gene A (CagA), a 125-140 kDa protein encoded by the *cag* pathogenicity island, is the main pathological hallmark of *H. pylori* infection (*cagPAI*) (Tohidpour, 2016; Ansari et al., 2020). CagA is also the first bacterial oncoprotein, and *H. pylori*-mediated adenocarcinoma is the world's second most lethal cancer form. Within the gastric epithelial cells, CagA undergoes cytoplasmic translocation and interacts with various proteins in phosphorylation-dependent and independent ways (Suyapoh et al., 2021; Youssefi et al., 2021). The link between *H. pylori* infection and the development of duodenal ulcers and stomach cancer has been extensively researched worldwide (Vogelmann and Amieva, 2007; Alipour, 2020). Underdeveloped nations, 70 to 90 per cent of people have *H. pylori* by the age of ten, but infection rates in wealthy countries range from 25 to 50 per cent. *H. pylori* cause more than 60% of stomach malignancies. Globally, gastric malignancies account for more than 8.2% of cancer fatalities globally (Leja et al., 2019; Raza et al., 2020). This case-control study aims to determine the prevalence of (*H.pylori*-CagA) and assess the levels of CD14 and CD163 in (*H.pylori*+) infected with duodenal ulcer (DU) and gastric cancer (GC).

## **Methods**

### **Study design and patients**

This study was carried out in AL-Sader Medical City, microbiology department, endoscopy unit, and tumors department in AL-Najaf Governorate, Iraq. Twenty

seven *H. Pylori*-positive patients, 22 duodenal ulcer patients, 20 gastric cancer patients, and 20 healthy individuals as controls were included in this work.

### ***H. Pylori*-positive patients diagnosis**

Stool antigen tests have been used in the diagnosis of *H. Pylori*-positive patients with 94% sensitivity and 97% specificity with a 100% positive detection rate at 1 ng/mL of pylori antigen in stool specimens (Wang et al., 2015; Pichon et al., 2020; Alhasnawi Aljanaby, 2022b). The test was done according to the kit provided by the manufacturing company (CTK Biotech, Inc. China).

### **Diagnosis of *H. Pylori*-CagA strains**

Diagnosed based on a qualitative reverse-phase Enzyme Immuno Assay technique according to the IgG ELISA Kit provided by the manufacturing company (BT LAB, CO, Cat.NO ED1335Hu. China). The results were calculated according to the optical density (OD) as follows:  $OD_{\text{sample}} < \text{Cutoff Value} = \text{Negative}$ , and  $OD_{\text{sample}} \geq \text{Cutoff Value} = \text{Positive}$ .

### **Diagnosis of duodenal ulcers and gastric cancer**

All patients with duodenal ulcer and gastric cancer have been diagnosed by a physician using an endoscope and biopsy, respectively, in the department of tumours at AL-Sader Medical City (Figure.1).



Figure 1. Endoscopy of the duodenal ulcer in *H. Pylori*-positive patient

### **ELISA**

Five ml of blood were collected from each individual and centrifuged at 7,000 rounds per minute for 15 min. Two mL of serum have been obtained to measure the concentration of human CD16 (Cat. No E0283Hu) and human CD163 (Cat. No E0246Hu) by an ELISA test (Aljanaby et al., 2022a; Alhasnawi and Aljanaby, 2022a) according to the kits provided by the manufacturing company (BT LAB, CO. China).

## Statically analysis

Graphpad-prism V.10 computer software was used in this study. The mean standard error value of CD14 and CD163 has been measured using the T-test, unpaired, one-tailed, and significant digits at 0.05. P-values less than 0.05 were considered statistically significant (Hasan et al., 2021; Alhasnawi and Aljanaby, 2022b; Aljanaby et al., 2022a; Aljanaby et al., 2022b).

## Ethics approval

We confirmed that we obtained all ethics approvals from all individuals in this work, which includes blood and stool sample collection and all tests related to patients infected with duodenal ulcer and stomach cancer, including endoscope and biopsy.

## Results

### *H.pylori*-CagA strains

Out of total 69 *H.pylori*-positive patients, there were 8 *H.pylori*-CagA positive strains distributed as follows: one strain (3.7%) was recorded in patients infected with *H.pylori* only, two strains (9%) were found in *H.pylori*-positive patients with duodenal ulcer, and five strains (25%) were detected in *H.pylori*- positive patients with gastric cancer (Figur 2).

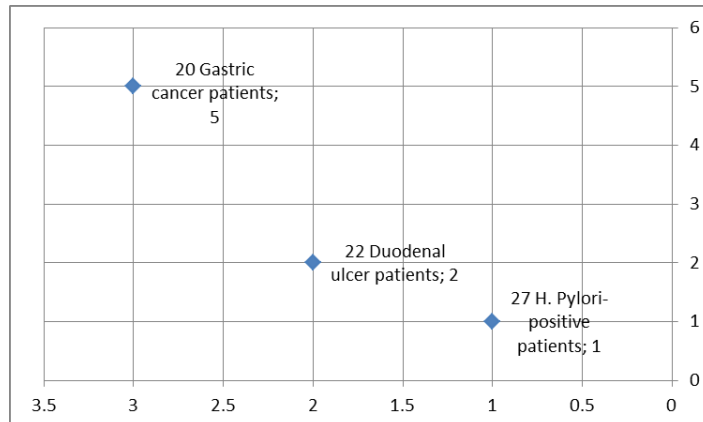


Figure 2. Total numbers of *H.pylori*-CagA strains

## CD14

Figure 3 showed that CD14 serum concentration was significantly higher ( $P \leq 0.0320$ ) in *H. pylori*-positive patients ( $1.2989 \pm 0.07$ ) than in controls ( $1.0547 \pm 0.07$ ). Also, there was a significant increase ( $P \leq 0.0036$ ) in *H.pylori*-positive patients infected with a duodenal ulcer ( $2.5630 \pm 0.45$ ) compared with control. The CD14 serum level in *H.pylori*-positive patients infected with gastric cancer ( $2.5015 \pm 0.37$ ) was significantly ( $P \leq 0.0005$ ) higher than control. On the other hand, a significant increase has been shown ( $P \leq 0.0044$  and  $P \leq 0.0008$ )

between *H. pylori*-positive patients and *H. pylori*-positive patients infected with a duodenal ulcer and infected with gastric cancer, respectively. While the results proved there were no significant differences ( $P \leq 0.9189$ ) in CD14 serum concentrations between *H. pylori*-positive patients infected with a duodenal ulcer and *H. pylori*-positive patients infected with gastric cancer.

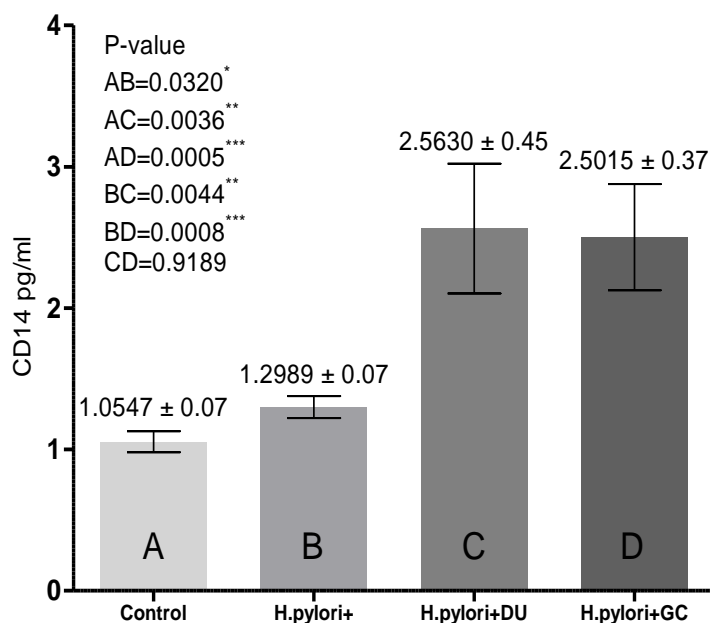


Figure 3. CD14 serum concentration in *H. pylori*-positive patients and control. DU: duodenal ulcer, GC: gastric cancer

### CD163

The CD163 serum concentration was found to be significantly higher ( $P \leq 0.0124$ ) in *H. pylori*-positive patients ( $4.5327 \pm 0.75$ ) than in controls ( $2.1930 \pm 0.17$ ). There was a highly significant increase ( $P \leq 0.0001$ ) in *H. pylori*-positive patients infected with a duodenal ulcer ( $9.6389 \pm 1.65$ ) compared with the control. The CD163 serum concentration in *H. pylori*-positive patients infected with gastric cancer ( $8.5502 \pm 1.55$ ) was significantly higher ( $P \leq 0.0002$ ) than control. A significant increase has been shown ( $P \leq 0.0046$  and  $P \leq 0.0157$ ) between *H. pylori*-positive patients and *H. pylori*-positive patients infected with a duodenal ulcer and infected with gastric cancer, respectively. On the other hand, the result demonstrated no significant difference ( $P \leq 0.6461$ ) between *H. pylori*-positive patients infected with a duodenal ulcer and *H. pylori*-positive patients infected with gastric cancer (Figure 4).

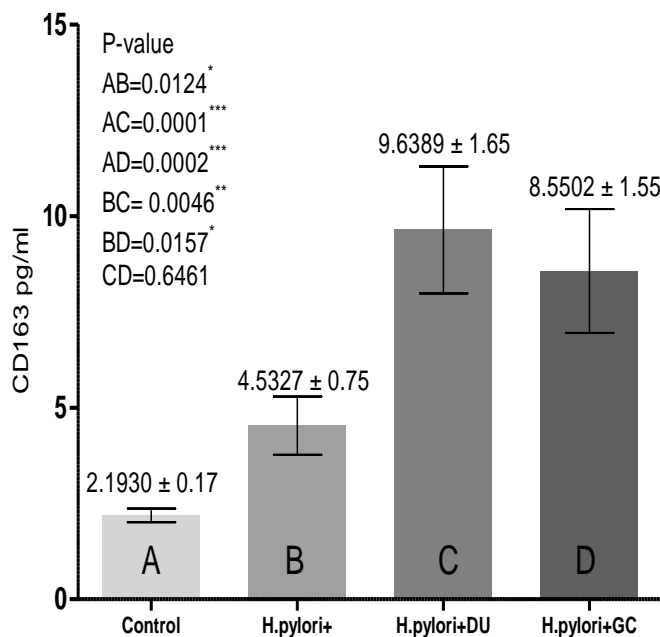


Figure 4. CD163 serum concentration in *H.pylori*-positive patients and control. DU: duodenal ulcer, GC: gastric cancer

## Discussion

Since its *H.pylori* infection is labile, meaning it changes based on the acid levels in the stomach; therefore, when Marshall inhibited acid in his stomach before swallowing *H.pylori*, the illness vanished two weeks later when acid secretion had returned to normal spontaneously (Marshall et al., 1985; Hobsley et al., 2008). The results of the current study proved that the prevalence of *H.pylori*-CagA strains was higher in *H.pylori*-positive patients with gastric cancer (25%) than in *H.pylori*-positive patients with duodenal ulcer (9%) while there was 3.7% in patients infected with *H.pylori* only. Our findings agree with many researchers who demonstrated that *H.pylori*-CagA strains were more prevalent in East Asia and there was positive correlation between *H.pylori*-CagA strains infection and gastric cancer (Satomi et al., 2006; Azuma, 2004; Nowińska and Dziegiel, 2010; Myint et al., 2018; Shetty et al., 2021; Nguyen et al., 2021). A cytotoxin-associated gene pathogenicity island (cagPAI) type IV secretion system (T4SS) is found in most but not all *H. pylori* strains (Viala et al., 2004). The *tfs4* gene cluster, which encodes another T4SS, contains duodenal ulcer promoting gene A (*dupA*) (Pachathundikandi et al., 2019). More severe gastritis and increased mucosal IL-8 expression have been associated with *dupA*-positive strains. Disease correlations have varied according to reports from throughout the world. Some research found links between duodenal ulcers and stomach cancer, but others could not substantiate these findings (Lee et al., 2013; Outlioua et al., 2020; Saaed et al., 2021). Among the most common causes of gastritis is *H. pylori* infection; gastric cancer may develop eventually due to this illness (Wang et al., 2013; Amieva and Peek, 2016; Serena et al., 2018). As a fact, *H.pylori* is

responsible for about 75% of duodenal ulcers, 17% of gastric ulcers and many cases of gastric cancer (Ford et al., 2020). Therefore, it is classified as a human carcinogen (Robinson and Atherton, 2021). CagA is *H.pylori* primary virulence factor, closely associated with gastric inflammation and carcinogenesis (Hatakeyama, 2014; Xu et al., 2020). Less than 3% of *H. pylori*-positive patients develop gastric cancer, suggesting that there is a unique way of interaction between *H. pylori* virulence factors and the host's immune response that is still not fully understood (Sukri et al., 2020). Our study found that CD14 and CD163 serum levels increased in *H.pylori*-infected patients as compared with *H.pylori*-negative individuals; this is consistent with previous observations (Fehlings et al., 2012; Rudnicka et al., 2013; Hou et al., 2019). It is well known that *H. pylori* triggers a TH-1 immune response in the host, and that M1-like monocytes can produce pro-inflammatory cytokines, triggering a TH-1 immune response (Quiding-Järbrink et al., 2010; Moyat et al., 2015; Zhang et al., 2018; Hou et al., 2019). Several layers of protective mucus cover the gastric mucosa, such as mucins secreted by cells (Padra et al., 2019). *H. pylori* adhere to mucins through the interaction of the bacterial protein blood group antigen-binding adhesion with disulfide-linked Lewisb blood group antigens (Radziejewska et al., 2014). Many types of cytokines are released by gastric epithelial cells in response to the *H.pylori* infection, including IL-6, IL-8, tumor necrosis factor  $\alpha$ , IL-1 $\beta$ , and IL-12 (Cook et al., 2014; Chonwerawong and Ferrero, 2017). Neutrophils, B and T lymphocytes, natural killer cells, natural killer T cells, macrophages, mast cells, and dendritic cells are all attracted to these cytokines, which cause the production of reactive oxygen and nitrogen species (White et al., 2015). Higher expression of pro-inflammatory genes due to host gene polymorphisms is linked to an increased risk of illness (Zhuang et al., 2015; Zhang et al., 2016). Increased creation of reactive oxygen and amplified inflammatory damage are both caused by dysregulation of autophagy, a mechanism for eliminating damaged cells (Eslami et al., 2019).

Human stomach colonization by *H.pylori* and its role in causing gastric cancer is one of the finest examples of the complex relationship between human cells, microbes, and their environment, and this is also an issue of enormous medical significance due to the world's widespread incidence and lethality of gastric cancer (Amieva and Peek, 2016; Lee et al., 2016; Sugano, 2019). Evidence indicates that more detailed interaction between host and pathogen genotypes can change the risk of gastric cancer; on the bacterial side, the cag pathogenicity island (PAI) is a well-studied and well-characterized virulence determinant of *H.pylori*; strains containing the cag PAI are associated with a higher risk of distal gastric cancer than those strains lacking this virulence factor (Amieva and El-Omar, 2008; Polk and Peek, 2010). Human genetics has shown that specific polymorphisms in genes encoding inflammatory cytokines can significantly increase the risk of gastric cancer among patients infected with *H.pylori* CagA strain (Waskito et al., 2018; Saniee et al., 2021).

## Conclusions

Patients with *H.pylori*-CagA infection are at risk of developing a duodenal ulcer and stomach cancer. CD14 and CD163 may serve as potential biomarkers for assessing the severity of *H.pylori* infection. The presence of elevated levels of

CD14 and CD163 might be used as an early indicator of duodenal ulcer and gastric cancer in patients infected with *H.pyloti*.

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