ARTICLE / INVESTIGACIÓN

Histological changes and diagnostic value of immunoglobulin G/M to *Helicobacter pylori* in gastric cancer patients

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Abstract: Stomach cancer is believed to be one of the most common cancers that lead to death. In Iraq, stomach cancer occupies the seventh place of cancer occurrence in both sexes and is counted as one of the ten most common cancers. The current study is designed to explore the link between *Helicobacter pylori* (*H.pylori*) infection and the development of incidences of stomach cancer. In addition, related age and gender were also studied. Histological examinations of stomach biopsies were performed in suspected people to evaluate stomach cancer occurrences. Of the 40 patients with stomach cancer, the infection of *H. pylori* was emphasized in 34 (66.66%) with serum IgG/IgM, which reflected a significant frequency for infection of *H. pylori* in stomach cancer patients. The study also showed that males with *H. pylori* infection record a higher percentage than female patients with stomach cancer. Moreover, the results revealed that age is also connected to *H. pylori* infection. Based on the above findings, monitoring infected people with *H. pylori* might be an excellent strategy to control stomach cancer occurrences.

Key words: Stomach cancer, Infectious diseases, IgG, IgM, *H. pylori*.

Introduction

Stomach cancer, in general, is considered one of the world's most significant public health problems and the most prominent challenges facing the scientific community in the current century due to the high rates of infection and mortality of cancer. Stomach cancer is one of the most common cancers that can lead to death and is also shown to be connected with cancer worldwide[1,2]. The development of this type of cancer is linked to several risk factors, and the incidence of this disease lags by age, socioeconomic conditions, and geographical location[3]. Indeed, stomach cancer is ranked seventh with 3.67% among all other body cancers. Carcinoma of the gastric adenocarcinoma accounts for 90–95% of all other gastric carcinomas, and in Lorraine 1965, gastric carcinomas can progress to the intestinal type[4].

Stomach cancer is the fifth most common, after lung, colon, and prostate cancer. It is the third cancer leading to deaths worldwide, at the end of 782,685 cases, or 8.2%. With the changes in the International Agency for Research on Cancer of the World Health Organization (World Health Organization WHO Soba), nearly one million good cases of stomach cancer are recorded5 annually. Gastric adenocarcinoma of the epithelial area is 90-95% of other stomach cancer[6,7].

Many environmental and genetic factors are linked to stomach cancer development, all of which can play a role in stomach cancer[8]. For instance, cigarette smoking and drug abuse[9,10], Nutritional factors[11,12], Obesity[13], and occupational exposure[14]. Moreover, other potential risk factors that might increase the risk of stomach cancer incidences are gastric surgery, gastroesophageal reflux disease, physical inactivity, non-steroidal anti-inflammatory drugs (NAIDs) and exposure to radiation[15-17]. The infection of *H. pylori* might be a risk factor connected to stomach cancer development. In 1994, the International Agency for Research on Cancer classified *H. pylori* among the causes of cancer of the class or first degree (Class 1 carcinogen). *H. pylori* is a gram-negative, air-loving, motile bacterium with 4-6 flagella helping penetrate the gastric mucosa of both the stomach and duodenum[18]. *H. pylori* infection rates increase with age due to the increasing cumulative effect, and the development of stomach cancer in those people might be connected to *H. pylori* infection[19-22]. It has been established that infection with *H. pylori* is accompanied by a decrease in hydrochloric acid (HCl) secreted by parietal cells, creating a suitable environment for survival. After *H. pylori* enters the stomach cavity, it secretes the urease enzyme that divides the urea compound into ammonia (NH3) and carbon dioxide (CO2)[23]. These components provide an alkaline environment, which leads to surrounding them with an alkaline medium equivalent to gastric acidity. Accordingly, they are not affected by stomach acid like other types of bacteria; in addition to this enzyme, they produce many different enzymes, such as catalase enzyme[24].

H. pylori infection and taking (NAIDs) are the most common cause of gastric ulcers. Gastric ulcers occur in the surface epithelium, extend to the mucous layer in the stomach wall, and, over time, can infect the peritoneum. Several studies have documented that people with gastric ulcers and duodenal ulcers have a higher risk of developing stomach cancer[25]. Therefore, monitoring people with *H. pylori* infection might pave the way to control stomach cancer.

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Materials and methods

Study design

The current study was performed in the Thi-Qar province/ southern Iraq. The ethical approval for this study was obtained from the Al-Nassrya Health Department.

The present study included 51 patients with stomach cancer who had undergone total or partial gastrectomy at the Cancer Center of Al-Hussein Teaching Hospital of the Dhi-Qar Health Department. Diagnosis of stomach cancer was performed by the specialist medical staff at Al-Hussein Teaching Hospital based on clinical examinations and stomach biopsy. Furthermore, serum IgG/IgM sensitivity was performed to detect *H. pylori* infection.

Histological examinations

Diagnosis of stomach cancer was done by taking total or partial gastrectomy of suspected people with stomach cancer. (4%) formaldehyde fixed tissue samples were subjected to serial dehydration and paraffin embedding. Then, (H&E) stained samples were evaluated under light microscopy. The evaluations of stomach cancer grading were done according to standard protocol[26].

Estimation of IgG/IgM sensitivity of H. pylori

H.pylori was identified using the *H. pylori* antibody (IgG/IgM) diagnostic Kit (RC11, ANAMOL, Kolgaon) and based on the manufacturer's instructions. The test line was provided with anti-IgG and anti-IgM, and the purple color on the test line was used to indicate positive *H. pylori*.

Statistical analysis

Statistical tests were done using the Chi-square test, and data were introduced as a percentage. $P < 0.05$ is considered statistically significant between the groups.

Results

Histological study

For stomach cancer diagnosis, a stomach biopsy was carried out in suspected people with stomach cancer. Histological examinations showed a clear-cut destruction in the architectures of harvested tissue samples. Variations in the size and shape of the malignant cells characterized these destructions. For example, the histological results showed pleomorphic irregular cells and their infiltration and penetration to different degrees within the layers of the stomach wall, as shown in (Figure 1). As for the histological pattern, the results of the current study show that epithelial adenocarcinoma is the most common type.

Age group

In the present study, we found that the age group (≥ 50 years old) recorded a remarkable increase in *H.pylori* infection of patients with stomach cancer, which represented 79.41% (Figure 4), compared to the age group (30-39

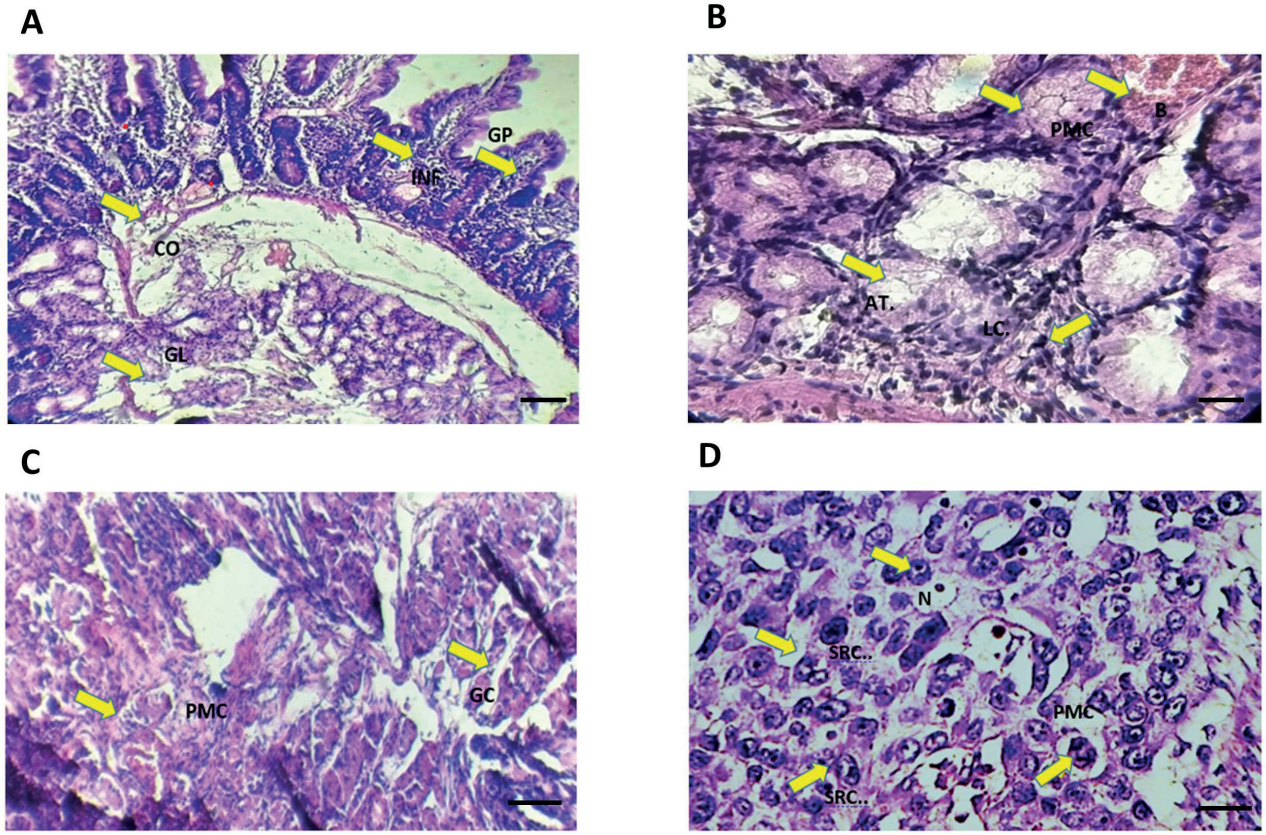


Figure 1. Cross-section of gastric cancer tissue using hematoxylin & eosin. Scale bar = 50 µm. A) changes in the lumen of glandular structures (GL) with cases of congestion (CO) and extensive infiltration of lymphocytes (LC) In gastric pits (GP). B) hemorrhage (B) with inflammatory cell infiltration (INF) in the Fundus region with atrophy and disappearance of gastric glands (AT.). C) glandular cavities)GC) and invasion of the muscular layer by polymorphous carcinomas (PMC). D) the invasion of polymorphous tumor cells (PMC) represented by signet ring cells (SRC) with terminal site nucleus and dense chromatin(N).

and 40-49) that correspond to the percentages 11.76% and 8.83%, respectively (Figure 2).

Gender

The results showed a remarkable superiority of males over females in the samples of stomach cancer patients infected with *H. pylori*. The frequency of the infection of *H. pylori* increased significantly ($p < .05$) in males with stomach cancer, compared to females with stomach cancer and corresponding to the percentages 70.59% and 29.41%, respectively, as shown in (Figure 3).

IgG/M detection of *H.pylori* infection

In this study, the sensitivity of IgG/M to the infection of *H. pylori* in patients with stomach cancer was examined using the sensitivity to IgG/M (Figure 4 and Table 2). We found that the frequency of infection of *H. pylori* was significantly higher in the IgG/M positive group compared to the IgG/M negative group (Table 2). It is essential to mention here that the infection of *H. pylori* recorded the highest percentage (76.41%) in the age group (≥ 50) of patients with stomach cancer as compared with another age group (Figure 4).

Discussion

It is well established that stomach cancer is the most common cancer and one of the leading causes of death worldwide, particularly in developing countries[1,2]. The possible incidence of stomach cancer can be due to different risk factors. In the current study, our findings showed that the infection of *H. pylori* significantly correlates to stomach cancer incidences. Moreover, the results also found a significant link between age and gender and *H. pylori* infection. Thus, detecting people with *H. pylori* infection could be an excellent way to control stomach cancer occurrences.

Various risk factors have been demonstrated to be connected to the development of stomach cancer[9,11,27]. It has been shown that the age factor is significantly associated with infection of *H.pylori* and the product of stomach cancer[28]. In the current study, we found that *H.pylori* infection was higher (79.41%) in the age group (≥ 50) of patients with stomach cancer. Our results aligned with accumulating data showing that age factor is significantly associated with *H. pylori* infection[28]. A recent study has explored that *H. pylori* infection plays an essential role in the development of sto-

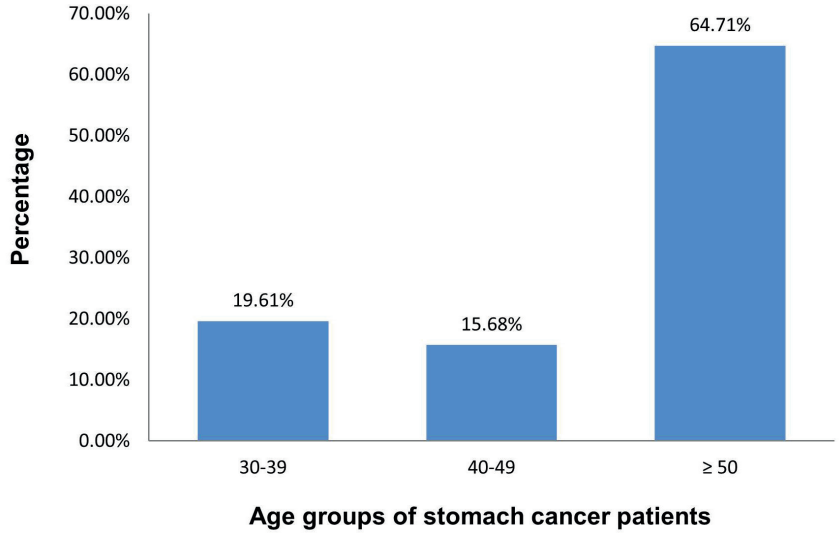
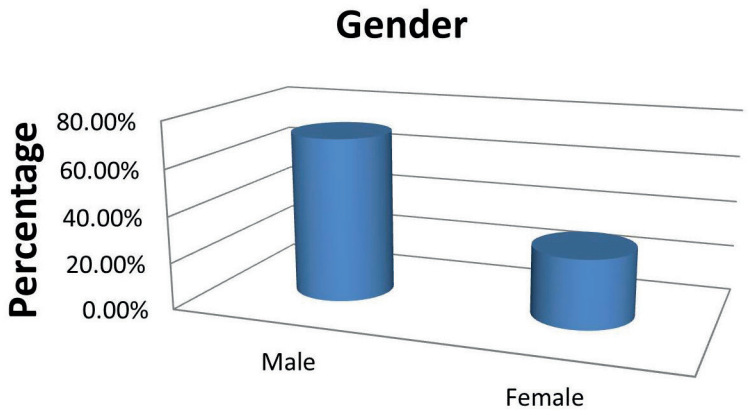


Figure 2. Age groups of patients with stomach cancer. Age criteria were divided into three groups (30-39, 40-49, ≥ 50). Data were presented as a percentage.



	Male	Female
Gender	70.59%	29.41%

Figure 3. Distribution of H. pylori infection among males and females of stomach cancer patients. Data were introduced as a percentage. The chi-square test was used for comparison between the groups. The significant difference between the groups was considered as P-value £ 0.05.

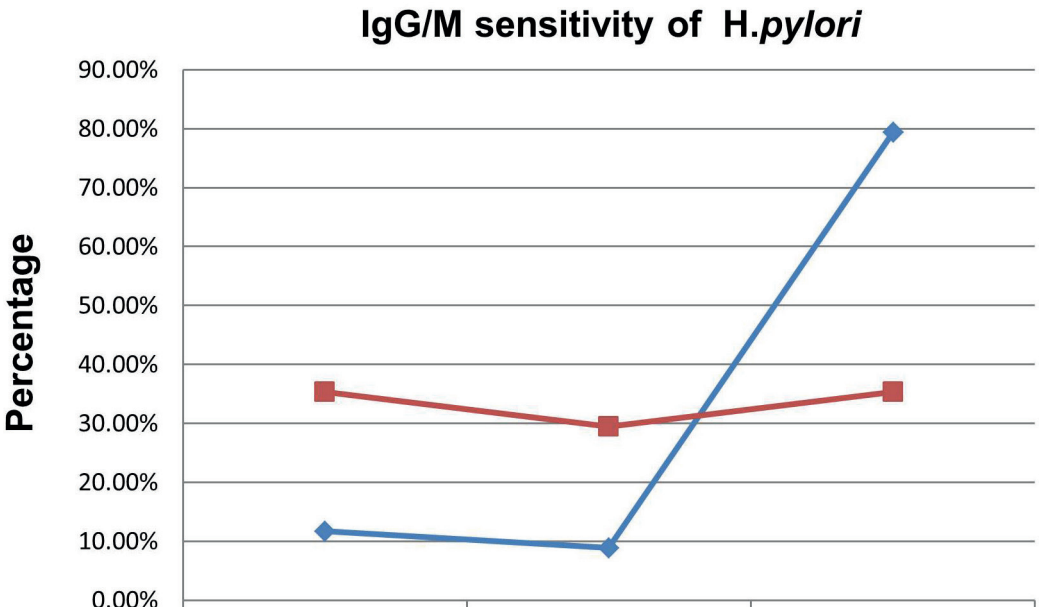


Figure 4. Percentages of IgG/M of *H. pylori* infection between age groups of stomach cancer patients. The blue line is the positive infection of *H. pylori*. The red line indicates a harmful infection of *H. pylori*. Data were presented as a percentage. The chi-square test was used for comparison between the groups. The significant difference between the groups was considered as P-value £ 0.05.

	Observed	Expected	Difference	Difference Sq.	Diff. Sq. / Exp Fr.
Male	36	25.5	10.50	110.25	4.32
Female	15	25.5	-10.50	110.25	4.32
					8.647

The chi-squared value is 5.667. The p-value is .01729. The significance of the results is at $p < .05$.

Table 1. Statistical differences between male and female patients with stomach cancer.

IgG/M	Observed	Expected	Difference	Difference Sq.	Diff. Sq. / Exp Fr.
Positive	34	25.5	8.50	72.25	2.83
Negative	17	25.5	-8.50	72.25	2.83
					5.667

The chi-squared value is 8.647. The p-value is .00328. The significance of results at $p < .05$.

Table 2. Statistical differences between IgG/M positive and negative of patients with stomach cancer.

mach cancer[29], which could explain the risk of age factor to stomach cancer incidences.

Gender is another factor suspected to have a link with *H. pylori* infection participating in the development of stomach cancer[30]; thus, in the present study, we examined the correlation between gender and the condition of *H. pylori*. The current data showed that males with stomach cancer recorded a higher percentage (70.59%) of *H. pylori* infection than females (Figure 3). Our data aligned with a recent study showing that the favorable ratio of *H. pylori* infection was significantly higher in the male group (43.68%) than in the female group (28.8%). Thus, periodic examinations for males could help avoid the infection with *H. pylori*, which might also protect against stomach cancer incidences.

It is well known that infection of *H. pylori* could be developed and cause chronic inflammation, which can increase the possibility of stomach cancer occurrences[31]. Moreover, *H. pylori* infection has also been considered the most com-

mon risk factor for stomach cancer[26,30,32]. We showed that the sensitivity of IgG/M to the disease *H. pylori* in patients with stomach cancer was higher in the IgG/M positive group than in the IgG/M negative group (Figure 4 and Table 2). The results also demonstrated that the infection of *H. pylori* was significantly increased (76.41%) in the age group (≥ 50) of patients with stomach cancer as compared with another age group (Figure 4). Therefore, this could explain why the infection with *H. pylori* might extensively participate in stomach cancer incidence connected with age factor. The exact mechanism of *H. pylori*-induced stomach cancer incidence is not entirely understood. However, the explanation of this condition could be due to the penetration of *H. pylori* to the mucous layer by flagella; scratching results in stimulating host cells to produce mediators of inflammation such as increased tumor necrosis factor (TNFα) and interleukins that promote the attraction of inflammatory cells[22]. These bacteria also have many strains that differ in virulence factors as a result of differences in two types of genes; the first is suspected to be responsible for the incidence of stomach cancer and associated with cytotoxicity (Cytotoxin associated gene) (CagA) ، while the other gene is the vacuolating cytotoxicity gene (Vaculating cytotoxin gene) (Vag A), which is responsible for creating vacuoles and perforations in the stomach tissue, which result in the activation of programmed death of stomach epithelial cells[30,33].

Conclusions

The present data demonstrate that age and gender strongly correlate to the infection of *H.pylori* that participates in the incidences of stomach cancer. Furthermore, our findings also show that the condition of *H.pylori* is extensively involved in stomach cancer occurrences. Accordingly, infection of *H.pylori* could have a high risk of stomach cancer incidences, and therefore, monitoring people with *H.pylori* infection might control the development of stomach cancer.

Acknowledgments

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Conflicts of Interest

The authors announced that the present study has no conflict of interest.

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