The prevalence of *Helicobacter pylori* cagA (+ve) among patients with gastric cancer: an Egyptian study

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Introduction and aims
Gastric cancer is currently the second most common cause of cancer-related death in the world and the fifth most common cancer and the fourth leading cause of cancer-related death in Europe. It has been evident for more than the past 20 years that H*elicobacter pylori* is involved in the development of gastric adenocarcinoma. The cagA gene of *H. pylori* is the main virulence factor that leads to the development of gastric adenocarcinoma through the derangement of cellular architecture and signaling. The objective of our work is to study the prevalence of cagA among patients with gastric cancer.
Patients and methods
This descriptive study was done on 60 patients with gastric cancer underwent serum anti-*H. pylori* IgM and anti-cagA IgG assessment, computed tomography, upper endoscopy, and biopsy taking, and if needed, computed tomography-guided biopsy, followed by histopathological examination.
Results
A total of 34 (56.67%) patients were cagA +ve and 26 (43.33%) patients were cagA –ve, with no statistically significant difference regarding sex or age.
Conclusion
H. pylori cagA plays a significant role in development of gastric cancer, so we recommend not only *H. pylori* screening but also cagA virulence strain.

Keywords:
cagA, gastric cancer, *H*elicobacter *pylori*, incidence of gastric cancer in eastern and western countries

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Introduction

Gastric cancer is currently the second most common cause of cancer-related death in the world and the fifth most common cancer and the fourth leading cause of cancer-related death in Europe. In Egypt, the frequency of gastric cancer is 2% in males and 1.5% in females from the newly diagnosed cases, with patients' median age being 54 years [1].

It has been evident for more than 20 years that H*elicobacter* pylori is involved in the development of gastric adenocarcinoma; in 1994, the WHO concluded that *H. pylori* is a definite or class I carcinogen in humans. *H. pylori* is responsible for ~75% of all noncardiac gastric cancers and 63.4% of all stomach cancers worldwide [2].

A strain-specific *H. pylori* gene cagA (cytotoxin-associated gene A) is a component of the cag pathogenicity island. Several genes within this island encode products that are homologs of proteins of the type IV bacterial secretion pathway. The cagA gene of *H. pylori* is the main virulence factor that leads to the development of gastric adenocarcinoma through the derangement of cellular architecture and signaling [3].

The *cag* cytotoxin-associated gene pathogenicity island (cag PAI) plays an important role in *H. pylori* pathogenesis and is not expressed in all strains. Approximately 60% of *H. pylori* strains isolated in Western countries carry the *cag* PAI, whereas almost all of the East Asian strains isolated are *cag* PAI positive [4].

Therefore, the aim of this study is to determine the prevalence of cagA virulence factor in patients with gastric cancer infected with *H. pylori*, to examine the association of cagA virulence factor of *H. pylori* with gastric cancer, and to prove the increased incidence of gastric cancer in patients infected with *H. pylori* strains that have cagA virulence factor.

Patients and methods

This study was conducted in Internal Medicine Department, Kasr El Ainy Hospital and National

Cancer Institute, Cairo University, between January 2017 and October 2018, in patients of age group 22–78 years old. There were 30 males and 30 females. This paper based on a study done in Kasr-Al Ainy for fulfillment of master degree in internal medicine (Kept in records of the Ethical committee files in Cairo University). All the participants are of Egyptian origin. Written consent was taken from each participant or a responsible family member after fully explaining the possible complications of the diagnostic procedures.

Our study aimed to determine the prevalence of cagA *H. pylori* antigen in patients with gastric cancer.

Inclusion criteria

The following were the inclusion criteria:

(1) Age group must not be less than 18 years old.
(2) All patients must have a histologically and/or cytological confirmed diagnosis of gastric cancer.
(3) All patients with positive anti-*H. pylori* IgM.
(4) All patients have a performance status of ECOG scale less than or equal to 2 with life expectancy of at least 6 months.
(5) All patients should have compliance, mental state, and geographic proximity that allow adequate follow-up, and they have to provide written informed consent before any study-specific procedure.

Exclusion criteria

The following patients were excluded:

(1) Pregnant and breastfeeding patients.
(2) Patients with a currently active second malignancy.

All patients were subjected to the following: full history taking, complete clinical examination, laboratory investigations, and *H. pylori* workup.

All serum samples obtained from patients with gastric cancer will be tested to detect anti-*H. pylori* IgM and anti-cagA IgG.

The serum samples were stored at −80°C until serological testing, which was performed using *H. pylori* line IgA/IgG immunoblot GB, (Druckdatum, Germany), according to the manufacturer's instructions.

Test principles

Pathogen antigen proteins are transferred into a nitrocellulose membrane by a special spraying process. The nitrocellulose membrane is then cut up into individual strips. Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immune complexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alkaline phosphatase-conjugated anti-human IgG or IgA antibodies, correspondingly. After unbound conjugated antibodies have been removed by a further washing step, visualization of the antigen-antibody complex (of the bound antibodies) is accomplished by the addition of a noncolored substrate, which forms blue-violet precipitates at each site (antigen bands) where the conjugated anti-human antibodies have bound. Depending on the observed band pattern, one can interpret the presence of specific IgG or IgA antibodies, correspondingly.

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean±standard deviation (SD). Qualitative data were expressed as frequency and percentage. (SPSS Statistics is a software package used for interactive, or batched, statistical analysis. Long produced by SPSS Inc., it was acquired by IBM (USA) in 2009.

Results

This is a descriptive study on 60 patients with gastric cancer who were diagnosed in National Cancer Institute, Cairo University, at the age group of 22–78 years old with mean age of 54.47 years. There were 30 males and 30 females (Tables 1–4).

Table 1 Demographic data distribution of the study group

	Statistics
Age	
Range	22–78
Mean±SD	54.47±12.78
Sex [*n* (%)]	
Male	30 (50)
Female	30 (50)
Complaint [*n* (%)]	
Dyspepsia	2 (3.33)
Dysphagia	1 (1.67)
Epigastric pain	16 (26.67)
Hematemesis	17 (28.33)
Melena	5 (8.33)
Recurrent vomiting	18 (30)
Weight loss	1 (1.67)

Table 5 shows statistically significant difference between groups regarding complaints.

Table 2 Laboratory descriptive data of the study group

Descriptive statistics	Range	Mean±SD
CBC		
Hb	3.90–12.80	9.43±1.66
PLT	168.00–619.00	354.12±107.35
TLC	2.50–13.00	6.34±2.52
Liver function		
AST	12.00–57.00	23.34±11.19
ALT	6.00–61.00	18.67±10.51
ALP	55.00–146.00	85.05±22.04
GGT	10.00–64.00	31.23±11.10
Bil	0.20–1.10	0.60±0.26
Albumin	2.40–4.60	3.40±0.49
Kidney function		
Urea	7.00–66.00	26.60±12.11
Creat	0.30–1.70	0.78±0.24
UA	3.50–6.60	4.96±1.01
Electrolytes		
Na	128.00–148.00	136.23±4.80
K	2.80–5.20	4.11±0.60
Ca	6.90–10.10	8.66±0.73
Phos	2.50–4.20	3.19±0.49
Anti-*Helicobacter pylori* IgM	1.30–4.20	2.54±0.78

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; Bil, bilirubin; Ca, calcium; CBC, complete blood count, Creat, creatinine; GGT, gamma-glutamyl transferase; Hb, hemoglobin; K, potassium; Na, sodium; Phos, phosphorus; PLT, platelet; TLC, total leukocyte count; UA, uric acid.

Tables 6 and 7 show statistically significant difference between groups regarding anti-*H. pylori* IgM (Figs 1, 2).

Discussion

It has been evident for more than 20 years that *H. pylori* is involved in the development of gastric adenocarcinoma; in 1994, the WHO concluded that *H. pylori* is a definite or class I carcinogen in humans. *H. pylori* is responsible for approximately 75% of all noncardia gastric cancers and 63.4% of all stomach cancers worldwide. The cagA gene of *H. pylori* is the main virulence factor that leads to the development of gastric adenocarcinoma through the derangement of cellular architecture and signaling [1,2].

In Egypt, a high prevalence of *H. pylori* infections has been reported, ranging from 70 up to 88% in the general population [5].

So, the aim of this study is to determine the prevalence of cagA virulence factor in patients with gastric cancer

Table 3 Anti-cagA IgG distribution of the study group

Anti-cagA IgG	*n* (%)
Positive	34 (56.67)
Negative	26 (43.33)
Total	60 (100.00)

Table 4 Comparison between cagA IgG (positive and negative) regarding demographic data

			t test	
	Positive	Negative	t/χ^2	*P* value
Age				
Range	22.00–78.00	30.00–74.00	–1.501	0.139
Mean±SD	52.32±13.96	57.27±10.67		
Sex [*n* (%)]				
Male	18 (52.94)	12 (46.15)	0.271*	0.602
Female	16 (47.06)	14 (53.85)		
Complaint [*n* (%)]				
Dyspepsia	2 (5.88)	0 (0.00)	13.873*	**0.031**
Dysphagia	0 (0.00)	1 (3.85)		
Epigastric pain	12 (35.29)	4 (15.38)		
Hematemesis	6 (17.65)	11 (42.31)		
Melena	5 (14.71)	0 (0.00)		
Recurrent vomiting	8 (23.53)	10 (38.46)		
Weight loss	1 (2.94)	0 (0.00)		

t, independent sample *t* test. *P* value more than 0.05 (NS). *P* value less than 0.05 (S). χ^2, χ^2 test. *χ^2 – Chi-square test.

Table 5 Comparison between cagA IgG (positive and negative) regarding anti-*Helicobacter pylori* IgM

			t test	
Anti-*Helicobacter pylori* IgM	Positive	Negative	*t*	*P* value
Range	1.30–4.20	1.50–3.20	3.228	0.002
Mean±SD	2.81±0.82	2.20±0.57		

Table 6 The frequency and percentage of CT abdomen and pelvis findings in relation to cagA +ve

CT abdomen and pelvis	Anti-cagA IgG Positive [*n* (%)]
Circumferential mural thickening in pyloric antrum	1 (2.94)
Mass at the greater curvature	1 (2.94)
Mass in the prepyloric region	1 (2.94)
Mass involving fundus and lesser curvature	0 (0.00)
Mass involving pylorus and lesser curvature	0 (0.00)
Mass involving the greater curvature	0 (0.00)
Mural thickening in gastric fundus and body	0 (0.00)
Pyloric mass	3 (8.82)
Circumferential mural thickening gastric wall	2 (5.88)
Circumferential thickening in pylorus	1 (2.94)
Circumferential thickening of the gastric antrum	1 (2.94)
Diffuse gastric wall thickening	1 (2.94)
Diffuse wall thickening at greater curvature	2 (5.88)
Fundal mass	1 (2.94)
Gastric antrum mass	o (2.94)
Gastric mural thickening	1 (2.94)
Gastric mural thickening involving body an fundus	1 (2.94)
Gastric mural thickening involving body and antrum	1 (2.94)
Gastric mural thickening involving fundus	2 (5.88)
Gastric pylorus soft tissue mass	1 (2.94)
Malignant gastric tumor at the junction between fundus and body	1 (2.94)
Malignant gastric tumor involving the body and pylorus	1 (2.94)
Mass at lesser curvature	1 (5.88)
Mild splenomegaly	1 (2.94)
Mural thickening of gastric wall	5 (14.71)
Normal	2 (8.82)
Total	34 (100.00)

Table 7 The frequency and percentage pathological findings in relation to cagA +ve

Pathology	anti-cagA IgG Positive [*n* (%)]
Adenocarcinoma grade 2	15 (44.12)
Adenocarcinoma grade 3	7 (20.59)
Anaplastic carcinoma	4 (11.76)
Mucinous adenocarcinoma	2 (5.88)
Signet ring carcinoma	4 (11.76)
Undifferentiated carcinoma	2 (5.88)

infected with *H. pylori*, to examine the association of cagA virulence factor of *H. pylori* with gastric cancer, and to prove the increased incidence of gastric cancer in patients infected with *H. pylori* strains that have cagA virulence factor.

In our study, we included 60 Egyptian patients proved to have gastric carcinoma and also infected with *H. pylori*.

The present study showed that the age range was 22–78 years old, with mean±SD of 54.47±12.78 years. This age group was approximately near to the age group of a study done by Liu *et al.* [6], which was done on patients with gastric cancer, where the age range, 33–83 years.

Our study found that 34 (56.67%) patients are cagA +ve and 26 (43.33%) patients are cagA –ve, with no statistically significant difference regarding sex or age. These results are in agreement with the results of a study done by Meine *et al.* [7], which was conducted on 29 patients with gastric cancer and showed that 62.1% of patients were infected by cagA-positive *H. pylori*, with no statistically significant difference regarding sex.

The current study showed that 35.29% (12 patients) of the patients complained of epigastric pain, which represents the highest percentage of all complaints, followed by recurrent vomiting (23.53%, eight patients) and then hematemesis (17.65%, six patients) in cagA +ve patients. These results are in agreement with a study done by Güdücüoğlu *et al.* [8], which was done on 99 *H. pylori*-infected patients with gastric cancer, complaining of epigastric pain, and the results showed that the positivity rates for *H. pylori* cagA was 78%, which is a higher percentage than our results.

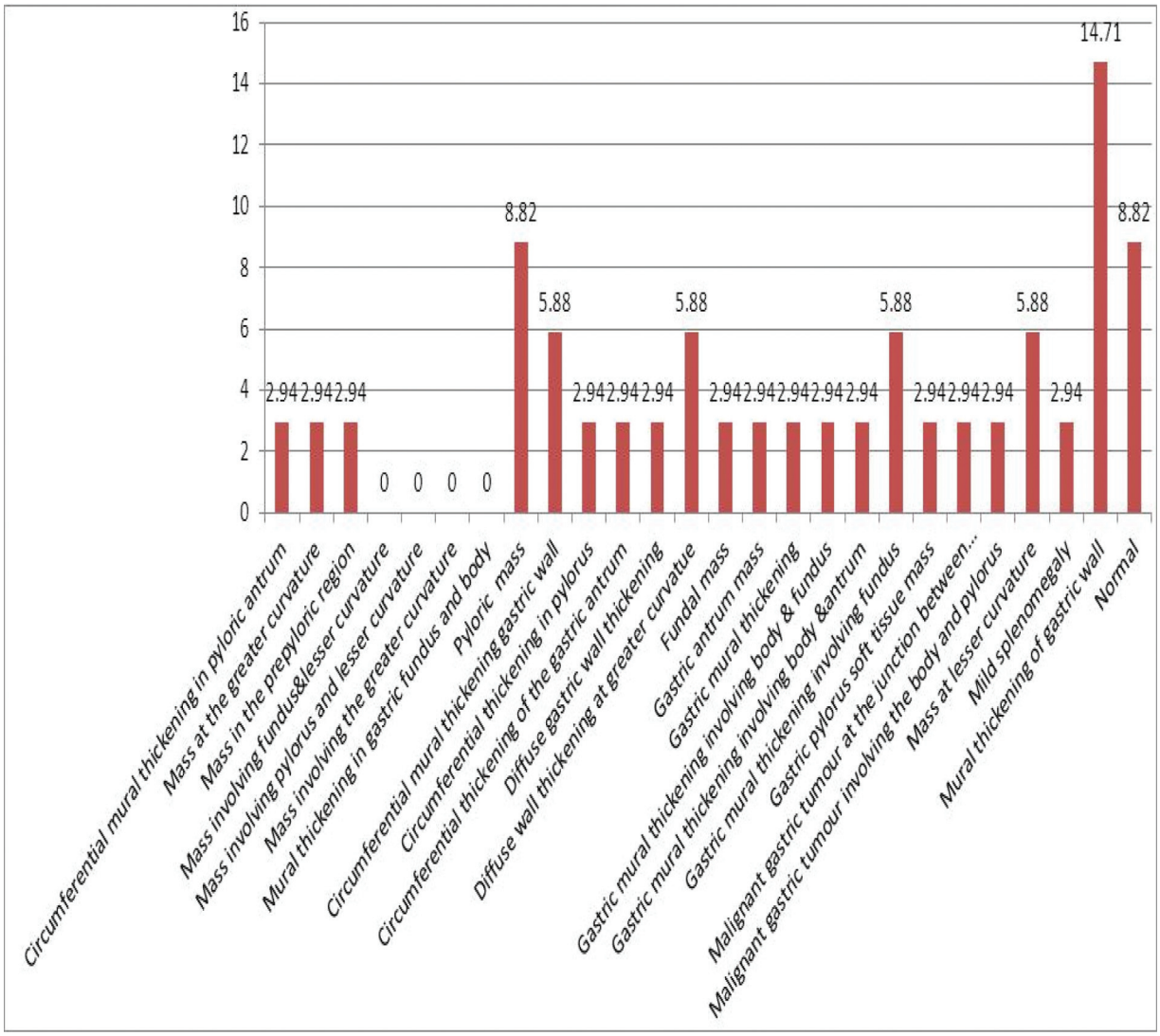
It is encouraging to compare this result with that found by Erzin *et al.* [9] who concluded that the prevalence of cagA was 73.6% in their study done on 33 gastric cancer and *H. pylori*-infected patients complaining of dyspepsia.

Moreover, our results are in agreement with the study done by Said Essa *et al.* [10] on 99 *H. pylori*-infected patients, including 90 dyspeptic patients (30 each with gastric cancer, peptic ulcer, and nonulcer dyspepsia) and nine nondyspeptic healthy controls. They concluded that anti-cagA antibodies were more prevalent among dyspeptic patients with gastric cancer or peptic ulcer (73.3%) compared with those with nonulcer dyspepsia (40%).

The current study also found a statistically significant difference between cagA +ve and –ve patients regarding anti-*H. pylori* IgM titer, with range of 1.30–4.20 and mean±SD of 2.81±0.82 for +ve patients and range of 1.50–3.20 and mean±SD of 2.20±0.57 for –ve patients, with *P* value of 0.002.

This is against the study done by Suzuki and colleagues [11], which reported that the risk of gastric cancer is different between high and low cagA antibody titers. They examined 299 patients with gastric cancer and 1048 matched controls. Among seropositive patients for *H. pylori* antibody, those with low cagA antibody titers had higher and more significant risk [relative risk

Figure 1



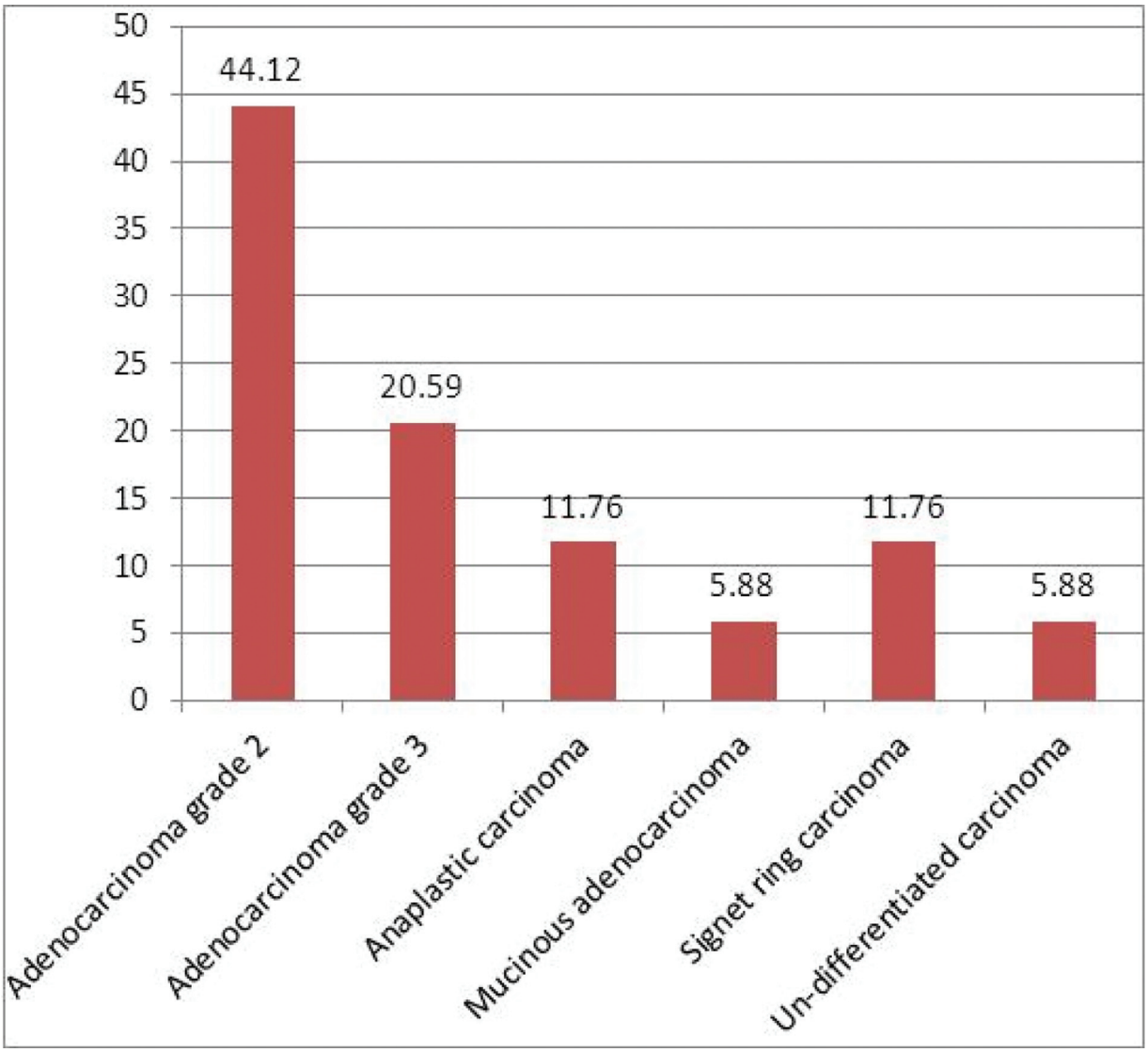
The frequency and percentage of computed tomography abdomen and pelvis findings in relation to cagA +ve, with mural thickening of gastric wall having the highest percentage (14.07%) followed by pyloric mass (8.82%).

(RR), 3.9; $P<0.001$) for future gastric cancer than those with cagA seronegativity (RR, 2.2; $P=0.0052$) or high cagA antibody titers (RR, 2.0; $P=0.0022$) [12].

The current study observed that in upper endoscopic findings, pyloric mass has the highest percentage (16.2%) followed by mass at the greater curvature and fundal ulcer (10.8% each) in cagA +ve patients. These results are concordant with the study conducted by Peleteiro *et al.* [13], which was done on 41 cardia and 339 noncardia cancer cases undergoing gastrectomy and 380 community controls. The associations between *H. pylori* infection and cardia and noncardia cancers were further compared. No positive relation was found for *H. pylori* infection, but cagA-positive strains were associated with an increased risk of noncardia cancer (odds ratio=1.60, 95% confidence interval=1.17–2.18).

Moreover, this matched with the study done by Suzuki and colleagues, which reported that the risk of gastric cancer is different between high and low cagA antibody titers. They examined 299 noncardia gastric cancers and 1048 matched controls. Among seropositive patients for *H. pylori* antibody, those with positive cagA antibody had higher and more significant risk (RR, 3.9; $P<0.001$) for future noncardia gastric cancer than those with cagA seronegativity (RR, 2.2; $P=0.0052$) [12].These results are also in acceptance with the study done by Filipec Kanizaj T and colleagues [14], which was done on 30 patients with gastric adenocarcinoma .Upper gastrointestinal endoscopy was performed, and 4 mucosal biopsy samples were obtained and assessed according to updated Sydney protocol. CagA seropositivity was significantly more often present in patients with higher activity grade in the antrum ($P=0.025$), and this proves that cagA is

Figure 2



The frequency and percentage of pathological findings in relation to cagA +ve, with adenocarcinoma grade 2 having the highest percentage (44.12%) followed by adenocarcinoma grade 3 (20.59%).

linked with pathogenesis of pyloric masses in gastric cancer [15].

In contrast to our study, Ren and colleagues [16] performed a study on 196 gastric cardia cancer cases, and the rate of cagA positivity was 82.1%, indicating an association between cagA and gastric cardia cancer [17].

Conclusion

This study suggests that cagA plays a significant role in the development of gastric cancer in patients who were infected by *H. pylori* and can be used as a screening marker for early detection of gastric cancer.

So we recommend that cagA should be used in any patient with symptoms and signs related to *H. pylori* infection as screening marker for early detection of gastric cancer and for regular follow-up of *H. pylori*-infected patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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