ORIGINAL ARTICLE

Association Between CagA EPIYA Motifs with Diverse Gastroduodenal Outcomes in Egyptian Patients Infected with Helicobacter pylori

Running title: CagA EPIYA Motifs and diverse gastroduodenal outcomes

Rabab Fouad [1], Waleed Fouad Fathallah [1] Doaa Ghaith [2], Mohamed Saif Elnasr [1], Walied Elhossary [1,] Pezhman Alavinejad[1]

Affiliations:
1. Department of Endemic Medicine and Hepatology, Faculty of Medicine, Cairo University, Cairo, Egypt
2. Clinical and Chemical Pathology department, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence

Walied Elhossary
Address:
Faculty of Medicine, Cairo University
Endemic Medicine Department
1 Al-Saray Str, Al-Manial, Faculty of Medicine
Cairo University, Cairo 11559, Egypt
Phone number: +201114215471
E-mail: Walied.elhossary@cu.edu.eg

Abstract

Background and Aims: *Helicobacter pylori CagA* gene is a major virulence factor that undergoes tyrosine phosphorylation in a region holding differing numbers of repeat sequences (EPIYAs) resulting in modulation of the inflammatory response. The aim of this cross-sectional study is to determine the correlation between *CagA* EPIYA motifs with diverse gastroduodenal outcomes.

Methods: Gastric biopsies were collected from 54 Egyptian patients (11 patients with PUD and 43 chronic non atrophic gastritis). Molecular detection of *H. pylori*, *CagA* gene with determination of the EPIYA motifs in *CagA* positive cases were done.

Results: Out of the 54 *H. pylori* positive cases, *CagA* gene was detected in 31 patients. EPIYA-ABC was the most presented pattern in 22 cases (71 %) and the least common pattern was EPIYA-ABCCC, which was positive only in one case (3.2%). Both EPIYA-AB and EPIYA-ABCC were presented in 4 cases for each (12.9% for each).

Conclusion: There was a significant statistical correlation between the presence of *CagA* gene and both PUD and GU. Furthermore, the structure of the variable region of the *CagA* gene in Egyptian strains was Western type with a variable number of EPIYA-C.

Keywords: *Helicobacter pylori*; *Cag A*; EPIYA motifs; Peptic ulcer disease; Gastric ulcer

Introduction

Helicobacter pylori is known as a main cause of most gastroduodenal diseases including gastric and duodenal ulcers, gastric adenocarcinoma and mucosa-associated lymphatic tissue lymphoma. However, the clinical outcomes succeeding H. pylori infection are different in diverse populations depending on multiple factors such as the host genetic susceptibility, environmental conditions and divergent H. pylori pathogenicity [1-3].

CagA is one of the virulence factors of H. pylori such as lipopolysaccharide, peptidoglycan, vacuolating cytotoxin, and gamma glutamyl transpeptidase. CagA may undergo tyrosine phosphorylation in a region holding differing numbers of repeat sequences (EPIYAs) resulting in modulation of the inflammatory response e.g. abnormal gastric epithelial cell proliferation, cytoskeletal abnormalities or even stimulation of cellular oncogenes [3-4].

Based on the EPIYA motifs, H. pylori was subcategorized as Western or East Asian strains. Western H. pylori strains have EPIYA-A, EPIYA-B, and EPIYA-C segments, and the EPIYA-C site is often repeated. The common EPIYA polymorphic types are primarily ABC, ABCC, and ABCCC. On the other hand, East Asian H. pylori strains contain EPIYAA, EPIYA-B, and EPIYA-D motifs. The most common EPIYA polymorphic type is ABD [5, 6].

Phosphorylated CagA combines Src homology 2 phosphatase (SHP-2), changing SHP-2 structure and interfering with host cell signaling pathways, finally leads to epithelial structure disorder. Amino acid differences at the C-terminal residues within the CagA EPIYA-D and -C segments may lead to variable SHP-2 affinities. SHP-2 combines EPIYA-D more strongly than EPIYA-C. Consequently, the affinity of the East Asian-type CagA to SHP-2 is expressively more than that of the Western-type CagA. As a result, East Asian-type CagA induces more cytoskeleton variations, and is more likely to be associated with gastric cancer [7].

In Africa generally and Egypt specifically, there is a gap of knowledge about the pattern of EPIYA and its possible effect in the African enigma of gastric cancer. In our study, we aimed to determine the correlation between CagA EPIYA motifs with diverse gastroduodenal outcomes and to define; for the first time in Egypt, the distribution of the different EPIYA patterns in the positive CagA cases.

2. Material and Methods

2.1 Study design and patients

During the period from December 2016 till February 2017, this case–control study was conducted with patient group (PG), comprising a total of 12 peptic ulcer (7 gastric ulcers, 3 duodenal ulcers and 2 gastroduodenal ulcers) patients, and a control group (CG), comprising a total of 44 chronic gastritis patients, were recruited in this study. Based on Beltrán-Anaya and his colleagues[1], the sample size was calculated using by GPower 3.1. With an α error of 0.05 and a power of 0.8, a minimum of 12 patients in the patients group should be required to detect the effect of multiple C EPYIA pattern between PUD group and the control group. In the CG, 44 H. pylori strains were isolated from the antrum and corpus biopsy specimens. While the patient group included 12 patients with PUD.

The study recruited patients above 18 years old presenting to Kasr Al Ainy endoscopy unit, Cairo university, Egypt. While we excluded patients with previous history of H. pylori eradication treatment, or had a history of antibiotics, antisecretory drugs, bismuth salts or sucralfate intake within 4 weeks prior to the screening. Patients who received immunosuppressive or non-steroid anti-inflammatory treatment were excluded from the study. The study was approved by the ethical committee of endemic gastroenterology and hepatology department of Cairo University, Faculty of Medicine. All the patients signed an informed consent to participate in the study.

2.2 Endoscopy and Biopsy collection

Upper gastrointestinal endoscopies were performed after an overnight fast for all the patients in endoscopy unit in Kasr Al Aini hospital by Olympus endoscope (SQ260 Olympus with serial number 2225070). In patients with endoscopic findings consistent with gastritis two biopsies from antrum and body were taken for histopathological assessment and two antral biopsies were taken for molecular isolation of H. pylori. While in PUD patients at least two biopsies were collected from the ulcer edges for histopathological assessment with another two antral biopsies were taken for molecular isolation of H. pylori. The histopathological specimens were immediately fixed in 10% formalin, while the other biopsies were placed in a buffered solution (glycerol broth) stored at −80°C for the molecular diagnosis of H. pylori.

2.3 Histological examination

For histological analysis, the formalin-fixed biopsies were embedded in paraffin, and 4-μm sections were stained with hematoxylin-eosin. Histopathological findings were used to determine each patient's diagnosis. Gastritis was classified according to the updated Sydney system.

2.4 Helicobacter pylori detection

DNA was extracted from mononuclear cell layer using Gene JET Genomic DNA Purification Kit supplied by Thermo Scientific (catalogue number MAN0012663) according to manufacturer's specifications. The specific presence of the H. pylori 16S rRNA gene was assessed according to the methods previously described by Hammar, et al.[2], Table 1. For all reactions, DNA samples from the cagA-positive ATCC43504 and J99 H. pylori strains were used as positive controls. For negative controls, DNA was substituted with sterile deionized water.

2.5 CagA gene amplification

H. pylori 16S rRNA gene-positive samples were subjected to PCR to detect the cagA gene using the primers described previously by Rota, et al. [3], Table 1. These oligonucleotides amplified a 298-bp fragment within the constant region.

2.6 Amplification of the 3' -Variable Region of the cagA Gene and determination of the EPIYA pattern

All positive patients for CagA gene were then submitted to investigation of variable region (EPIYA) polymorphisms by amplification the 3′ variable region of the CagA gene by the primers described previously by Yamaoka, et al. [4], were used, Table 1. Products of 500 to 850 bp were obtained depending on the type and number of repeats of the EPIYA-C motif in the cagA gene. The PCR products were separated by electrophoresis on 2% agarose gel that was stained with ethidium bromide and visualized under a UV transilluminator. Positive controls of the different patterns of the EPIYA motif were used to confirm the PCR results.

Table 1. Primers used in the study

Gene	Nucleotide sequence	Reference
Forward	5'-(TGGCGTGTCTATTGACAGCGAGC)-3'	[2]
Reverse	5'-(CCTGCTGGGCATACTTCACCATG)-3'	
CagA / Con Forward	5'-GTGCCTGCTAGTTTGTCAGCG -3'	[3]
CagA / Con Reverse	5'-TTGGAAACCACCTTTTGTATTAGC-3'	
CAG1	5'-ACCCTAGTCGGTAATGGGTTA- 3'	[4]
CAG2	5'-GTAATTGTCTAGTTTCGC-3'	

2.6.1 According to EPIYA pattern, we divided the CagA positive isolates into two groups:

▪ No more than one EPIYA-C motif (including EPIYA-AB and EPIYA-ABC)

▪ Multiple EPIYA-C motifs (including EPIYA-ABCC and EPYIA-ABCCC).

2.6.2 After endoscopic and histopathological examination patients were grouped into 3 groups according to the diagnosis:

- **Patient group (Peptic ulcer disease):** The endoscopic examination showed peptic ulcer disease (more than 3 mm). On other hand, histopathological examination showed no evidence of atrophy, intestinal metaplasia or dysplasia.
- **Control group (Chronic non-atrophic gastritis):** The endoscopic examination showed gastritis regardless to the extent. On other hand, histopathological examination showed chronic active gastritis with no evidence of atrophy, intestinal metaplasia or dysplasia.

2.7 Statistical analysis

Pre-coded data are entered on the computer using "Microsoft Office Excel Software" program (2016) for windows. Data are then transferred to the Statistical Package of Social Science Software program, version 21 (SPSS) to be statistically analyzed.

Data are summarized using mean and standard deviation for quantitative variables and frequency and percentage for qualitative ones. Comparisons between groups are performed using independent sample T-test for quantitative variables and Chi square test or Fisher's exact test for qualitative ones. P values equal to or less than 0.05 are considered statistically significant and if less than 0.01 considered highly significant. Graphs are used to illustrate some information.

2. Results

3.1 Population characteristics

Of the 56 studied patients, 44 (78.6%) were diagnosed with chronic non atrophic gastritis and 12 (21.4%) with peptic ulcers. The age of patients ranged from 19 to 72 years old with mean 39.67 years old. The female gender participated more in the 2 groups. More than 55 % of the patients came from urban areas. There was no statistically significant association between any of the studied demographic features (age, gender and residence) and CagA gene of H. pylori.

3.2 CagA status of Helicobacter pylori infections

The H. pylori CagA gene was found in 31 (55.4 %) of the 56 infected patients. There was a significant statistical correlation between the presence of CagA gene and both PUD and GU table 2.

Table 2. The diverse gastroduodenal outcomes in Cag A positive patients

	Cag A (variable region)				P value	OR	95% CI
	Positive (n=33)		negative (n=25)				
	N	%	N	%			
Gastritis	19	57.6	24	96.0	0.008	0.134	0.027-0.666
PUD	10	30.3	1	4.0	0.016	10.435	1.235-88.133
GU	6	18.2	0	0.0	0.032	NA	NA
DU	5	15.2	1	4.0	0.222	4.286	0.468-39.270
GC or PRCA	4	12.1	0	0.0	0.126	NA	NA

PUD: peptic ulcer disease; GU: gastric ulcer; DU: duodenal ulcer; GC: gastric carcinoma; PRCA: pure red cell aplasia.

3.3 EPIYA segments and EPIYA-C motif numbers

The PCR products amplified from CagA-positive samples showed four electrophoretic patterns that corresponded to the following combinations of EPIYA motifs: ABC, ABCC, ABBC, and ABBCCC. The EPIYA-D motif was not detected. These patterns of variable area CagA gene means that CagA gene is of Western type in Egyptian population. The EPIYA-ABC segment was detected in 22 (71%) patients, while the other motifs were detected as follow: EPYIA-AB in 4 (12.9%), EPYIA -ABCC in 4 patients (12.9%) and only one patient with EPIYA-ABCCC pattern (3.2%). Furthermore, we divided the Cag A positive isolates according to EPIYA pattern into two groups:

No more than 1 EPIYA-C motif (including EPIYA-AB and EPIYA-ABC) with 26 out of 31 patients (83.87%)

Multiple EPIYA-C motifs (including EPIYA-ABCC and EPYIA-ABCCC) with 5 patients (16.13%).

There was a significant statistical difference in correlation between no more than EPIYA-C motif group and chronic non-atrophic gastritis table 3.

Table 3. The diverse gastroduodenal outcomes with different EPIYA patterns

	CagA EPIYA motifs				P value	OR	95% CI
	No more than 1 EPIYA-C motif (n= 27)		Multiple EPIYA-C motifs (n= 6)				
	N	%	N	%			
Gastritis	19	70.4	0	0	0.025	11.875	1.190-118.498
PUD	6	22.2	4	66.7	0.053	0.143	0.021-0.979
GU	4	14.8	2	33.3	0.295	0.348	0.047-2.576
DU	3	11.1	2	33.3	0.216	0.250	0.031-1.999
GC or PRCA	2	7.4	2	33.3	0.142	0.160	0.017-1.482

PUD: peptic ulcer disease; GU: gastric ulcer; DU: duodenal ulcer; GC: gastric carcinoma; PRCA: pure red cell aplasia.

Otherwise, no significant relation was detected between the PUD and multiple EPYIA-C patterns.

Discussion

Helicobacter pylori infect the stomach of around one half of the population worldwide [5-8]. In our study, out of the 56 H. pylori infected patients, 31 isolates (55.4 %) were positive for CagA gene. This prevalence is convenient with El-Khlousy and her colleagues in 2016 who found that 17 out of 37 (45.9 %) H. pylori isolates in Egyptian dyspeptic patients were positive for CagA gene [9]. The epidemiological prevalence of CagA-positive H. pylori infection in western countries is nearly 60% [10] and the prevalence is about 90% in Asian countries [11]. This study showed no significant association between any of the studied demographic features (age, sex and residence) and the presence of CagA gene. But a significant association was detected between CagA gene and both peptic ulcer disease and gastric ulcer.

Several studies showed that the CagA-positive strains are directly associated with acute gastritis, gastric ulcer, and gastric cancer development [7,12-14].While in Egypt, no significant association were found between CagA and the severity of the gastritis [9]. Therefore, our results have proved the crucial role of CagA in pathogenesis of H. pylori associated

PUD and GU, which was already proved globally by meta-analyses [15,8].

All CagA-positive patients harbored EPIYA A, B or C only in our study, which means that Egyptian H. pylori strains are similar to the Western strains that have EPIYA C motifs [16] and not EPIYA-D like Asian strains [17]. These results came in agree with the studies conducted in Middle East countries like Iraqi, Iran and Turkey where EPIYA region of CagA does not include EPIYA-D motif [18-21]. I Furthermore, we found that the most frequently detected EPIYA pattern of CagA gene was EPIYA-ABC, which was found in 22 (71%) patients out of 31 CagA positive patients. While the least presented pattern was the EPIYA-ABCCC, as only one patient expressed it (3.2%). These results are compatible with previous studies conducted in Middle East and Europe. In Africa, a study was conducted in Casablanca population. Here was the prevalence of cagA gene 37%. The risk of intestinal metaplasia was higher in CagA-positive strains. The EPIYA pattern were determined. The EPIYA-ABC was the most prevalent pattern (58%) [25]. In 2015, Honarmand and his colleagues conducted a study in Iran on 168 H. pylori CagA strains. The frequency of ABC was 93.50%, ABCCC 5.40%, ABC+ABCCC 0.6% and ABCC 0.6% [18]. In the same year, Kocazeybek and his colleagues investigated the EPIYA pattern in 142 Cag A positive H. pylori strains in Turkey. EPIYA-ABC was detected in 89 patients (62.7 %), EPIYA-ABCC in 27 strains (19 %), EPIYA-ABCCC in 7 patients (4.9%) and 19 strains (13.4%) without any EPIYA-C motifs [19]. In Europe, Ferreira and his colleagues studied the EPIYA pattern in Portugal. with the following distribution of CagA types: ABC (60.0%), AB (20.0%), ABCC (16.4%) and ABCCC (3.6%) [22].

Our patients were grouped into 2 groups: Control group (Chronic non-atrophic gastritis) and Case group (PUD). In addition, CagA positive isolates were divided the into two groups; no more than 1 EPIYA-C motif (including EPIYA-AB and EPIYA-ABC) and multiple EPIYA-C motifs (including EPIYA-ABCC and EPYIA-ABCCC). There is no statistically significant correlation between any of the studied demographic features and EPIYA pattern of CagA gene. But there is a significant correlation between no more than EPIYA-C motif group and chronic non-atrophic gastritis.

Although in-vitro studies suggest a role for the polymorphic CagA EPIYA-containing region in the pathogenicity of H. pylori, studies with human subjects have shown conflicting results [23]. In Turkey, Kocazeybek and his colleagues conducted a study on 142 patients and concluded that multiple EPIYA-C repeats increases the gastric cancer risk by 30.6-fold and the DU risk by 8.9-fold versus the chronic gastritis [19]. On other side, Honarmand and his colleagues in Iran found that H. pylori CagA positive strains that have the three EPIYA-C repeats is significantly associated with GU, but there was no significant association between the number of CagA EPIYA-C segment and DU or gastric cancer [18]. In Europe, Ferreira's study on 169 CagA positive H. pylori strains had shown that the magnitude of risk for gastric carcinoma and for gastric precancerous lesions increases with increasing number of EPIYA C motifs in the infecting H. pylori strains [22].

Given that in well established gastric cancer is not colonised with Helicobacter pylori. Therefore, "hit-and-run" process of CagA action should be included in the multistep progress to gastric cancer. This is why, a genetic and epigenetic alterations for CagA-directed cancer should be required for "hit-and-run" pathogenesis of gastric cancer [27].

In 2021, a meta analysis were conducted in Iranian population. Here 1762 Iranian patients were included. A significant correlation between severe clinical outcomes of Helicobacter pylori infection and CagA genotypes ABCC and ABCCC. Here could be concluded the positive relation between the increased risk for gastric cancer and the increased copies of EPIYA-C. Furthermore, a strong correlation between the EPIYA-ABCCC motif and gastric cancer. [26].

Li and his colleagues conducted a large meta-analysis for 23 studies. The aim of this meta-analysis was to evaluate whether 1 CagA EPIYA-D motif or multiple EPIYA-C phosphorylation sites were associated with PUD or GC risk. Finally, Li concluded that in Asia 1 EPIYA-D motif is significantly associated with increased GC risk and multiple EPIYA-C motifs are associated with increased PUD and DU risk. On other side, in the United States and Europe multiple EPIYA-C motifs are associated with increased GC risk [8].

The diverse results between the previous studies could be explained from different aspects. As we all know, the pathogenesis of the H. pylori associated gastroduodenal disease is usually complex and multifactorial that depends not only on the diversity of H. pylori strains but also on host immune response and environmental factors.

As regard H. pylori strains diversity, the vacA gene is present in most H. pylori strains; however, considerable differences in vacuolating activities are observed between strains. The VacA has different polymorphic forms. These diverse forms of VacA are associated with variable clinical outcomes. Considerable genetic variations are found in: The s (signal) region with alleles s1a, slb, slc, or s2; the m (middle) region with m1 or m2 alleles; and the i (intermediate) region with type i1 or i2 alleles H. pylori strains having combination VacAs1/m1 or vacAs1/m1/i1 showed the highest risk of progression to GC than vacA s2/m2 or vacAs2/m2/i2 strains [24]. El-Khlousy and his colleague found that the vacA s1gene was identified in H. pylori strains from 6/37(16.2%), vacA s2 was identified from 12/37 (32.4%) and one isolate was positive for both vacA s1 and s2. The vacA m1a subtype was identified in only one (2.7%) isolate and the vacA m2 type from 13/37 (35.1%). The vacA s1m1 genotype was identified in only one isolate (2.7%), the vacA s1m2 genotype was identified in three (8.1%) isolates, the vacA s2m1 was not identified, while the vacA s2m2 genotype was identified in 10 (27.02%). So far, the most aggressive VacA genotype (s1m1) is found only in one isolate (2.7%) [9].

These findings are explaining the low virulence of the H. pylori strains in Egypt. On other hand, many factors are still not well studied in Egypt like host immune response variations (IL-1 β polymorphism, IL-8, IL-10 and TNF- α) beside the influence of the different dietary habits, gastric microbiota and helminthic co-infection on the aggressiveness of H. pylori in Egyptian society. Finally, the variations in the study design and sample size are all factors that could explain these variations between the studies.

In conclusion, we have found that the Egyptian H. pylori isolates have a Western type of CagA gene. Infection with CagA positive H. pylori strains are significantly associated with PUD and GU. Furthermore, infection by the H. pylori CagA positive strains that have the no more than one EPIYA-C is significantly associated with chronic non-atrophic gastritis. Meanwhile, there was no significant association between the number of CagA EPIYA-C segment and PUD or GC.

References

1. Beltrán-Anaya FO, Poblete TM, Román-Román A, et al. The EPIYA-ABCC motif pattern in CagA of Helicobacter pylori is associated with peptic ulcer and gastric cancer in Mexican population. BMC gastroenterology. 2014 Dec;14(1):223.
2. Hammar M, Tyszkiewicz T, Wadström T, et al. Rapid detection of Helicobacter pylori in gastric biopsy material by polymerase chain reaction. Journal of Clinical Microbiology. 1992 Jan 1;30(1):54-8.
3. Rota CA, Pereira-Lima JC, Blaya C, et al. Consensus and Variable Region PCR Analysis ofHelicobacter pylori 3′ Region of cagA Gene in Isolates from Individuals with or without Peptic Ulcer. Journal of clinical microbiology. 2001 Feb 1;39(2):606-12.
4. Yamaoka Y, Kodama T, Kashima K, et al. Variants of the 3′ Region of the cagA Gene inHelicobacter pylori Isolates from Patients with DifferentH. pylori-Associated Diseases. Journal of clinical microbiology. 1998 Aug 1;36(8):2258-63.
5. Ramzy I, Elgarem H, Hamza I, et al. Genetic mutations affecting the first line eradication therapy of Helicobacter pylori-infected Egyptian patients. Revista do Instituto de Medicina Tropical de São Paulo. 2016;58.
6. Camilo V, Sugiyama T, Touati E. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2017 Sep;22:e12405.
7. Nejati S, Karkhah A, Darvish H, et al. Influence of Helicobacter pylori virulence factors CagA and VacA on pathogenesis of gastrointestinal disorders. Microbial pathogenesis. 2018 Apr 1;117:43-8.
8. Li Q, Liu J, Gong Y, et al. Association of CagA EPIYA-D or EPIYA-C phosphorylation sites with peptic ulcer and gastric cancer risks: a meta-analysis. Medicine. 2017 Apr;96(17).
9. El-Khlousy M, Rahman EA, Mostafa S, et al. Study of the clinical relevance of Helicobacter pylori virulence genes to gastric diseases among Egyptian patients. Arab Journal of Gastroenterology. 2016 Jun 1;17(2):90-4.
10. Chiurillo MA, Moran Y, Cañas M, et al. Genotyping of Helicobacter pylori virulence-associated genes shows high diversity of strains infecting patients in western Venezuela. International Journal of Infectious Diseases. 2013 Sep 1;17(9):e750-6.
11. Sheu SM, Sheu BS, Yang HB, et al. Presence of iceA1 but not cagA, cagC, cagE, cagF, cagN, cagT, or orf13 genes of Helicobacter pylori is associated with more severe gastric inflammation in Taiwanese. Journal of the Formosan Medical Association= Taiwan yi zhi. 2002 Jan;101(1):18-23.
12. Matos JI, de Sousa HA, Marcos-Pinto R, et al. Helicobacter pylori CagA and VacA genotypes and gastric phenotype: a meta-analysis. European journal of gastroenterology & hepatology. 2013 Dec 1;25(12):1431-41.
13. Batista SA, Rocha GA, Rocha AM, et al. Higher number of Helicobacter pylori CagA EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer. BMC microbiology. 2011 Dec;11(1):61.
14. McColl KE, El-Omar E, Gillen D. Helicobacter pylori gastritis and gastric physiology. Gastroenterology Clinics of North America. 2000 Sep 1;29(3):687-703.
15. Wang D, Li Q, Gong Y, et al. The association between vacA or cagA status and eradication outcome of Helicobacter pylori infection: A meta-analysis. PloS one. 2017 May 11;12(5):e0177455.
16. Sgouras DN, Trang TT, Yamaoka Y. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2015 Sep;20:8-16.
17. Higashi H, Tsutsumi R, Fujita A, et al. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proceedings of the National Academy of Sciences. 2002 Oct 29;99(22):14428-33.
18. Honarmand-Jahromy S, Siavoshi F, Malekzadeh R, et al. Multiple repeats of Helicobacter pylori CagA EPIYA-C phosphorylation sites predict risk of gastric ulcer in Iran. Microbial pathogenesis. 2015 Dec 1;89:87-92.
19. Kocazeybek BS, Caliskan R, Cetin SE, et al. Patterns of EPIYA motifs among cagA-positive Helicobacter pylori strains: a case–control study in a Turkish population with Eurasian geographical features. Journal of medical microbiology. 2015 Oct 1;64(10):1117-23.
20. Salih BA, Guner A, Karademir A, et al. Evaluation of the effect of cagPAI genes of Helicobacter pylori on

AGS epithelial cell morphology and IL-8 secretion. Antonie Van Leeuwenhoek. 2014 Jan 1;105(1):179-89.
21. Kalaf EA, Al-Khafaji ZM, Yassen NY, et al. Study of the cytoxin-associated gene a (CagA gene) in Helicobacter pylori using gastric biopsies of Iraqi patients. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association. 2013 Mar;19(2):69.
22. Ferreira RM, Machado JC, Leite M, et al. The number of Helicobacter pylori CagA EPIYA C tyrosine phosphorylation motifs influences the pattern of gastritis and the development of gastric carcinoma. Histopathology. 2012 May;60(6):992-8.
23. Ohnishi N, Yuasa H, Tanaka S, et al. Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proceedings of the National Academy of Sciences. 2008 Jan 22;105(3):1003-8.
24. .....
25. Cover TL. Helicobacter pylori diversity and gastric cancer risk. MBio. 2016 Mar 2;7(1):e01869-15.
26. Jouimyi, Mohamed R., Boura H., et al. "The EPIYA-ABCC motif of Helicobacter pylori cagA gene and gastric carcinogenesis in Casablanca population." *African Health Sciences* 22.1 (2022): 573-80.
27. Keikha, Masoud, and Mohsen Karbalaei. "EPIYA motifs of Helicobacter pylori cagA genotypes and gastrointestinal diseases in the Iranian population: a systematic review and meta-analysis." *New Microbes and New Infections* 41 (2021): 100865.
28. Takahashi-Kanemitsu A, Knight CT, Hatakeyama M. Molecular anatomy and pathogenic actions of Helicobacter pylori CagA that underpin gastric carcinogenesis. Cell Mol Immunol. 2020 Jan;17(1):50-63. doi: 10.1038/s41423-019-0339-5. Epub 2019 Dec 5. PMID: 31804619; PMCID: PMC6952403.