e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(12); 1247-1254

Original Research Article

Expression Analysis of Genes of Type IV Secretory System of H. Pylori in Different Gastrointestinal Diseases

Feeshan Ahmed[1], Vijay Kumar Ramnani[2], Aleem A khan[3]

[1]PhD Scholar, Department of Microbiology, LN Medical College Bhopal Madhya Pradesh

[2]Professor and Head, Department of Microbiology, LN Medical College Bhopal Madhya Pradesh

[3]Head of Department, Central laboratory for stem cells and Translational medicine, Center for liver Research and Diagnostics, Deccan College of Medical Sciences, Kanchanbagh Hyderabad

Received: 25-09-2023 / Revised: 28-10-2023 / Accepted: 30-11-2023

Corresponding author: Dr. Vijay Kumar Ramnani

Conflict of interest: Nil

Abstract:

Helicobacter pylori (H. pylori) has been classified as a class I carcinogen which infects approximately half of the world's population with certain geographical variations. This has made H. pylori infection as a global public health issue. Differential disease outcome has further led more interest in understanding the molecular pathogenesis of H.pylori. Advancements in the proteomics, transcriptomics and accessibility to partial or complete H. pylori genome sequences helped in understanding the complex gene regulation networks of H. pylori. Yet, the precise molecular mechanism through which the H. pylori infection can cause a significant clinical result remains unknown. It was believed that a combination of factors like host genetic factors, environmental factors, and specific bacterial virulence genes are involved in H. pylori pathogenesis. Therefore, the main objective of the present study was to determine the molecular pathogenesis of H. pylori by examining the transcript expression of type 4 secretion system genes in a variety of patients with gastrointestinal disorders (GIDs), by extracting DNA/RNA from biopsies, and amplifying the relevant genes using PCR. Further, the type 4 secretion system gene expression patterns were correlated with different grade of gastric diseases, to elucidate the role of H. pylori's molecular pathogenesis. The findings of the research revealed that GID patients had significantly higher type 4 secretion system gene expression. Specifically, of the 240 DNA samples examined, 240 (100%), 209 (87.1%), 225 (93.7%), and 234 (97.5%) of the subjects had hrgA, cagA, cagE, and cagT, respectively. A predictive biomarker for early gastric cancer diagnosis may be developed using the correlated expression analysis of transcripts from type 4 secretion system genes.

Keywords: H. pylori, Cag A, Gastric cancer, Gene expression, Predictive Biomarker.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

The pathogenicity of Helicobacter pylori is associated mainly with H. pylori type IV secretion systems (T4SSs). These T4SSs represent remarkable molecular transporter machines with an extracellular pilus structure present in H.pylori which are commonly composed of multiple structural proteins spanning the inner and outer membranes. By definition, these T4SSs exhibit central functions as the secretion of effector proteins in mammalian host target cells. In recent years, numerous features on the molecular functionality of these T4SSs were disclosed. H. pylori encodes up to four T4SSs on its chromosome, namely the Cag T4SS present in the cag pathogenicity island (cagPAI), the ComB system, as well as the Tfs3 and Tfs4 T4SSs, some of which exhibit unique T4SS functions. In the H. pylori-associated diseases pathogenic mechanisms, several strain-specific virulence factors were reported, such as cag A (cytotoxin-associated gene A), cag T, cag E, vac A (vacuolating cytotoxin A) and hrg A, genes. One of the main virulence factors is Cag A, which is associated with higher risk of gastric cancer and peptic ulcer. Cag A protein can interact with intercellular proteins and activate signalling pathways through both tyrosine phosphorylation-dependent or independent mechanisms. (Quigley, E. M. et al 1987, Chen S, et al 2001) The interindividual differences in risk of H. pylori-induced gastric diseases involve significant heterogeneity of both host genetics and H. pylori strain virulence factors. About 70% of H. pylori strains have been found with cag PAI gene. The cag A gene comprises 40 kb of chromosomal DNA with 32 open reading frames (ORFs), such as cag1 to cag 26, cag A to cag Z. The pathogenic mechanism of Cag A protein involves aberrant cellular signaling which may result in gastric cell malignancy (Fischer et al 2011, Hatakeyama et al 2005). Previously it was observed that the

expression of cag A gene varies among H. pylori strains that might be due to several host interactions which are opt for bacteria survival and its pathogenicity [Torres J et al 1998, Batista SA 2011]. Several in vivo studies have been reported the aberrant expression of virulent genes in development of gastric cancers (T. Franco et al 2005, Franco AT, et al 2008), however, there are no studies on correlation of virulent gene expression and host gastric cancer development from Indian population. The current study aimed to evaluate the H. pylori virulence gene cag A expression and correlate the expression pattern with gastric cancer development and other inflammatory responses.

In a study evidence indicates that the risk of gastric cancer or premalignant lesions is higher in persons infected with cag A-positive H. pylori strains than in persons infected with cag A-negative strains (Blaser MJ et al 1995, Plummer M, et al 2007.). The increased risk of gastric cancer observed with cag A-positive strains (which often contain the entire cag PAI) is attributed to the cellular effects of Cag A combined with an enhanced gastric mucosal inflammatory response (Suzuki N, et al 2012).

Cag A was described as the first bacterial oncogene but as with many other oncogenes in humans, the story became even more complex. The disease-associated factor Cag A is also associated with health, and its lack can lead to disease. Although cag A+ H. pylori strains were preferentially associated with both ulcers and gastric cancer, we and others found that these same strains had an inverse (protective) relationship with premalignant and malignant conditions of the esophagus, specifically Barrett esophagus and esophageal adenocarcinoma (Chow WH, et al 1998).

The present study was undertaken to know the association of Cag PAI -genes and different gastrointestinal diseases.

Methodology

Study Design

This study was carried out from 2019 to 2022 at Centre for liver research and diagnosis laboratories and Dept of Gastroenterology, Deccan college of Medical sciences and allied hospitals, Hyderabad, India. Study protocol was approved by the Institutional Ethics Committee, Deccan College of Medical Sciences, and Hyderabad, India. A total of 515 subjects in the age range 18 to 60 years who had undergone upper gastrointestinal endoscopy were included in the study. An informed consent was taken from all patients and other demographic data which include questionnaire on their place of origin, profession, diet, smoking and alcohol habits had recorded. Clinical history regarding the duration and frequency of abdominal pain, heartburn, vomiting, nausea and previous history was also recorded. Subject less than 16 Years and on antimicrobial therapy and H2 receptor blockers, proton pump inhibitors, and NSAID prior to endoscopy were excluded from study.

Subjects infected with HIV, HCV and HBV, suffering with any other type of cancers (other than Gastric cancer) or any other co-morbid diseases and infected with active or latent TB were also excluded from the study.

The endoscopic findings of each patient were classified into 5 categories:

1. Gastric Adenocarcinoma,
2. Peptic Ulcer Disease,
3. Duodenal Ulcer,
4. GERD
5. Gastritis

DNA Isolation Gastric biopsy:

Gastric Biopsies of all 515 subjects was collected during the procedure of upper gastrointestinal endoscopy by using sterile forceps and appropriate measures were taken to ensure aseptic collection of the specimen. Biopsy was collected in phosphate buffered saline (PBS) for genomic DNA isolation.

DNA Isolation:

Genomic DNA was isolated from all samples as per the standard protocol by the Cetyl Trimethyl Ammonium Bromide method. (Clayton et al 1998). All the extracted DNA samples were screened for 16 S rRNA PCR for the presence of H.pylori.

Screening of H. pylori

16S rRNA PCR

DNA was amplified using 16S rRNA PCR to determine the presence of H. pylori. The Polymerase Chain Reaction (PCR) was carried out by using 16S rRNA primers and the DNA isolated from biopsy specimens of the subjects. The amplified product gives a 534 bp band upon agarose gel electrophoresis using a 1.5 % agarose gel at 120v, shown in Figure:

Table 1: Primers utilized to identify the Helicobacter pylori-specific 16S rRNA

Target gene	Primer sequence (5’→3’)	Product size (bp)
16SrRNA of H. pylori	F- 5’- TAAGATCAGCCTATGCC - 3’ R- 5’- TCCCACGCTTTAAGCGCAAT - 3’	534 bp

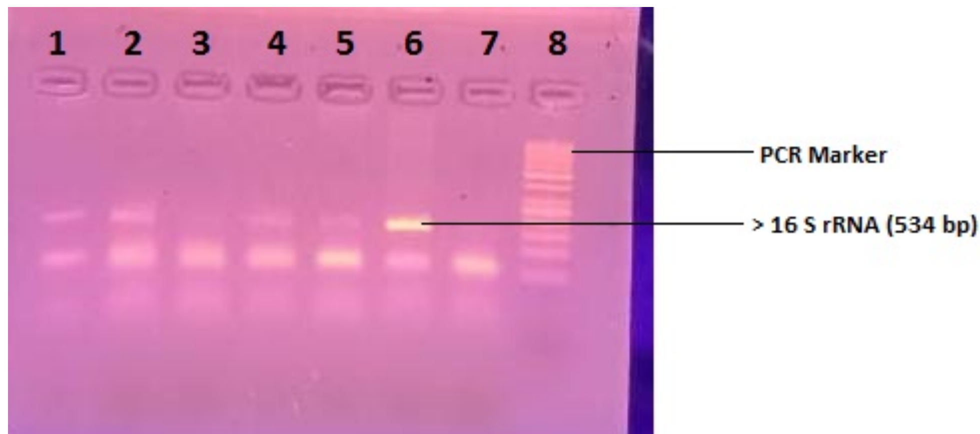

Figure 1: Gel imaging displaying the 16s rRNA gene's amplified result.

Here, Lane 1,2,4,5 and 6: Amplified 16s rRNA gene, product of size 534 bp,

Lane 8: PCR marker (New England Bio labs)

PCR Conditions:

The reaction conditions for the16S rRNA gene amplification with both the primers were optimized and are as follows 950 C for 5 min (Initial denaturation step), 940 C for 30 sec 560 C for 30 sec, 40 cycles 720 C for 30sec, 720 C for 5 min (Final extension step).

Positive and negative controls are kept for the validation of the PCR performed. The negative control consists of the reaction mixture without the DNA template. The positive control contains the H. pylori DNA as the template.

Electrophoresis Conditions:

PCR products were visualized after electrophoresis at 120v on 1.5% agarose gel containing 1µl /ml ethidium bromide under UV light. The PCR product size was 534 bp.

Analysis of type 4 secretary genes H. pylori

Following type 4 secretary genes of H. pylori involved in Gastric pathogenesis.

- **Cytotoxin-associated gene A (Cag A)**
- **Cytotoxin-associated gene E (Cag E)**
- **Cytotoxin-associated gene T (Cag T)**
- **(Hrg A)**
- **Vacuolating cytotoxin A (Vac A)**

Primers and Target genes

Oligonucleotide primers selected to detect the cagE, cagA, cagT, hrgA and vacA genes of Helicobacter pylori

Table 2: oligonucleotide primers chosen for identifying the Helicobacter pylori genes cagE, cagA, cagT, hrgA, and vacA

Target gene	Primers pairs	Sequence (5'→3')	Amplicon size (bp)	Reference
cagE	cagE-F	GCGATTGTTATTGTGCTTGTAG	329	Ikenoue et al. (2001)
	cagE-R	GAAGTGGTTAAAAAATCAATGCCCC		
cagT	cagT-F	ATGAAAGTGAGAGCAAGTGT	842	S.K. Tiwari et al. (2007)
	cagT-R	TCACTTACCACTGAGCAAAC		
cagA	cagA-F	GATAACAGGCAAGCTTTTGA	499	S.K. Tiwari et al. (2007)
	cagA-R	CTGCAAAAGATTGTTTGGCA		
hrgA	hrgA-F	TCTCGTGAAAGAGAATTTCC	594	Ando et al. (2002)
	hrgA-R	TAAGTGTGGGTATATCAATC		
vacA	vacA-F	ATGGAAATACAACAAACACAC	259 (s1) / 286(s2)	Mukhopadhyay et al. (2000)
	vacA-R	CTGCTTGAATGCGCCAAAC		

Polymerase Chain Reaction (PCR)

PCR was carried out on 240 H. pylori positive samples using Cag A, Cag E, Cag T, Hrg A and Vac A Oligonucleotide specific as mentioned above (table).

PCR Amplification: 19 ml of the reaction mixture containing 1X PCR buffer (50 mmol KCl, 10 mmol Tris-HCl [pH 8.3], 1.5% [v/v] Triton X-100), 1.5 mmol MgCl2, 200 mmol concentrations of each dNTP, 10 pmol of each primer, and 1 U of Taq polymerase (Invitrogen Life Technologies, Karlsruhe, Germany) were combined with 1 mL of the template DNA. The PCR amplification process involved a 5-minute initial denaturation at 95°C, 40 cycles total, with one cycle consisting of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C. The last cycle comprised a 10-minute

extension step to guarantee that the PCR products are fully extended. A thermocycler was used to perform the amplification. (USA: Watertown, MA; M J Research). On a 1.5% agarose gel stained with ethidium bromide for visibility, the PCR products were examined. As a positive control, H. pylori DNA (type ATCC 26695) were utilized, whereas water was utilized as a negative control.

Results:

Molecular diagnosis results (16S rRNA PCR)

The patients with H. pylori infection were confirmed by H. pylori species specific 16srRNA gene amplification. Out of total 515 screened gastrointestinal tissue biopsies, 240 where found positive for H. pylori. Gastrointestinal disease based analysis showed, Out of 240 H. pylori 75 patients were found gastric adenocarcinoma, 40 and 45 patients were found peptic ulcer, duodenal ulcer respectively. Further 50 patients were found to suffer from gastroesophageal reflux disease (GERD) and 30 were diagnosed to have Gastritis.

Table 3: Combined result of all 240 isolates for each of the five type H .pylori genes

No of subjects with different GI disorder n=240	Cag A	Cag E	Cag T	Hrg A	Vac A	
					S1 allele	S2 allele
Gastric adino carcinoma (n=75)	75(100%)	75(100%)	75(100%)	75(100%)	62(82.7%)	13(17.3%)
Peptic ulcer (n=40)	40(100%)	40(100%)	40(100%)	40(100%)	40(100%)	-
Duodenal ulcer (n=45)	36(80%)	35(77.7%)	39(86%)	45(100%)	30(66.7%)	15(33.3%)
GERD (n=50)	38(76%)	50(100%)	50(100%)	50(100%)	28(56%)	22(44%)
Gastritis (n=30)	20(66.6%)	25(83.3%)	30(100%)	30(100%)	19(63.3%)	11(36.7%)
Total n=240	209(87.1%)	225(93.7%)	234(97.5%)	240(100%)	179(74.5%)	61(25.5%)

Based on the 16S rRNA amplification, it was found that all 240 participants tested positive for H. pylori. The existence of an active H. pylori infection was confirmed by amplifying all of the DNA samples using 16S rRNA-specific oligonucleotide primers, which produced a product of 534 bp characteristic to H. pylori. Following a 16S rRNA positive test, each of the following genes was examined individually: cagA, cagE, cagT, vacA signal region (s1–s2), and hrgA. Out of the 240 DNA samples that were analyzed, 240 (100%), 209 (87.1%), 225 (93.7%), and 234 (97.5%) of the participants had hrgA, cagA, cagE, and cagT, respectively. Of the vacA-positive subjects, vacAs1 and s2 have been identified in 179 (74.5%) and 61 (25.5%), respectively.

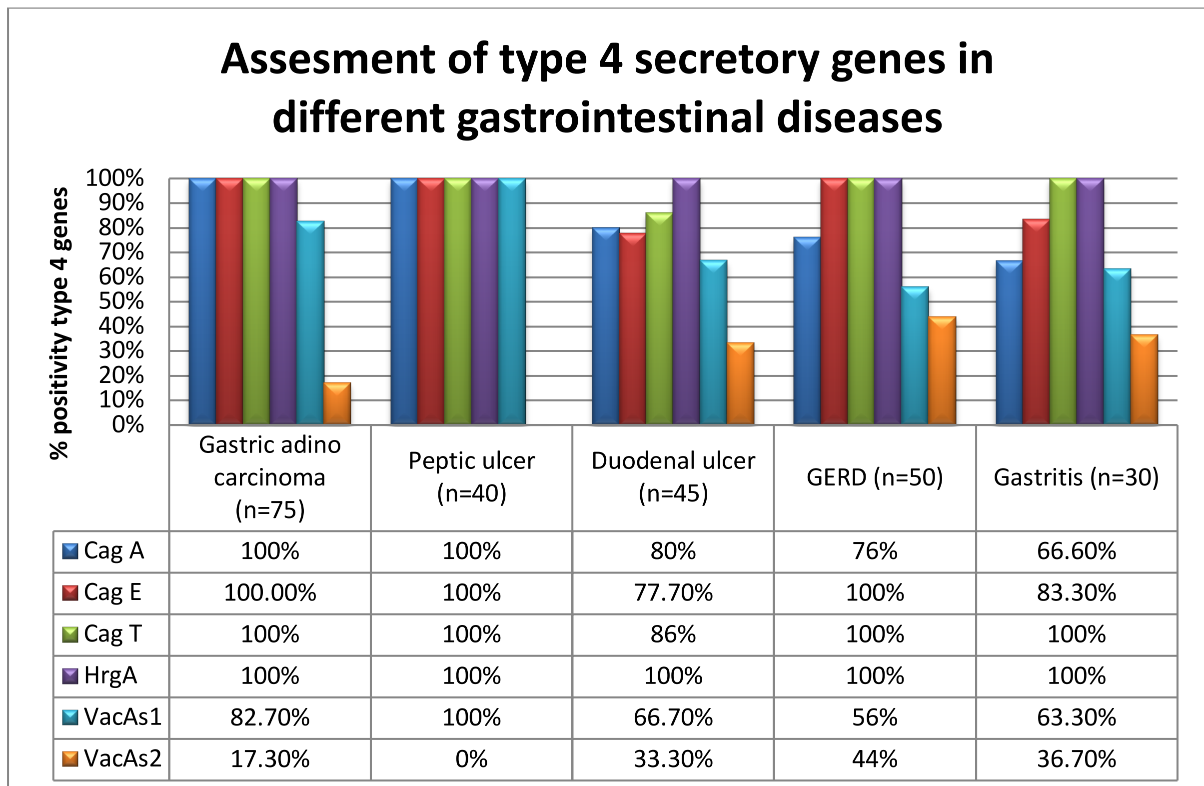

Figure 2: Type 4 secretory gene positive percentage in various gastrointestinal disorders

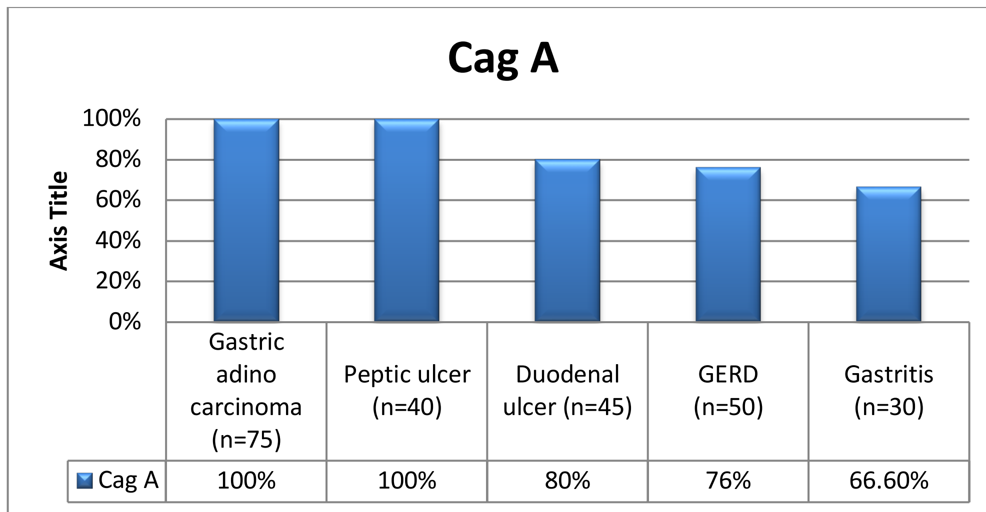

Figure 3: Cag A percentage positivity in various gastrointestinal diseases

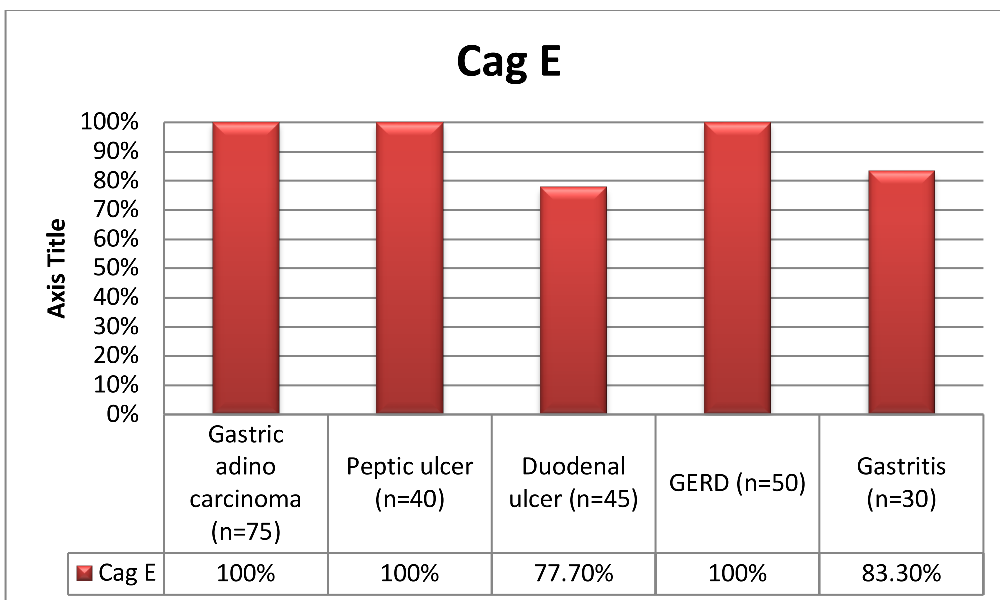

Figure 4: Depicting the percentage of positive Cag E in various gastrointestinal diseases

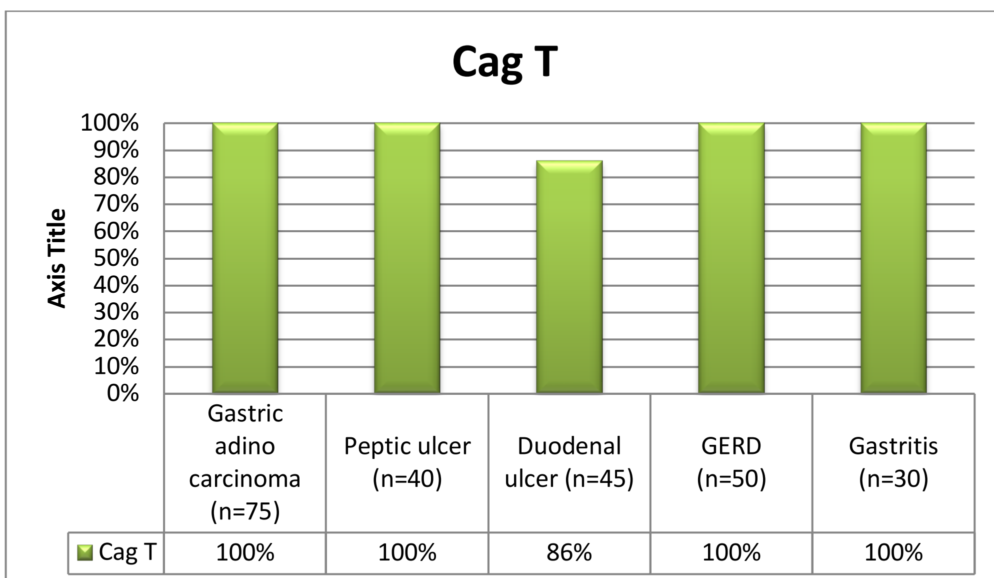

Figure 5: Shows the percentage of positive Cag T in different gastrointestinal conditions

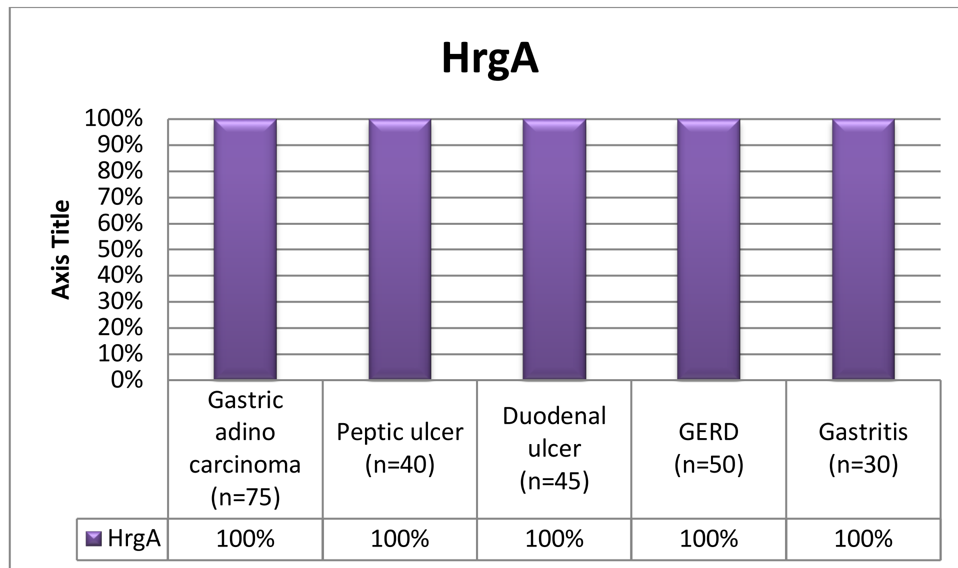

Figure 6: Hrg A positive percentage across a range of gastrointestinal disorders

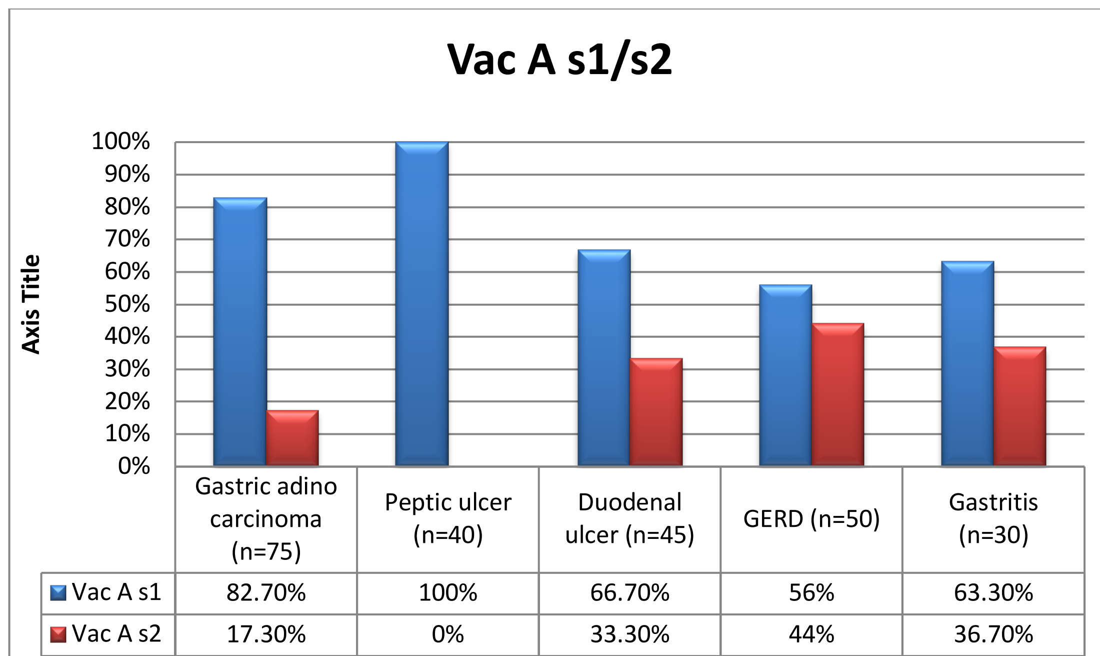

Figure 7: Shows the percentage of Vac A s1/s2 positive in different gastrointestinal disorders

Discussion

The presence of Helicobacter pylori in the stomach tissue was confirmed by 16S rRNA amplification. In this study 16S rRNA PCR amplification is the "standard" for confirming the presence of an active H. pylori infection. 16S rRNA primers have been employed in multiple investigations that support increased sensitivities and specificities. (Ho S, et al. 1991, Weiss J, et al. 1994.).

In the course of our analysis, the biopsy specimens of all 515 patients revealed 240 positive results for 16S rRNA PCR amplification in 5 different categories of gastrointestinal diseases. The results of the 16S rRNA amplification show a 534 bp product size. The visibility of the desired band with each primer set on an agarose gel, indicates that amplification with distinct primers spanning diverse areas of cag PAI produced a positive result. Based on the data of our study, the hrgA gene was the most often amplified virulence marker across all 240 (100%) samples, followed by cagT (97.5%), cagE (93.7%), and cagA (87.1%). S1 alleles were found in 74.5% and S2 alleles in 25.5% of the 240 samples that were included, and all of them tested positive for vacA. In our investigation peptic ulcer demonstrated 100% significant value to all 5 genes. A latest study indicates that strains obtained from ulcer patients had a notably greater frequency of all individual genes and intact cagPAI than strains obtained from patients without ulcers. (Markovska, R et al 2018). Compared to individuals with GERD

and Gastritis, patients with overt disease presentations such as gastric cancer, duodenal ulcer, and peptic ulcer had a higher prevalence of the s1 subtype of the vacA gene; these findings were determined to be statistically significant ($P < 0.01$).

The fact that the hrgA gene was 100% prevalent in all disease groups, regardless of clinical category, is another significant finding of this study. This study's result is significantly different from that of (Ando et al. 2002), who found that patients with stomach cancer had a greater prevalence of hrgA separated from them than did patients with cancer in Asia. Further investigation into the cause of this inconsistent outcome in a large sample of H. pylori from various geographic locations is necessary.

A recent study by Jenk et al. found that while the presence of the complete cag PAI is strongly linked to duodenal ulcers, the analysis of several cag PAI genes, such as cagA, cagE, and cagT, does not consistently predict the clinical outcome of H. Pylori infection. While cagE was not consistent with cagT, it was entirely consistent with cagA in the study conducted by Jenk et al. In a different investigation, cagE and cagT were found to have 96.6% consistency in the case of people with duodenal ulcers by Ikenoue et al.

On the other hand, our study, which included 40 patients with peptic ulcers and 45 patients with duodenal ulcers infected with H. pylori, demonstrated that, when compared to cagA, cagE and cagT were the most reliable markers to confirm the presence of cag PAI. This was because cagE was detected in 93.7% of the subjects and cagT in 97.5% of the total cases included in the study. According to Barrozo, R.M, et al. 2013, CagT is essential for translocation of the effector protein CagA into the epithelial cells. In contrast to previous studies (Ogura K, et al 2000.) that focused on the presence of cagE or cagA as likely virulence markers influencing the disease's fate, the current study therefore indicated for the first time that cagT could be the key gene controlling the disease's outcome.

It was found that nearly every sample of ulcer-affected participants had cagT, and a considerable proportion of patients in other groups also carried the cagT locus. Only one locus (cagT) is linked to the severe consequence of H. pylori infection, which is duodenal and peptic ulcers, despite a thorough investigation of the status of cag PAI and the features of what is known as a key pathogenicity factor, i.e., the cagA gene. Because the genes in the cag PAI have been passed down as a whole during evolutionary events that have occurred after their integration, it is well known that these genes encode proteins that are extremely similar in their function. Rather than being caused by a single virulence factor, virulence is more likely the outcome of a confluence of factors, such as the presence of cag PAI together with those encoding cytotoxin production and adhesion expression. (Covacci A, et al 1997) However, no meaningful correlation could be found in the current investigation between the cag PAI isolation status and the study groups' enrollment. Rather, a significant determinant called cagT was discovered, which may contribute to the development of the disease's severe form. Our study showed that the genes involved in type 4 secretary system of h. pylori have higher positivity in our severe gastrointestinal diseases.

Reference:

1. Ando, T., Wassenaar, M., Peek, R.M., Aras, R.A., Tschumi, A.I., van Doorn, L.J., Kusugami, K. and Blaser, M.J. (2002) A Helicobacter pylori restriction endonuclease-replacing gene, hrgA, is associated with gastric cancer in Asian strains. Cancer Res. 2002; 62: 2385–2389.
2. Barrozo RM, Cooke CL, Hansen LM, Lam AM, Gaddy JA, et al. (2013) Functional Plasticity in the Type IV Secretion System of Helicobacter pylori. PLOS Pathogens. 2013; 9(2): e1003189.
3. Batista SA, Rocha GA, Rocha AM, et al. Higher number of Helicobacter pylori Cag A EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer. BMC Microbiology. 2011 Mar; 11:61.
4. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. 1995. Infection with Helicobacter pylori strains possessing cag A is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 55:2111–2115.
5. Chen S, Zhang R and Duan G: Pathogenic mechanisms of the oncoprotein Cag A in H. pylori-induced gastric cancer (Review). Oncol Rep 36: 3087-3094, 2016 Receptor signaling. Infect. Immun. 2001;69: 1714–1721.
6. Chow WH, et al. An inverse relation between cag A+ strains of Helicobacter pylori infection and risk of esophageal and gastric cardia adenocarcinoma. Cancer Res. 1998; 58:588–90.
7. Clayton CL, Mobley HLT. Methods in Molecular Medicine Helicobacter pylori protocols. Totowa: Humana Press Inc, 1998: 33.
8. Covacci A, Falkow S, Berg DE, Rappuoli R. Did the inheritance of a pathogenicity island modify the virulence of Helicobacter pylori? Trends Microbiol. 1997; 5: 205–8.
9. Fischer, W. Assembly and molecular mode of action of the Helicobacter pylori Cag type IV

secretion apparatus. FEBS J. 2011; 278: 1203–1212.
10. Franco AT, Israel DA, Washington MK, Krishna U, Fox JG, Rogers AB, Neish AS, Collier-Hyams L, Perez-perez GI, Hatakeyama M, Whitehead R, Gaus K, O'Brien DP, Romero-Gallo J, Peek RM Jr. Activation of beta-catenin by carcinogenic Helicobacter pylori. Proc Natl Acad Sci U S A. 2005 Jul 26;102(30):10646-51.
11. Franco AT, Johnston E, Krishna U, et al. Regulation of gastric carcinogenesis by Helicobacter pylori virulence factors. Cancer Res. 2008;68(2):379-387.
12. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. Cell. 2011; 144: 646–674.
13. Hatakeyama, M. & Higashi, H. Helicobacter pylori Cag A: a new paradigm for bacterial carcinogenesis. Cancer Sci. 2005; 96: 835–843 (2005).
14. Helicobacter pylori [Clinical significance of infection with cag A and vac A positive Helicobacter pylori strains]. Srp Arh Celok Lek. 2004 Nov-Dec; 132(11-12):458-62. Serbian.
15. Helicobacter pylori vacA s1m1 genotype but not cagA or babA2 increase the risk of ulcer and gastric cancer in patients from Southern Mexico
16. Ho S, Hoyle J, Lewis F et al. Direct polymerase chain reaction test for the detection of Helicobacter pylori in humans and animals. J. Clin. Microbiol. 1991; 29: 2543–4.
17. Ikenoue T, Maeda S, Ogura K et al. Determination of elicobacter pylori virulence by simple gene analysis of the cag pathogenicity island. Clin. Diag. Lab. Immunol. 2001; 8: 181–6.
18. Jenks PJ, Megraud F, Labigne A. Clinical outcome after infection with Helicobacter pylori does not appear to be reliably predicted by the presence of any of the genes of the cag pathogenicity island. Gut 1998; 43: 752–8.
19. Markovska, R.; Boyanova, L.; Yordanov, D.; Stankova, P.; Gergova, G.; Mitov, I. Status of Helicobacter pylori cag pathogenicity island (cagPAI) integrity and significance of its individual genes. Infect. Genet. Evol. 2018; 59: 167–171.
20. Mimuro, H. et al. Helicobacter pylori dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. Cell Host Microbe. 2007; 2: 250–263.
21. Mukhopadhyay, A.K., Kersulyte, D., Jeong, J.Y., Datta, S., Ito, Y., Chowdhury, A., Chowdhury, S., Santra, A. et al. Distinctiveness of genotypes of Helicobacter pylori in Calcutta, India. J Bacteriol. 2000; 182: 3219–3227.
22. Plummer M, van Doorn LJ, Franceschi S, Kleter B, Canzian F, Vivas J, Lopez G, Colin D, Muñoz N, Kato I. Helicobacter pylori cytotoxin-associated genotype and gastric precancerous lesions. J Natl Cancer Inst. 2007; 99:1328–1334.
23. Quigley EM, Turnberg LA. pH of the microclimate lining human gastric and duodenal mucosa in vivo: studies in control subjects and in duodenal ulcer patients. Gastroenterology. 1987 Jun 1;92(6):1876-84.
24. Suzuki N, Murata-Kamiya N, Yanagiya K, Suda W, Hattori M, Kanda H, Bingo A, Fujii Y, Maeda S, Koike K, Hatakeyama M. 2015. Mutual reinforcement of inflammation and carcinogenesis by the Helicobacter pylori CagA oncoprotein. Sci Rep. 2015; 5:10024.
25. Tegtmeyer, N.; Neddermann, M.; Asche, C.I.; Backert, S. Subversion of host kinases: A key network in cellular signaling hijacked by Helicobacter pylori Cag A. Mol. Microbiol. 2017; 105: 358–372.
26. Tiwari SK, Khan AA, Manoj G, Ahmed S, Abid Z, Habeeb A, Habibullah CM. A simple multiplex PCR assay for diagnosing virulent Helicobacter pylori infection in human gastric biopsy specimens from subjects with gastric carcinoma and other gastro-duodenal diseases. J Appl Microbiol. 2007 Dec;103(6):2353-60.
27. Torres J, Perez-Perez GI, Leal-Herrera Y and Munoz O: Infection with CagA+ Helicobacter pylori strains as a possible predictor of risk in the development of gastric adenocarcinoma in Mexico. Int J Cancer. 1998;78: 298-300.
28. Vaziri, F.; Peerayeh, S.N.; Alebouyeh, M.; Maghsoudi, N.; Azimzadeh, P.; Siadat, S.D.; Zali, M.R. Novel effects of Helicobacter pylori Cag A on key genes of gastric cancer signal transduction: A comparative transfection study. Pathog. Dis. 2015; 73: ftu021.
29. Weiss J, Mecca J, Silva E, Gassner D. Comparison of PCR and other diagnostic techniques for detection of Helico-bacter pylori infection in dyspeptic patients. J. Clin. Micro- biol. 1994; 32: 1663–8.
30. Wroblewski, L.E.; Peek, R.M., Jr. Helicobacter pylori: Pathogenic enablers-toxic relationships in the stomach. Nat. Rev. Gastroenterol. Hepatol. 2016, 13, 317–318.
31. Yamaoka, Y. Mechanisms of disease: Helicobacter pylori virulence factors. Nat. Rev. Gastroenterol. Hepatol. 2010; 7: 629–641.