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Association of *H.pylori cagA* Gene with Duodenal Ulcer & Gastric Carcinoma in Bangladeshi Patients

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Abstract **Background:** Prolong infection with *Helicobacter pylori* may lead to chronic inflammation of gastroduodenal mucosa which in turns develops into severe diseases like peptic ulcer and gastric carcinoma. Bacterial virulence factor Cytotoxin Associated gene (*cagA*) is found to be responsible for developing such severe diseases in different countries. So this study was conducted to assess the relationship between occurrence of several gastroduodenal diseases and the presence of *H. pylori cagA* gene in Bangladeshi patients. **Methods:** Endoscopic gastroduodenal biopsy sample of 113 dyspeptic patients from different districts of Bangladesh were studied. *H. pylori* infection was detected by Rapid urease test, PCR of *ureC* gene and histological staining (Geimsa). Gastroduodenal disease was diagnosed by histopathological examination and *cagA* gene was detected by PCR. **Result:** *H. pylori* infection was identified among 48% (54/113) patients. Fifty seven percent of *H. pylori* infected patients were found to be *cagA* gene positive. *cagA* gene is significantly associated with Duodenal ulcer (p= .024) and Gastric carcimoma (p < .001). However, a further larger study is required to confirm this finding.

Keywords: *H. pylori infection, cagA gene, duodenal ulcer, gastric carcinoma*

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1. Introduction

Helicobacter pylori is a curved Gram negative bacteria that is colonizes in the gastric mucosa of about half of the world's population where it causes a variety of clinical outcomes ranging from asymptomatic carriage to gastritis, peptic ulcers, and cancer [1]. However, less than 20% of infected patients present with clinical symptoms suggesting that, the disease severity is dependent on interactions between the host and the environment, and bacterial virulence [2].

Many putative virulence factors have been identified in *H. pylori* that contribute to its pathogenesis. The 128-kDa cytotoxin-associated gene encoded antigen A (*cagA*) and vacuolating cytotoxin antigen gene (*vacA*) are known as the most important ones [3,4].

The *cagA* gene is a strain-specific *H pylori* gene and has been widely recognized as a marker for strains that confer increased risk for peptic ulcer disease [5] and gastric cancer [6]. The *cagA* gene is present downstream of a 40-kb cluster of virulence genes known as the cag pathogenicity island (cag- PAI). These virulence genes encode a type IV secretion system that forms a syringe-like structure to translocate the *cagA* protein into the gastric epithelial cells. The cag-PAI has also been implicated in the induction of IL-8 in cultured gastric cells [7]. This property contributes to the virulence capability of the *cag* positive strains by enhancing their proinflammatory power.

In contrast to the *cagA* gene, nearly all *H. pylori* strains around the world has possesed the *vacA* gene [8]. A strong correlation has been found between the presence of the *cagA* gene with expression of the vacuolating cytotoxin activity [9]. Furthermore, it has been found that most strains possessing *cagA* also possess the more virulent vacuolating form of *VacA* [10].

A number of recent studies have suggested that, infection with cag PAI-positive strains of *H. pylori* may significantly increase the risk of developing severe gastric mucosal inflammation, duodenal ulceration and gastric cancer and its precursor lesions [11,12].

Although, serological methods to detect specific antibodies to the *H. pylori cagA* protein would be more suitable [13] than genotype identification as it needs endoscopic gastroduodenal biopsy samples, Genotype identification is more accurate procedure as subjects infected with *H. pylori* strains containing the *cagA* gene do not always induce serum *cagA* antibody [14]. Moreover, it has been suggested that host immunological responses to *H. pylori* may vary in different populations [15].

H. pylori has a global distribution but geographical differences in the prevalence of *cagA* status among *H. pylori* isolates have been reported [16]. Even within country, the prevalence rate may vary between distinct geographic region [17]. Moreover no study was done to determine the association between *cagA* gene and gastroduodenal diseases in our country.

So the aim of this study was to determine the association between the presence of *cagA* genotype in *H. pylori* and the severity of different gastroduodenal diseases in a group of Bangladeshi patients.

2. Methods

The study was conducted in Department of Microbiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.

2.1. Patients and Gastric Biopsy Samples

The present study included 113 Bangladeshi patients who underwent upper Gastrointestinal endoscopic examination at outpatient department of Department of Gastroenterology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka, and Dhaka Medical college Hospital, Dhaka between March, 2015 to February 2016. Out of 113 patients 65 patients were endoscopically reported as gastritis followed by 18 duodenal ulcer, 17 gastric ulcer and 13 gastric carcinoma. Patient aged from 18 to onward presenting with symptoms of dyspepsia more than 1 month were included in the study. Patients who received *H. pylori* eradication treatment in the previous 2 months [18], Elderly individuals who had age more than 65 years, had severe medical or surgical illnesses or had used proton pump inhibitors, nonsteroidal anti-inflammatory drugs, colloidal bismuth compounds, or antibiotics within 4 weeks of enrollment were excluded from the study [19].

The study populations were categorized into 2 groups based on the case definition used in this study: *H. pylori* positive patients (n = 54) & *H. pylori* negative patients (n = 59). Patients were considered as *H. pylori* positive when positive results were obtained in at least two of the three tested methods (Rapid urease test, histology for *H. pylori* and PCR for *ureC* gene) and considered as negative when the results of all diagnostic tests were negative. Gastroduodenal diseases was diagnosed by endoscopic and histopathological examinations and established in accordance with the Sydney System Classification.

From each patient three pieces of gastric tissue were taken from nonlesional mucosa of the lesser curvature side of the antrum and three pieces from midbody for *H. pylori* detection (Pentax Video-Endoscopy EG/3485). Additional biopsies were taken from margins of malignant looking ulcers or proliferative growths for histopathological examination to confirm the diagnosis. Two specimen, each from the antrum and body were fixed in 10% buffered formalin and send to the Pathology Department of BSMMU and DMC hospital for histopathological examination. One specimen each from the body and antrum were examined for the presence of *H. pylori* by rapid urease test and one specimen from the antrum and body were preserved in 1.5 ml microcentrifuge tube containing 1 ml phosphate buffer solution for detection of *H. pylori ureC* gene and *cagA* gene by PCR. All biopsy samples were stored at -20°C until DNA extraction from the samples were performed.

2.2. DNA Extraction and PCR for ureC and cagA Gene

DNA from gastric tissues was extracted by using the QlAamp (QIAGENÒ) DNA Mini Kit according to the manufecturer's instructions. For confirming the presence of *H. pylori* DNA in tissue, the *ureC* gene was identified by PCR using the following primers [20]. (Figure 1)

Primers	Primer sequence (5′-3′)	Product size
ureC-F	AAGCTTTTAGGGGTGTTAGGGGTTT	
ureC-R	AAGCTTACTTTCTAACACTAACGC	294 bp

H. pylori cagA gene was detected by using following primers designed by Yamaoka et al. (1997) [21] (Figure 2).

Primers	Primer sequence (5′-3′)	Product size
cagA-F	GATAACAGGCAAGCTTTTGAGG	
cagA-R	CTGCAAAAGATTGTTTGGCAGA	349 bp

The PCR was performed as previously reported in another study [22]. The DNA were denatured at 94°C for 4 minutes, followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds with a final extension at 72°C for 7 minutes.

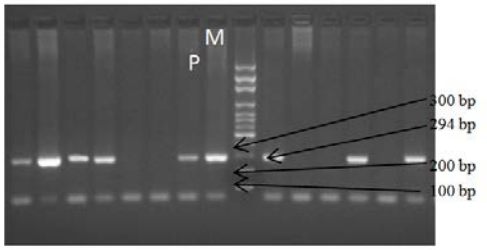

Figure 1. Amplification of 294 bp product of *H. pylori ureC* gene. (M = ladder marker, Lane-p = positive results).

This work was ethically approved by Institutional Review Board, BSMMU; process number BSMMU/2015/10008. Written, informed consent was obtained from all patients.

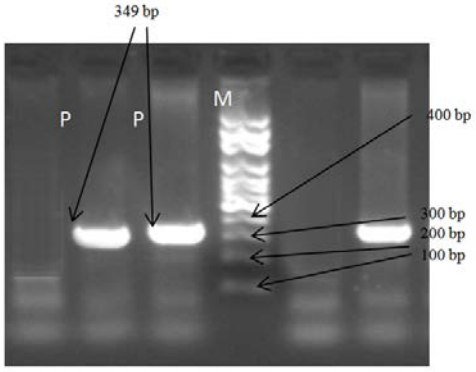

Figure 2. Amplification of 349 bp product of H. *pylori cagA* gene. (M =ladder marker, Lane-p = positive results)

2.3. Statistical Analysis

The sample size was calculated by using the following formula. A 95% confidence interval was used. The seroprevalence of *H. pylori* infection in Bangladesh was 92% and acceptable error was 5%. The statistical analysis was based on the creation and categorization of variables and was performed by using SPSS version 20.0 (Statistical Program for the Social Sciences Inc., Chicago, USA). The Chi-square test or Fisher's exact test were used to compare between proportions. Values of $p < 0.05$ were considered statistically significant.

3. Results

The Out of 113 patients, majority (65) were endoscopically reported as gastritis followed by 18 duodenal ulcer, 17 gastric ulcer and 13 gastric carcinoma. The study populations were between the ages 18- 65 years, with mean age 39.4±12.8 years. Majority of gastritis patients (15, 23%) were in age group between 21-30 years whereas 35.3% (6/17) of gastric ulcers and 46.2 % (6/13) of gastric carcinoma patients were in 41-50 years of age group. Thirty three percent (6/18) patients of duodenal ulcer were in age group between 60-65 years (Table 1). 72 (63.7%) of the study population were male and 41 (36.3%) were female with male female ratio of being 2:1. The relation between endoscopic findings and histopathologial findings are shown in Table 2. Endoscopic findings of the study population were significantly associated with histopathological findings (p<.001) (Table 2). In the present study 47.8% (54/113) were defined as *H. pylori* positive cases and 52.2 % (59/113) were considered as *H. Pylori* negative cases (Table 3).

Table 1. Endoscopic findings of the study population in relation to age (n =113)

Age groups (in years)	Endoscopic findings			
	Gastritis **n (%)**	Gastric ulcer **n (%)**	Duodenal ulcer **n (%)**	Gastric carcinoma **n (%)**
< 20 (n=5)	4(6.2)	0(0)	1(.06)	0(0)
21-30 (n=20)	15(23)	1(.06)	4(22.2)	0(0)
31-40 (n=22)	14(21.5)	5(29.4)	3(16.7)	0(0)
41-50 (n=18)	10(15.4)	3(17.6)	2(11.1)	3(23)
51-60 (n=26)	12(18.4)	6(35.3)	2(11.1)	6(46.2)
>60 (n=22)	10(15.4)	2(11.7)	6(33.3)	4(30.8)

Table 2. Relation between Endoscopic findings and histopathological findings among study population (N =113)

Endoscopic findings n (%)		Histopathological findings						Total cases
	NGM n (%)	G n (%)	CGU n (%)	DU n (%)	IM n (%)	GD n (%)	GC n (%)	
Gastritis n=65	13 (20)	48(73.8)		0(0)	4(6.2)	0(0)	0(0)	65(57.5)
Gastric ulcer n=17	0(0)	0(0)	12(70.6)	0(0)	3(17.6)	1(5.9)	1(5.9)	17(15)
Duodenal ulcer n=18	0(0)	0(0)	0(0)	17(94.4)	1(5.6)	0(0)	0(0)	18(15.9)
Gastric carcinoma n=13	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	13(100)	13(11.5)
Total n=113	13(11.5)	48(42.4)	12(10.6)	17(15)	8(7)	1(.08)	14(12.4)	113(100)

p< .001*. *p* value was determined by Chi square test. (*= statistically significant).
NGM= Normal gastric mucosa, G = Gastritis, CGU = Chronic Gastric ulcer, DU = Duodenal ulcer, IM = Intestinal metaplasia, GD = Gastric dysplasia, GC = Gastric carcinoma

Table 3. Distribution of study population as per case definition (N=113)

Study population	No of cases	Percentage
***H. pylori* positive cases**	54	47.8
***H. pylori* negative cases**	59	52.2

The rate of *H. pylori* infection in different histopathologically confirmed gastroduodenal diseases are demonstrated in Table-4. Gastric carcinoma is significantly associated with *H. pylori* infection (p=.046). Table-5 has revealed the frequency of positive cagA gene in different gastroduodenal diseases among *H. pylori* positive cases. Out of 54 *H. pylori* positive patients, 31 (57.4%) were *cagA* gene positive.

The *cagA* gene is significantly associated with duodenal ulcer cases (p=.024) and gastric carcinoma (p=<.001).

Table 4. Rate of *H. pylori* infection in different gastrointestinal diseases confirmed by histopathology (n=113)

Histopathological findings	*H. pylori* positive cases n (%)	*H. pylori* negative cases n (%)	*p-value*
Normal Gastric mucosa (n =13)	1(8)	12 (92)	.136
Gastritis (n=48)	23 (48)	25(52)	.068
Chronic Gastric ulcer (n=12)	5 (41.67)	7 (58.33)	.053
Duodenal ulcer (n=17)	10 (58.82)	7 (41.18)	.056
Intestinal metaplasia (n=8)	6 (75)	2(25)	.167
Gastric dysplasia (n=1)	1 (100)	0(0)	.500
Gastric carcinoma (n=14)	8 (57.14)	6 (42.86)	.046*

Figures within parenthesis mention percentage (%)
p value was determined by Chi square test. (*= statistically significant).

Table 5. Frequency of positive *cagA* gene in different gastroduodenal diseases among *H. pylori* positive cases (N =54)

Histopathological findings	No of cases	*H.pylori* positive cases n (54)		*p-value*
		cagA positive case (n= 31)	*cagA* negative case (n= 23)	
Normal Gastric mucosa	1	0 (0)	1(100)	NS
Gastritis	23	10 (43.5)	13 (56.5)	NS
Chronic Gastric ulcer	5	2 (40)	3 (60)	.067
Duodenal ulcer	10	7 (70)	3 (30)	.024*
Intestinal metaplasia	6	4 (66.67)	2 (33.33)	.102
Gastric dysplasia	1	1 (100)	0 (0)	NS
Gastric carcinoma	8	7 (87.5)	1 (12.5)	<.001*
Total	54	31(57.41)	23(42.39)	

Figures within parenthesis mention percentage (%). p value was determined by Chi square test. NS= Not significant.

4. Discussion

In the present study 47.8% was diagnosed as *H. pylori* positive cases but the prevalence of *H. pylori* in Bangladesh is reported to be 42% by 2 years of age with a rapid increase to 67% by 10 years of age [23]. On the other hand, Rahman et al. (2009) [24] has found *H. pylori* positive in 53.3% cases by RUT and in 43.4% cases by histology. In another study about 92% adult population in Bangladesh was seropositive for *H. pylori* [25]. The present study has found lower *H. pylori* detection rate in comparison to a study done by Hanif et al. (2010) [26] in Pakistan who found 68% *H. pylori* positive by RUT, 62% by PCR for *ureC* gene and 64% by histological staining. On the other hand, 91% of the studied dyspeptic patients were positive for *H. pylori* in an Egyptian study by Amer et al. [27] Prevalence of *H. pylori* infection varies between and within countries depending on socioeconomic factors, different demographic distribution of the organism among various regions, previous antibiotic consumption [28,29] and the method of detection of infection because gastroduodenal biopsy based tests may give false negative results due to sampling error [30]

Among the histopathology confirmed intestinal metaplasia patients, 75% were *H. pylori* positive cases but In the current study, 39.3% of the gastritis and 58.8% of the duodenal ulcer patients were infected with *H. pylori*. This findings correlate with the findings reported by Helaly et al. (2009) [31] in Egypt who found 41.1% of the gastritis patients and 54.5% of duodenal ulcer patients to be *H. pylori* positive. In spite of high prevalence rate of *H. pylori* infection among dyspeptic patients, a low incidence of gastric adenocarcinoma has been found in the present study as reported by Miwa et al, 2002 [32].

In the present study, *H. pylori* infection was predominant among the gastric carcinoma patients (p=.046). Yamagata et al. (2000) [33] in Japan observed similar findings among the age-adjusted gastric cancer patients. Only a minority of infected patients develops severe diseases and variations in clinical outcome may be due to the considerable genetic diversity of the *H. pylori* strains that cause infection, and host factors.

The prevalence of *cagA* positive *H. pylori* varies among different geographic regions. The primary geographic influence has an important role in the adaptation of organism to the environment and climatc conditions [34]. In this study, out of 54 *H. pylori* positive cases 31(57.4%) were cagA gene positive. This finding correlates with the findings of several studies, where *cagA* positivity rate was reported 61% in China [35], 61.6% in Tunissia [36] and 65.9% in Brazil [37]. Almost similar finding was reported by Rahman et al. [38] (2003) in Bangladesh who found 68.4% *cagA* gene positive among 57 culture positive *H. pylori* cases. *H. pylori cagA* positive strains are more virulent causing higher level of gastric mucosal inflammation in gastritis and gastric cancer [39]. In the present study, in relation to different gastroduodenal diseases *cagA* gene was positive in 70% of duodenal ulcer and 41.7% of gastritis patients. It has been reported that, *cagA* was present among 75% of the strains from the patients with duodenal ulcer disease and 55% of strains from patients with gastritis in Bangladeshi patients [38]. The *cagA* was significantly associated with duodenal ulcer cases (p =.024) in this study. This finding is in agreement with studies done by Nomura et al. (2002) [5] and Yamoka et al. (1999) [40] in Japan who found strong association between *cagA* gene and peptic ulcer diseases but in contrast, no association was found between *cagA* status and duodenal ulcer in Singapore [41]. The large variation in the *H. pylori* genome that amplifies the *cagA* gene from *H. pylori* isolated in one country failed to detect *cagA* from another country [42]. Moreover, there may be several distinct form of *cagA* gene with an uneven geographical distribution that may provide a marker for difference in virulence among *cagA* positive *H. pylori* strain. This may be the reason for difference in *cagA* status in different countries. Moreover, selection criteria of patients and large diverse study groups with respect to genotypes and clinical symptoms are also considered as important factor for variation in results in different regions [43]

A high frequency of *cagA*-positive strains was observed in intestinal metaplasia and gastric dysplasia patients (Table 5), indicating that a statistical association could be reached by increasing the number of patients in future studies.

In this study, 87.5% (7/8) of the gastric carcinoma patients were infected with *cagA* gene positive strains. Zohu et al (2004) [44] reported 100% of gastric carcinoma patients were *cagA* positive. A significant association is observed between *cagA* status and gastric carcinoma (p <.001) in the present study. It has been suggested that, the persons infected with *cagA* positive *H. pylori* are at considerably increased risk of gastric cancer than with uninfected subjects [45].

5. Conclusion

Our present study demonstrated that, the *cagA* gene is significantly associated with duodenal ulcer disease and gastric carcinoma in Bangladeshi patients. One of the major limitations of our study was that we could not compare our result with serological test as anti *cagA* antibody cannot be detected due to limitation of time and

budget. Besides *H. pylori* colonizer was not identified as asymptomatic patients were not included in the study. Though our study provides a significant association between infections by *cagA* gene-positive strains of *H. pylori* and severe clinical outcomes, but future multicentre studies on a large scale with a full characterization of Bangladeshi *H pylori* isolates are required to confirm our findings and overcome above mentioned limitations.

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References

[1] Atherton J. (2006). The pathogenesis of *H pylori*-induced gastro-duodenal diseases. *Annu. Rev. Pathol*;1: 63-96.

[2] Kamali-Sarvestani E, Bazargani A, Masoudian, Lankarani K, Taghavi AR, Saberifiroozi M (2006). Association of *H. pylori* cagA and vacA Genotypes and IL-8 Gene Polymorphisms with Clinical Outcome of Infection in Iranian Patients with Gastrointestinal Diseases, *World Journal of Gastroenterology* ; 12 (32): 5205-5210.

[3] Graham DY & Yamaoka Y (1998). H. pylori and cagA: relationships with gastric cancer, duodenal ulcer, and reflux esophagitis and its complications, *Helicobacter*; 3: 145-151.

[4] Atherton JC, Cover TL, Twells RJ, Morales MR, Hawkey CJ, Blaser MJ (1999). Simple and accurate PCR-based system for typing vacuolating cytotoxin alleles of *Helicobacter pylori*, *Journal of Clinical Microbiology*; 37: 2979-2982.

[5] Nomura AM, Perez-Perez GI, Lee J, Stemmermann G, Blaser MJ (2002). Relation between Helicobacter pylori cagA status and risk of peptic ulcer disease. *American Journal of Epidemiology*; 155: 1054-1059.

[6] Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH et al. (1995). Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach, *Cancer Research*; 55: 2111-2115.

[7] Fischer W, Püls J, Buhrdorf R, Gebert B, Odenbreit S, Haas R (2001). Systematic mutagenesis of the Helicobacter pylori cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8, *Molecular Microbiology*; 42: 1337-1348.

[8] Chen XJ, Yan J, Shen YF. (2005). Dominant CagA/VacA genotypes and coinfection frequency of *H. pylori* in peptic ulcer or chronic gastritis patients in Zhejiang Province and correlations among different genotypes, coinfection and severity of the diseases. *Chin. Med. J. Engl.* 118: 460-467.

[9] Sharma S, Tynnyry M, Miller G. (1995). Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. *Infect. Immunol*. 63: 1681-1687.

[10] Argent RH, Thomas RJ, Letley DP, Ritting MG, Hardie KR, Atherton JC. 2008. Functional association between the Helicobacter pylori virulence factors VacA and CagA. J. Med. Microbiol. 57: 145-150.

[11] Essa AS, Nouh MA, Ghaniam NM, et al. (2008). Prevalence of cagA in relation to clinical presentation of *Helicobacter pylori* infec¬tion in Egypt. *Scand J Infect Dis*; 40: 730-733.

[12] Destura RV, Labio ED, Barrett LJ, et al. (2004). Laboratory diagnosis and susceptibility profile of *Helicobacter pylori* infection in the Philippines; *Ann Clin Microbiol Antimicrob*; 3: 25-30

[13] Figueiredo C, Quint W, Nouhan N, van den Munckhof H, Herbrink P, Scherpenisse J, et al. (2001). Assessment of Helicobacter pylori vacA and cagA genotypes and host serological response. *J. Clin. Microbiol*. 39: 1339-1344.

[14] Shiota S, Matsunari O, Watada M, Yamaoka Y. (2010). Serum *Helicobacter pylori* CagA antibody as a biomarker for gastric cancer in east-Asian countries. *Future Microbiol*. 5: 1885-1893.

[15] Saverymuttu S. (2001). Genetic diversity in the *Helicobacter pylori* cag pathogenicity island and effect on expression of anti-CagA serum antibody in UK patients with dyspepsia. J. Clin. Pathol. 54: 219-223.

[16] Saber T,. Ghonaim MM, Yousef AR, Khalifa A, Qurashi HA, Shaqhan M et al. (2015) Association of Helicobacter pylori cagA Gene with Gastric Cancer and Peptic Ulcer in Saudi Patients, J. Microbiol. Biotechnol., 25(7): 1146-1153.

[17] Fock KM and Ang TL (2010). Epidemiology of Helicobacter pylori Infection and Gastric Cancer in Asia, *Journal of Gastroenterology and Hepatology*; 25 (3): 479-486.

[18] Ye BD, Kim SG, Park JH, Kim JS, Jung HC, Song IS. (2009) The interleukin-8-251 A allele is associated with increased risk of noncardia gastric adenocarcinoma in *Helicobacter pylori* infected Koreans, *J Clin Gastroenterol*, 43, 233e9.

[19] Tongtawee T, Kaewpitoon S, Kaewpitoon N, Dechsukhum C, Loyd RA, Matrakool L, (2015) .Correlation between Gastric Mucosal Morphologic Patterns and Histopathological Severity of *Helicobacter pylori* Associated Gastritis Using Conventional Narrow Band Imaging Gastroscopy, *Biomedical Research International* :1-7.

[20] Lu JJ, Perng CL, Shyu RY, Chen CH, Lou Q, Chong KF et al. (1999).Comparison of Five PCR Methods for Detection of *Helicobacter pylori* DNA in Gastric Tissues, *Journal of Clinical Microbiology*; 37 (3): 772-774.

[21] Yamaoka Y, Kita M, Kodama T, Sawai N, Kashima K, and Imanishi J (1997). Induction of Various Cytokines and Development of Severe Mucosal Inflammation by cagA Gene Positive *Helicobacter pylori* Strains, *Gut*; 41 (4): 442-451.

[22] Taguchi A, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, Niwa Y, Goto H, (2005). Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan; *Cancer Epidemiol Biomarkers Prev*, 14: 2487-2493.

[23] Clemens J, Albert MJ, Rao M, Huda S, Qadri F, Van Loon FP et al. (1996). Sociodemographic, Hygienic and Nutritional Correlates Helicobacter Pylori Infection of Young Bangladeshi Children, Pediatric Infectious Disease Journal; 15 (12): 1113-1118.

[24] Rahman SH, Rahman MA, Arfin MS, Alam MM, Bhuiyan TM, Ahmed N et al. (2009). Helicobacter pylori Infection and Strain Types in Adult Dyspeptic Patients Undergoing Endoscopy in a Specialized Hospital of Dhaka City, Bangladesh Journal of Medical Microbiology; 3(1): 4-9.

[25] Ahmad MM, Rahman M, Rumi AK, Islam S, and Hug.F, Chowdhury MF et al. (1997). Prevalence of Helicobacter pylori in asymptotic populatio -a pilot serological sudy, Bangladesh Journal of Epidemiology; 7 (4): 251-254.

[26] Hanif M, Zaidi P, Rasool A, Hameedl A, Ahmed L (2010). Cytotoxin genes of Helicobacter pylori in gastroduodenal disease patients of Karachi, Asia-Pacific Journal of Molecular Biology and Biotechnology; 18(3): 333-340.

[27] Amer FA, El-Sokkary RH, Elahmady M, Gheith T, Abdelbary EH, Elnagar Y et al. (2013). *Helicobacter pylori* genotypes among patients in a university hospital in Egypt: identifying the determinants of disease severity; *Journal of Microbiology and Infectious Diseases* ; 3 (3): 109-115.

[28] Bazzoli M, Al-Qurain A, Al-Quorain A. (1999). *Campylobacter pylori* in Saudi Arabia under upper gastrointestinal endoscopy. *Saudi Med. J*. 8: 516-518.

[29] Cabrita J, Oleastro M, Matos R, Manhente A, Cabral J, Barros R, et al. (2000). Features and trends in Helicobacter pylori antibiotic resistance in Lisbon area, Portugal 1990- 1999. *J. Antimicrob. Chemother*. 46: 1029-1031.

[30] Perez - Perez GI, Rothenbacher D, Brenner H (2004). Epidemiology of *Helicobacter pylori* infection. *Helicobacter*; 9(s1): 1-6

[31] Helaly GF1, El-Afandy NM, Hassan AA, Dowidar NL, Sharaf SM Diagnostic Value of Housekeeping [glmM] GeneExpression in Antral Biopsies in Comparison to Rapid Urease Test and Histological Detection of *Helicobacter Pylori* Infection Egyptian *Journal of Medical Microbiology*, October 2009 Vol. 18, No. 4.

[32] Miwa H, Go MF, Sato NH (2002). *H. pylori* and gastric cancer: the Asian enigma, *The American journal of gastroenterology*; 97(5): 1106-1112.

[33] Yamagata H, Kiyohara Y, Aoyagi K, Kato I, Iwamoto H, Nakayama K et al. (2000). Impact of Helicobacter pylori Infection on Gastric Cancer Incidence in a General Japanese Population, *Archives of Internal Medicine*; 160(13): 1962-1962.

[34] Benenson S, Halle D, Rudensky B, Faber J, Schlesinger Y, Branski D et al. (2002). Helicobacter pylori genotypes in Israeli children: the significance of geography, *Journal of pediatric gastroenterology and nutrition*; 35(5): 680-684.

[35] Aziz F, Chen X, Yang X, Yan Q (2014). Prevalence and correlation with clinical diseases of *Helicobacter pylori* cagA and vacA genotype among gastric patients from Northeast China. *BioMedical research international*; 2014: Article ID 142980.

[36] Hussein NR, Mohammadi M, Talebkhan Y, Doraghi M, Letley DP, Muhammad MK (2008). Differences in virulence markers between *Helicobacter pylori* strains from Iraq and those from Iran: potential importance of regional differences in H. pylori-associated disease, *Journal of clinical microbiology*; 46(5): 1774-1779.

[37] Essawi T, Hammoudeh W, Sabri I, Sweidan W, Farraj MA (2013). Determination of Helicobacter pylori virulence genes in gastric biopsies by PCR. *ISRN gastroenterology*, Article ID 606258: 1-4.

[38] Rahman M, Mukhopadhyay AK, Nahar S, Datta S, Ahmad MM, Sarker S et al. (2003). DNA-level characterization of Helicobacter pylori strains from patients with overt disease and with benign infections in Bangladesh, *Journal of clinical microbiology*; 41(5): 2008-2014.

[39] Hatakeyama M & Higashi H (2005). *Helicobacter pylori* CagA: a new paradigm for bacterial carcinogenesis, *Cancer science*; 96 (12): 835-843.

[40] Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY (1999). Relation between clinical presentation, Helicobacter pylori density, interleukin 1beta and 8 production, and cagA status, *Gut*; 45: 804-811.

[41] Zheng PY, Hua J, Yeoh KG, Ho B (2000). Association of peptic ulcer with increased expression of Lewis antigens but not cagA, iceA, and vacA in *Helicobacter pylori* isolates in an Asian population, *Gut*; 47(1): 18-22.

[42] Miehlke S, Kibler K, Kim JG, Figura N, Small SM, Graham DY et al. (1996). Allelic variation in the cagA gene of Helicobacter pylori obtained from Korea compared to the United States, *American Journal of Gastroenterology*, 91(7).

[43] Ghotaslou R, Milani M, Akhi MT, Nahaei MR, Hasani A, Hejazi MS, Meshkini M. (2013). Diversity of *Helicobacter pylori* cagA and vacA genes and its relationship with clinical outcomes in Azerbaijan, Iran. *Adv. Pharmaceut. Bull*. 3: 57-62.

[44] Zhou J, Zhang J, Xu C and He L. (2004), cagA pylori genotype and variants in Chinese Helicobacter pylori strains and relationship to gastroduodenal diseases. *Journal of Medical Microbiology*, 53: 231-235.

[45] Parsonnet J, Replogle M, Yang S, Hiatt R (1997). Seroprevalence of CagA-Positive Strains among Helicobacter pylori-Infected, Healthy Young Adults. *Journal of Infectious Diseases*, 175 (5): 1240-1242.