IJMRP

International Journal of Medical Research Professionals

Original Article

EPIYA Motif Polymorphism of *cagA* Gene in *Helicobacter Pylori* Isolated From Patients Suffering with Gastroduodenal Diseases

Roy CK[1*], Ahmed S[2], Sattar ABN[3], Islam SMS[4], Sattar H[5]

[1]Senior Lecturer (Microbiology), Laboratory Based Department, Faculty of Medicine, University Kuala Lumpur Royal College of Medicine Perak, Ipoh, Perak, Malaysia.

[2]Professor, [3]Associate Professor, [5]Chairman & Professor, Department of Microbiology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

[4]Lieutenant Colonel, Medicine Specialist & Gastroenterologist, Combined Military Hospital, Bogra, Bangladesh.

Article History
Received: 11 Jan 2016
Revised: 13 Jan 2016
Accepted: 14 Jan 2016

***Correspondence to:**
Chandan Kumar Roy
Senior Lecturer,
Faculty of Medicine,
Universiti Kuala Lumpur
Royal College of
Medicine Perak, No 3.
Jalan Greentown, Ipoh
30450, Perak,
chandan@unikl.edu.my

ABSTRACT

Introduction: EPIYA motif diversity may provide a useful tool for prediction of *H. pylori* pathogenic activity and accurate determination of number and type of *cagA* EPIYA motifs could identify the virulent *H. pylori.* The aim of this study was to detect *H. pylori cagA* gene polymorphism.

Materials: This cross sectional study was conducted in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh during the period of March, 2014 to February, 2015. A total of 78 patients with dyspeptic symptoms and had endoscopic findings of any inflammation, ulcer or growth in gastroduodenal mucosa were enrolled in the study. Endoscopic biopsy specimens were collected and rapid urease test, *H. pylori* histology and *ureC* PCR done. *H. pylori cagA* gene was detected in *ureC* positive specimens and EPIYA motif polymorphism was determined. *H. pylori cagA* gene sequencing was done and analyzed.

Results: EPIYA motif in *H. pylori cagA* positive cases showed all were western type *cagA* EPIYA ABC and there was no East Asian EPIYA ABD motif. Significant number of gastroduodenal cases (57.9%) had 3 copies of EPIYA (ABC type), 26.3% had 4 copies (ABCC type), while remaining 10.5% had AC and 5.2% AB type EPIYA motif. Most of the duodenal ulcer cases (75%) and chronic gastritis cases (57.1%) followed by 50% intestinal metaplasia and adenocarcinoma cases were EPIYA ABC motif.

Discussion: Only Western type *cagA* EPIYA motif was found and there was no East Asian EPIYA motif. Predominant EPIYA motif was EPIYA ABC and some were EPIYA ABCC which has the risk of developing gastric carcinoma.

KEYWORDS: *H. pylori*, *cagA*, EPIYA polymorphism.

INTRODUCTION

Several invasive and noninvasive techniques are currently used for detection of *H. pylori* infection, such as rapid urease test (RUT), urea breath test, culture, serological tests and histological methods.[1] Molecular approaches based on DNA amplification by polymerase chain reaction (PCR) have been developed for the detection of *H. pylori* in gastric biopsy specimen.[2] *H. pylori* is genetically more diverse than most other bacterial species and the genetic identification by using housekeeping *H. pylori* genes is needed to accurately identify *H. pylori* and several virulence genes, such as *cagA* and *vacA*, can be used as a tool to predict the risk of developing various gastroduodenal diseases resulting from *H. pylori* infection.[3,4] For the genetic identification of *H. pylori*, several PCR methods that employ the 16S rRNA gene, *rpoD*, *ureA*, *ureB*, and *ureC* have been used. Among them, *ureC* gene is known to be specific to *H. pylori*. PCR-based detection of the *ureC* gene appears to be the most promising for detection of *H. pylori.*[5]

Helicobacter pylori strains can be divided into two major types based on their ability to produce a 120–145 kDa immunodominant protein called cytotoxin associated gene A (*cagA*) antigen.[6] The *cagA gene that* encodes *cagA* is localized at one end of the *cag PAI*, a 40-kb DNA segment that was most likely incorporated into the *H. pylori* genome by a process of horizontal transfer.[7] *H. pylori* strains possessing the *cagA* gene were linked with an increased risk of developing gastric

cancer and peptic ulcer. The risk of developing gastric cancer in *H. pylori* infected *cagA*-positive subjects is six fold higher than that in *cagA*-negative subjects.[8] More than 90% of isolated strains from East Asia including Korea, Japan, and China are known to harbor *cagA,* while 50%-60% of isolated strains from Western countries are positive for it.[9] In Bangladesh a study showed 81% children were seropositive for both *cagA* and *vacA* detected by an in-house Western Blot analyses.[10]

The 3′-end region of *cagA* where the tyrosine phosphorylation sites are located are highly polymorphic.[11,12] Phosphorylation occurs on specific tyrosine residues within repeating penta amino acid Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, present at the C-terminus of the protein.[13] Four different CagA EPIYA motifs, EPIYA-A, -B, -C, and -D have been defined based on the amino acid sequences surrounding the EPIYA residue.[14] CagA proteins nearly always possess an EPIYA-A and an EPIYA-B, followed by various number of EPIYA-C repeats in Western-type or EPIYA-D motifs in East Asian type strains.[15] It has been suggested that the considerable variation in number of repeating EPIYA-C or -D motifs determines the biological activity of *cagA.*[13]

Several studies have been done to see the association between gastroduodenal diseases and *cagA* gene of *H. pylori.* In Meta-analysis in Southeast Asian populations showed that both Western and East Asian-type strains of *H. pylori cagA* were found in Southeast Asia and a significant relationship between *cagA* status with development of gastroduodenal diseases.[16] Another study showed that there is a significant statistical link between gastric carcinoma and Western type EPIYA ABD motif in South Korean population.[14]

CagA strains possessing multiple number EPIYA C segments predisposes to precancerous lesions and gastric cancer.[13,17] The A-B-C pattern of EPIYA sequences in Indian strains of *H. pylori* represents a common ancestral root of origin with Europeans.[18] Western type EPIYA ABC pattern present in 100% *H. pylori* strains isolated from patients with various gastroduodenal diseases in India.[19] Western specific *cagA* EPIYA-C motif identified in *cagA* positive *H. pylori* strains isolated from patients and asymptomatic individual in West Bengal, India.[20] Again western type *cagA* predominate in dyspeptic patients in Pakistan.[21] In Bangladesh a study was done in ICDDR,B in which the phylogenetic analysis of the 5′ end of the *cagA* gene indicates that Bangladeshi isolates are more closely related to *H. pylori* isolates from India are different from isolates from East Asia.[22]

As large number of populations are seropositive for *H. pylori* in Bangladesh so, it is important to know the number and pattern of *cagA* EPIYA motifs for identifying the *H. pylori* infected patients who tend to develop severe gastroduodenal diseases. Therefore, EPIYA motif diversity may provide a useful tool for prediction of *H. pylori* pathogenic activity and accurate determination of number and type of *cagA* EPIYA motifs could identify the virulent *H. pylori* causing severe gastroduodenal diseases in Bangladesh. So, this study was designed to detect *H. pylori* cagA gene polymorphism in gastric biopsy specimen from patients with gastroduodenal diseases.

MATERIALS AND METHODS

This cross sectional study was conducted in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka during the period of March, 2014 to February, 2015. A total of 78 patients with dyspeptic symptoms who attended endoscopic examination in the Department of Gastroenterology of BSMMU and Dhaka Medical College Hospital and had endoscopic findings of any inflammation, ulcer or growth in gastroduodenal mucosa were enrolled in the study. Clinical history of those patients was recorded in predesigned data sheet. All the patients who satisfied the inclusion criteria were included in the study. Patients with history of partial gastric resection, *H. pylori* eradication therapy or treatment with antibiotics, bismuth containing compounds, H_2-receptor blockers or proton pump inhibitors within 4 weeks prior to enrolment were excluded from the study.

The study was approved by Institutional Review Board, BSMMU and Ethical Review Committee of Dhaka Medical College. Informed consent was obtained from each patient prior to endoscopy and biopsy specimen collection. No conflict of interest was related with the study.

Endoscopy and biopsy

Upper gastrointestinal endoscopy was done aseptically by a skilled endoscopist using a standard forward viewing video endoscope (Olympus GIF, Japan) under topical Lignocaine anesthesia. Six biopsy specimens were collected from an area of inflammation in the antrum and body of stomach from each patient. Three biopsy specimens were taken from the gastric antrum and three from the gastric body. One specimen each from the antrum and body were fixed in 10% buffered formalin and send to the Department of Pathology of BSMMU for histopathological examination. One specimen each from the body and antrum were examined for the presence of *H. pylori* by rapid urease test and the remaining were preserved in 1.5ml microcentrifuge tube containing 1ml phosphate buffer solution for PCR detection of *H. pylori ureC* gene. All biopsy samples were stored at -20°C prior to DNA extraction.

Histopathological procedure

All biopsy specimens for histological examination were fixed in 10% formalin, embedded in paraffin wax on the oriented edge, and cut into 5 μm thick sequential

sections. All tissue sections were stained with Hematoxylin & Eosin (H&E) stains and modified Giemsa stain and examined under microscope by an experienced histopathologist. Giemsa stained slides were examined for the detection of *H. pylori*.

Rapid urease test

Gastric biopsy specimen was placed immediately into a screw capped tube containing 0.5 ml of urea solution (0.5 gm urea was dissolved in 10 ml distilled water and mixed with 10 drops of 0.1% phenol red). A positive result was taken by a change in the colour of the solution from yellow to pink within twenty four hours after incubation at 37^{0}C [23]. Known urease positive bacterial organism *Klebsiella* species was used as positive control and urease negative organism *Escherichia coli* as negative control.

DNA extraction from gastric tissues

DNA from gastric tissues was extracted by using the QIAamp (QIAGEN) DNA Mini Kit according to the manufacturer's instruction. Purified DNAs were stored at -20ºC prior to the procedures.

PCR assay

PCR amplification was done to detect *H. pylori ureC* gene, *cagA* gene, number and pattern of cagA EPIYA motif and *cagA* 3´variable region. For the detection of *H. pylori* DNA in gastric tissues, the *ureC* gene was identified by PCR using the primers [5] (forward 5´ AAGCTTTTAGGGGTGTTAGGGGTTT 3´ and reverse 5´ AAGCTTACTTTCTAACACTAACGC 3´ with a 294-bp size product. For detecting the presence of the *cagA* gene, PCR was performed by using primer pairs: forward (*cagA*F) 5′-GATAACAGGCAAGCTTTTGAGG-3′ and reverse (*cagA*R) 5′- CTGCAAAAGATTGTTTGGCAGA -3′ with a 349-bp size product [4]. The forward primer *cagA*28F (5´ TTCTCAAAGGAGCAATTGGC 3´) and reverse primers *cagA*P1C (5´ GTCCTGCTTTCTTTTTATTAACTTKAGC 3´), equimolar mixture of *cagA*P2CG (5´ TTTAGCAACTTGAGCGTAAATGGG3´) and *cagA* P2TA(5´TTTAGCAACTTGAGTATAAATGGG 3´), *cagA*P3E (5´ ATCAATTGTAGCGTAAATGGG 3´) and *cagA*-PD (5´ TTGATTTGCCTCATCAAAATC 3´) were used to amplify the EPIYA motif encoding the sequences A, B, C and D respectively [14,15]. Amplification of the *cagA* 3´ variable region was performed using primers *cag*2 (5´ GGAACCCTAGTCGGTAATG 3´) and *cag*4 (5´-ATCTTTGAGCTTGTCTATCG 3´).

DNA sequencing

To determine the type and number of EPIYA motif, direct sequencing of *cagA* 3´ variable region primer based amplified PCR product was performed. Sequencing was done by Sanger dideoxy sequencing. For automated DNA sequencing, PCR products were purified by using Thermo Scientific GeneJet PCR Purification Kit prior to cycle sequencing. The purified DNA obtained was quantified by spectrophotometer (Nanodrop, ND 1000; Japan). After determining the concentration of DNA six samples were selected for sequencing. Purified products were sequenced using a BigDye Terminator Cycle Sequencing Kit in an ABI 3130 Genetic Analyzer (Applied Biosystems). The sequences obtained were aligned using the CAP3 Sequence Assembly Program (available from: http://pbil.univ-lyon1.fr/cap3.php). After alignment, nucleotide sequences were transformed into amino acid sequences using the Blastx program (available from: http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared to previously published *CagA* gene sequence of strains *H. pylori* 26695 (AE000511) in the GenBank (http//www.ncbi.nlm.nih.gov/Genbank/).

RESULTS

H. pylori were detected in biopsy specimens of study population by rapid urease test, *H. pylori* histology and amplification of *ureC* gene by PCR. Out of 78 cases, 33 (42.3%) were positive for *H. pylori* by rapid urease test, 31 (39.7%) by PCR for *ureC* gene and 27 (34.6%) by *H. pylori* histology.

Table 1: Detection of *H. pylori* in biopsy specimens by rapid urease test, *H. pylori* histology and amplification of *ureC* gene by PCR. (N=78)

Name of the test	No. of cases (n)	(%)
Rapid urease test	33	42.3
Histology for *H. pylori*	27	34.6
***ureC* gene by PCR**	31	39.7

Among 31 *ureC* positive cases, all 31(100%) were shows positive rapid urease test and 27 (87.1%) were found positive in *H. pylori* histology. On the other hand, out of 47 PCR negative cases two (4.3%) cases were positive by only rapid urease test and all cases were negative by *H. pylori* histology. Out of 31*ureC* positive cases 19 (61.3%) were *cagA* gene positive. Among the 19 *cagA* gene positive cases 7 (43.8%) were found in chronic gastritis, 4 (66.7%) in duodenal ulcer and 02 (66.7%) in chronic gastric ulcer. All cases of Adenocarcinoma and intestinal metaplasia were positive for *cagA* gene. *cagA* gene is significantly associated with duodenal ulcer and chronic gastritis cases (p=0.04).

EPIYA motif in *H. pylori cagA* positive cases showed all were western type *cagA* EPIYA ABC and there was no East Asian EPIYA ABD motif. Majority of gastroduodenal cases (57.9%) had 3 copies of EPIYA (ABC type), 26.3% had 4 copies (ABCC type), while remaining 10.5% had AC and 5.2% AB type EPIYA motif. Most of the duodenal ulcer cases (75%) and chronic gastritis cases (57.1%) followed by 50% intestinal metaplasia and adenocarcinoma cases were EPIYA ABC motif. Fifty percent cases of chronic gastric

ulcer and adenocarcinoma composed of ABCC type EPIYA motif. On the other hand, ABCC type EPIYA motifs were found in 25% cases of duodenal ulcer and intestinal metaplasia. There was no significant association among numbers of EPIYA-C motif and types of gastroduodenal diseases.

Table 2: *H. pylori ureC* gene status in relation to rapid urease test and detection of *H. pylori* by histology (N=78)

H. pylori *ureC* gene	Rapid urease test		*H. pylori* Histology	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Positive (n=31)	31 (100)	00 (0)	27 (87.1)	04 (12.9)
Negative (n=47)	02 (4.3)	45 (95.7)	00 (0)	47 (100)
Total (N=78)	**33 (42.3)**	**45 (57.7)**	**27 (34.6)**	**51 (65.4)**

Table 3: *H. pylori cagA* gene among *ureC* positive cases in relation with histopathological findings (N=31)

Histopathological findings	*H. pylori ureC* gene Positive	*H. pylori cagA* gene Positive (%)
Chronic Gastritis	16	07 (43.8)
Duodenal ulcer	06	04 (66.7)
Chronic gastric ulcer	03	02 (66.7)
Intestinal metaplasia	04	04 (100)
Adenocarcinoma	02	02 (100)
Total	**31**	**19 (61.3)**

Table 4: Distribution of *cagA* EPIYA motif and clinical outcomes in *H. pylori cagA* positive cases (N=19)

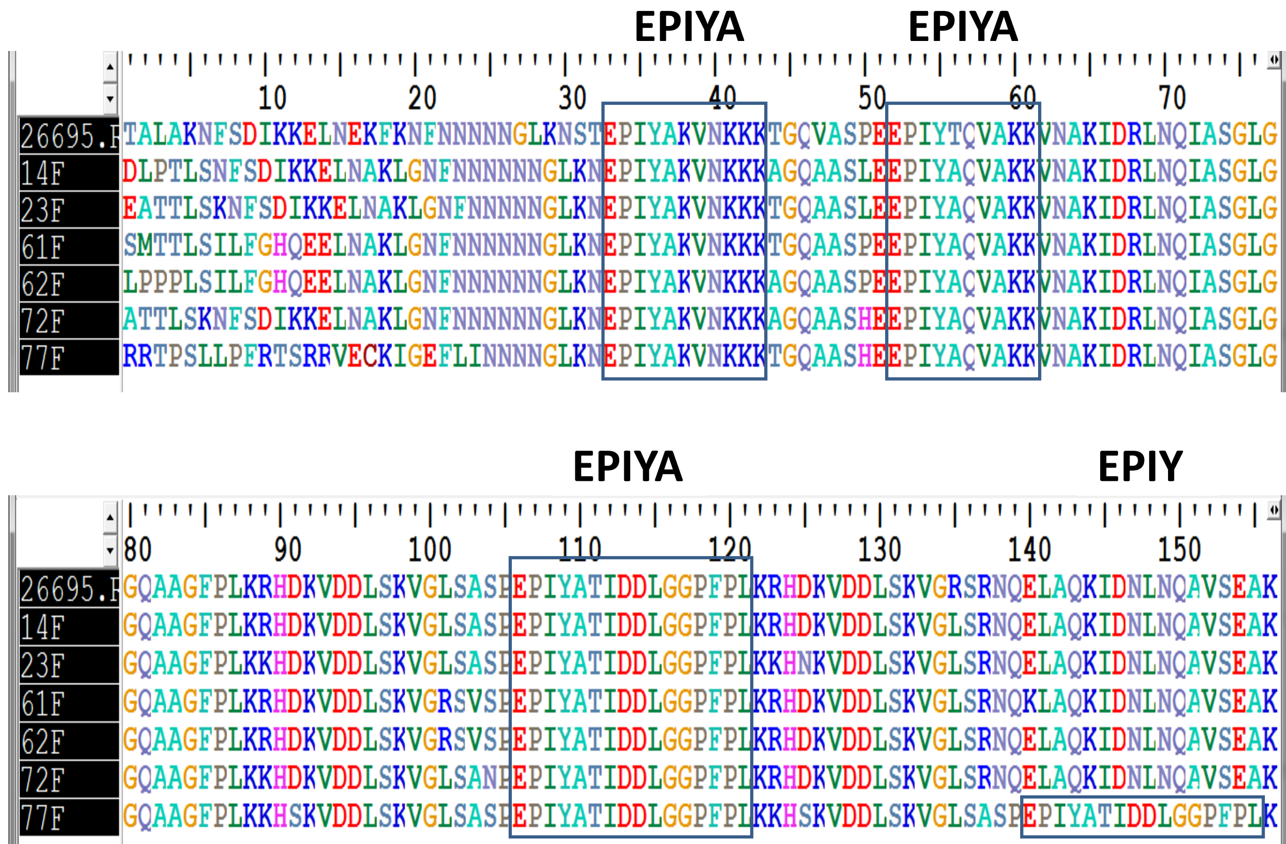
Histopathological findings	CagA EPIYA motif (%)				
	AB	AC	ABC	ABCC	ABD
Chronic gastritis (n=7)	01 (14.3)	01 (14.3)	04 (57.1)	01 (14.3)	00 (0)
Duodenal ulcer (n=4)	00	00	03 (75)	01 (25)	00 (0)
Ch. Gastric ulcer (n=2)	00	00	01 (50)	01 (50)	00 (0)
Intestinal metaplasia (n=4)	00	01 (25)	02 (50)	01 (25)	00 (0)
Adenocarcinoma (n=2)	00	00	01 (50)	01 (50)	00 (0)
Total (N=19)	**01 (5.2)**	**02 (10.5)**	**11 (57.9)**	**05 (26.3)**	**00 (0)**

Sequence analyses confirmed that three types of EPIYA motifs were observed: EPIYA-A for EPIYAKVNKKK (A/T/V/S) GQ; EPIYA-B for EPIY(A/T)(Q/K)VAKKVNAKI; and EPIYA-C for EPIYATIDDLGGPFPL. No East Asian type of EPIYA-D (EPIYATIDFDEANQAG) was found.

DISCUSSION

Detection of *Helicobacter pylori* in the clinical specimens was the important laboratory step of the present study as *cagA* gene detection and finding the polymorphism among these genes were done only in the clinical specimens that contain the specific DNA of *H. pylori*. Culture is the most authentic method for the detection of *H. pylori* but it is cumbersome and needs special setup and was not possible to perform in this study. Genetic identification is the suitable alternative of culture for detection of *H. pylori* in clinical specimens. For the genetic identification several PCR methods that employ the 16S rRNA gene, *rpoD, ureA, ureB* and *ureC* have been used. Among them *ureC* gene is known to be specific most promising for detection of *H. pylori.*[5] In this study the other popular detection methods of *H. pylori* detection (rapid urease test and histopathology) also applied along with *ureC* gene detection to assess their performance. Among 78 biopsy specimens tested, *H. pylori* was detected in 33 (42.3%) cases by positive rapid urease test, 31 (39.7 %) cases by PCR for *ureC* gene and 27 (34.6%) cases were detected by histology. Similar findings were reported in a study where investigators found *H. pylori* positive in biopsy specimens by rapid urease test, *ureC* gene PCR and histology in 43.3%, 44% and 42.5% respectively.[24] Another study in Bangladesh reported that *H. pylori* positive in 53.3% cases by rapid urease test and in 34.4% cases by histology.[25] In the present study, only 2(4.3%) cases were found positive by rapid urease test but both PCR and histology were negative which is similar to a study in Belgium reported one case was positive alone by rapid urease test where both PCR and histology were negative.[26] The reason might be the presence of other urease-positive bacteria in the gastric tissues or reflux of alkaline bile into the stomach[27] or the contamination of biopsy with saliva as false-positive results can occur bacteria from the oral flora that produce urease.[28] In this study 4 (11.8%) cases were both PCR and rapid urease test positive but histologically negative for *H. pylori*. This finding was comparable to a study where investigators found two cases PCR positive but histology negative.[24] Those situations may be explained by the fact that, *H. pylori* is not evenly distributed throughout the gastrointestinal tissues, so biopsies can miss the site of infection.[29]

Fig 1: Alignment of amino acid sequences among cagA strain (showing the EPIYA motifs) from 6 *H. pylori* strains including the *H. pylori* reference strain 26695.



In this study *H. pylori* was detected by the detection of *ureC* gene by PCR in 35.6% cases of gastritis and 54.5% cases of duodenal ulcer which is comparable to a study where *H. pylori* was positive by *ureC* gene PCR in 41.1% and 50% cases of gastritis and duodenal ulcer respectively.[27]

H. pylori cagA positive strains are more virulent causing higher levels of gastric mucosal inflammation in gastritis and gastric cancer. The prevalence of *cagA* positive *H.pylori* varies from one geographic region to another. The rate differs from very high in East Asian countries to low from Western Europe countries.[30] In this study, out of 31 *ureC* positive *H. pylori* cases, 19 (61.3%) were *cagA* gene positive. This finding consistent with the findings of previous studies showing *cagA* positivity rate 61% in China and 65.9% in Brazil.[31] In a study, investigators of Bangladesh reported 68.4% *cagA* gene positive among 57 culture positive *H. pylori* cases.[22] Some other studies reported high positivity rate of *cagA* gene in Korea, India and Japan.[32-34] Lower positivity rate of *cagA* than the present study was reported in a study conducted in Pakistan.[21] In the present study in relation to diseases *cagA* gene was positive in 66.7% cases in each of duodenal ulcer and gastric ulcer patients compared to 43.8% cases of gastritis. These findings correlate with the findings of the previous study in Bangladesh.[22] *cagA* gene was significantly associated with duodenal ulcer cases ($p < 0.05$). This finding is similar with the studies conducted in Japan[4] and Cuba[35] where investigators found strong association between *cagA* gene and peptic ulcer diseases, but in contrast to a study done in Singapore reported that there was no association between *cagA* status and duodenal ulcer.[36] For this difference in the *cagA* status, one possibility is the large genomic variations in the *H. pylori* genomes that amplifies the *cagA* gene from *H. pylori* isolated in one country failed to detect *cagA* in isolates from another country and also some forms of the *cagA* gene are associated with severe gastroduodenal diseases.[37] In this study *cagA* was positive in 100% cases gastric carcinoma and intestinal metaplasia patients.

Determination of the number and types of *H. pylori cagA* EPIYA motifs has been suggested by some researchers as a way to predict clinical outcome of *H. pylori* associated pathologies and as a prognostic tool by others.[38] This is the study conducted in Bangladesh for first time that identified the types of *cagA* EPIYA motif. In the present study Western type *cagA* EPIYA-C motif was found in all 19 *cagA* positive *H. pylori* cases but there was no East Asian *cagA* EPIYA-D motif. Similar findings were reported from India and Colombia.[19] In contrast, these results differed from that of Korea where the East Asian type *cagA* (EPIYA-D) is dominant[39] and some other countries like Malaysia[40] and Thailand[41] where both types of *cagA* (Western and East Asian) are present.

Among 19 *cagA* positive cases majority (11, 57.9%) were EPIYA ABC motif followed by 5(26.3%) EPIYA ABCC, 2(10.5%) AC and 1(5.2%) AB motif. This results is comparable with the results of previous study done in Columbia.[38] In some other studies EPIYA ABC motif was higher than the present study in South Africa[42], USA and France.[43]

The multiple numbers of EPIYA motifs, especially EPIYA-C, is thought to be related to the development of gastroduodenal diseases. *cagA* positive *H. pylori* strains with multiple EPIYA-C motifs are reported to be associated with a higher gastric cancer risk than strains with only one EPIYA-C motif.[14,44,45] In the present study more than one EPIYA–C (ABCC) was found in 26.3% of *cagA* positive cases which is comparable to the findings reported in South Africa[42], Columbia[38] and France.[43] Another study done in USA where investigators found lower positivity of EPIYA ABCC motif than the present study.[43] Geographic variations in the frequency of *H. pylori* strains with one or two EPIYA-C repeats may be explained by different acidic conditions in the gastric mucosa, which could be related to differences among populations in the frequencies of cytokine gene polymorphisms that attenuate gastric acid secretions.[47] However, there is no association between the number of EPIYA-C motifs and acid secretion.[46] Present study showed that the variation in the EPIYA motifs in *cagA* protein was not directly associated with the outcome of the disease caused by *H. pylori* and that there is no relation between the number of EPIYA-C motifs and the gastroduodenal diseases (P>0.05). These results coincided with those studies in Iran and Iraq.[45,49] Higher number of EPIYA C segments was associated with gastric carcinoma in Italy and Brazil[44], otherwise no association was observed in Colombia.[38] These differences might be due to different study designs, sample size, populations and geographical diversity of *H. pylori* markers of pathogenicity.

In the present study sequencing of the *cagA* 3´repeat region confirmed that PCR-based methods well matched the sequence data. The alignment of the deduced protein sequences confirmed that Western type EPIYA-C and no East Asian EPIYA-D was found (Figure-1). One advantage for sequencing is that it can detect even one amino acid mutation, which is impossible by PCR-based methods. As mentioned above, the important differences between EPIYA-C and EPIYA-D is only one amino acid exchanges (i.e. EPIYA-C is EPIYATID**D** and EPIYA-D is EPIYATID**F**). This study shows the pattern of *cagA* EPIYA motif in endoscopic biopsy specimen from patients with gastroduodenal diseases. This is the first study in Bangladesh that shows Western type *cagA* EPIYA motif was found and there is no East Asian EPIYA motif. Predominant EPIYA motif was EPIYA ABC and some were EPIYA ABCC which has the risk of developing gastric carcinoma.

CONCLUSION

Large number of populations are seropositive for *H. pylori* in Bangladesh and it is important to determine the number and pattern of *cagA* EPIYA motif to identify the infected patients who prone to develop severe gastroduodenal diseases. Therefore, EPIYA motif diversity may provide a useful tool for prediction of *H. pylori* pathogenic activity and accurate determination of number and type of *cagA* EPIYA motifs could identify the virulent *H. pylori.* It is interesting to found in the present study that Western type *cagA* EPIYA-C motif was in all 19 *cagA* positive *H. pylori* cases but there was no East Asian *cagA* EPIYA-D motif. This finding demand further study to be conducted with more clinical specimens and phylogenetic analysis of the *H. pylori* genome should be carried out to determine the ancestry relationship among the overall international isolates. The multiple numbers of EPIYA motifs, especially EPIYA-C, is thought to be related to the development of severe gastroduodenal diseases and associated with higher gastric cancer risk and was found in 26.3% of *cagA* positive cases in the present study which also demand more studies to be conducted further to estimate the actual risk of severe gastroduodenal diseases.

ACKNOWLEDGEMENT

Funding: Department of Microbiology, Bangabandhu Sheikh Mujib Medical University, Shahbagh, Dhaka-1000, Bangladesh.

DNA sequencing: Dr. Latiful Bari, Associate Professor (Microbiology) and Principle Scientist, Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000. Bangladesh.

CONFLICT OF INTEREST: None declared.

REFERENCES

1.Krogfelt KA, Lehours P, Megraud F. Diagnosis of Helicobacter pylori Infection. Helicobacter. 2005; 10 Suppl 1:5–13.

2.Pacheco N, Mago V, Gómez I, Gueneau P, Guelrud M, Reyes N, Pericchi LR, Domínguez-Bello MG. Comparison of PCR and common clinical tests for the diagnosis of H. pylori in dyspeptic patients. Diagn Microbiol Infect Dis. 2001; 39:207-10.

3.Atherton JC, Cao P, Peek RM, Jr Tummuru MK, Blaser MJ . Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem. 1995; 270(30):17771–77.

4.Yamaoka Y, El-Zimaity HM, Gutierrez O, Figura N, Kim JC, et al., Relationship between the cagA 3 repeat region of Helicobacter pylori, gastric histology, and susceptibility to low pH. Gastroenterology 1999; 117:342–349.

5.Lu JJ, Perng CL, Shyu RY, Chen CH, Lou Q, Zhou S et al., Comparison of Five PCR Methods for Detection of Helicobacter pylori DNA in Gastric Tissues. J Clin Microbiol. 1999; 37: 772- 74.

6.Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D,Timothi R et al., Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA. 1993; 90: 5791–95.

7.Tummuru MK, Cover TL, Blaser MJ. Cloning and expression of a high-molecular-mass major antigen of Helicobacter pylori: evidence of linkage to cytotoxin production. Infect Immun. 1993; 61:1799–809.

8.Yamaoka Y, Orito E, Mizokami M, Gutierrez O, Saitou N. Helicobacter pylori in North and South America before Columbus. FEBS Lett. 2002; 517:180–184.

9.Kim SY, Woo CW, Lee YM. Genotyping CagA, VacA subtype, IceA1, and BabA of Helicobacter pylori isolates from Korean patients, and their association with gastro-duodenal diseases. J Korean Med Sci. 2001; 16: 579-84.

10.Sarker SA, Rahman MM, Mahalanabis D, Bardhan PK, Hildebrand P. Prevalence of Helicobacter pylori infection in infants and family contacts in a poor Bangladesh community. Dig Dis Sci. 1995; 40: 2669-72.

11.Yamaoka Y, Kodama T, Graham DY, Kashima K. Comparison of four serological tests to determine the CagA or VacA status of Helicobacter pylori strains. J. Clin. Microbiol. 1998; 36:3433-34.

12.Yamazaki S, Yamakawa A, Okuda T, Ohtani M, Suto H, et al,. Distinct diversity of vacA, cagA, and cagE genes of Helicobacter pylori associated with peptic ulcer in Japan. J. Clin. Microbiol. 2005; 43:3906–16.

13.Higashi H, Tsutsumi R, Muto S. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. Science. 2002; 295: 683–86.

14.Jones KR, Joo YM, Jang H, Yoo YJ, Lee HS, Chung IS et al,. Polymorphism in the CagA EPIYA motif impacts development of gastric cancer. J. Clin. Microbiol. 2009; 47(4): 959-68.

15.Sgouras DN, Panayotopoulou EG, Papadakos K, Martinez-Gonzalez B, Roumbani A,. CagA and VacA polymorphisms do not correlate with severity of histopathological lesions in Helicobacter pylori-infected Greek children. J Clin Microbiol. 2009; 47: 2426-34.

16.Sahara S, Sugimoto M, Vilaichone RK, Mahachai V, Miyajima H. Role of Helicobacter pylori cagA EPIYA motif and vacA genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. .BMC Infectious Diseases. 2012; 12:223 Page 2 of 13 available at http://www.biomedcentral.com/1471-2334/12/223. (Accessed 17.01.2015).

17.Naito M, Yamazaki T, Tsutsumi R, Higashi H, Onoe K. Influence of EPIYA-Repeat Polymorphism on the Phosphorylation-Dependent Biological Activity of Helicobacter pylori CagA. Gastroenterology. 2006; 130(4): 1181-90.

18.Devi S, Ahmed M, Francalacci P, Hussain MA, Akhter Y. Ancestral European roots of Helicobacter pylori in India.. BMC Genomics. 2007; 8:184–94.

19.Tiwari SK , Sharma V, Sharma VK, Gopi M, Saikant R. Phylogenetic analysis, based on EPIYA repeats in the cagA gene of Indian Helicobacter pylori, and the implications of sequence variation in tyrosine phosphorylation motifs on determining the clinical outcome. Genetics and Molecular Biology. 2011; 34, 2, 280-85.

20.Chattopadhyay S, Patra R, Chatterjee R, Alam J, Ramamurthy T, Mukhopadhyay AK et al,. Distinct repeat motifs at the C-terminal region of CagA of Helicobacter pylori strains isolated from diseased patients and asymptomatic individuals in West Bengal, India. Gut Pathogens. 2012 ; 4:4 Page 2 of 12 available at http://www.gutpathogens.com/content/4/1/4. (Accessed 10.09.2014).

21.Khan A, Farooqui A, Raza Y, Rasheed F, Manzoor H. Prevalence, diversity and disease association of Helicobacter pylori in dyspeptic patients from Pakistan. J Infect Dev Ctries. 2013; 7(3):220-28.

22.Rahman M, Mukhopadhyay AK, Nahar S, Datta S, Ahmad MM. DNA-level characterization of Helicobacter pylori strains from patients with overt disease and with benign infections in Bangladesh. J Clin Microbiol. 2003; 41:2008-14.

23.Wong BCY, Wong WM, Wang WH, et al,. An evaluation of invasive and non-invasive tests for the diagnosis of Helicobacter pylori infection in Chinese. Aliment pharmacol Ther. 2001; 15:505-11.

24.Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis- the updated Sydney system. Am J Surg Pathol. 1996; 20: 1161-81.

25.Argent RH, Zhang Y, Atherton JC. Simple method for determination of the number of Helicobacter pylori CagA variable‑region EPIYA tyrosine phosphorylation motifs by PCR. J ClinMicrobiol. 2005;43:791‑5.

26.Poudel A, Regmi S, Poudel S, Joshi P. Correlation between endoscopic and histopathological findings in gastric lesion. Journal of universal college of Medical Sciences. 2013; 11: 36-41

27.Helaly GF, El-Afandy NM, Hassan AA, Dowidar NL, Sharaf SM. Diagnostic Value of Housekeeping [glmM] Gene Expression in Antral Biopsies in Comparison to Rapid Urease Test and Histological Detection of Helicobacter Pylori Infection. Egyptian Journal of Medical Microbiology. 2009; 18(4):119 -30.

28.Brooks HJ, Ahmed D, McConnell MA, Barbezat GO. Diagnosis of helicobacter pylori infection by polymerase chain reaction: is it worth it?. Diagn Microbiol Infect Dis. 2004; 50:1-5.

29.Rahman SHZ, Rahman MA, Arfin MS, Alam MM, Bhuiyan TM. Helicobacter pylori Infection and Strain Types in Adult Dyspeptic Patients Undergoing Endoscopy in a Specialized Hospital of Dhaka Cit Bangladesh. J Med Microbiol. 2009; 03 (01): 4-9.

30.Lage AP, Godfroid E, Fauconnier A, Burette A, Butzler JP. Diagnosis of Helicobacter pylori infection by PCR: comparison with other invasive techniques and detection of cagA gene in gastric biopsy specimens. J Clin Microbiol. 1995; 33: 2752-56.

31.Aziz F, Chen X, Yang X and Yan Q. Prevalence and correlation with clinical disease of the Helicobacter pylori cagA and vacA genotype among gastric patients from northeast China. Biomed Reasearch International; 2014:

Atricle ID142980, 7pages, avilable at http://dx.doi.org/10.1155/2014/14298 (Accessed 6.8.2014)

32.Park D, Lee K, Jin S, Lee J, Min J. Phenotypic differences of gastric cancer according to the Helicobacter pylori infection in Korean patients. J. Gastric Cancer. 2010; 10:168-74.

33.Mishra KK, Srivastava S, Dwivedi PP, Prasad KN. Genotypes of Helicobacter pylori isolated from various acid peptic diseases in and around Lucknow, Current Science. 2002; 83(4): 605-607.

34.Hussein NR, Mohammadi M, Talebkhan Y, Doraghi M, Letley DP, et al., Differences in virulence markers between Helicobacter pylori strains from Iraq and those from Iran: potential importance of regional differences in H. pylori-associated disease. J. Clin. Microbiol. 2008; 46:1774–79.

35.Torres L, Melián K, Moreno A, Alonso J, Sabatier C. Prevalence of vacA, cagA and babA2 genes in Cuban Helicobacter pylori isolates. World J Gastroentrol. 2009; 15(2):204-10.

36.Zheng PY, Hua J, Yech KG, and Ho B. 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. 2000; 47: 18–22.

37.Miehllkhe S, Lehn N, Meining A. Helicobacter pylori reinfection is rare in peptic ulcer patients cured by antimicrobial therapy. European Journal of Gastroenterology and Hepatology. 1996; 8(12):1161-63.

38.Acosta N, Quiroga A, Delgado P, Bravo MM, Jaramillo C. Helicobacter pylori CagA protein polymorphisms and their lack of association with pathogenesis. World J Gastroenterol. 2010; 16: 3936-43.

39.Choi KD, Kim N, Lee DH, Kim JM, Kim JS. Analysis of the 3'variable region of the cagA gene of Helicobacter pylori isolated in Koreans. Dig.Dis.Sci. 2007; 52(4):960–66.

40.Schmidt H, Andres S, Kaakoush N, Engstrand L, Eriksson L. The prevalence of the duodenal ulcer promoting gene (dupA) in Helicobacter pylori isolates varies by ethnic group and is not universally associated with disease development: a case-control study. Gut Pathog. 2009; 1:5.

41.Chomvarin C, Phusri K, Sawadpanich K, Mairiang P, Namwat W. Prevalence of cagA EPIYA motifs in Helicobacter pylori among dyspeptic patients in Northeast Thailand. SoutheastAsian J Trop Med Public Health. 2012; 43(1):446-49

42.Argent RH, Kidd M, Owen RJ, Thomas RJ, Limb MC. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of Helicobacter pylori. Gastroenterology. 2004; 127: 514–23.

43.Yamaoka Y, Osato MS, Sepulveda AR, Gutierrez O, Figura N, et al., Molecular epidemiology of Helicobacter pylori: separation of H. pylori from East Asian and non-Asian countries. Epidemiol Infect. 2000; 124: 91-96.

44.Basso D, Zambon CF, Letley DP, Stranges A, Marchet A. Clinical Relevance of Helicobacter pylori cagA and vacA gene polymorphisms. Gastroenterology. 2008; 135:91-9.

45.Shokrzadeh L, Baghaei K, Yamaoka Y, Dabiri H, Jafari F. Analysis of 3'-end variable region of the CagA gene in Helicobacter pylori isolated from Iranian population. J Gastroenterol Hepatol. 2010; 25(1):172-77.

46.Argent H, Thomas R, Letley D, Rittig M, Hardie K. Functional association between the Helicobacter pylori virulence factors vacA and cagA. J Med Microbiol. 2008; 57:145-50.

47.Snaith A and El-Omar EM. Helicobacter pylori Host genetics and disease outcomes. Expert rev Gastroenterology Hepatol. 2008; 2:577-85.

48.Queiroz DM, Cunha RP, Saraiva IE, Rocha AM. Helicobacter pylori virulence factors as tools to study human migrations. Toxicon. 2010; 56(7):1193-97.

49.Kalaf EA, Al-Khafaji ZM, Yassen NY, AL-Abbudi FA, Sadwen SN. Study of the Cytoxin-associated gene a (CagA gene) in Helicobacter pylori using gastric biopsies of Iraqi patients. Saudi J Gastroenterol. 2013; 19:69-74.

Copyright: © the author(s) and publisher IJMRP. This is an open access article distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cite this article as: Roy CK, Ahmed S, Sattar ABN, Islam SMS, Sattar H. EPIYA Motif Polymorphism of *cagA* Gene in *Helicobacter Pylori* Isolated From Patients Suffering with Gastroduodenal Diseases. Int J Med Res Prof. 2016, 2(1); 117-24.