Original Article

Helicobacter pylori virulence genes in patients with gastroduodenal diseases

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Abstract

Background: Helicobacter pylori cause various gastroduodenal diseases. Infection is acquired mostly from feco-oral route, resides in gastric epithelium by forming a protective environment that evades host immune damage. Virulence factors and host response determine the severity of disease and duration of infection. The objective of this study was to identify cytotoxin associated gene A, vacuolating cytotoxin gene A and urease gene C by polymerase chain reaction in helicobacter pylori positive gastric lesions obtained during endoscopy in symptomatic patients.

Methods: This is a cross sectional, hospital based observational study carried out at gastroenterology department, Bir hospital during November 2019 to January 2021. Giemsa stain was done to identify helicobacter pylori in gastric biopsies. Cytotoxin associated gene A, vacuolating cytotoxin gene A and urease C gene were identified by polymerase chain reaction among the positive lesions. Data was entered in Microsoft Excel 19 and descriptive analysis was done.

Results: Gastritis (n=114, 67.8%) was the commonest lesion identified. Helicobacter pylori was positive among 65 gastric biopsies (38.6%) by Giemsa stain. Higher frequency of cytotoxin associated gene A (n=63, 96.9%) compared to vacuolating cytotoxin gene A (n=50, 76.9%) was detected in these positive lesions. Urease C gene was detected in these 63 patients (37.5%) by polymerase chain reaction.

Conclusions: Cytotoxin associated gene A was the commonest virulence gene in gastroduodenal diseases. Polymerase chain reaction had a good diagnostic role for identification of helicobacter pylori.

Introduction

Helicobacter pylori (H. pylori) infection causes various gastroduodenal diseases like peptic ulcer disease, gastritis, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma and is common in poor communities and overcrowding.[1,2] Prevalence of H. pylori in Nepal range between 16%-70% according to various invasive and non- invasive detection methods.[3,4]

H. pylori discharges multiple virulent toxins through cytotoxin associated gene A (CagA) and vacuolating cytotoxin A (VacA), results in host tissue damage through various inflammatory mediators along with its invasive nature.[5] These virulence factors are diverse among H. pylori isolates from different geographic areas and ethnic groups, which may explain the differences in disease incidence and severity.[6]

The aim of this study was to identify H. pylori (UreC gene), its virulence genes (CagA and VacA) by polymerase chain reaction method (PCR) and find their association with gastroduodenal lesions.

Methods

This was a prospective, cross sectional, hospital based observational study done at gastroenterology department at Bir hospital during November 2019- January 2021 with H. pylori positive gastroduodenal lesions detected at endoscopy among symptomatic patients. Ethical clearance (reference number 931/076/077) was obtained before conduction of study. The sample size was estimated by modified Cochran's formula[8] as: $n= Z^2 P (1-P)/d^2$, where n =

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required sample size, Z = desired reliability level at 95% confidence interval (1.96), P= estimated prevalence (H. pylori positive in gastric biopsy Samples=16%) d = maximum tolerable error (10%=51, 5% =206). The sample size calculated is 206. H. pylori positivity in gastric biopsies in year 2018 AD in Bir hospital was 60 (unpublished data), in view of less case load per year, corrected sample size formula is applied for study. Corrected sample size = Calculated sample/1+Calculated sample/Estimated sample of disease per year. The sample size calculated is 52.

All symptomatic patients >18 years with gastroduodenal lesions detected during endoscopy were included whereas patients receiving proton pump inhibitors for last 2 weeks, receiving antibiotics for last 1 month, on nonsteroidal anti-inflammatory drugs for last 2 months, active bleeding at endoscopy, post-surgery involving stomach and pregnant ladies were excluded.

The endoscopic findings were classified into four major gastroduodenal diseases: gastritis, peptic ulcer (duodenal ulcer and benign looking gastric ulcer), gastric cancer (carcinoma, lymphoma) and mixed lesions (combination of ulcer, gastritis and polyp or mass). For every patient, two sets of biopsy specimens were obtained from three different gastric sites: 1 from the incisura angularis, 1 from the greater curvature of the corpus and 1 from the greater curvature of the antrum, having similar sensitivity compared with five biopsies according to modified Sydney protocol (considered as gold standard)[9,10] using a disinfected endoscope. One set of biopsy was preserved in 1ml of 10% formalin and subjected to histopathological examination including Giemsa stain and the second set was preserved in 1 ml of sterile saline solution and then stored in −20°C at laboratory for DNA extraction and PCR till analysis. This second set of biopsy was analyzed for presence of UreC gene for molecular identification of bacilli, CagA and VacA virulence genes if biopsy staining was positive for H. pylori (figure1). Paraffin blocks of first biopsy set were assessed for CagA and VacA genes, only if were negative in second set of biopsies which accounted four samples. Mixed lesions in endoscopy were grouped in according to predominant histological features. For suspected gastric cancer, mixed lesions and gastric ulcers in endoscopy: biopsies were obtained from lesions as well as antrum as per our study protocol. Data analysis was done with Statistical Package for the Social Sciences version 25.

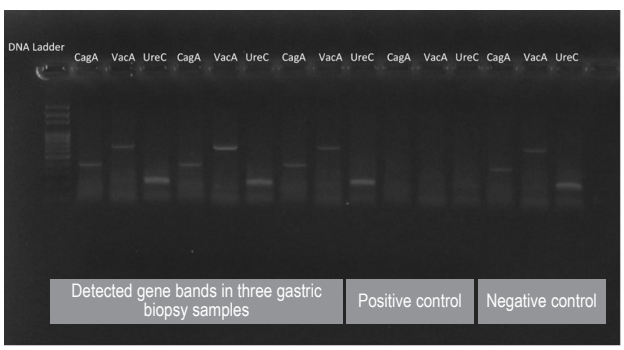

Figure 1: PCR of detected genes

Results

During the study period, 1169 patients underwent endoscopy examination for gastrointestinal symptoms. Among them, 382 patients had gastroduodenal lesions. After exclusion, 168 patients were eligible for biopsy examination. 103 patients (61.31%) were male. The median age of participants was 46 years (age range 20-80years). Majority of patients complained of abdominal pain/ burning (86.90%) followed by anorexia (n=108, 64.29%) and nausea (n=104, 61.90%).

Among the 168 patients with endoscopic lesions only 65 patients had Giemsa stain positive for H. pylori in gastric biopsy samples (38.69%). Gastritis (67.86%), duodenal ulcer (19.04%), gastric ulcer (16.07%) and malignant appearing lesions (16.67%) were detected respectively. H. pylori detection rate with Giemsa stain was highest for gastritis (37.71%) followed by duodenal ulcer (31.25%) as shown in table 1.

Table 1: H. pylori detection by Giemsa stain

Lesions	Total Patients (N = 168)	H. pylori	
		Positive (n, %)	Negative (n, %)
Gastritis	114 (67.86%)	43 (37.71%)	71 (62.28%)
Gastric ulcer	28 (16.67%)	6 (21.43%)	21 (77.77%)
Duodenal ulcer	32 (19.04%)	10 (31.25%)	22 (68.75%)
Gastric cancer	28 (16. 67%)	6 (21.42%)	22 (78.57%)

Molecular analysis (PCR) of detected H. pylori (n=65) in gastric biopsy demonstrated CagA gene in 63 patients (96.9%) and VacA in 50 patients (76.9%). Significant association of CagA gene was seen in all major gastroduodenal lesions compared to VacA gene implicating its major pathogenic role for chronic infection and causation of disease. CagA gene was present in all H. pylori detected in peptic ulcer lesions and gastric cancer and 95% of gastritis as shown in table 2. VacA gene was detected in 83%, 75% and 76% of bacilli detected in gastric cancer, peptic ulcer and gastritis respectively. Both of these genes were present in higher frequency in severe symptomatic lesions (ulcer disease and carcinoma).

Table 2: Virulence genes in gastroduodenal lesions

Lesions	H. pylori positive (N=65)	CagA (%)	VacA (%)	P value
Gastritis	43 (66.15%)	41 (95.34%)	33 (76.74%)	0.009
Peptic Ulcer	16 (24.61%)	16 (100%)	12 (75%)	0.013
Gastric Cancer	6 (9.23%)	6 (100%)	5 (83.33%)	0.224
Total	65 (100%)	63 (96.9 %)	50 (76.92%)	0.001

Comparison of UreC gene detection by PCR with Giemsa stain revealed similar identification rate for bacilli in all histological lesions. Only two patients with gastritis were negative for UreC gene. All patients with UreC gene were positive for CagA virulence gene. This implies usefulness of PCR method to identify H. pylori in infected individuals with fair accuracy (table 3).

Table 3: Detection of H. pylori by PCR method among Giemsa positive gastric biopsy

Histological lesions UreC gene PCR Giemsa positive for H. pylori

Histological lesions	UreC gene PCR	Giemsa positive for H. pylori
Gastritis	41 (65.07%)	43 (66.15%)
Peptic Ulcer	16 (25.39%)	16 (24.61%)
Gastric Cancer	6 (9.52%)	6 (9.23%)
Total	63 (100%)	65 (100%)

Discussion

Diagnosis and treatment of H. pylori associated gastroduodenal diseases remains a challenge even in high prevalent areas. Chronic infection is the rule, which initiates and elaborates persistent inflammation in gastric epithelium through interaction of many bacterial virulence factors and host response to infection.[11] Bacterial factors like flagella, urease enzyme and various virulence genes like CagA, VacA, CagE, blood group antigen-binding adhesin (BabA), outer membrane protein (OMP), induced by contact with epithelium gene (iceA) stimulate the host immune system to elaborate the inflammatory cytokines results in epithelial damage.[12,13] Various invasive and noninvasive tests are recommended to diagnose the presence of this bacilli with good sensitivity and specificity.[14] Molecular method (PCR) detects active as well as dead bacilli and can be done in stool and biopsy samples. It is helpful in identifying the H. pylori strain, its important virulence genes for disease causation and is currently recommended to detect antibiotic resistance to guide the choice of therapy.[15,16]

Age and gender distribution in this study was similar whereas H. pylori prevalence of 38.69% of eligible patients was higher (23.9%) than previous study at our center and similar to study from a teaching hospital - 38.4% (56/146).[17,18] Major lesions identified during endoscopy were gastritis (68%), duodenal ulcer (19%), gastric ulcer (16%) and malignant appearing lesions (16%). Studies from tertiary level hospitals have demonstrated antral gastritis in range from 58% - 79% as the commonest lesion in endoscopy.[19,20] H. pylori detection was 38.6%, which was lower compared to the population study from Sherpa community in Nepal (70.5%) and higher than previous study at our centre (23%).[21] Giemsa stain was positive for H. pylori in 38% in gastritis, 31% in duodenal ulcer and 21% in gastric ulcer respectively in our study, which was lower than similar study where duodenal ulcer and gastric ulcer had the highest bacilli colonization (85.7% and 84%) which was significantly higher than in gastritis, duodenitis and gastric cancer (61.8%, 69.2%, and 60%, respectively).[22] The lower prevalence of bacilli detection could be due to use of PPI therapy as over the counter drugs which patients may not have known as major anti H. pylori drug and previous use of medications before visiting our centre.

Our study showed CagA in 97% and VacA in 77% among detected helicobacter bacilli with significant difference between these two virulence genes among different gastroduodenal lesions. Peptic ulcer and gastric cancer had CagA detected in all, though VacA was also positive in approximately 77% in overall detected samples. Significantly higher association of CagA gene was demonstrated than VacA in all these lesions which implies H. pylori strain causing symptomatic disease had CagA as major virulent factor in our patients. These virulence genes detection rate was similar to a study from a teaching hospital in the country.[23] Lavanya Jeyamani et al. from India also detected H. pylori infection in 37% (61/165) of endoscopic lesions with all strains having CagA and 54% having VacA, which was positive in all peptic ulcer disease similar to our study.[24]

UreC gene was positive in 63/168 samples (37.5%) suggesting a good diagnostic role as compared to Giemsa stain for bacilli identification. Mishra KK et al. detected H. pylori in 53%, 43%, 48% and 50% of patients by PCR, rapid urease test, culture and histological examination respectively with sensitivity and specificity of PCR being the highest: 95% and 100% when compared with both culture and histology.[25]

Conclusion

Helicobacter pylori was detected in 38.6% of gastric biopsies in patients with gastroduodenal diseases. CagA gene was significantly higher (97%) than VacA gene (77%) in causation of these diseases. Polymerase chain reaction had a good diagnostic role in identification of helicobacter pylori.

Conflict of interest:

None

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References

1. Suerbaum S, Michetti P. Helicobacter pylori Infection. New England Journal of Medicine. 2002 Oct 10;347(15):1175–86. doi: 10.1056/NEJMra020542. PMID: 12374879.

2. Sanders MK, Peura DA. Helicobacter pylori-Associated Diseases. Curr Gastroenterol Rep. 2002 Dec;4(6):448–54. doi: 10.1007/s11894-002-0019-x. PMID: 12441033.

3. Thapa R, Lakhey M, Yadav P, … PKJ of N, 2013 undefined. Histopathological study of endoscopic biopsies. PMID: 24362659.

4. Sherpa T, Sherpa K, … GNTNZ, 2012 undefined. The prevalence of Helicobacter pylori infection in Sherpa residents of the Upper Khumbu, an isolated community in Eastern Nepal. PMID: 23254498.

5. Epidemiology of, and risk factors for, Helicobacter pylori infection among 3194 asymptomatic subjects in 17 populations. The EUROGAST Study Group. Gut 1993 Dec 1;34(12):1672–6. doi: 10.1136/gut.34.12.1672. PMID: 8282253; PMCID: PMC1374460.

6. Suzuki R, Shiota S, Yamaoka Y. Molecular epidemiology, population genetics, and pathogenic role of Helicobacter pylori. Infection, Genetics and Evolution. 2012 Mar;12(2):203–13. doi: 10.1016/j.meegid.2011.12.002. Epub 2011 Dec 17. PMID: 22197766; PMCID: PMC3294018.

7. Quantitative Research By Example Ioan Gelu Ionas Version 1.0.0 {β}. Page 36-37.

8. Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test: Histology. Ann Transl Med. 2015 Jan 1;3(1):10. doi: 10.3978/j.issn.2305-5839.2014.11.03. PMID: 25705642; PMCID: PMC4293485.

9. Essawi T, Hammoudeh W, Sabri I, Sweidan W, Farraj MA. Determination of Helicobacter pylori Virulence Genes in Gastric Biopsies by PCR. Int Sch Res Notices. 2013 Apr;2013. doi: 10.1155/2013/606258. PMID: 23691338; PMCID: PMC3649278.

10. Yamaoka Y, Ansari S. Current understanding and management of Helicobacter pylori infection: An updated appraisal [version 1; referees: 3 approved]. Vol. 7, F1000Research. F1000 Research Ltd; 2018. doi: 10.12688/f1000research.14149.1. PMID: 29946428; PMCID: PMC5998008.

11. Feldman Mark, Friedman S. Lawrence, Brandt J.Lawrence, Chung T. Raymond, Rubin T. David WMC. Gastrointestinal and Liver Disease. 11th ed. Philadelphia: Elsevier; 2021. page 784-786.

12. Roesler BM, Rabelo-Gonçalves EMA, Zeitune JMR. Virulence factors of helicobacter pylori: A review. Vol. 7, Clinical Medicine Insights: Gastroenterology. Libertas Academica Ltd.; 2014. p. 9–27. doi: 10.4137/CGast.S13760. PMID: 24833944; PMCID: PMC4019226.

13. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. American Journal of Gastroenterology. 2017 Feb 1;112(2):212–39. doi: 10.1038/ajg.2016.563. Epub 2017 Jan 10. PMID: 28071659.

14. Owen RJ. Molecular testing for antibiotic resistance in Helicobacter pylori. Vol. 50, Gut. BMJ Publishing Group; 2002. p. 285–9. doi: 10.1136/gut.50.3.285. PMID: 11839700; PMCID: PMC1773144.

15. Jaka H, Rhee JA, Östlundh L, Smart L, Peck R, Mueller A, et al. The magnitude of antibiotic resistance to Helicobacter pylori in Africa and identified mutations which confer resistance to antibiotics: Systematic review and meta-analysis. BMC Infect Dis. 2018 Apr 24;18(1):193. doi: 10.1186/s12879-018-3099-4. PMID: 29699490; PMCID: PMC5921563.

16. Mandal A, Kafle P, Puri P, Chaulagai B, Sidhu J, Hassan M, et al. An association of Helicobacter pylori infection with endoscopic and histological findings in the Nepalese population. J Family Med Prim Care. 2019;8(3):1227. doi: 10.4103/jfmpc.jfmpc_82_19. PMID: 31041278; PMCID: PMC6482781.

17. Miftahussurur M, Sharma RP, Shrestha PK, Suzuki R, Uchida T, Yamaoka Y. Molecular Epidemiology of Helicobacter pylori Infection in Nepal: Specific Ancestor Root. Ahmed N, editor. PLoS One. 2015 Jul 30;10(7):e0134216. https://doi.org/10.1371/journal.pone.0134216.

18. Poudel A, Regmi S, Poudel S, Joshi P. Correlation between Endoscopic and Histopathological Findings in Gastric Lesions. Journal of Universal College of Medical Sciences. 2013;1(3):37–41. DOI:10.3126/jucms.v1i3.8763

19. Shrestha R, Batajoo K, Koirala K, Shiv Raj K. Helicobacter pylori infection among patients with upper gastrointestinal symptoms: prevalence and relation to endoscopy diagnosis and histopathology. J Family Med Prim Care. 2014;3(2):154. doi: 10.4103/2249-4863.137663. PMID: 25161975; PMCID: PMC4139998.

20. Jeyamani L, Jayarajan J, Leelakrishnan V, Swaminathan M. CagA and VacA genes of Helicobacter pylori and their clinical relevance. Indian J Pathol Microbiol. 2018 Jan 1;61(1):66–9. doi: 10.4103/IJPM.IJPM_234_17. PMID: 29567886.

21. Mishra KK, Srivastava S, Dwivedi PP, Prasad KN, Ayyagari A. UreC PCR based diagnosis of Helicobacter pylori infection and detection of cag A gene in gastric biopsies. Indian J Pathol Microbiol. 2002 Jan 1;45(1):31–7. PMID: 12593561.