



IDENTIFICATION OF HELICOBACTER PYLORI STRAIN BY PCR IN ENDOSCOPIC SAMPLES FROM PEPTIC ULCER PATIENTS

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Abstract: Worldwide studies have shown that *H. Pylori* infections are strongly associated with peptic ulcer patients. Various studies have shown that there is a definitive impact on genotypic classification of *H. Pylori* on treatment strategy and the prevalence of *H. Pylori* is not homogeneous and varies depending on the country of origin, ethnic background and socio economic status. There is no reported evidence of genotype specific *H. Pylori* detection in our population and so the focus of the study is to classify *H. Pylori* detection in our population which would help in the effective treatment of *H. Pylori* infection. The study included 32 patients with peptic ulcer disease who were posted for gastric endoscopy in the surgery OPD. Gastric antral biopsy was collected for all the patients by endoscopy. Culture of the biopsy was done by sheep blood agar. Genotyping of *H. Pylori* was done by PCR. 16S rRNA and Cag A genes of *H. Pylori* are said to be commonly associated with peptic ulcer. The study showed the prevalence of 16S rRNA in all the 32 patients, which confirms the presence of *H. Pylori*, but Cag A strain was negative for all cases, thus necessitating the implementation of specific genotype oriented treatment approach.

Key Words: Peptic ulcer, *H. Pylori*, Cag A, 16S rRNA

INTRODUCTION

Peptic ulcer is defined as discontinuity in gastric epithelium due to erosions greater than 0.5cm. Almost 90% of the individuals are infected with *H. Pylori* out of which only 15% of persons may have the disease. The mortality rate is 2-4% of the infected patients due to complications [1,2,3]. Whites are less affected. No sex predilection is seen, but it can occur in any age group. Genotypes includes (BMC Microbiology, 8: 175) 99 [1491 genes], 26695 [1587 genes], strain-A, J99/26695. All genomes contain 2 copies of genes for 16S rRNA, 23S rRNA and 5S rRNA. Genetic or phenotypic diversity is of clinical significance. The subtypes include CagA, VacA (All strains are non-conserved strains), iceA, ureA. Out of which 60% of *H. Pylori* infection in USA and Europe possess CagA and vacA strains. CagA gene in cagA strain is responsible for the secretion of picB, a protein homologous with type3 secretory proteins which are required for *H. Pylori* to induce gastric epithelial cells to secrete IL-8 (inflammatory maker) causing gastritis and ulcer [4,5]. Antibiotic sensitivity differs for different strains. So, this made the necessity for us to identify the strain prevalent in our region which will help in the effective management of the disease and thus eradication of the infection. Since cagA was found to be predominant in the earlier studies which was the common cause for ulcer in Western countries. We designed our study to explore whether cagA could be prevalent in our population [6,7].

MATERIALS AND METHODS

Patients with peptic ulcer, gastritis, duodenal ulcer, undergoing upper GI endoscopy were enrolled in the department of surgery, RMMCH in the month of February and March 2009 were selected. Patients with history of gastric surgery, receiving steroids or immunomodulation treatment were excluded. Upper GI endoscopy was performed and biopsy from gastric antrum was taken and collected in vial containing normal saline. The culture was grown from the gastric biopsy in sheep blood agar. Extraction of genomic DNA was done from the colonies of the culture. This was used as a template for PCR [8].

PCR Components

PCR primers were designed on the basis of published sequences of *H. Pylori* 16S rRNA, CagA.

16S rRNA

Forward-5'-GCT AAG AGA TCA GCC TAT GTC C-3'

Back ward-5'-TGG CAA TCA GCG TCA GGT AAT G-3'

Cag A

Forward-5'-GAT AAC AGG CAA GCT TTT GAG G-3'

Backward-5'-CTG CAA AAG ATT GTT TGC GAG-3'

Master mix containing Taq polymerase, Dntp, MgCl₂, Forward prime, Reverse Primer, Template DNA, and Distilled water were pipetted into a 200µl PCR tube according to the table given below [9]. (Table 1). These PCR tubes were then kept in the thermocycler and the target DNAs were amplified by 30 cycles according to

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the table given below. Then the amplified PCR products were stored at 4°C until electrophoresis [10]. Amplification of *H. Pylori* genomic DNA sequences was carried out in a total volume of 50 µl containing PCR buffer (50mM KCl, 10mM Tris HCl [pH 8.3]), 1.5mM MgCl₂, 200 µM (each) deoxynucleotides, 2U of AmpliTaq Polymerase and 1 µl of extracted DNA as template. The 16srRNA, cagA primers were used at a final concentration of 0.5 µM, each reaction mixture was overlaid with 25 µl of light mineral oil and was amplified for 30 cycles. Analysis of PCR products was done by submarine gel electrophoresis. DNA bands were analyzed in gel documentation [11, 12, 13]. (Table 2)

Table 1: Components in PCR

Components	Volume	Volume	Final Concentration
5X Master Mix	10 µl	5 µl	IX
Forward Primer	15 µl	15 µl	0.4 µM
Reverse Primer	15 µl	15 µl	0.4 µM
Template DNA	1 µl	1 µl	5-100 ng
Distilled water	Up to 50 µl	Up to 25 µl	

Table 2: Components in Cycling Condition

Operation	Temperature	Time	Cycles
Initial Denaturation	95 °C	3-5 min	1
Denaturation	94 °C	1 min	30
Annealing	55 °C	1 min	
Elongation	72 °C	2 min	
Final Elongation	72 °C	7 min	1
	Held at 4 °C		

RESULTS

The sensitivity of the PCR assay for the detection of *H. Pylori* 16srRNA and cagA was investigated by performing PCR. Biopsy samples from 32 patients were cultured and from the DNA extracted PCR was carried out. All the 32 samples were positive for 16srRNA and cagA was negative in all the samples (Table 3).

Table 3: Number of Patients positive for 16srRNA and CagA

Number of Patients	16srRNA Positive	CagA Positive
32	32	Nil

DISCUSSION

All the 32 Urease positive samples had shown positive for 16srRNA indicating *H. Pylori* infection. CagA was negative in all the samples. This could be due to Absence of CagA strain in our population (or) Presence of a mutant strain which was not picked up by the primer we used based on the reference.

Our culture results revealed that *H. Pylori* showed sensitivity to Levofloxacin. Previous studies on cag A positive *H. Pylori* infections have shown sensitivity to levofloxacin. So it is presumed that a mutant Cag A strain may be present in our population but it needs further study and sequencing.

CONCLUSION

PCR offers high sensitivity in identification of *H. Pylori* strain. All the endoscopic samples have shown the presence of 16srRNA while all samples were negative for CagA.

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