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RAPID COMMUNICATION

Gastric juice for the diagnosis of *H pylori* infection in patients on proton pump inhibitors

Javed Yakoob, Shahid Rasool, Zaigham Abbas, Wasim Jafri, Shahab Abid, Muhammad Islam, Zubair Ahmad

Javed Yakoob, Shahid Rasool, Zaigham Abbas, Wasim Jafri, Shahab Abid, Muhammad Islam, Department of Medicine, Aga Khan University Hospital, Karachi 74800, Pakistan
Zubair Ahmad, Department of Pathology, Aga Khan University Hospital, Karachi 74800, Pakistan
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Correspondence to: Javed Yakoob, MBBS, PhD, Section of Gastroenterology, Department of Medicine, Aga Khan University Hospital, Stadium Road, Karachi 74800, Pakistan. yakoobjaved@hotmail.com
Telephone: +92-21-4864679 Fax: +92-21-4934294
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Abstract

AIM: To determine the efficacy of gastric juice polymerase chain reaction (PCR) for the detection of *H pylori* infection in comparison with histology and gastric antral biopsy PCR in patients on a proton pump inhibitor (PPI).

METHODS: Eighty-five consecutive patients with dyspeptic symptoms were enrolled. Gastric biopsies for histology, PCR and gastric juice were collected at endoscopy for PCR of the *H pylori* urease C gene (ure C). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive and negative likelihood ratio for PCR of gastric juice for the *H pylori* ure C gene was compared to histology and gastric antral biopsy *H pylori* ure C PCR in patients with and without PPI.

RESULTS: Gastric juice PCR was positive in 66 (78%) patients. Histology showed *H pylori* associated gastritis in 57 (67%). Gastric biopsy PCR was positive in 72 (85%). In patients not taking PPI, the sensitivity, specificity, PPV, NPV, accuracy and positive and negative likelihood ratio for gastric juice PCR were 89%, 72%, 91%, 67%, 90%, 85%, 3.1 and 0.1 respectively. In patients on PPI these values were 86%, 100%%, 100%, 29%, 86%, 9.5 and 1.4, respectively.

CONCLUSION: Gastric juice PCR for the diagnosis of *H pylori* infection has increased sensitivity compared to histology with PPI. The use of gastric juice PCR is recommended to confirm *H pylori* status in patients taking PPIs.

INTRODUCTION

H pylori is a spiral gram negative microaerophilic bacterium that infects the human gastric mucosa and is associated with gastritis, gastroduodenal ulcer disease, gastric carcinoma and mucosal associated lymphoid tissue lymphoma[1,2]. The prevalence of *H pylori* is high in developing countries. A recent study revealed an early colonization/infection of infants with *H pylori* and a prevalence of 67% at 9 mo of age in a peri-urban community in Karachi, Pakistan[3]. *H pylori* serology was positive in 58% of our general population in a previous study but is likely to actually be higher[4]. Currently, several diagnostic tests of varying sensitivity and specificity are available for determining the presence of *H pylori*, which include rapid urease test (RUT), histology, culture, urea breath test (UBT), and serology. Isolation of *H pylori* from gastric biopsy specimens constitutes the most specific way to establish the diagnosis of infection and to study the genotype of the infecting strains, however, it is a time consuming process. RUT and histology are still commonly used for the diagnosis of *H pylori* infection in our country as other modalities of *H pylori* testing, such as UBT and facilities for *H pylori* stool antigen tests, are not widely available.

In Pakistan, self-prescription is common and medications are available over the counter without prescriptions[5]. It is known that acid reducing drugs; e.g, proton pump inhibitor (PPI), histamine-2 receptor blocker (H2RB), antibiotics and bismuth compounds reduce the sensitivity and specificity of the diagnostic tests for *H pylori*[6,7]. In our previous studies, we demonstrated that histology is comparatively less affected by PPI than RUT[8,9]. Polymerase chain reaction (PCR) is a highly

sensitive technique that can detect very small amounts of DNA. The DNA molecule is chemically stable and can survive in the environment for long periods[10]. PCR may be useful, therefore, in detecting the presence of *H pylori,* even when the organism is in a nonculturable state. In previous studies, gastric juice PCR has been evaluated as a highly specific and rapid method for the detection of *H pylori*[11,12]. An efficient and accurate diagnosis of *H pylori* infection is important when seeking to cure patients with persistent gastric symptoms in which *H pylori* infection is suspected. The aim of this study was to determine the efficiency of gastric juice PCR for the detection of *H pylori* infection in patients on PPI and compare it with histology and gastric biopsy PCR.

MATERIALS AND METHODS

Patients

Eighty-five consecutive patients with dyspeptic symptoms attending the gastroenterology outpatient clinic from February-November 2006 were enrolled. There were 58 (68%) males and 27 (32%) females. The age range was 17-70 years with a mean age of 36.8 ± 11. Patients were divided into two groups: (1) those who received PPI (mainly omeprazole 20 mg once a day) for at least 4 wk before undergoing esophagogastroduodenoscopy (EGD); (2) patients with no previous treatment with antibiotics, PPI, H2RB and bismuth compounds. Patients in each respective group also did not use other drugs. Compliance with treatment was ascertained during an outpatient visit before the endoscopy. The study was approved by the Ethics Review Committee of Aga Khan University Hospital. Informed consent was obtained from all patients for EGD with biopsies from the antrum and corpus of the stomach and aspiration of gastric juice. EGDs were performed after an 8 h fast. A sample of gastric juice (5 mL) was aspirated at endoscopy by means of a sterile cannula used for endoscopic retrograde cholangiopancretography (ERCP), passed through the suction channel and collected in a disposable sterile syringe. After each examination the endoscopes were washed with 2% glutaraldehyde and disinfected with 70% ethanol followed by rinsing with sterile water after each examination. Biopsy forceps were sterilized by autoclaving to ensure lack of cross contamination using the endoscopic equipment. All patients received conscious sedation with intravenous midazolam and topical pharyngeal anesthetic spray. Sterilized biopsy forceps were used to obtain gastric biopsy specimens from the antrum and mid of the corpus. Two biopsy specimens were removed from each site for histology and dispatched in a formalin containing container. Biopsy for PCR was dispatched in normal saline. Sensitivity, specificity, PPV, NPV, accuracy, positive and negative likelihood ratio for gastric juice *H pylori* ure C gene PCR were compared against histology and gastric biopsy PCR in patients with and without PPI to establish the efficiency of this diagnostic approach.

Histology

Gastric biopsy specimens from each site for histopathology were stained with hematoxylin and eosin and Giemsa stain for the detection of *H pylori*; the degree of gastritis as determined by hematoxylin and eosin (HE) stain was scored in accordance with the Sydney system[13].

Extraction of genomic DNA from gastric juice

Extraction of genomic DNA from gastric juice was carried out as previously described[11]. A 5 mL of gastric juice aspirate was buffered to a neutral pH with 5 mL of Tris (0.67 mol/L, pH 7.4). Each sample was then concentrated by centrifugation at 10000 × *g* for 20 min. The supernatants were removed and the pellets were resuspended in 100 μL of sterile distilled water. One hundred μL of lysis buffer [100 mmol/L NaCl, 10 mmol/L Tris-HCl (pH 8.0), 25 mmol/L EDTA, 0.5% sodium dodecyl sulfate], and 5 μL of proteinase K (10 g/L) were added. Incubation was carried out at 50℃ for 20 h; this was followed by phenol-chloroform extraction and ethanol precipitation. The resulting pellet was allowed to dissolve in 35 μL of TE buffer 10 mmol/L Tris-HCl (pH 7.4) and 0.1 mmol/L EDTA (pH 8.0) for 20 h at 37℃. Samples were stored at -20℃ before PCR amplification was performed. DNA content and purity was determined by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer (Beckman DU-600, USA).

Extraction of DNA from gastric biopsy

Briefly, gastric tissue was homogenized to uniformity in 500 mL of sterile water and centrifuged at 12000 × *g* for 3 min. Five hundred μL of lysis buffer [100 mmol/L NaCl, 10 mmol/L Tris-HCl (pH 8.0), 25 mmol/L EDTA, 0.5% sodium dodecyl sulfate], and 10 μL of proteinase K (10 g/L) were added. Incubation was carried out at 56℃ for 20 h; this was followed by phenol-chloroform extraction and ethanol precipitation. The resulting pellet was allowed to dissolve in 40 μL of TE buffer [10 mmol/L Tris-HCl (pH 7.4) and 0.1 mmol/L EDTA (pH 8.0)] for 20 h at 37℃. Samples were stored at -20℃ before PCR amplification was performed. DNA content and purity was determined by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer (Beckman DU-600, USA).

PCR for ure C

PCR was performed using extracted DNA as the template and urease gene C for primers. Forward primer (5'-TG GGACTGATGGCGTGAGGG-3') and reverse primer (5'-AAGGGCGTTTTTAGATTTTT-3') were prepared from the urease gene sequence according to the report of Labigne *et al*[14]. PCR amplification was carried out in a total volume of 50 μL containing 2 μL of 2 mmol/L dNTPs, 1 μL containing 50 ρmol of primer 1, 1 μL containing 50 ρmol of primer 2 (synthesized by ABI Automatic synthesizer), 1 unit of Taq DNA polymerase (Promega), 5 μL of 10 × PCR reaction buffer, 3 mmol/L of $MgCl_2$, 2 μL of DNA template containing 0.5 ng of extracted DNA and total volume rounded to 50 μL by double distilled water. The reaction was carried out in a Perkin Elmer 9700 thermal cycler. The amplification cycle consisted of an initial denaturation of target DNA at 95℃ for 5 min and then denaturation at 94℃ for 1 min, primer

Table 1 Comparison of histology, gastric biopsy and juice PCR for the diagnosis of *H pylori* infection with and without PPI *n* (%)

	Medication		*P* value
	On PPI *n* = 37	Without PPI *n* = 48	
Histology			
H pylori positive gastritis	23 (62)	34 (71)	0.24
H pylori negative gastritis	14 (38)	14 (29)	
Gastric juice PCR			
Positive	30 (81)	36 (75)	0.50
Negative	7 (19)	12 (25)	
Gastric biopsy PCR			
Positive	35 (95)	37 (77)	0.02
Negative	2 (5)	11 (23)	

annealing at 56℃ for 1 min and extension at 72℃ for 1 min. The final cycle included an extension step for 5 min at 72℃ to ensure full extension of the product. Samples were amplified through 35 consecutive cycles. Negative reagent control reactions were performed with each batch of amplifications, consisting of tubes containing distilled water in place of the DNA samples. Five μL of PCR product was electrophoresed on a 1.5% agarose gel to ensure homogeneity and yield. PCR amplification resulted in a homogeneous DNA fragment of the expected size of 820 bp for ure C gene.

Statistical analysis

The statistical package for social science SPSS (Release 11.5, standard version, copyright © SPSS; 1989-99) was used for data analysis. The descriptive analysis was done for demographic and clinical features. Results were presented as mean ± SD for quantitative variables and number (percentage) for qualitative variables. Odd ratio (OR) and 95% confidence interval (95% CI) were estimated to check the strength of association. Sensitivity, specificity, PPV, NPV, accuracy and negative and positive likelihood ratio were determined for PCR and histology.

RESULTS

Thirty seven (43.5%) patients were on PPI while 48 (56.5%) were not taking any medications. Abdominal pain was present in 60 (71%) and dyspepsia 25 (29%). Symptoms were equally common in two groups. The endoscopic diagnosis was pangastric erythema in 56 (66%), antral erythema in 25 (29.4%), gastric ulcer 1 (1.2%), gastric carcinoma 2 (4.2%), and duodenal ulcer 1 (1.2%). The age range of these patients was 25-70 years with mean age 43.5 ± 13.2.

Comparison of histology, gastric biopsy and juice PCR with and without PPI

Histology showed *H pylori* associated gastritis in 57 (67%) and *H pylori* negative gastritis in 28 (33%). On PPI, 23 (62%) had *H pylori* positive gastritis $P = 0.24$ (Table 1). Gastric juice ure C PCR was positive in 66 (78%) and negative in 19 (22%). On PPI, gastric juice PCR was positive in 30 (81%) $P = 0.50$ (Table 1). Gastric biopsy ure C PCR was positive in 72 (85%) and negative in 13 (15%). On PPI, gastric biopsy PCR was positive in 35 (95%) with $P = 0.02$ (Table 1).

Table 2 Comparison of gastric juice PCR and histology using gastric biopsy PCR as the gold standard *n* (%)

	PCR of gastric biopsy		*P* value
Over all (*n* = 85)			
Histology	Positive	Negative	
Positive	56 (78)	1 (8)	< 0.001
Negative	16 (22)	12 (92)	
Gastric juice PCR			
Positive	63 (87)	3 (23)	< 0.001
Negative	9 (13)	10 (77)	
On PPI (*n* = 37)			
Histology			
Positive	23 (66)	0	0.17
Negative	12 (34)	2 (100)	
Gastric juice PCR			
Positive	30 (86)	0	0.003
Negative	5 (14)	2 (100)	
No PPI (*n* = 48)			
Histology			
Positive	33 (89)	1 (9)	< 0.001
Negative	4 (11)	10 (91)	
Gastric juice PCR			
Positive	33 (89)	3 (27)	< 0.001
Negative	4 (11)	8 (73)	

Comparison of histology and gastric juice PCR with gastric biopsy PCR

Seventy two (85%) were positive by ure C PCR of gastric biopsy compared to 57 (67%) by histology and 66 (78%) by gastric juice ure C PCR with $P < 0.001$ and $P < 0.001$, respectively (Table 2). For patients taking PPI, 23 (62%) were positive by histology while 30 (81%) were positive by gastric juice PCR with $P = 0.17$ and $P = 0.003$, respectively (Table 2). For patients not taking PPI, histology was positive in 34 (71%) while 36 (75%) were positive by gastric juice PCR with $P < 0.001$ and $P < 0.001$, respectively (Table 2). For patients taking PPI, the sensitivity, specificity, PPV, NPV, accuracy and positive and negative likelihood ratio for gastric juice PCR and histology were 86%, 100%, 100%, 29%, 86%, 9.5 and 1.4 and 73%, 100%, 100%, 14%, 0.6 and 2.7, respectively (Table 3).

DISCUSSION

Gastric juice represents a pooled source of events in the entire gastric microenvironment, and it may be valuable for studying *H pylori* whose mucosal distribution is patchy and variable. This is even more relevant in the setting of developing countries, where *H pylori* possibly exists as a dynamic mix of quasi-species. A single biopsy sample may not be able to detect the presence of *H pylori*, whereas gastric juice, being a more global sample, may overcome this limitation because the gastric juice reflects the actual microenvironment and the global level of infection in the stomach. *H pylori* has a very potent urease activity, and because of this highly specific activity of the urease enzyme *H pylori* are able to hydrolyze the urea present in

Table 3 Comparison of gastric juice PCR and histology with and without PPI using gastric biopsy PCR as the gold standard

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Likelihood ratio LR+	Likelihood ratio LR-	Accuracy (%)
Over all (*n* = 85)							
Histology	77	92	98	43	9.6	0.25	80
Gastric juice PCR	87	77	95	53	3.7	0.16	85
On PPI (*n* = 37)							
Histology	73	100	100	14	0.6	2.7	74
Gastric juice PCR	86	100	100	29	9.5	1.4	86
No PPI (*n* = 48)							
Histology	89	90	97	71	0.89	0.12	85
Gastric juice PCR	89	72	91	67	3.17	0.15	85

the stomach. This serves to protect the organism from the harmful effects of gastric acid and the ammonia generated protects the organism by buffering gastric acid[15,16]. The proton pump inhibitor reduces gastric acid secretion and inhibits urease activity[17,18]. When the secretion of acid is suppressed, *H pylori* in the presence of urea increases the pH of its local environment to alkaline values and are unlikely to survive in a culturable form[19].

This study has demonstrated that for patients taking PPI gastric juice *H pylori* PCR with a specific primer for ure C was more sensitive than histology to detect *H pylori* infection 86% *vs* 73% (Table 3). Gastric juice PCR was able to detect positively 7 (19%) patients who were negative on histology. This could be due to a patchy distribution of the *H pylori*, obtaining biopsies from an uninfected sites resulting in false negatives on histology and PPI activity against *H pylori*[20]. The histology-negative, PCR-positive subjects were older, with a mean age of 43.5 ± 13.2 years. This is in keeping with a previous study[21]. *H pylori* infection associated atrophy and intestinal metaplasia progresses with age[22,23]. Thus, older patients may be more liable to have false-negative results from the commonly biopsied sites in our practice. Although, the overall sensitivity of the gastric juice PCR as seen in the present study is low. This might be attributed either to the lack of *H pylori* in the gastric juice or the presence of some inhibitor of PCR decreasing sensitivity of the technique. In this study, we did not choose to compare RUT with gastric juice PCR as it is already known that PPI reduces the sensitivity of RUT[7-9]. In patients on PPI, the biopsy specimen may contain low bacterial density of viable cells giving a negative urease test. This will also lead to the lack of *H pylori* identification on histology. Similarly, culture of *H pylori* from gastric mucosal biopsies is likely to be negative with PPI as they are not only known to effect the distribution of *H pylori* within the stomach but are also detrimental to *H pylori* in both the antrum and the corpus[7,24].

Proton pump inhibitors have been reported to modify the level of *H pylori* gastritis[20,25,26]. The sensitivity, NPV, accuracy and positive likelihood ratio of gastric juice PCR was greater and the negative likelihood ratio was less for *H pylori* in patients on PPI, while specificity and PPV was similar to histology (Table 3). A previous study by Dickey *et al* showed histological examination sensitivity, specificity, PPV, NPV and diagnostic accuracy were reduced on acid reducing drugs[7]. In their study, five (83%) of the histology-negative, seropositive patients taking PPI had histological changes consistent with *H pylori* gastritis even though no *H pylori* were detected[7]. In our study, the detection rate of *H pylori* was greater by gastric juice PCR on PPI (Tables 1-3). Of the various tests that are available for *H pylori* detection, histological examination of gastric biopsy is considered the most accurate method of diagnosis[27]. If more than one gastric biopsy tissue is used it might improve the test sensitivity without compromising its specificity. Obtaining a serological test in these cases in our population, will not help in deciding whether to treat or not, as a positive result with serology does not tell whether the patient has a current infection or had a past infection that is now cured. The drawbacks of treating these patients who are not actively infected include among many others contribution to antibiotic resistance.

This is the first study to investigate influence of PPI on the results of PCR of gastric juice and histological examinations while using gastric biopsy PCR as the gold standard. In the presence of PPI, gastric juice PCR was more sensitive than histology. Seven (19%) subjects who were negative for *H pylori* by histology and positive by the gastric juice PCR assay should be regarded as having ongoing infections. These patients would have benefited from antimicrobial therapy. However, the results of this study needs to be confirmed in a larger group of patients. In conclusion, the use of the gastric juice PCR can be recommended to exclude *H pylori* infection in patients taking PPI. However, it can also be used as an additive test to confirm the *H pylori* status in patients having histological changes consistent with *H pylori* gastritis though negative for *H pylori*.

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COMMENTS

Background

An efficient and accurate diagnosis of *H pylori* infection is important when seeking to cure patients with persistent gastric symptoms in which *H pylori* infection is suspected. In patients on acid reducing drugs such as proton pump inhibitors (PPI), H-2 receptor blockers (H-2RB) *etc*, the accuracy of the rapid urease test, urea breath test and histology are known to be less accurate for the diagnosis of *H pylori* infection.

Research frontiers

The development of new types of test or targets to test for the *H pylori* infection in patients with prior use of PPI *etc* is important considering the morbidity and mortality associated with this infection.

Innovations and breakthroughs

This study determined the efficiency of gastric juice polymerase chain reaction (PCR) for the detection of *H pylori* infection in patients on PPI and compared it with histology and gastric biopsy PCR.

Applications

It showed gastric juice PCR for the diagnosis of *H pylori* infection had an increased sensitivity compared to histology in patients on PPI. The use of the gastric juice PCR can be recommended to confirm the *H pylori* status in patients taking PPIs.

Terminology

PPI: proton pump inhibitors are a group of drugs whose main action is pronounced and long-lasting reduction of gastric acid production. They are the most potent inhibitors of acid secretion available today.

Peer review

The paper means a real advance in the methodology of diagnosis in this field. The conclusions are valuable. The design is original. The methodology is correct and the results are well presented. Statistical analysis is adequate.

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