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Multiplex PCR for detection of *Helicobacter pylori* infection in gastric biopsies with lower inflammatory score

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ABSTRACT

Background: No gold standard has yet been established for the diagnosis of *H. pylori* infection. A multiplex polymerase chain reaction (mPCR) was developed in this study for rapid, sensitive and specific detection of *H. pylori* from gastric biopsies.

Methods: *H. pylori* infections were determined by in-house rapid urease test (iRUT), culture, histology and multiplex PCR.

Results: A total of 140 (60.9%) from 230 patients were positive for *H. pylori* infection. *H. pylori* were detected in 9.6% (22/230), 17% (39/230), 12.6% (29/230) and 60% (138/230) of biopsy specimens by culture, iRUT, histology and mPCR, respectively. mPCR identified *H. pylori* infection in 100% of biopsies with positive histology and culture. All biopsies with positive iRUT yielded positive PCR except two cases. mPCR also detected *H. pylori* in additional 116, 101 and 109 biopsies that were negative by culture, iRUT and histology, respectively. Positive samples by mPCR showed lower average in *H. pylori* density, activity and inflammation scores. The Indians showed the highest prevalence of *H. pylori* infection compared to the Chinese and the Malays. In addition, Chinese patients with older age were significantly infected compared to other ethnicities.

Conclusion: PCR was able to detect the highest numbers of positive cases although the lowest average scores were recorded in the activity, inflammatory and *H. pylori* density.

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KEYWORDS
Helicobacter pylori; diagnosis; multiplex PCR; inflammatory scores

Introduction

H. pylori infections are a major public health concern of the global community. About half of the world populations are infected with the organism [1] and many of them remain asymptomatic.[2] Infections can result in gastritis and some infected individuals developed peptic ulcer or gastric adenocarcinoma.[3,4] Accurate detection of the organism is essential for patient management. *H. pylori* eradication results in a marked reduction in the rate of recurrence of peptic ulcer and prevention of gastric cancer.[5,6] In addition, *H. pylori* treatment can potentially prevent gastric cancer by reducing the progress of precancerous lesion defined as atrophy, intestinal metaplasia or dysplasia to invasive cancer. Therefore, methods that accurately detect *H. pylori* infection in patients with dyspepsia symptoms are of major importance.

Numerous techniques have been developed to identify *H. pylori* infection, but none is definitive. Current diagnostic tests for *H. pylori*, however, still involve invasive gastric endoscopy and detection of the organism in gastric biopsy. *H. pylori* has been identified in gastric tissues by a rapid urease test, staining (histological examination), and culturing. Rapid urease test require an adequate numbers of bacteria because urease production by the bacterium is reduced in patients who taking proton pump inhibitor (PPI), antibiotics or bismuth compound. [7] *H. pylori* are fastidious and slow growing organisms, often difficult and time-consuming to isolate by culture which require 3–7 days of incubation.[8] Furthermore, *H. pylori* viability will reduce prior treatment with antibiotics, PPI or during transportation.[9] The isolation of *H. pylori* in culture certainly indicates the presence of the bacteria, however negative cultures do not prove its absence. Histology is easily affected by factors such as site of the biopsy taken, number and the size of biopsy, stain used and requires expertise personnel.

Molecular tests offer excellent ways for diagnosis of *H. pylori* infection and do not require the bacteria to be alive when tested and even the organism is present in low numbers. Many polymerase chain reaction (PCR) methods targeting putative *H. pylori* specific genes have been reported.[10–12] In this study, three genes were targeted for amplification namely a conserved region flanked by genus-specific primer binding sites in

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Helicobacter 16S rDNA and species-specific sequences, *ureA* and *hpaA* gene. *H. pylori* was definitively distinguished from *Campylobacter* and other bacterial genera on the basis of 16S rRNA studies.[13–15] The *ureA* gene was widely used for identifying *H. pylori* by PCR [12,16,17] and encode for urease enzyme. *H. pylori* adhesin A (HpaA) is a surface-located lipoprotein.[18,19] The expression of the HpaA protein has previously been found to be highly conserved among *H. pylori* isolates. [20] Furthermore, genomic studies show no significant sequence homologies of HpaA with other known proteins.[21,22] Therefore, the aim of this study was to develop a multiplex PCR method for rapid, sensitive and specific detection of *H. pylori* directly from biopsy samples. The study demonstrated the feasibility of using mPCR to detect *H. pylori* infection, especially in biopsies with mild inflammation. Results obtained were also analysed according to patients' characteristics.

Materials and methods

Study population

Two hundred and thirty consecutive patients with symptoms of dyspepsia, who underwent oesophagogastroduodenoscopy (OGDS) at Universiti Kebangsaan Malaysia Medical Centre (UKMMC) from March 2012 until April 2013, were recruited in this study. Exclusion criteria were the patients who received antibiotics or non-steroidal anti-inflammatory drugs four weeks prior endoscopy and patients with evidence of malignancy and immunosuppression. This study was approved by Medical Ethic Committee of the university (UKM 1.5.3.5/244/UKM-GUP-2011-307). Informed consent was obtained from patients before the procedure. Four antral biopsy specimens were obtained from each patient for in-house rapid urease test (iRUT), culture, histopathology examination and mPCR analysis.

In-house rapid urease test (iRUT)

iRUT was considered positive when the gel contained 2% urea with phenol red as a pH indicator, result in colour changes from yellow into red or pinkish when there is an increase in pH of the medium. When a biopsy specimen containing *H. pylori* is introduced to a urea-rich medium, the urease produce by *H. pylori* breaks the urea into carbon dioxide and ammonia. The ammonium ion increases the pH which is detected by the indicator phenol red.

H. pylori culture

Biopsy specimens for culture were transported to the laboratory in Brucella broth (BBL, Becton Dickson, USA) containing 15% glycerol at 4 °C and immediately inoculated onto Columbia agar base (Oxoid, Basingstoke, UK) containing 7% sheep blood supplemented with Dent's supplement (Oxoid, Basingstoke, UK). During inoculation, biopsies were smoothly grind using sterile wire loop while streaking was carried out. The plates were incubated at 37 °C for 5–7 days under microaerophilic conditions which is generated by using microaerophilic gas pack (CampyGen, Oxoid, Basingstoke, UK). Culture was recorded as positive when *H. pylori* growth was identified (small, circular, smooth and translucent colonies that show curved Gram-negative rods under Gram-stained examination) that give positive results for urease, catalase and oxidase test.

Histopathological examination (HPE)

Gastric biopsies were fixed in 10% formalin and paraffin-embedded section were cut and stained with haematoxylin-eosin and when necessary, sections were also stained with Warthin-Starry for better visualisation of *H. pylori*. Severity of gastritis was recorded based on the updated Sydney system [23] and graded from 0 to 3 (equating to none, mild, moderate and marked).

DNA extraction and mPCR analysis

DNA was extracted from biopsies using Invitek RTP® Bacteria DNA mini kit (Stratec Molecular, Berlin) according to the manufacturer's instructions. Optimisation of the PCR components and conditions were carried out to determine the optimum conditions for mPCR. Concentration of primers (10 pmol), $MgCl_2$ (1.5 mM), *Taq* DNA polymerase (1 unit), dNTPs mix (0.2 mM) and 1× PCR buffer were used. mPCR was carried out for detection of 179 bp fragment of *hpaA* gene, 422 bp fragment of 16S rDNA and 627 bp fragment of *ureA*. The sequences of primers used were as previously described.[24,25] The optimum amplification conditions were achieved consisting an initial denaturation of target DNA at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 1 min and extension at 72 °C for 1 min. The final cycle included extension for 10 min at 72 °C. Five microlitre of mPCR products were subjected to electrophoresis on 2% (wt/vol) agarose gel in 90 mA for 40 min using submarine horizontal electrophoresis apparatus. The gels were stained with FloroSafe DNA staining solution (Axil Scientific Pte. Ltd., Singapore) and PCR bands were visualised under ultraviolet light. The specificity of the mPCR assay was evaluated by testing with reference *H. pylori* strains, i.e. ATCC 700824 (J99 strain) and ATCC 700392 (26695 strain) as positive controls. Other bacterial species were also tested which include five strains from American Type Culture Collection (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* BAA 1706, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853) and three strains from clinical isolates (*Acinetobacter* spp., *Clostridium difficile* and *Proteus mirabilis*).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) version 21. Data were analysed using Pearson chi-square and Yate's correction for categorical data of positivity rates by different methods. HPE scores and mean age were analysed using Mann-Whitney *U* test. *P* value less than 0.05 was considered significant.

Results

Study population characteristics

A total of 230 consecutive patients consisting of 110 male (47.8%) and 120 female (52.2%) aged range from 17 to 82 years, mean age 52.37 ± 15 years with dyspeptic symptoms were recruited in this study. Within this population, 99 (43%) were Malays, 96 (41.7%) were Chinese, 25 (10.9%) were Indian and 10 (4.3%) were from other ethnic groups (4 Bangladeshi, 2 Myammese, 2 Punjabis, 1 indigenous and 1 Caucasian). The patients were classified into four groups according to endoscopic finding: normal ($n = 2$), non-ulcer dyspepsia (NUD) ($n = 162$) [gastritis; $n = 136$, duodenitis, $n = 2$, gastritis and duodenitis; $n = 24$], peptic ulcer disease (PUD) ($n = 35$) [duodenal ulcer; $n = 2$, gastric ulcer; $n = 27$, duodenal and gastric ulcer; $n = 6$], and others ($n = 29$) [hiatus hernia; $n = 19$, fundal polyps; $n = 1$, Barrett's oesophagus; $n = 5$, gastro-esophageal reflux disease (GERD); $n = 6$].

According to HPE, stomach within the normal limits, chronic gastritis, chronic active gastritis and precancerous lesions were observed in 3, 192, 29 and 4 patients, respectively. Histopathological results for two biopsies were not available. The grading of gastritis was as follows: mononuclear inflammation was assessed as absent ($n = 14$), mild ($n = 178$), moderate ($n = 31$) and marked ($n = 5$), neutrophil infiltration identified as absent ($n = 200$), mild ($n = 22$), moderate ($n = 5$) and marked ($n = 1$), *H. pylori* was determined as absent ($n = 199$), mild ($n = 17$), moderate ($n = 8$) and marked ($n = 4$). Sixteen patients presented with intestinal metaplasia and 24 with atrophy.

H. pylori detection

H. pylori were detected in 9.6% (22/230), 17% (39/230), 12.6% (29/230) and 60% (138/230) of biopsy specimens by culture, iRUT, histology and mPCR, respectively. Patient was considered to be infected with *H. pylori* if either iRUT or culture or histological examination or mPCR methods gave positive results. As shown in Figure 1, a total of 140 biopsies gave positive results for at least one of the methods examined. Positive growth of *H. pylori* observed in 22 biopsy samples and all gave positive results by mPCR. Twenty-nine samples were both positive by mPCR and histological staining. *H. pylori* were detected in 39 biopsies by iRUT however, two of them were negative by mPCR. A total of 138 samples were positive by mPCR. Patient samples were considered to be positive for *H. pylori* by mPCR amplification if the 179 bp fragment of *hpa*A gene, 422 bp fragment of 16S rDNA and 627 bp fragment of *ureA* were amplified (Figure 2). Overall, 44 samples were positive by more than one tests. In addition, mPCR detected additional 116, 101 and 109 biopsies that were negative by culture, iRUT and histology, respectively (Table 1). Positive and negative predictive values of mPCR in respect to other methods were calculated as shown in Table 2. mPCR detected all positive samples with culture and histology (PPV = 100%), however PPV of mPCR was 94.9% for iRUT positive samples. mPCR shows similar NPV for negative samples by culture, iRUT and histology with 44.2, 47.1 and 45.8%, respectively.

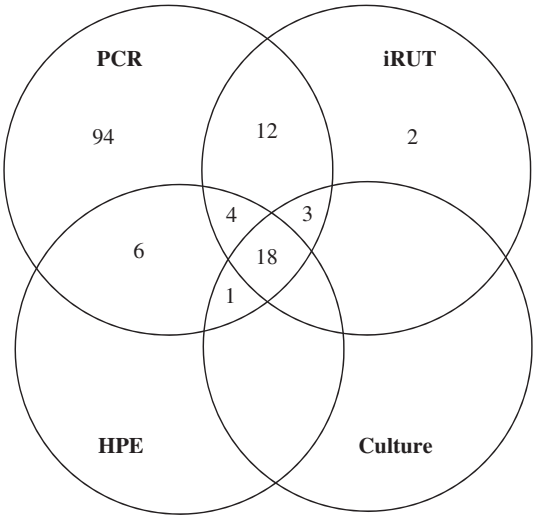

Figure 1. Distribution of *H. pylori*-positive biopsy specimens according to the testing methods.

The relationship between histopathological features of the biopsies and each test method was shown in Table 3. The average score for all HPE characteristics were greater in culture-positive, iRUT-positive and mPCR-positive compared to negative biopsy specimens. The average activity (assessed by neutrophil infiltration) and chronic inflammatory (assessed by MNC inflammation) scores were significantly greater in mPCR-positive than in mPCR-negative biopsy specimens. Similarly, both scores were higher in iRUT-positive and culture-positive biopsy specimens than in negative specimens. Among positive samples, the average activity and chronic inflammatory scores showed decreasing trend in culture, followed by iRUT and the lowest in mPCR. This shows that mPCR method was able to detect the highest numbers of positive cases although the lowest average scores were recorded in the activity, inflammatory, and *H. pylori* density compared to other methods. Even though the results were included positive biopsies by culture or iRUT, the numbers of positive biopsies by PCR alone were too large (more than 100 biopsies) compared to culture and iRUT. This will not give a significant difference if the results of positive culture and iRUT were excluded. Although

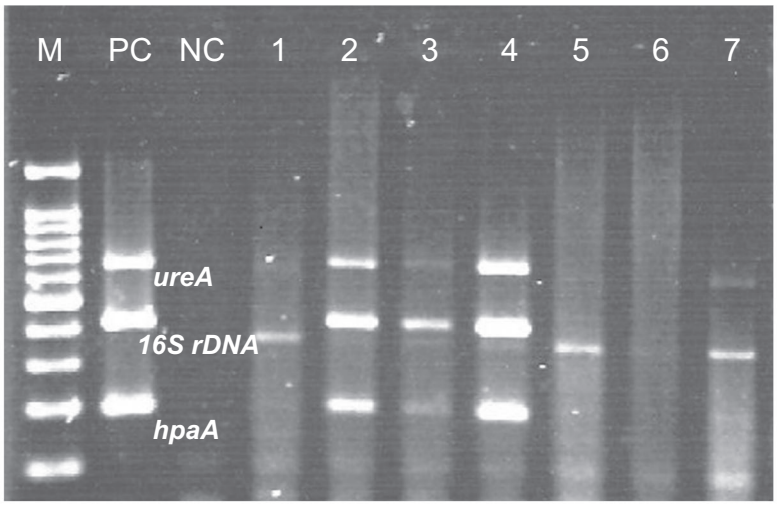

Figure 2. PCR amplification of *hpaA*, 16S rDNA and ureA of *H. pylori*. Lane 2, 3 and 4; biospies positive for *H. pylori*, Lane 1, 5, 6 and 7; biopsies negative for *H. pylori*, PC; positive control (DNA of *H. pylori* ATCC 700824), NC; negative control (DNA of Proteus mirabilis), M; 100 bp DNA ladder.

Table 1. Number of samples tested by mPCR compared to other diagnostic methods.

	Samples tested with mPCR		
Test method	Positive	Negative	Total
Culture			
Positive	22	0	22
Negative	116	92	208
iRUT			
Positive	37	2	39
Negative	101	90	191
Histology			
Positive	29	0	29
Negative	109	92	201

Table 2. Positive and negative predictive values of mPCR compared to other methods.

	Results for mPCR
Method	% (no. of samples with value/total no.)
Culture[a]	
PPV	100 (22/22)
NPV	44.2 (92/208)
iRUT[b]	
PPV	94.9 (37/39)
NPV	47.1 (90/191)
Histology[c]	
PPV	100 (29/29)
NPV	45.8 (92/201)

Notes: PPV – positive predictive value; NPV – negative predictive value.
[a]PPV: compared with culture positive, NPV: compared with culture negative.
[b]PPV: compared with iRUT positive, NPV: compared with iRUT negative.
[c]PPV: compared with HPE positive, NPV: compared with HPE negative.

culture method showed the highest in activity, inflammation and *H. pylori* density, the positivity of the method was the lowest compared to iRUT and PCR. Higher scores for intestinal metaplasia and atrophy were observed in culture-positive samples compared to iRUT and PCR.

H. pylori prevalence

Table 4 shows the characteristics of patients enrolled in this study. Generally, the *H. pylori* prevalence rate among males was higher than female. Among ethnic groups, the Indians had the highest infection rate (76%), followed by the Chinese (60.4%) and the Malays (57.6%). However, these differences were not statistically significant. Among the *H. pylori*-positive patients, peptic ulcer disease was high compared to patients with non-ulcer dyspepsia. Furthermore, patients with peptic ulcer disease in *H. pylori*-positive were higher compared to *H. pylori*-negative patients although the difference was not statistically significant.

Table 5 shows the characteristic of *H. pylori*-positive patients from different ethnic groups with various gastric diseases. The mean age for Chinese was 59.43 ± 12.61 years, Indians was 52.00 ± 13.93 years and Malays was 47.25 ± 14.46 years. It shows that the Chinese patients with older age were significantly more prevalent to be infected compared to the Indians and the Malays. The distribution of *H. pylori* infection was higher among the male patients from the Malays and the Indians compared to the Chinese, whereas Chinese females were predominantly being infected compared to the Malays and the Indians. However, the difference was not statistically significant. Peptic ulcer disease was diagnosed more in the Chinese and the Malays compared to the Indians. Majority of the Indians had non-ulcer dyspepsia.

Discussion

Results of the present study shows higher prevalence rate of *H. pylori* infection compared to previous studies. [26,27] In those studies, *H. pylori* infection was detected using conventional methods i.e. culture, rapid urease test and histology. However, in the present study mPCR was included as one of the testing method and it was proved that mPCR increased the percentage of *H. pylori* detection.

Table 3. Summary of the average histolopathological examination scores for each method.

Testing methods	No (%) of test	*H. pylori* density	*P*	Neutrophil infiltration	*P*	MNC inflammation	*P*	IM	*P*	Atrophy	*P*
Culture											
Positive	22 (9.6%)	1.5	<0.01	1.0	<0.01	1.7	<0.01	0.2	0.035	0.4	<0.01
Negative	208 (90.4%)	0		0.1		1.1		0.1		0.1	
iRUT											
Positive	39 (17.0%)	0.9	<0.01	0.6	<0.01	1.6	<0.01	0.1	0.949	0.2	0.054
Negative	191 (83.0%)	0		0.1		1.0		0.1		0.1	
PCR											
Positive	138 (60.0%)	0.3	<0.01	0.2	<0.01	1.2	<0.01	0.1	0.450	0.1	0.246
Negative	92 (40.0%)	0		0		1.0		0.1		0.1	

Notes: MNC – mononuclear, IM – intestinal metaplasia. Statistical analysis for positivity rates of *H. pylori* detection: culture vs. iRUT: Yate's correction, $P < 0.01$; culture vs. PCR: Pearson chi-square, $P < 0.01$; iRUT vs. PCR: Pearson chi-square, $P < 0.01$.

Table 4. Characteristics of the patients with and without *H. pylori* infection.

Patients' characteristics	*H. pylori* status: Positive ($n = 140$)	*H. pylori* status: Negative ($n = 90$)	*P*
Age (years)			
Mean (±s.d)	52.54 ± 14.75	52.11 ± 15.43	
Range	17–82	24–81	
Gender			
Male ($n = 110$)	72 (65.5%)	38 (34.5%)	0.173
Female ($n = 120$)	68 (56.7%)	52 (43.3%)	
*Ethnic**			
Malays ($n = 99$)	57 (57.6%)	42 (42.4%)	0.239
Chinese ($n = 96$)	58 (60.4%)	38 (39.6%)	
Indians ($n = 25$)	19 (76.0%)	6 (24.0%)	
Others ($n = 10$)	6 (60.0%)	4 (40.0%)	
*Disease group***			
Normal ($n = 2$)	1 (25.0%)	3 (75.0%)	
NUD ($n = 162$)	97 (59.9%)	65 (40.1%)	0.202
PUD ($n = 35$)	25 (71.4%)	10 (28.6%)	
Others ($n = 31$)	17 (58.6%)	12 (41.4%)	

*Statistical analysis was computed for Malays, Chinese and Indians.
**Statistical analysis was computed for NUD and PUD.

Here, we developed a unique mPCR assay to detect *H. pylori* in gastric biopsy specimens and compared the positivity to other standard routine techniques (culture, urease test and histology). The ability of PCR to detect *H. pylori* in gastric biopsies has been previously demonstrated.[12] In the present study, mPCR identified *H. pylori* infection in 100% of patients who had positive histology and culture. All biopsies except two were positive for iRUT showed positive mPCR results. This might be explained by the patchy distribution of *H. pylori* in gastric mucosa.[28] Presence of non-*H. pylori* urease-producing organisms in the stomach has been reported [29] and this might be resulted in positive iRUT.

mPCR method developed in this study for detection of *H. pylori* in biopsy samples shows high sensitivity (PPV: 94.9–100%). Low specificity (44.2–47.1%) of the mPCR method found when referring to the NPV was hampered by the high numbers of negative samples as detected using other methods. The positive and negative predictive values are very dependent on the prevalence of the infection in the community or on the group of patients studied.[30] In fact, mPCR shows very specific for *H. pylori* when tested using other bacterial strains (data not shown, see PCR analysis in Methods).

The histopathological features of the biopsies show that majority of these patients had low average inflammation scores and might not be suspected to have *H. pylori* gastritis. However, mPCR method was able to detect the highest numbers of positive cases although the lowest average scored was recorded in the activity, inflammatory and *H. pylori* density, compared to the other test methods. Culture method identified only 9.6% of *H. pylori* infection but the average score for *H. pylori* density, activity and inflammatory was the highest. This is in agreement with the facts that the presence of *H. pylori*

Table 5. Characteristics of *H. pylori*-positive patients from different ethnicities.

Patients' characteristic	Ethnic groups: Malay ($n = 57$)	Chinese ($n = 58$)	Indian ($n = 19$)	Others ($n = 6$)
Mean age ± s.d (years)[a]	47.25 ± 14.46	59.43 ± 12.61	52.00 ± 13.93	37.83 ± 8.06
Gender[b]				
Male ($n = 72$)	32 (56.1)%	25 (43.1%)	10 (52.6%)	5 (83.3%)
Female ($n = 68$)	25 (36.8%)	33 (56.9%)	9 (47.4%)	1 (16.7%)
Disease groups				
Normal ($n = 1$)	0	1 (1.7%)	0	0
NUD ($n = 97$)	37 (64.9%)	38 (65.5%)	17 (89.5%)	5 (83.3%)
PUD ($n = 25$)	11 (19.3%)	12 (20.7%)	1 (5.3%)	1 (16.7%)
Others ($n = 17$)	9 (15.8%)	7 (12.1%)	1 (5.3%)	0

Statistical analysis:
[a]Chinese vs. Indian; $P = 0.047$, Malay vs. Chinese; $P < 0.01$, Malay vs. Indian, $P = 0.225$.
[b]Pearson chi-square; $P = 0.365$.

is in association with the recruitment of neutrophils in the biopsy specimens.[31,32]

In the present study, mPCR able to detect *H. pylori* positivity in quite a huge numbers of culture-negative biopsies. This shows that culture-negative biopsies contained *H. pylori* DNA that can be amplified by PCR. *H. pylori* do exist in two forms, an actively dividing spiral form and a coccoid form. Coccoid forms have been described as 'viable but non-culturable'.[33] When cultured under favourable condition *in vitro*, *H. pylori* will present as bacillary appearance but exposure to unfavourable conditions result in the conversion of this bacillary form to coccoid form. Environment outside the human stomach is unfavourable to *H. pylori* and stimulates its conversion to coccoid, non-culturable form. These coccoid forms are not culturable by standard laboratory method but these form may be viable and possibly infectious [34] however, their DNA can be amplified by PCR. Urease production has also been one of the cornerstones in the diagnosis of *H. pylori*-associated disease. However, urease activity decreased in the coccoid form of *H. pylori* result in negative urease test. [35] The presence of sufficient numbers of bacteria is needed to ensure large amount of urease production for detection by rapid urease test.[36] A low average scores in iRUT for activity, inflammatory and *H. pylori* density were found compared to the culture method. However, 18 additional cases that were negative by culture were positive by iRUT. The diagnostic yield of rapid urease test is said to be increased by over 5% if more than a single biopsy were used.[37] In this study, mPCR method detected *H. pylori* in patients who had negative iRUT and culture methods with a majority of these cases had low average inflammation scores.

Differences in prevalence of *H. pylori* infection between races and gender have been reported in Malaysia. In this study, *H. pylori* infection among non-Malays was higher than the Malays. The Indians had the highest infection rate (76%), followed by the Chinese (60.4%) and the Malays (57.6%). This finding was concordance with previous reports.[26]. Malays consistently had the lowest prevalence group infected by *H. pylori*.[38,39] The great importance of *H. pylori* infection is the association with peptic ulcer disease. Patients with peptic ulcer disease in *H. pylori*-positive patients were higher compared to *H. pylori*-negative patients. The present study shows that the majority of patients with peptic ulcer disease (71.4%) were infected with *H. pylori*. The disease is high in the Chinese, followed by the Malays and the least in the Indians. *H. pylori* strains carrying virulence factors predominantly infected the Chinese and non-virulent *H. pylori* strains were predominantly found in the Indians and the Malays.[40,41] This finding suggested the occurrence of infection with less virulent *H. pylori* strains [40] or the presence of specific protective antibodies among the Indians.[42]

Generally, the prevalence rate among the males was higher than the female even though the difference was not statistically significant. Ohtani et al. [43] observed that gender-based differences have been observed in delaying onset of intestinal dysplasia and less development of intestinal inflammation in females infected with *H. pylori* compared to males. However, in this study more Chinese females were infected with *H. pylori* compared to the male patients could be due to the bigger number of Chinese females recruited in this study. Chinese patients with older age were significantly more prevalent to be infected compared to the Malays and the Indians. This might be due to allelic variation and genotypic differences in both the bacterium and its human host.

In conclusion, study showed that the majority of these patients had low average inflammation scores and might not be suspected to have *H. pylori* gastritis. However, *H. pylori* infection in these patients can be detected by mPCR method. mPCR detected the highest numbers of positive cases compared to the other test methods and we recommend adding mPCR method in routine diagnosis of *H. pylori* infection. Correct diagnosis is important in view of the high prevalence of *H. pylori* in this region. Other test methods might miss a low-level infection of *H. pylori*. Furthermore, PCR method also can be applied in detection of antibiotic resistance genes direct from biopsies for appropriate antibiotic of choice and screening of the virulence genes for the purpose of clinical evaluation. Early diagnosis is very important in helping appropriate treatment and management of the patients. This work represents an advance in biomedical science because mPCR increases diagnosis of *H. pylori* infection in samples with non-culturable *H. pylori* organisms and mild inflammation where it is undetectable by other methods (Table 6).

Table 6. Summary.

What is known about this subject:
- Routine diagnosis of *H. pylori* infection involve culture, urease test and histology which showed lower positivity cases.
- Culture and histology considered as gold standard for diagnosis of *H. pylori* but the methods are time consuming and need expertise.
- Histopathological features of biopsies in patients with low average inflammation scores were not suspected to have *H. pylori* gastritis.

What this paper adds:
- Multiplex PCR detects the highest numbers of positive *H. pylori* in biopsies compared to culture, urease test and histology with high sensitivity.
- Multiplex PCR offer excellent ways for diagnosis of *H. pylori* in a rapid manner which do not require live bacteria and high numbers of organisms.
- Multiplex PCR able to detect *H. pylori* infection in most of biopsies with lower average scores of the activity, inflammatory and *H. pylori* density.

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Disclosure statement

The authors declared that they have no conflict of interest.

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