

## Radar plot analysis of *Helicobacter pylori* detection in North Peninsular Malaysia



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### ABSTRACT

**Introduction:** *Helicobacter pylori* (*H. pylori*) infection is one serious disease that could result in complications such as gastritis, peptic ulcer and gastric cancer. Both invasive and non-invasive methods have been used to detect the infection, however the best detection depends on the clinical setting. One way to determine this is by graphical radar plot analysis, as it provides practical and useful data for many clinical aspects. The aim of this study was to investigate the prevalence rate of *H. pylori ureA* genotypes infection with a graphical technique.

**Method:** We conducted a cross-sectional study in which data was collected from patients with infection symptoms in north peninsular Malaysia. All eligible subjects were required to perform a simple clinical procedure to provide their cell and saliva samples. Subjects were also examined by the medical staff based on their symptoms. Detection of *H. pylori* in the samples were conducted with three different detection methods such as culture, biopsy, saliva and the combination of these three methods.

**Results:** Biopsies were cultured and revealed 19.2% (48/250) of the patients had *H. pylori* infection. However, when PCR and culture were combined, the prevalence of *H. pylori* infection jumped by three times to 59.6% (149/250), indicating that PCR on biopsies and saliva was more sensitive. Males who were not Malay and Malay males in the age range of 41 to 60 were found to have significantly different rates of *H. pylori* infection (p 0.05), according to age group. According to ethnicity, 49.5% (28/58) Malay men were infected compared to 68.1% (62/91) non-Malaysia men (p0.05), while there was no difference between 64.2% (34/53) non-Malaysia women and 51.1% (23/45) Malay women.

**Conclusion:** This study found that saliva method is accurate for the identification of *H. pylori*. This approach has several advantages that could be helpful to patients worldwide, thus diagnosis and treatment can be done earlier to prevent complications.

**Keywords:** Radar plot, *Helicobacter pylori*, Malaysia.

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### INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a Gram-negative and fastidious bacterium that colonizes the mucosa lining of the human being and causes several diseases such as gastritis, peptic ulcer and gastric cancer. International Agency for the Research on Cancer (IARC) classified Hp as a Group 1 carcinogen in 1994.<sup>1</sup> *H. pylori* as a human pathogenic bacteria have a few routes of transmission and consider to be oral-oral, faecal-oral, gastro oral or mother to baby.<sup>2</sup>

The diagnosis of *H. pylori* infection requires accurate diagnostic methods. Several diagnostic methods, both invasive (biopsy, histology, culture, PCR and urease) and non-invasive (saliva, serology, faecal antigen and urea breath test) have

been developed.<sup>3</sup> However, non-invasive technique is more convenient to determine the presence of *H. pylori* infection and considered inexpensive. Therefore, a lot of advantages when implementing non-invasive method. For saliva method, it is implemented after discoveries of *H. pylori* occurrence in supragingival dental plaque.<sup>4</sup> Most of saliva testing consists of saliva isolation and anti-gen. Similar to urine or urea breath test, it has its own anti-gen to detect *H. pylori*. There are available testers in market for these both methods,<sup>5,6</sup> which is made the procedure ease and simple without the need of complex procedure, likes, biopsy procedure. Also, it is relatively inexpensive compared to endoscopy,<sup>7,8</sup> culture<sup>9,10</sup> and

biopsy procedure.<sup>11,12</sup> Hence, a screening with appropriate test and treatment are important for the effective management of *H. pylori* infection.

The aim of this study was to determine the prevalence of *H. pylori ureA* genotypes infection with a graphical technique. The radar plot is one of graphical technique to displays a particular suitable trends or analysis. This graph is efficient and practical for a wide range of data, including a disease diagnosis, age, symptoms, and other aspects. 13 The ideas, as well as the specifics of each individual variable, will make major and immediate sense on the radar map. The radar plots form can present a wide range of data on a single graph and can hold considerable data.

## METHOD

### Data collection

This study was conducted for all patients from the north peninsula Malaysia. We conducted a cross-sectional study, in which few stages were involved in the set up of the research methodology with pre-caution consideration. There were 250 subjects participated in the study with age ranging from 15 to 90 years old. Our inclusion criteria were those who had infection symptoms and their data of symptoms collected, gave their consent to perform clinical procedures in the study. Patients who had history of malignancy, long-term treatment on chronic diseases or antibiotic use for gastrointestinal and hepatological infection in the last 3 months were excluded. All subjects have given their personal consent letters to confirm their participation in this study. Table 1 shows the characteristic of the subject based on their age and gender. Subjects were required to perform a simple clinical procedure that must provide their cell and saliva samples to the researchers. Also, subjects were examined through anamnesis and physical examination by the medical staff based on their symptom. The used of these humans was reviewed and approved by the National Medical Research Register of Advancing Medical Research in Malaysia [Reference number: NMRR-16-2406-31887]

The samples were then studied using four methods in order to determine the infection. The detection methods involved culture, biopsy, saliva and the combination of these three results.

### Biopsy samples

Three antrum biopsies were obtained from dyspepsia patient. Biopsies were used for the diagnosis of *H. pylori* infection with the Rapid Urease Test (RUT), culturing on blood agar and Polymerase Chain Reaction (PCR) of each antrum biopsy, respectively.

### Culturing samples

A biopsy sample was transferred onto a Stuart transport medium during an endoscopic surgery (Oxoid, UK). This was done to guarantee that the bacteria

would be culturable and alive in the lab for the ensuing studies. *H. pylori* was

**Table 1. Subjects according to age classes and genders**

Age	Male	Female	Total
15-20	1	7	8
21-30	12	13	25
31-40	26	10	36
41-50	34	30	64
51-60	29	18	47
61-70	33	14	47
70<	17	6	23
<b>Total</b>	<b>152</b>	<b>98</b>	<b>250</b>

**Table 2. Sequence of ureA gene *H. pylori***

Name	Sequence
ureA - F	5'- GCC AAT GGT AAA TTA GTT - 3'
ureA - R	5' - CTC CTT AAT TGT TTT TAC - 3'

**Table 3. Number of results for each method**

Detection Method	Number of results
Diagnosis	250
Kultur	250
Biopsy	250
Saliva	250
Total	1250

**Table 4. Detection results based on 4 detection methods and the combination methods from 10 subjects**

No	Gender	Age	Diagnosis	Culture	Biopsy	Saliva
1	M	33	PU	-	√	√
2	M	35	NU	-	√	√
3	M	36	PU	-	√	-
4	M	36	PU	-	-	√
5	F	37	PU	-	-	-
6	F	38	NU	-	-	-
7	M	38	PU	-	-	-
8	M	39	NU	-	-	-
9	F	39	NU	√	√	-
10	M	40	NU	√	√	-

\* PU indicates Peptic Ulcer and NU indicates non-Ulcer detection

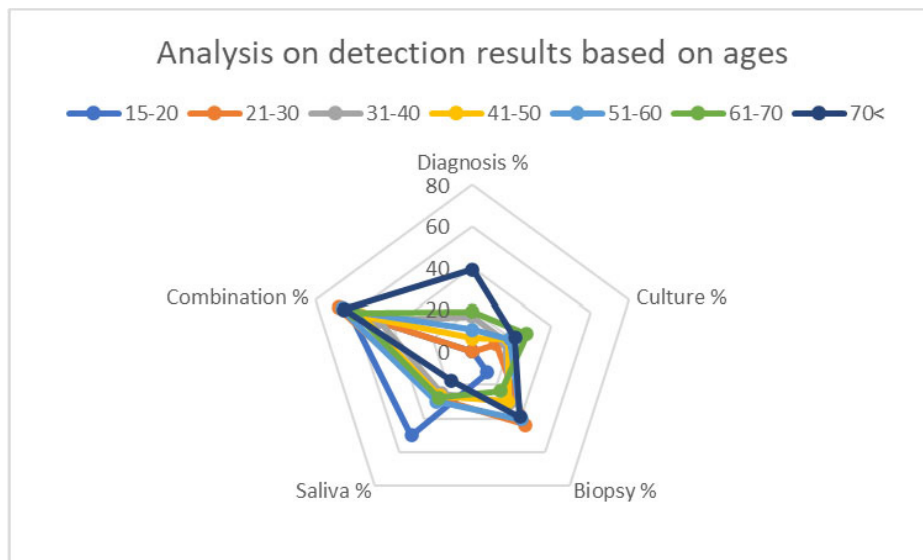
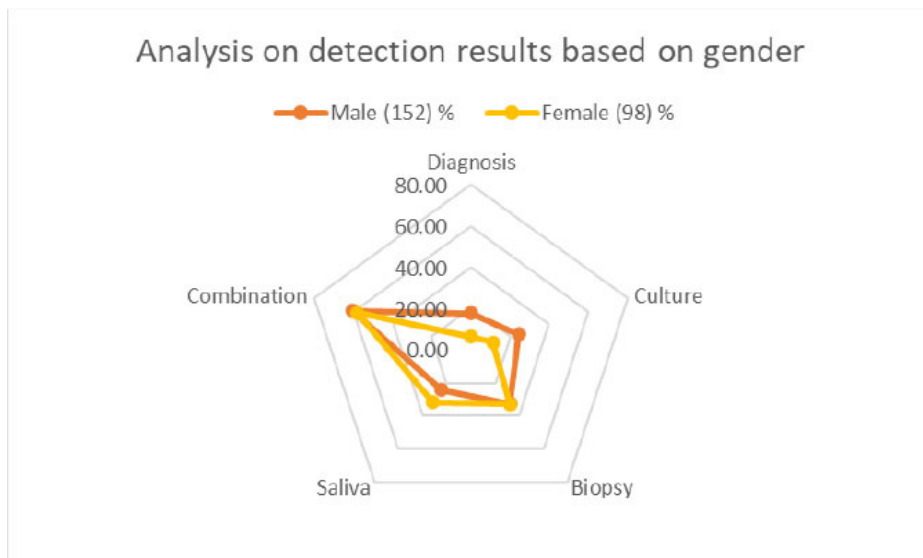
\* - indicates negative and √ indicates positive detection

**Table 5. Overall detection results from 250 subjects based on ages**

Age	No. of subject	Diagnosis		Culture		Biopsy		Saliva	
		No. of positive	%	No. of positive	%	No. of positive	%	No. of positive	%
15-20	8	0	0.00	0	0.00	1	12.50	4	50.00
21-30	25	0	0.00	3	12.00	11	44.00	7	28.00
31-40	36	6	16.67	6	16.67	11	30.56	9	25.00
41-50	64	4	6.25	12	18.75	19	29.69	17	26.56
51-60	47	5	10.64	9	19.15	19	40.43	14	29.79
61-70	47	9	19.15	13	27.66	11	23.40	13	27.66
70<	23	9	39.13	5	21.74	9	39.13	4	17.39

**Table 6.** Overall detection results from 250 subjects based on genders

Method	Male (152)		Female (98)	
	No. positive	%	No. positive	%
Diagnosis	27	17.76	6	6.12
Culture	37	24.34	11	11.22
Biopsy	49	32.24	32	32.65
Saliva	37	24.34	31	31.63
Combination	92	60.53	57	58.16

**Figure 1.** Radar analysis based on ages.**Figure 2.** Radar analysis based on genders.

transported using a variety of agar types, including eugon agar, which contains 5–10% (v/v) human blood. 14 Typically, a growth medium was streaked with the biopsy material. 15 After that, the medium was incubated at 37 °C with a mixture of ambient oxygen, 10% CO<sub>2</sub>, and 100% humidity. The development of small,

translucent colonies revealed the presence of *H. pylori* after four to seven days of incubation. Gram stain and urease tests had to be run in order to confirm these colonies. Fundamentally, the antimicrobial resistance testing was made possible by the culture of gastric biopsy specimens, which had a 100% specificity.

### Polymerase Chain Reaction Biopsy and Saliva Sample

One antrum biopsy and saliva sample were then preceded with the determination of *H. pylori* infection by use of Polymerase Chain Reaction (PCR). By this method, DNA of biopsy and saliva sample were extracted by following the procedure of Wizard Genomic DNA Purification (Promega, USA). The ureA gene was then used to determine *H. pylori* infection by a specific sequence of ureA (Table 2), PCR mixture and amplification temperature.

### Data Analysis

The combination of detection method involved the invasive (culture and PCR biopsy) and non-invasive (PCR saliva) techniques of *H. pylori* infection to increase the percentage of *H. pylori* detection.

## RESULTS

### Detection Results

The detection results from 250 subjects were described in a table to perform the analysis. Based on the clinical procedure (3 methods) and diagnosed (1 method) by medical staff, there were 1000 detection results obtained and those results were classified as shown in Table 3. It means, of each endoscopic patient, 4 different detections were use which is a total 250 patients equivalent with 1000 detection result. The results showed positive or negative detection results. Interestingly, there have been different results (non-detected and detected) based on the different methods for the same subject. Table 4 shows the example of the detection results based on the conducted procedures for 10 subjects.

### Analysis based on age

As reported, senior citizens at age 50 years old and above are commonly infected by *H. pylori* bacteria in the United State of America (USA).<sup>16</sup> This study shows similar pattern between Malaysia and USA, where people who were positively infected by these bacteria wer commonly among the age of 50 years old and above. The overall results are shown in Table 5 and Figure 1. Table 5 shows results in numbers and Figure 1 shows the results in illustration form. The results shows clearly that the

high numbers of infection by *H. pylori* were from age range of 51-60, 61-70 and above 71 years old with positivity rate of 65.96%, 59.57% and 65.22%, respectively. The percentage were calculated based on each age category using equation (1) as shown below:

$$\% \text{ positive} = \frac{\text{no. of positive patients}}{\text{total no. of patients}} \times 100\% \quad (1)$$

Also, as shown in [Figure 1](#), biopsy and saliva detection methods obtained high detection results compared to culture and physical diagnosed by medical staff. This can conclude that the methods were more promising and reliable to detect the infection.

### Analysis based on gender

For detection analysis based on genders, the results are shown in [Table 6](#) and [Figure 2](#). Similar to analysis based on age, [Table 6](#) describes the results in terms of number and [Figure 2](#) describes the results in terms of illustration. Based on 250 subjects, it showed that male subjects had higher numbers compared to female subjects with participation of 152 and 98, respectively.

For male results, it showed that culture, biopsy and saliva methods had high number of detections with percentage of 24.34%, 32.24% and 24.34%, respectively. While, female results showed that biopsy and saliva method had high number of detections with percentage of 32.65% and 31.63%, respectively. If similar results from the previous analysis were counted, biopsy and saliva method were more convincing for detection.

The missing data in this study were due to negative detection during data collection, resulting in a 13.2%, 19.2%, 32.4% and 27.2% for diagnosis, culture, biopsy and saliva detection respectively for both gender.

In [Figure 2](#), it shows the pattern of detection results. Both, male and female results show almost similar shape, which could be concluded that the detection results are almost similar for male and female based on the detection methods.

## DISCUSSION

Based on our finding, we proved that people at age of 50 years old and above are commonly infected by *H. pylori*. The same pattern happens in USA,<sup>16</sup> and

around the world as reported by Pilotto & Franceschi.<sup>17</sup>

In this study, four detection methods have been conducted based on 250 subjects. The results show that biopsy and saliva method perform with high detection for both detections based on genders and ages. As reported by previous researchers, biopsy is one promising detection method that has been used in many years due to its high accuracy.<sup>18</sup> Now, saliva detection method also can be used to detect *H. pylori* at initial stage. Benefits from this method can be rapid procedure, cost effective and high accurate of detection. Our research is not without limitation. We have not conducted analysis on the rate of resistant *H. pylori* infection, which could potentially affect the priority of detections by the three different methods. Future research may focus on this matter to consider the best method to detect resistant infection by *H. pylori*. Nonetheless, our research promoted the use of simple method of detection, since infection usually happens in developing countries, hence, inexpensive procedure of detection is an excellent solution.

## CONCLUSIONS

Our research has found that saliva method for *H. pylori* detection is an accurate method, beside the invasive method. Along this method, it provides many benefits, which may help patients worldwide. Early detection can save people life, and treatment can be given before the infection is becoming worse.

## CONFLICT OF INTEREST

Authors of this article declared no conflict of interest was involved in the writing of this article.

## AUTHORS CONTRIBUTION

All four authors contributed in even proportion to the conducting of the research until the writing of the reports and manuscript.

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