Dissertation Title:

A study to determine the correlation between number of endoscopic gastric biopsy specimen and sensitivity of CLO test in Helicobacter Pylori infection in Hospital Universiti Sains Malaysia

By

Dr Kenneth Voon Kher Ti

Dissertation Submitted in Partial Fulfillment of the Requirement for the degree of Master of Medicine (Surgery)

UNIVERSITI SAINS MALAYSIA

2015

ACKNOWLEDGEMENT

I would like to convey my gratitude to all the people involved in the process of completing this dissertation. I would especially like to thank my main supervisor Dr Nizam Mohd Hashim (Department of Surgery), co-supervisors: Dr Syed Hassan Syed Abd Aziz (Department of Surgery and Head of Endoscopy Unit), and Dr Syarifah Emelia (Department of Pathology) for their guidance, advice and time spent in the process of preparation, writing and correction of this research. I would also like to extend my gratitude to Dr Wan Ariffin of Biostatistic Unit for his diligent review and assistance in planning for the methodology and statistical analysis of this study, and Dr Lee Yong Yeh (Gastroenterologist) for his invaluable advice and active participation in patient recruitment. To all the faculty members of the Department of Surgery, I would like to express my gratitude for their continuous support in the process of patient recruitment and advice on the preparation of this dissertation. Not to forget the supporting staffs of Endoscopy Unit and Pathology Laboratory in their contribution towards preparing specimens for diagnostic studies.

ABBREVIATIONS

1	Campylobacter-like organism	CLO
2	Oesophagogastroduodenoscopy	OGDS
3	Helicobacter pylori	H. pylori
4	Hospital Universiti Sains Malaysia	HUSM
5	Histopathological Examination	HPE
6	Clinical research form	CRF
7	Haematoxylin & Eosin	H&E
8	Mucosa-associated lymphoid tissue	MALT
9	National Cancer Registry	NCR
10	Gastroesophageal Reflux Disease	GERD
11	Non-steroidal anti-inflammatory drugs	NSAIDs

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

BACKGROUND: Northeastern peninsular state of Kelantan has an exceptionally low prevalence of Helicobacter Pylori infection; yet receive high volume of patients referred for Oesophago-gastroenteroscopy (OGDS) for upper gastrointestinal symptoms. CLO test is the most commonly used initial diagnostic test, but sensitivity is widely variable especially in the background of low prevalence. Amount of tissue biopsy and site of gastric biopsy may be crucial in maximizing the detection rate in this region.

METHOD: 150 patients with upper gastrointestinal symptoms undergoing elective OGDS in Hospital Universiti Sains Malaysia (HUSM) were included. Four gastric mucosa biopsies were taken from each patient using a standard 2.8mm biopsy forcep, at the pre-pyloric region within 3cm from pylorus, each bite adjacent to each other. One biopsy specimen was compared with two biopsy specimens on a CLO test well, and positive results were recorded when there was colour change at 1 hour, 3 hour, 6 hour and 24 hour. The fourth specimen was sent for histopathological examination with Haematoxylin & Eosin staining, followed by Warthin-Starry staining as control to diagnose Helicobacter pylori. Data entry and analysis was done using SPSS software version 20. Mc Nemar's test was used to compare the overall sensitivity of CLO test and sensitivity at each time between both groups to determine the earliest detection.

RESULTS: Overall prevalence rate of Helicobacter pylori infection was 13.3%. Demographic pattern is consistent with previous local studies, but this study recorded a higher prevalence rate of 10.7% among Malay ethnic patients. Presentation with black

tarry stool has the highest prevalence rate of infection at 22.2%, whereas endoscopic diagnosis of gastric ulcer has the highest prevalence rate of infection at 32%. Overall sensitivity of both group were equal at 75% with no statistical significance. However, speed of CLO test becoming positive was slightly higher in two biopsy group at 1 hour and 3 hour, recording sensitivities of 37.5% and 68.8% respectively, compared to single biopsy group with sensitivities of 25.0% and 37.5% respectively, without any statistical significance.

CONCLUSION: This study confirmed the low prevalence of Helicobacter pylori infection in this region but suggested that the method applied may improve the detection rate, particularly among ethnic Malay population with low bacterial load in their gastric mucosa. Sensitivity of CLO test is similar with either single or double biopsy specimens, but speed of CLO test becoming positive appeared to be slightly higher with double biopsy specimens, even though statistically not significant. Both presenting symptoms and endoscopic diagnosis are poor indicator of Helicobacter pylori status in this region. However, prevalence according to racial distribution is consistent with national pattern.

ABSTRAK

LATARBELAKANG: Negeri Kelantan yang terletak di timur utara Semenanjung Malaysia mempunyai prevalen jangkitan *Helicobacter Pylori* yang sangat rendah. Namun demikian, bilangan pesakit yang mengalami gejala penyakit gastrousus atas serta dirujuk untuk *Oesophago-gastroduodenoscopy* (*OGDS*) adalah tinggi. Ujian *CLO* merupakan kaedah yang paling biasa digunakan untuk mencapai diagnosis, walaupun sensitivitinya berubah secara meluas terutama sekali dalam populasi yang berprevalen rendah. Jumlah biopsi tisu dan tempat biopsi gastrik dijangka penting untuk meningkatkan kadar pengesanan di kawasan ini.

KAEDAH: 150 pesakit yang mengalami gejala gastrousus atas menjalani penyiasatan *OGDS* secara elektif di Hospital Universiti Sains Malaysia (HUSM) menyertai kajian ini. Empat biopsi mukosa gastrik diperoleh daripada setiap pesakit dengan menggunakan forcep biopsi standard 2.8mm. Lokasi biopsi ditetapkan di kawasan pra-*pyloric*, dalam lingkungan 3cm daripada *pylorus*, setiap biopsi adalah bersebelahan antara satu sama lain. Satu spesimen biopsi dibandingkan dengan dua spesimen biopsi dalam *kit* ujian *CLO*. Keputusan positive dicatatkan apabila terdapat perubahan warna pada 1 jam, 3 jam, 6 jam and 24 jam. Specimen keempat dihantar untuk pemeriksaan histopatologi. Pewarnaan *Haematoxylin & Eosin* diikuti dengan pewarnaan *Warthin-Starry* digunakan sebagai kawalan untuk mengesahkan diagnosis jangkitan *Helicobacter pylori.* Perisian *SPSS* versi 20 digunakan untuk kemasukan data and analisis. Ujian *Mc Nemar* digunakan untuk membandingkan antara 2 kumpulan dari segi sensitiviti ujian CLO secara keseluruhan dan sensitiviti pada setiap waktu yang ditetapkan bagi menentukan pengesanan terawal.

KEPUTUSAN: Prevalen jangkitan *Helicobacter pylori* pada keseluruhan adalah 13.3%. Corak demografi adalah konsisten dengan kajian-kajian tempatan sebelum ini. Kajian ini mendapati kadar prevalen adalah lebih tinggi di kalangan pesakit berbangsa Melayu, iaitu pada 10.7%. Pesakit yang mengalami gejala najis hitam mempunyai kadar prevalen yang tertinggi pada 22.2%. Dari segi diagnosis endoscopi, ulser gastrik mempunyai kadar prevalen tertinggi pada 32%. Sensitiviti secara keseluruhan untuk kedua-dua kumpulan adalah sama iaitu 75% dan tidak menunjukkan pengertian statistik yang bermakna. Namun demikian, kelajuan ujian CLO menjadi positif adalah lebih tinggi dalam kumpulan 2 biopsi pada jam pertama and ketiga, dengan sensitiviti 37.5% dan 68.8% masing masing, di mana pada waktu yang sama, sensitiviti dalam kumpulan 1 biopsi adalah 25% dan 37.5% masing-masing. Keputusan ini tidak menunjukkan pengertian statistik yang bermakna.

KESIMPULAN: Kajian ini mengesahkan prevalen jangkitan *Helicobacter pylori* adalah rendah di rantau ini. Walau bagaimanapun, kajian ini menunjukkan bahawa kaedah yang digunakan berpotensi meningkatkan kadar pengesanan jangkitan ini, terutamanya di kalangan populasi berbangsa Melayu yang sememangnya mempunyai beban bakteria yang rendah dalam mukosa gastrik. Sensitiviti ujian CLO adalah sama bagi satu mahupun dua biopsi, tetapi kelajuan ujian CLO menjadi positif adalah lebih untuk kumpulan dua biopsi tanpa menunjukkan pengertian statistik yang bermakna. Jenis gejala gastrousus atas dan diagnosis endoskopi bukan penunjuk status *Helicobacter Pylori* yang tepat di rantau ini. Prevalen jangkitan berdasarkan distribusi kaum adalah konsisten dengan corak taburan kebangsaan.

TABLE OF CONTENTS

1. INTRODUCTION

Helicobacter Pylori was discovered by Warren and Marshall in 1982 (Marshall and Warren, 1984). It is a gram negative campylobacter like bacteria that predominantly colonizes human gastric epithelium and is one of the most common gastric infections worldwide (Malfertheiner *et al., 2009*). Over the last 3 decades, many different diagnostic tools have been developed. Diagnostic methods can be divided into invasive and non-invasive methods. Invasive methods include endoscopy and biopsy for either rapid urease test or histopathological staining, whereas non-invasive methods includes urea breath test, serology and stool antigen test (El-Zimaity, 2000; Ji and Li, 2014; Malfertheiner *et al.,2009*).

Campylobacter-like Organism (CLO) test is the most commonly used rapid urease test for gastric biopsy specimen and is the initial test of choice for diagnosis of Helicobacter Pylori at endoscopy (Stabile *et al.*, 2005; Malfertheiner *et al.*, 2007). It incorporates a gel containing urea, with phenol red as a pH indicator. Biopsy specimen is inoculated and allowed to incubate. If Helicobacter pylori organisms are present in the patient's sample, urease will hydrolyze the urea in the gel leading to an accumulation of ammonium ions ($NH4^{+}$). This causes a rise in pH which is detected by a pH indicator in the test system changing from yellow to magenta (Laine *et al.*, 1996; Siddique *et al.*, 2008).

Gastric biopsy for CLO test is done using non-needle biopsy forceps, includes only part of mucosal layer, which is sufficient for diagnosis of H. Pylori as these microorganisms reside at the superficial mucosal layer only. There is no evidence that obtaining deeper biopsy (i.e. to include the submucosal layer) will improve the yield of H. Pylori organisms (El-Zimaity, 2000; Bernstein *et al.*, 1995). Therefore, it is well established that gastric biopsy for CLO testing requires only a non-needle biopsy forcep. There is a wide range of non-needle biopsy forceps, ranging from small 1.8mm diameter forcep suitable for paediatric size flexible endoscopes or transnasal OGDS to large 'jumbo' size forceps with 3.3mm forceps (Bernstein *et al.*, 1995).

The ideal test for diagnosis of H. Pylori at endoscopy should have excellent sensitivity, specificity, cost-effective and provides rapid results. This will benefit both physicians and patients as diagnosis and treatment can be given to patients immediately on the day of endoscopy (Malfertheiner *et al.*, 2007; Laine *et al.*, 1996). Therefore, it should be of great interest for most researchers and clinicians to study on all possible variables to ensure this diagnostic test is of excellent value. Of the factors mentioned above, sensitivity of CLO test is still of great research interest as it ranges from as low as 75% to as high as 93% (Stabile *et al.*, 2005; Laine *et al.*, 1996; Yousfi *et al.*, 1996). This leads to concern of higher rates of false negative results causing misdiagnosis and failure to treat the infection.

One of the major concerns is the variability of equipments and endoscopic biopsy method used is various endoscopic units. Many studies have been carried out to standardize the technique and strategy of obtaining gastric biopsy to optimize the sensitivity of rapid urease testing (Yousfi *et al.*, 1996; El-Zimaity, 2000; Stolte and Meining, 2001; Jeon *et al.*, 2012; Laine *et al.*, 1996).

The overall improvement of CLO test sensitivity will benefit health care system in general, and specifically by:

1. Improving the rate of detection of H. Pylori infection among patients presented with dyspepsia, thereafter eradication therapy can be instituted immediately. Most recent clinical practice guidelines advocate starting eradication therapy immediately during the day of endoscopy (Stabile *et al.*, 2005; Malfertheiner *et al.*, 2007).
2. Effective eradication strategy can reduce the risk of future complication, reduce the time of follow-up and reduce the need for repeat OGDS (Stabile *et al.*, 2005).
3. Improving the technique of endoscopic biopsy for diagnosis of Helicobacter Pylori, whereby the reliance on more time consuming histopathological examination can be significantly reduced (Stabile *et al.*, 2005; El-Zimaity, 2000).

In the last 10 years, 2 studies have been carried out in Hospital Universiti Sains Malaysia to determine the local prevalence of H. Pylori infection using endoscopic

biopsy as the main diagnostic modality. Both studies reported exceptionally low prevalence compared to regional and worldwide prevalence (Sasidharan *et al.*, 2008; Yeh *et al.*, 2009). As we are still struggling to explain the reason for such low prevalence, we are faced with a large burden of patients under Hospital Universiti Sains Malaysia presenting with significant upper gastrointestinal symptoms such as epigastric burning pain, dyspepsia and malaena. Endoscopy Unit in Hospital Universiti Sains Malaysia have performed between 350 and 400 elective upper oesophageal-gastroduodenoscopies (OGDS) per year from 2010-2012 (3 years).

This study will revisit the endoscopic biopsy technique and diagnostic modalities of H. Pylori commonly used in this hospital, and explore the possibilities of improving the diagnostic yield of these modalities to improve detection rate in a background of low prevalence population.

2. LITERATURE REVIEW

2.1 REGIONAL HELICOBACTER PYLORI PREVALENCE AND PREVIOUS LOCAL STUDIES

Global prevalence of Helicobacter Pylori infection is in the range of 40% - 56% (El-Zimaity, 2000; Stabile *et al.*, 2005; Malfertheiner *et al.*, 2007). In Malaysia, there is a geographical and racial variation in prevalence of Helicobacter Pylori infection (Kaur and Naing, 2003; Goh, 1997; Sasidharan *et al.*, 2008; Yeh *et al.*, 2009). Goh et al., 1997 reported a prevalence of 49% diagnosed by endoscopic rapid urease test or histopathology, with Malay 16.4%, Chinese 48.5% and Indian 61.8%. A study of Northern Peninsular Malaysia population found that the prevalence is much lower at 23.5% reported by Sasidharan et al., 2008. In the population of Kelantan, 2 studies were carried out in Hospital Universiti Sains Malaysia (HUSM) within the last 10 years, showing exceptionally low prevalence of Helicobacter Pylori infection. Yeh *et al.*, 2009 reported 6.8% prevalence and Kaur and Naing, 2003 reported 13.5%.

In Yeh et al., 2009, 234 patients presented with upper gastrointestinal symptoms underwent OGDS, whereby endoscopic biopsy was taken from sites with endoscopic features of gastritis. It was noted that nearly half of the patients has histological diagnosis of chronic atrophic gastritis, which is associated with lower yield of Helicobacter Pylori organism. Kaur and Naing, 2003 studied on 52 patients,

whereby biopsies where taken at anterior and posterior wall of gastric antrum, according to the previous Sidney's system recommendation.

According to El-Zimaity, 2000, when atrophic changes occur, an environment that is unfavorable to the growth of *H.* pylori develops, and the organism can be found in a small percentage of endoscopic biopsy specimens. The yield for *H.* pylori infection is reduced when intestinal metaplasia is present, emphasizing the importance of obtaining biopsy specimens from the antrum and the corpus. This may explain the exceptionally low prevalence of Helicobacter Pylori infection from the above two studies

2.2 HISTOPATHOLOGICAL EXAMINATION AND SPECIAL STAINING

Gold standard for diagnosis of H Pylori is endoscopic biopsy and histopathological examination with special staining. Sensitivity and specificity of this diagnostic method ranges from 90% to 100% (El-Zimaity, December 2000; Cohen and Laine, 1997; Ji and Li, 2014). There are several special stainings used for confirmation of Helicobacter pylori, i.e Giemsa stain, Genta stain, Warthin-Starry stain, Diff-Quik stain and El-Zimaity staining, with equal sensitivity and specificity (El-Zimaity, December 2000). However, histopathological diagnosis requires longer time and higher cost (Siddique *et al.*, 2008).

2.3 CLO TEST

Eradication therapy of H. Pylori can be started based on endoscopic biopsy with positive rapid urease test kits i.e. CLO Test (Stabile *et al.*, 2005; Malfertheiner *et al.*, 2007). This is especially for patients who presents to endoscopy without pre-treatment. It is recommended as first line diagnostic test for endoscopically investigated patients. Overall CLO test sensitivity ranges from 75% to 93% and specificity from 95% to 100% (Stabile *et al.*, 2005; Yousfi *et al.*, 1996; Laine *et al.*, 1996). The reason for such a wide range of sensitivity is believed to be largely attributed to wide variability of technique and procedure of obtaining specimen for testing. Factors that influence the sensitivity of CLO test to gastric biopsy specimen are 1) sites of biopsy specimen, 2) number, and 3) size of biopsy specimen (El-Zimaity, December 2000).

2.4 SITE OF ENDOSCOPIC BIOPSY

Site of biopsies have been extensively studied. One prominent author proposed 3 biopsy specimens; one each from corpus-antrum junction, mid-corpus of greater curvature & antrum of greater curvature (El-Zimaity, 2000). Another prominent guideline, the updated Sidney's system proposed 2 biopsies from antrum, 2 biopsies from corpus and 1 biopsy from incisura (Stolte and Meining, 2001). Prepyloric region is the most common region being sampled as it has a false negative rate of less than 3% in detection of helicobacter pylori as applied by Laine and colleagues, where biopsies were taken contiguously at the pre-pyloric region,

approximately 3cm from pylorus, without any specific aspect of the wall (Genta and Graham, 1994).

Using the biopsy method proposed and practiced by Laine and colleagues, this study is looking to determine the optimal amount of gastric mucosa tissue to be taken at specified sites recommended above endoscopically, therefore improving the sensitivity of rapid urease CLO test. By answering this question, we will be able to recommend a standardized procedure to obtain optimal tissue mass specifically for rapid urease test biopsy.

2.5 TYPE OF ENDOSCOPIC BIOPSY FORCEPS

Biopsy for rapid urease test only requires specimen consist of partial thickness mucosa, therefore only non-needle forceps will be considered in our review. Types of forceps range from small forceps which fits into transnasal oesophago-gastroduodenoscope with diameter of 1.8mm to large jumbo-size forceps with diameter of 3.3mm (Bernstein *et al.*, 1995). It is well documented that larger diameter forceps are able to obtain tissue specimens with larger dimension and mass. A 3.3mm diameter jumbo forcep yields approximately double the volume of a standard 2.8mm biopsy forcep (Yousfi *et al.*, 1996), (Laine *et al.*, 1996; Jeon *et al.*, 2012; Bernstein *et al.*, 1995). According to Danesh et al., 1985 who studied extensively on various types of biopsy forceps, a jumbo size forcep is able to obtain a mean tissue mass of 15.51mg with SD of 2.09mg, whereas a standard cup 2.8mm diameter forcep, such as the one used in HUSM Endoscopy Unit, can

obtain a mean tissue mass of 5.93mg with SD of 0.71mg. Therefore, selection of a large diameter forcep can significantly increase the mass of tissue specimen (Bernstein *et al.*, 1995; Danesh *et al.*, 1985; Siddique *et al.*, 2008). A jumbo size forcep would require a large working channel of at least 3.4mm diameter, which is not available in a lot of endoscopy units, including HUSM. Another method of obtaining large mass of tissue specimen is by increasing the number of biopsies, in other words, repeated biopsy on the same site using the same forcep.

2.6 FOCUS OF STUDY: SIZE AND NUMBER OF ENDOSCOPIC BIOPSY

We embark to study on the optimal size or number of gastric biopsy specimen to achieve the best sensitivity rate for rapid urease test. Therefore, a search of studies regarding the relationship of biopsy size or number and CLO test sensitivity is done on several reputable databases. Evidence for this postulation is inadequate as only 4 studies being published so far, with conflicting results.

Yousfi *et al.*, 1996 on the contrary, concluded that the diagnostic yield of rapid urease test is not adversely affected by small biopsy specimen. This study reported overall slight increase of sensitivity of large biopsy specimen (92.1%) to CLO test compared to sensitivity of small biopsy specimen (88.5%) by directly comparing the sensitivity rates without statistical analysis. This study compared specimen taken with 1.8mm forcep and 3.3mm forcep.

Jeon *et al.*, 2012 compared rates of positive CLO test for specimens taken from 1.8mm forcep and 2.2mm forcep via transnasal OGDS in 100 patients. This study reported their results in terms of rate of positive CLO test in two arms. Rate of positive CLO test for biopsies taken with 1.8mm forcep was 33% compared to biopsies taken with 2.2mm forcep at 58%. Using Kappa statistic, ƙ value was 0.83 and p-value of 0.001. They concluded that the concordance rate of different biopsy size in relation to rate of positive CLO test was significant.

The 2 studies above were able to demonstrate slight increase in sensitivity or rate of positive CLO test when using large forcep compared to using smaller forcep. However, both studies use small caliber forceps via transnasal flexible endoscopy, which is not a common practice in our local endoscopy units. Both used different combinations of forcep sizes, making comparison slightly difficult. An indirect conclusion is that sensitivity of CLO test is related to the amount of tissue specimen obtained.

A more significant study was conducted by Loren Laine from University of Southern California, which studied on 102 patients, whereby biopsy specimens were taken with 2 types of forcep: 2.8mm and 3.3mm forceps to obtain 2 different sizes of mucosa tissue. They found that single bite using large forcep results in sensitivity of 80% compared to sensitivity of 75% from single bite with small forcep (p-value of 0.002). It is also found that sensitivity improved to 79% if 2 biopsies were taken using the small forcep (p-value of 0.001). This study concluded that increasing the size or number of tissue specimen for gastric mucosa biopsy will

increase the sensitivity of CLO test and will hasten the time to positivity of CLO test, and that multiple small biopsies are equivalent to single large biopsy in terms of CLO test sensitivity and time to positivity (Laine *et al.*, 1996).

Another significant study compared 1, 2, 3 and 4 biopsies taken at prepyloric antrum and tested the time taken to achieve positive CLO test. All biopsy were taken using standard size forcep. They concluded that the sensitivity of CLO test was increased by the number of biopsies taken and the time to achieve positive CLO test was shortened by increased number of biopsies. Sensitivity of CLO test was 96% when 4 biopsies were tested compared to 68% when 2 biopsies where tested (p-value <0.01), and 52% when 1 biopsy was tested (p-value <0.05). However, it should be highlighted that each arm of study were taken from different patients (Siddique *et al.*, 2008).

Here in HUSM, due to limitation of endoscopic equipment, biopsy by large cup forcep is not being done. Hence, the alternative proposed is to increase the number of biopsy specimen from prepyloric antrum to achieve higher yield of gastric mucosa tissue. It is believed that larger amount or mass of gastric mucosa tissue increases the yield of Helicobacter pylori organism to be tested in CLO test reagent (Bernstein *et al.*, 1995), (Siddique *et al.*, 2008).

3. OBJECTIVES

General objective:

To improve the detection rate of Helicobacter Pylori using rapid urease CLO test by increasing the number of endoscopic biopsy at specific location of the stomach

Specific objectives:

1. To determine the prevalence rate of Helicobacter Pylori infection using a new biopsy method; by taking gastric endoscopic biopsy at specified site: pre-pylorus antrum, within 3cm from pylorus.
2. To describe the prevalence rate of helicobacter pylori infection of demographic pattern, presenting symptoms and endoscopic diagnosis among patients undergoing Oesophago-gastro-duodenoscopy (OGDS) electively in HUSM.
3. To determine and to compare the sensitivity of CLO test between single gastric biopsy and two gastric biopsy specimen
4. To determine and to compare the earliest detection and sensitivity between single gastric biopsy and two gastric biopsy specimen

4. RESEARCH QUESTIONS

1. Does the implementation of biopsy at specified site (pre-pylorus antrum, within 3cm from pylorus along the greater curvature) increases the prevalence, hence detection rate of Helicobacter Pylori infection among patients in Hospital Universiti Sains Malaysia?

2. Do two gastric biopsy specimens increase the sensitivity of CLO test to detect H Pylori compared to single biopsy?

3. Do testing two gastric biopsy specimens in single test kit leads to reduced time for CLO test to become positive compared to single biopsy?

5. HYPOTHESIS

Research question 1

Alternative hypothesis (H_A): Implementation of biopsy at specific site can lead to higher detection rate of Helicobacter Pylori infection among patients in Hospital Universiti Sains Malaysia

Null hypothesis (Ho): Implementation of biopsy at specific site will not lead to higher detection rate of Helicobacter Pylori infection among patients in Hospital Universiti Sains Malaysia

Research question 2

Alternative hypothesis (H_A): There is a significant correlation between number of biopsy specimen and sensitivity of CLO test

Null hypothesis (Ho): There is no correlation between number of biopsy specimen and sensitivity of CLO test

Research question 3

Alternative hypothesis (H_A): There is a significant correlation between number of biopsy specimen and the speed of CLO test becoming positive

Null hypothesis (Ho): There is no correlation between number of biopsy specimen and the speed of CLO test becoming positive

6. METHODOLOGY

6.1 PATIENT SELECTION

This was a cross sectional study involving patients coming to Endoscopy Unit, Hospital Universiti Sains Malaysia (HUSM) for elective Oesophago-gastro-duodenoscopy (OGDS) for investigation of gastroduodenal symptoms from April 2013 to September 2014 (18 months).

Patients were explained regarding the study by the research team and consent taken after fulfilling inclusion and exclusion criteria as below:

Inclusion criteria:

1) Patients referred for elective Oesophago-gastro-duodenoscopy (OGDS) in Endoscopy Unit, Hospital University Sains Malaysia (HUSM) after reviewed by either Gastroenterology Unit or General Surgery Teams.
2) Patients investigated for gastroduodenal symptoms such as dyspepsia, gastro-esophageal reflux symptoms, acute or chronic epigastric pain, history of malaena or unexplained iron deficiency anaemia.

Exclusion criteria:

1) Patients presented with acute non-variceal or variceal upper GI bleeding, or actively bleeding peptic ulcers.
2) Patients presented with perforated gastric/duodenal ulcers
3) Patients with previous history of total or partial gastrectomy
4) Critically ill patients, haemodynamically unstable patients or endoscopies done bedside in high dependency unit or intensive care unit

6.2 DURATION OF STUDY

This is a cross-sectional study, whereby patients presented to Endoscopy Unit for elective ODGS from April 2013 to September 2014 (18 months) were recruited for the study. Patients were not required to be followed-up in this study protocol.

6.3 CONSENT FOR STUDY

Informed consent was taken in accordance to Declaration of Helsinki, with protocol and statement of informed consent approved by Ethics Committee of Universiti Sains Malaysia. (Appendix 1, 2 and 3)

Each patient was explained regarding the objectives of this study, the method and procedure of OGDS with biopsy, as well as the risk and complication of the procedure. It was emphasized that their treatment and follow-up plan was not being interfered and all routine reporting procedure will have been made available to their primary treating physician or surgeon to determine further treatment and follow-up.

Patients under the age of 18 years, consent was taken from their legal guardians.

6.4 SAMPLING METHOD

The patients were sampled according to purposive sampling where they are selected based on presence of symptoms requiring investigation by Oesophago-gastro-duodenoscopy (OGDS).

6.5 SAMPLE SIZE CALCULATION

Calculation of sample size was based on the 2nd objective, where sample size required for estimating sensitivity of CLO test using single biopsy versus two biopsy specimens was done. Calculation was done using Sample Size Calculation for Sensitivity and Specificity Studies with Excel 5 (written by Dr Lin Naing @ Mohd Ayub Sadiq, 2004).

Single biopsy group:

- Expected sensitivity based on literature: 0.75 (Laine et al., 1996)
- Expected specificity based on literature: 0.85 (Laine et al., 1996)
- Expected prevalence based on literature: 0.49 (Goh, 1997)
- Desired precision: 0.10
- Confidence level: 95%

To achieve the precision of 0.10 for Sensitivity, we needed a total sample size of 149 patients. With this sample size, the precision for Specificity will be 0.080

Two biopsies group:

- Expected sensitivity in this group: 0.90 (Laine et al., 1996)
- Expected specificity based on literature: 0.85 (Laine et al., 1996)
- Expected prevalence based on literature: 0.49 (Goh, 1997)
- Desired precision: 0.08
- Confidence level: 95%

To achieve the precision of 0.08 for Sensitivity, we needed a total sample size of 113 patients. With this sample size, the precision for Specificity will be 0.092

According to national statistic where prevalence for Helicobacter pylori infection was 49% based on Goh 1997. Therefore, a total of a total of 149 patients was required for this study, taking the larger number based on the above two calculations. Throughout the period of study from April 2013 to September 2014 (18 months) we managed to recruit 150 patients for this study.

6.6 STUDY PROTOCOL

All patients included underwent Oesophago-gastroduodenoscopy (OGDS) using standard flexible scope Olympus H180. Gastric mucosa biopsy was taken by standard cup forcep Olympus FB 25K non-needle fenestrated cup.

3 sets of antral gastric biopsy specimens were taken from each patient for study purpose. Endoscopic findings were documented. Each biopsy was taken from adjacent sites, all at greater curvature of gastric antrum, within 3 cm from pylorus as stated in the objective.

1. First specimen: single biopsy using 2.8mm forcep: labeled as Specimen A
2. Second specimen: 2 biopsies taken using 2.8mm forcep: labeled as Specimen B
3. Third specimen: single biopsy using 2.8mm forcep: labeled as Specimen C

Specimen A and B was applied to 2 separate CLO test reagent well. Product name: Clotest Rapid Urease Test Single Well (CLO 025), manufactured by Tri-Med Distributors Pty Ltd, Australia. Examination of the change of colour was done at 1 hour, 3 hour, 6 hour & 24 hour by endoscopy nurse / research assistant in charge.

Positive results were interpreted when change of colour of reagent from yellow into pink, magenta or orange within 24 hour at room temperature. Negative results were interpreted as no change of colour (yellow) after 24 hour.

Specimens labeled as C were sent for histopathological examination and special staining for definite diagnosis of Helicobacter Pylori infection. All specimens C were fixed in 10% Formalin and sent to pathology lab, where:

- Haematoxylin &Eosin (H&E) staining was done and examined for presence of inflammation and identification of Helicobacter pylori bacterium. If bacterium was not identified on H&E staining, Warthin-Starry stain was applied for identification of bacterium
- Microscopic examination by single pathologist (study pathologist) unaware of the CLO test results
- If there were presence of discrepancy between pathologist report and CLO test, slides were examined by a 2nd pathologist who was also blinded. Disputes were settled by consensus.

6.7 RESULTS

Data was collected in a clinical research form (CRF). (Appendix 4) CLO test interpretation was performed by endoscopy nurse acting as research assistance that was well trained in performing and interpreting CLO test results.

Histopathological conclusion was reported as presence or absence of Helicobacter Pylori infection. Reports were addressed to principle researcher for documentation.

CLO test was interpreted as positive or negative for each specimen A & B. Research assistant recorded time of test achieving positive results at 1 hour, 3 hour, 6 hour and 24 hour after test started for each specimens A & B.

Patients who tested positive for either one of the test (CLO or HPE positive) were diagnosed as having Helicobacter Pylori infection and results forwarded to their primary treating physicians or surgeons for definitive treatment.

Prevalence of Helicobacter Pylori infection was determined based on positive testing on either CLO testing or histopathological examination (HPE).

6.8 STATISTICAL ANALYSIS

Data entry, cleaning and analysis was done by using IBM SPSS Statistics version 20. Results were presented as frequency and proportion, sensitivity and specificity.

Overall prevalence were presented in percentage and is derived from the total number of patients diagnosed positive for Helicobacter Pylori (either CLO test or Histopathology positive) over the total number of patients recruited (N). Prevalence

rates for each demographic category, namely sex, race, endoscopic diagnosis and presenting symptoms were described.

For objective number 3, analysis was done by comparing sensitivity of CLO test from both arm and tested using Mc Nemar's test

	HPE +
CLO +	a_1
CLO -	c_1
	(a_1+c_1)

Sensitivity of CLO test using single biopsy (A)

$= a_1 / (a_1+c_1)$

	HPE +
CLO +	a_2
CLO -	c_2
	(a_2+c_2)

Sensitivity of CLO test using two biopsy (B)

$= a_2 / (a_2+c_2)$

Sensitivity was determined for both groups A & B

Single biopsy (A)	Sensitivity (A) %
Two biopsy (B)	Sensitivity (B) %

Mc Nemar's test was performed to compare sensitivity of both groups A & B

	CLO + (B)	CLO – (B)
CLO + (A)		
CLO – (A)		

For objective number 4, cumulative frequency and sensitivity of CLO test for each of the time period CLO test turned positive was calculated for both groups A & B. Sensitivity of CLO test for each time period between both groups were then compared with Mc Nemar's test for statistical significance.

7. RESULTS

7.1 Demographic pattern of study sample

A total of 150 patients were recruited in this study, with 89 (59.3%) male and 61 (40.7%) female patients. (Figure 1) Mean age was 51.3 years with standard deviation of 17.3 years. The youngest patient was 13 year old and the oldest was 89 year old. (Figure 2) Racial distribution in this study is similar to the general population in the state of Kelantan. The study sample consists of predominantly Malay at 87.3%, followed by Chinese at 10% and Indian at 0.7%. 2% of study sample were grouped together as others, which consist of 1 Siamese and 2 Nigerians. (Figure 3)

Presenting symptoms leading to decision of Oesophago-Gastro-Duodenoscopy (OGDS) were recorded among study sample. The most common presenting symptom was epigastric burning pain (50.7%), followed by history of dyspepsia (18.0%). 12% of patients complained of history of black tarry stool and 10% gave typical history of pain related to meals. Only 5.3% presented with iron deficiency anaemia without any pain and 4.0% complained of reflux symptoms. (Figure 4)

OGDS findings were recorded among study sample. 32.7% of study sample had endoscopic antral gastritis, followed by 17.3% having pan-gastritis and 18.0% with both gastritis and duodenitis. Gastric ulcers were found in 16.7% of study sample and duodenal ulcers in 0.7%, whereas combined gastric and duodenal ulcers were found in 3.3% of study sample. 10% of study sample were found to have hiatal

hernia or esophagitis as the cause of upper gastrointestinal symptoms. 1.3% of elective upper endoscopy was normal. (Figure 5)