DIAGNOSIS HELICOBACTER PYLORI BY RE-USED PRONTO DRY TEST

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การตรวจวินิจฉัยหาเชื้อเฮลิโคแบคเตอร์ไพโลริ ด้วยชุดตรวจพรอนโตดรายที่เคยใช้แล้วกลับนำมาใช้ใหม่

นายสมบูรณ์ ทรัพย์วงศ์เจริญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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สมบูรณ์ ทรัพย์วงศ์เจริญ : การตรวจวินิจฉัยหาเชื้อเฮลิโคแบคเตอร์ไพโลริด้วยชุดตรวจพรอนโตดรายที่เคยใช้แล้วนำมาใช้ใหม่ (Diagnosis helicobacter pylori by re-used Pronto Dry test) อาจารย์ที่ปรึกษา: ศจ.สุรศักดิ์ ฐานีวนิชสกุล,อาจารย์ที่ปรึกษาร่วม: นพ.อภิชาติ พลอยสังวาล , 60หน้า.ISBN 974-17-6948-2

วัตถุประสงค์ : เพื่อศึกษาหาความไว ความจำเพาะ ความแม่นยำของชุดตรวจพรอนโตดรายที่เคยใช้แล้วกลับนำมาใช้ใหม่ในผู้ป่วยที่มีอาการปวดแน่นท้องด้านบน ที่ต้องรับการตรวจด้วยการส่องกล้องตรวจทางเดินอาหาร

รูปแบบการทดลอง : การศึกษาแบบวิจัยเชิงพรรณนา แบบตัดขวาง การตรวจวินิจฉัย

สถานที่ทำการวิจัย : โรงพยาบาลราชวิถี

วิธีการศึกษา : คนไข้ที่มีอาการจุกแน่นท้องส่วนบน ที่จำเป็นต้องเข้ารับการตรวจวินิจฉัยด้วยการส่องกล้องตรวจหาพยาธิสภาพ และตรวจหาเชื้อเฮลิโคแบคเตอร์ไพโลริ จำนวน 202 ราย ผู้ป่วยทุกรายหลังจากตรวจด้วยกล้องส่องทางเดินอาหารเรียบร้อย ก่อนเอากล้องออก จะตัดชิ้นเนื้อบริเวณแอนทรัมและบอดี้ของกระเพาะอาหารตำแหน่งละ 3 ชิ้น ชิ้นขนาด 2-3 มิลลิเมตร หลังจากนั้นแบ่งส่วนชิ้นเนื้อเป็น 3 ส่วน ส่วนละ 2 ชิ้น เพื่อส่งตรวจทางอิมมูนฮิสโตเคม, ตรวจพรอนโตดรายชุดใหม่, ตรวจพรอนโตดรายที่เคยใช้และกลับนำมาใช้ใหม่ โดยจะเปรียบเทียบชุดตรวจพรอนโตดรายที่เคยใช้แล้วกลับนำมาใช้ใหม่ กับฮิทมูโนฮิสโตเคม และชุดตรวจพรอนโตดรายใหม่ อายุ, เพศ ผลการตรวจทางกล้องตลอดจนการตรวจแต่ละชนิดจะถูกเก็บบันทึกไว้

ผลการศึกษา : พบว่าชุดตรวจพรอนโตดรายที่เคยใช้แล้วกลับนำมาใช้ใหม่มีความไว 61.70% (95% CI47.29-74.70) ความจำเพาะ 96.77% (95% CI 92.99-98.81) และความแม่นยำ 88.61% (95% CI 83.66-92.46) และมีความสัมพันธ์กับชุดตรวจพรอนโตดรายใหม่ แคปปา 0.63 (95% CI 0.51-0.74) พบกลุ่มอาการที่ปวดแน่นท้องโดยไม่พบพยาธิสภาพผิดปกติทางกล้อง ความชุก 7.9% แต่ตรวจพบเชื้อเฮลิโคแบคเตอร์โพโลริ มีความชุก 23% ไม่พบภาวะแทรกซ้อนในการตรวจวินิจฉัยนี้

สรุป : ชุดตรวจพรอนโดที่เคยใช้และกลับนำมาใช้ใหม่ ให้ความไวปานกลางแต่จำเพาะสูง ไม่แนะนำให้นำกลับมาใช้ตรวจกรองหาเชื้อเฮลิโคแบคเตอร์ไพโลริ

สาขาวิชา การพัฒนาสุขภาพ ลายมือชื่อนิสิต.....

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD: UREASE TEST, RE-USED PRONTO DRY TEST, H. PYLORI.

SOMBOON SUBWONGCHAROEN: DIAGNOSIS HELICOBACTER PYLORI BY RE-USED PRONTO DRY TEST.

THESIS ADVISOR: PROF. SURASAK TANEEPANICHSKUL,

THESIS CO-ADVISOR: DR. APICHART PLOYSANGWAL, 60pp. ISBN 974-17-6948-2

Objective: To determine sensitivity, specificity, accuracy to diagnose H. pylori in dyspepsia patients by re-used Pronto Dry test.

Design: Diagnostic cross-sectional study.

Setting: Rajavithi Hospital

Method: 202 patients with symptom of dyspepsia that need endoscopic procedure to make diagnosis were enrolled in this study. After complete examination duodenum, stomach, 3 pieces of gastric mucosa each from antrum and body were obtained and randomly allocated for histology with immunohistochemistry , new Pronto Dry, re-used Proto Dry. Results of all these tests, age, sex were recorded.

Results: This Study showed that sensitivity, specificity, accuracy of re-used Pronto Dry test was 61.70% (95% CI47.29-74.70), 96.77% (95% CI 92.99-98.81), 88.61% (95% CI 83.66-92.46) respectively, and the kappa agreement between re-used Pronto Dry test and new Pronto Dry test was 0.63(95%CI 0.51-0.74).

Prevalence of functional dyspepsia was 7.9% and prevalence of H. pylori infection was 23%. There was no adverse event in this study.

Conclusion: Re-used Pronto Dry test could be used to diagnose H. pylori infection with intermediate sensitivity, and high specificity, therefore It is not recommended to use for screening test of H. pylori infection.

Field of study Health Development Student's signature .....

Academic year 2004 Advisor's signature .....

Co-advisor's signature .....

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CHAPTER I

BACKGROUND AND RATIONALE

Dyspepsia is defined as a constellation of symptoms that include upper abdominal pain or discomfort, which is intermittent or constant and may be associated with additional symptoms of nausea and vomiting *(1)*. While these symptoms may be associated with a wide range of specific clinical diagnosis (e.g., peptic ulcer disease (PUD), gastric cancer, and gastroesophageal reflux (GERD), among others), often no organic cause can be found (functional dyspepsia)*(2).* High-risk patients (as defined below) present with additional signs and symptoms, so called "alarm symptoms," suggestive of more significant organic causes. In the absence of such "alarm symptoms," provisional diagnosis based on history and physical exam alone are often inaccurate; leading to inappropriate management plans and/or a delay in establishing the correct diagnosis *(2)*. Endoscopic examination of the upper GI tract remains the "gold standard" for establishing (or excluding) PUD and other specific organic diseases/UGI pathologies. Endoscopy is the procedure of choice for the diagnostic evaluation of the UGI tract because of its ease, reliability, diagnostic superiority, and the ability to perform biopsies and/or therapeutic interventions. This is especially true

for patients presenting with dyspepsia and who are at high risk based on the presence of additional symptoms and/or physical signs. These high-risk patients include:

1. New onset dyspepsia in individuals over age 50 years old.
2. Dyspepsia associated with dysphagia and/or weight loss.
3. Those with evidence of gastrointestinal bleeding (occult blood, anemia, hematemesis, and/or hematochezia / melena.)
4. Those who have not responded to an appropriate trial of empiric therapy.
5. Those patients using NSAIDS or other ulcerogenic agents.
6. Those with signs or symptoms of UGI tract obstruction (e.g., early satiety, vomiting).
7. Those whose ethnic and/or racial background is associated with increased risk for UGI malignancies or other significant disease states.

Whether Helicobacter pylori plays a causative role in dyspepsia (and nonulcer dyspepsia) remains controversial. Many patients with new onset dyspepsia as an isolated symptom (epigastric pain/discomfort without weight loss, evidence of gross or occult bleeding, obstruction, perforation, or associated multisystem disease) may be treated empirically for H. pylori based on a positive test result for H. pylori. This is more commonly accepted for younger individuals (e.g., < 45-50 years old) However, for patients > 50 years old or any patients with the risk factors listed above, endoscopy should be the first-line approach. In Thailand most of endoscopic centers use urease test to detect H.pylori

infection in those cases that need endoscope for diagnosis of dyspepsia and CLO test is widely accepted as a rapid urease test because of its high sensitivity and specificity *(3)*. This test need to be stored in the refrigerator (36-48° F/2-8° C). The results have to be interpreted in 24 hourss. It was originally designed as a disposable test for the rapid detection of H. pylori before the patient leaves the endoscopy room. Although it is not really an expensive item in developed countries, but it may represent an extra medical expense in developing or underdeveloped countries, either to the patient or to the government. Therefore it will be benefit when it is possible to recycle a waste product and bring back to use again. In 1999 Lee CL et al *(4)* reported diagnosis H. pylori infection by 216 re-used CLO test pellets with sensitivity 98.6%, specificity 98.2%. Nowadays we use new rapid urease test (Pronto Dry) instead of CLO test because it could be kept in room temperature and interpreted within 1 hour and the sensitivity 98%, specificity 97%. For the best of my knowledge, no available data about re-used Pronto Dry test in clinical use to diagnose H. pylori infection.

CHAPTER II

LITERATURE REVIEW

Infection with H. pylori is associated with chronic gastritis in children as well as in adults *(5, 6)*. In children, H. pylori gastritis seems to be asymptomatic *(7, 8)*. H. pylori infection is related to dyspeptic symptoms in adult patients with a history of peptic ulcer or in patients with active ulcer. Meanwhile, there is no doubt that H. pylori infection plays a causal role in the development of duodenal and gastric ulcers and should be eradicated in case of diagnosis of such a condition *(9)*. It has been suggested that up to95% of duodenal and 70% of gastric ulcers are attributable to this infection. The epidemiologic evidence relating H. pylori infection with gastric cancer came initially from three nested case control studies which all showed that cancer patients had a higher H. pylori sero prevalence compared to controls, and the risk associated with a positive serology varied between 2.1 and 8.7 *(10, 11)*. Gastric cancer is one of the leading causes of cancer related deaths in the world. On a worldwide scale, crude gastric cancer rates are increasing (despite a decrease in age adjusted rates) because life span is increasing worldwide. Since 1994, infection with H. pylori has been classified as a class I carcinogen for humans by the International Agency

for Research on Cancer. However, only cancers located distally to the cardia (non-cardia adenocarcinomas) are related to H. pylori infection; cancers located proximally are not.

A meta-analysis of 19 cohort studies and case-control studies published in 1998 estimated a summary odds ratio of 1.92 indicating an approximately 2-fold risk of gastric cancer among infected individuals *(12)*. In addition to non-cardia adenocarcinoma, gastric B-cell lymphomas, which account for about 5% of all gastric malignancies, are also linked to a higher infection rate with H. pylori *(13)* and eradication of H. pylori usually leads to regression of mucosa-associated lymphoid tissue lymphomas. Warren and Marshall *(14)* were the first researchers to successfully culture the bacterium H. pylori from gastric biopsies in 1983. However, as early as 1893 spiralic bacteria were described in the stomachs of autopsied rabbits. In humans, the bacterium was first described in 1906. Since the first description of the organism in 1982 *(14)*.

Several methods have been proposed to detect this bacterium. These methods are either invasive (requiring endoscopy to obtain biopsies for urease test, culture, histology, and PCR) or noninvasive (based on the detection of antibodies to H. pylori or the urea breath test [UBT]). H. pylori prevalence varies with geographic region, and transmission among persons might be of primary importance *(15)*. The prevalence of infection in developing countries seemed very high, with almost the totality of the children being infected by a certain age, whereas the prevalence in developed countries seemed

considerably lower. Prevalence in adults ranged from 10-50% in the developed world and up to 80-90% in developing countries. Within each geographic region, there was also an increase within each geographic region, there was also an increase in prevalence with age. The prevalence of H. pylori infection in the studies conducted in Ulm (Germany), covering all pre-school children living within a defined study region, was 13% in 1996 *(16)* and 11% in the 1997 study and in 1998, in a random selection of 305 pre-school children, 13% *(17)*. In all of these independent studies, we found a considerable difference in prevalence according to ethnic origin of the children. Current knowledge implies that acquisition of H. pylori seems to occur predominantly in childhood and that once acquired the infection persists lifelong in most infected subjects *(18, 19)*. Infection seems to be relatively stable in juveniles and adults. There seems rarely to be a new infection in adulthood. If one considers the low risk of infection in children older than five in comparison to those under 5 years of age, the acquisition of the infection seems to occur mainly within the first 5 years of life but rarely within the first year of life *(20)*.

Humans are so far the only identified source of H. pylori infection. Animals seem not to be a relevant source of infection and contact with pets is not associated with an increased risk of infection in western countries *(21)*. Intrafamilial transmission seems to be the main route of infection, and DNA typing showed that within the members of a family the same strain of the bacteria, with otherwise high genetic diversity, is often found *(22)*. From a

higher prevalence of H. pylori infection in parents of infected children, person-to-person transmission within the family was suggested *(23, 24, 25)*.

DIAGNOSIS

A large number of tests are now available for the diagnosis of H. pylori infection. For the purpose of this presentation, we consider separately tests that require gastric tissue obtained through and endoscopic procedure (invasive tests) and laboratory test that can be performed without endoscopy (noninvasive tests). This distinction, although artificial, is practically useful.

Invasive Tests

Histopathologic Examination of Gastric Biopsy Specimens: Histochemical and Immunocytochemical Stains

Bacteria can be visualized in histologic preparations of gastric biopsy specimens stained with a variety of methods. H. pylori can be detected in routine hematoxylin and eosin-stained biopsy specimens. However, variations in the quality of the stain, the paucity of bacteria in some specimens, and the need for exceptional diligence on the part of observers make the hematoxylin and eosin stain a suboptimal choice for the specific task of detecting H. pylori. Numerous special stains are now available for the optimal detection of H. pylori *(26, 27)*. Several anti-H. pylori antibodies are commercially available for the

immunohistochemical detection of H. pylori in paraffin-embedded biopsy specimens. This method requires considerable expertise, but its sensitivity and specificity are high and some laboratories use it for routine clinical diagnosis *(28, 6)*. Immunohistochemistry may be particularly useful for the detection of the coccoid forms of H. pylori, but there is no evidence that detection of coccoid forms has any clinical utility. Because local laboratory conditions and financial constraints often determine the choice of a stain more than the individual histopathologist's preference, no universal recommendation is made as to what technique should be used. However, the revised Sydney System for the classification of gastritis states that "the use of a special stain is strongly recommended, particularly when the hematoxylin and eosin stain fails to reveal organisms in a biopsy specimen with chronic active inflammation. Thus, although many positive cases can be recognized in a good hematoxylin and eosin stain, careful examination of a special stain is deemed essential before declaring an inflamed biopsy specimen histologically negative for H. pylori *(29)*.

In Situ Hybridization

In situ hybridization may be used for the detection of H. pylori in paraffin-embedded sections *(30, 31)*. Although in situ hybridization may turn out to be the most specific and sensitive method for the visual detection of H. pylori in biopsy specimens, the high cost and difficulty of this procedure seem to make this an overly optimistic vision, especially when biopsies from different regions of the stomach must be examined for each patient.

Smear, Brush, and Touch Preparations

Smears of gastric mucus and exfoliated epithelial cells may be prepared by using techniques similar to those used to obtain specimens for cytologic examination. Smears are usually stained with Gram stain, and allow the detection of bacteria within minutes of the endoscopic procedure *(32, 33)*. This approach has largely been supplanted by the introduction of the rapid urease tests (RUTs).

Bacterial Culture

H. pylori is best cultured in a microaerophilic and humid atmosphere, usually 5% O_2; 10% CO_2; 80% to 85% N_2 99% to 100% humidity; and temperature between 33°C. Incubators containing 10% to 15% CO_2 or anaerobic jars can also be used to culture H. pylori. Most culture media use fresh horse or sheep blood, and both selective media, containing antibiotics such as vancomycin, trimethoprim, and amphoteriein B to suppress contaminants, and nonselective media are used. The highest rates of success are reported from laboratories that prepare their own media. Colonies appear within 14 days, usually 3 to 7 days It is considered imprudent to discard the plates before 14 days. Isolates are identified as H. pylori by Gram stain and biochemical identification based on positivity for urease, catalase, and oxidase. Because many clinical facilities are not equipped to perform the time consuming procedures necessary to culture H. pylori several methods for

transportation have been revised *(34, 35)*. H. pylori is sensitive to drying in aerobic conditions. Special techniques are necessary for storage of biopsy specimen and clinical isolates. The most widely used method consists of freezing the fresh biopsy specimens in a glycerol-containing media such as skim, milk, brucella broth, or cysteine-albini medium. Biopsy samples can be stored indefinitely at -70°C and for up to a week at -20°C. Pre immersion of the biopsy forceps in formalin does not reduce the yield of culture, so it is not critical to take biopsies for culture before those for repaid urease testing or histologic analysis.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a technique that allows the amplification of a deoxyribonucleic acid (DNA) template into multiple copies through sequential round of DNA replication by DNA polymerase. This permits the detection of a DNA fragment that can be resolved and visualized in agarose gels. A number of protocols have been devised, but few have been properly standardized. The practical usefulness of PCR for the detection of H. pylori infection has been difficult to define because this technique is much more sensitive than the other possible gold standards, such as the histopathologic detection of H. pylori. Thus, cases are frequently encountered in which no bacteria can be identified by histopathologic examination, yet the PCR yields a positive result *(36)*.

Rapid Urease Tests

These assays exploit the high urease content of H. pylori *(37, 38, 39)*. To perform the test, a fragment of gastric mucosa is placed into a medium containing urea and a pH indicator.

The urease produced by H. pylori hydrolyzes the urea, releasing ammonia, which raises the pH of the broth or agar. An appropriate indicator (e.g. phenol red) changes color as the pH increases. In the first commercially produced RUTs, the CLO test (TriMEd Specialties, Inc., Lenexa, KS), the original yellow gel capsule into which the specimen is placed becomes red within minutes to hours, depending on the quantity of bacteria present. Three tests are available commercially in the United States, but any laboratory can easily produce a successful medium . A homemade test can be made with a solution containing 2 g urea, 10 ml of 0.5% (w/v) phenol red, and 20 mg sodium oxide in 100 ml of 0.01 mol/L sodium phosphate buffer, pH 6.5 A 0.5-ml dram vial is filled with 50 μl of this solution and biopsy specimens are added in the endoscopy room. The test is positive if the medium changes from orange to definite pink. Two of the commercial tests use urea impregnated agar (hpfast [GI Supply] and CLOtest, Camp Hill, PA). The hpfast test uses a detergent to help release the urea and starts the reaction at a pH below non-H. pylori thus theoretically increasing the specificity. Another test uses a urea-impregnated semi permeable membrane for ammonia gas (PyloriTek, Serim, Elkhart. ID). The sensitivity and specificity

for these RUTs is between 90% to 95%. The specificity and sensitivity of the commercial tests compared with histopathologic examination are extremely high, in most cases approaching 100%. The speed of the reaction is related to the number of bacteria present. Thus, the most rapid results are obtained if several specimens, or a specimens, or a specimen taken with large-cup forceps, are used.

Noninvasive Tests

Serology

A correlation between the presence of H. pylori in the gastric mucosa, chronic active gastritis, and serum anti H. pylori antibodies was documented shortly after the discovery of the organism using crude antigen preparations in complement fixation, bacterial agglutination tests, immunoblottin techniques, and enzyme-linked immunosorbent assays. At present, the best commercially available enzyme immunoassays have sensitivities from 93% to 98% and specificities from 95% to 98% and are considered useful methods to assess for the presence of H. pylori infection *(40))*.

Stool Tests

Culture of H. pylori from stool samples has proven to be very difficult, and improved methods are needed. Recently, a noninvasive test to detect H. pylori antigen in stool specimens was introduced (Premier Platinum HpSA, Meridian Diagnostics, Inc., Cincinnati, OH). Preliminary data show good sensitivity and specificity. Large head-to-head

comparisons with other tests are needed to establish the place of this approach in the diagnostic armamentarium.

Urea Breath Tests

The urea breath tests (UBTs) represent one of the most important and innovative methods to detect H. pylori infection *(41, 42*). The basic principle of these tests relies on the ability of H. pylori to produce large quantities of urease . Thus, the ingestion of a solution containing urea is rapidly followed, in an infected person, by the production of NH_3 and CO_2 The latter rapidly appears in the person's breath. If the ingested urea is labeled with a detectable isotope, then the exhaled CO_2 will also be labeled and, therefore, measurable by an appropriate detection method.

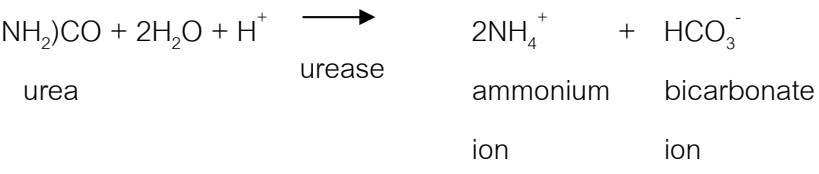
Limitation of Test for Presence of H. pylori

All the available tests to identify the absence of H. pylori infection have limitations. Rapid urease test require a high density of bacteria and have the highest likelihood of missing the presence of a low level of remaining infection (false-negative results.). A negative rapid urease test should not be taken as the sole evidence for cure. UBTs share the limitations of the biopsy urease test and also require a high density of bacteria but have an advantage over rapid urease test because they sample the entire gastric mucosa. Culture is theoretically the most sensitive test and is the most specific. H. pylori is a fastidious microorganism that many laboratories find difficult to isolate. Culture also requires transport from the endoscopy laboratory to the microbiology laboratory, and delay,

drying, and poor choice of transport media all serve to reduce the value of culture as a clinically useful diagnostic test. Methods to transport and store biopsies before culture have been published, but choice of culture media and culture conditions as well as expertise and experience of the laboratory still influence the results of culture. Histologic analysis is most often used as the gold standard for detection of H.pylori infection because in theory it is the most standardized procedure and provides an objective and permanent record of whether the bacteria are present or absent. The histopathologist has an additional advantage because, even when the bacteria are sparse, the other feature of H. pylori gastritis are usually evident. Despite these advantages, histopathology has serious potential and practical limitations.

Pronto Dry test

Pronto Dry (new rapid urease test) is intended for use for the detection of urease enzyme in gastric mucosal biopsy specimens for the presumptive determination of H. pylori in symptomatic patients. H. pylori produces large amounts of urease enzyme. Although urease primarily allows H. pylori to utilize urea as a nitrogen source, the breakdown of urea also produces high local concentrations of ammonia, which enable the organism to tolerate low pH (see reaction below).



Tests for gastric urease are specific for H. pylori because mammalian cells do not produce urease and very few microorganisms survive in the stomach, except for H. pylori. Pronto Dry consists of a dry filter paper containing urea, phenol red (a pH indicator), buffers and a bacteriostatic agent, in a sealed plastic slide. If the urease enzyme of H. pylori is present in an inserted tissue sample, the resulting decomposition of urea causes the pH to rise and the color of the dot turns from yellow to a bright magenta. The tests should be stored at room temperature. Pronto Dry has a shelf life of 24 months.

Reading the Pronto Dry.

If urease is present in the tissue, an expanding magenta color external ring will be note around the biopsy specimens, or the Pronto Dry will gradually change to a deep orange, then magenta color. A pink-magenta ring at 1 hour is a positive reaction. A negative result is when the external ring is still yellow 1 hour after insertion of the specimens. Subsequent color changes may occur, although in most cases a stable magenta or yellow color will be present.Pronto Dry test can diagnose H.pylori infection with 98%sensitivity,97% specificity.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Research question

3.1.1 Primary research question

Can Pronto Dry (new rapid urease test) test be re-used to diagnose H. pylori infection in the high risk dyspepsia patients with 93±3% sensitivity?

3.1.2 Secondary research question

How many functional dyspepsia patients and H.pylori infection are found in Rajavithi hospital?

3.2 Research objective

3.2.1 To determine sensitivity, specificity, positive predictive value, negative predictive value, accuracy, likelihood ratio of re-used Pronto Dry test for diagnosis H. pylori infection .

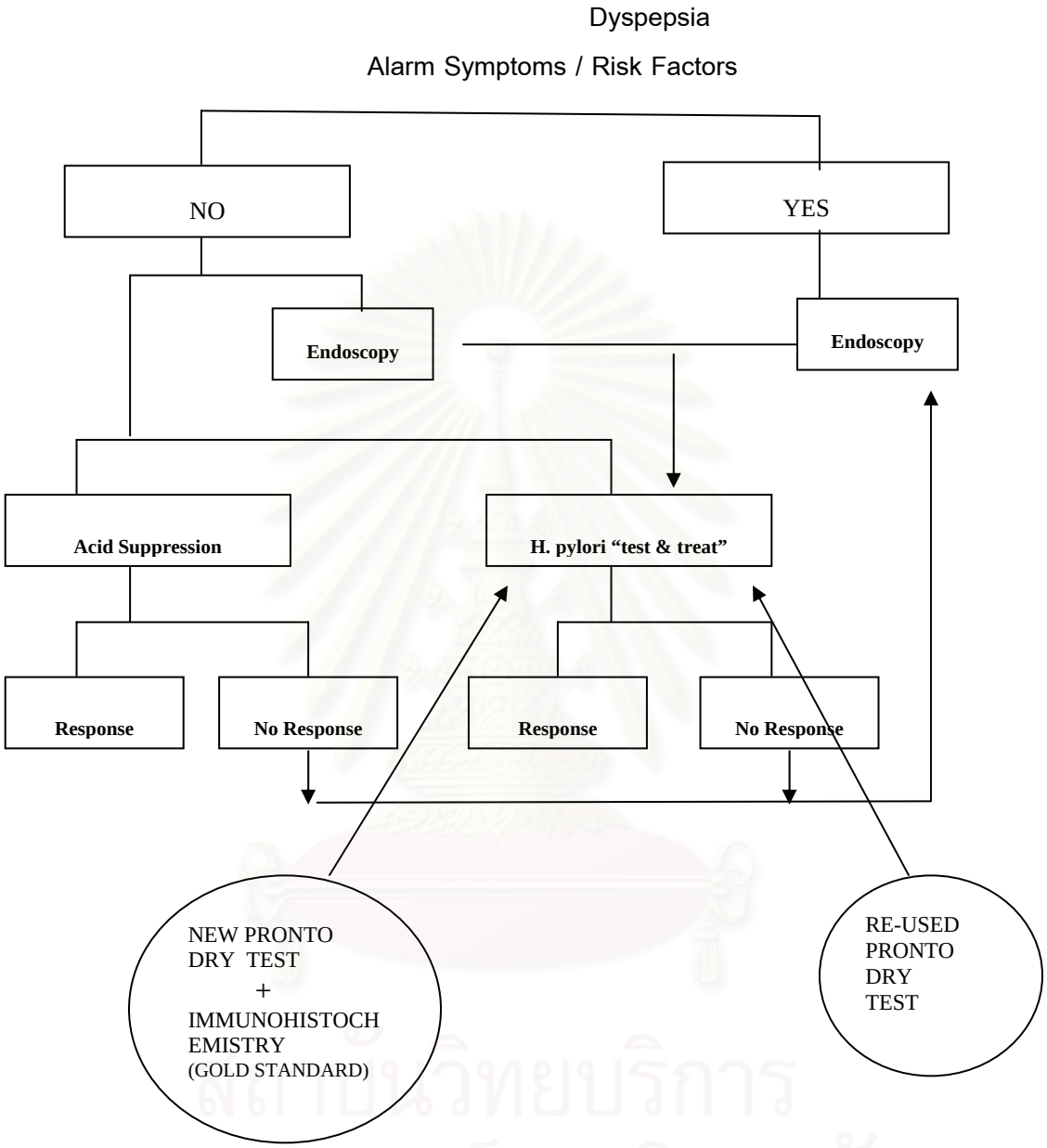
3.2.2. To determine prevalence of functional dyspepsia and H. pylori infection.

3.2.3 To determine agreement between new and re-used Pronto Dry test.

3.3 Hypothesis

none

3.4 Conceptual frame work



3.5 Keyword

Urease test ,Re-used ,Pronto Dry test,H. pylori.

3.6 Operation definition

3.6.1 Dyspepsia is defined as a constellation of symptoms that include upper abdominal pain or discomfort, which is intermittent or constant and any be associated with additional symptoms of nausea, vomiting, and with one of these following “alarming symptom” or high risk.

3.6.1.1 New-onset dyspepsia in individuals over age 50 years old.

3.6.1.2 Dyspepsia associated with dysphagia and/or weight loss.

3.6.1.3 Those who have not responded to an appropriate trial of empiric therapy.

3.6.1.4 Those patients using NSAIDS or other ulcerogenic agents.

3.6.1.5 Those with signs or symptoms of UGI tract obstruction (e.g., early satiety, vomiting).

3.6.1.6 Those whose ethnic and/or racial background is associated with increased risk for UGI malignancies or other significant disease states.

3.6.2 Functional dyspepsia is dyspepsia that no organic cause is found from gastroscope.

3.6.3 Re-used Pronto Dry is the Pronto Dry test (urease test) being used for one or two times and still showing negative result (yellow color).

3.6.4 New Pronto Dry is the Pronto Dry test (urease test) (opened from package).

3.6.5 Endoscopy is the fiberoptic scope used for diagnosis upper GI tract disease.

3.6.6 Positive Pronto Dry is determined by pink-magenta ring at 1 hour.

3.6.7 Negative Pronto Dry is determined by still yellow ring at 1 hour.

3.6.8 Gold standard a gold standard based on at least two diagnostic methods was used in most of the studies. According to the guidelines for clinical trials in *H pylori* infection, patients with at least two positive tests are classified as positive for *H pylori*. If culture alone was positive, because of its absolute specificity, the patient was also classified as positive. Therefore, patients were defined as positive for *H pylori* if: histology with immunohistochemistry and urease test were positive, or if culture was positive. Patients with any other possible combination of results were classified as *H pylori* negative.

In this study "Gold standard" based on 1) histology with immunohistochemistry 2) urease test. If both tests show positive results, patients were defined as positive for *H*. pylori. Patients with any other possible combination of results were defined as *H. pylori* negative.

3.7 Research design

Diagnostic test study, cross-sectional.

3.8 Research methodology

3.8.1 Target population

Thai patients with dyspepsia undertake endoscopic diagnosis and test for H. pylori infection with Pronto Dry test (urease test).

3.8.2 Sample population

Patients with dyspepsia in department of surgery Rajavithi Hospital need endoscopic diagnosis and test for H. pylori infection with Pronto Dry test.

3.8.2.1 Inclusion

3.8.2.1.1 Patients with dyspepsia (as operation definition) and need endoscopic method for diagnosis and test for H. pylori infection.

3.8.2.1.2 Age 15-85.

3.8.2.1.3 Patients with dyspepsia (as operation definition) in whom symptoms recur after therapy is completed.

3.8.2.2 Exclusion

3.8.2.2.1 Patients recently taken antibiotic, bismuth, proton pump inhibitor within 2 weeks.

3.8.2.2.2 Pregnancy.

3.8.2.2.3 GI bleeding.

3.8.2.2.4 Refuse to participate or continue study.

3.8.2.2.5 Poor medical condition could not tolerate endoscopic procedure.

3.8.3 Sample Size Calculation

The formula for descriptive study to calculate sample size is

$$N = \frac{Z^2 PQ}{d^2}$$

N = Number of patients diagnosed as H. pylori infection .

P = Expected sensitivity of test.

Q = 1- P

d = Error that can be accepted.

Prevalence of H. pylori infection diagnosed by urease test in Rajavithi Hospital = 49.7% *(43)*.

Expected sensitivity (P) = 0.93 Q = 0.07

Accepted error (d) = 0.05

$$N = \frac{(1.96)^2 (0.93 \times 0.07)}{(0.05)^2} = 100.03$$

Sample size = N = $\frac{100.03}{\text{Prevalence}} = \frac{201.26}{49.7}$

Sample size = 202 cases

3.9 Intervention

All eligible patients had to starve 6-8 hours before examination with fiberoptic gastroscope. They received local anesthetic spray around posterior pharyngeal wall before passing scope in to GI tract. After complete examination of the 1st, 2nd part duodenum, stomach. All gastroscope findings were recorded, then biopsy forcep was introduced into channel of gastroscope and 3 pieces (2-3mm)of gastric mucosa each from antrum and body of stomach were obtained and randomly allocated for histology with immunohistochemistry ,new Pronto Dry , re-used Pronto Dry test by nurse assistants that did not know details of research protocol. Some patients might require sedative drug during this procedure, 1 or 2 mg of midozolam was used in those cases. The patients could take liquid or light soft diet after finish the procedure. After one hour, Pronto Dry result was interpreted by physician blinded to detail of study. Results of histology with immunohistochemistry will be reported within one week after endoscope procedure. Treatment is based on the results of the new Pronto Dry test and histopathology.

3.10 Outcome measurement

Demographic and base line characteristics

- Age

- Gender

- Provisional diagnosis

Main outcome

- Test results of re-used Pronto Dry

- Test results of new Pronto Dry

- Results of histology with immunohistochemisry

Outcome variable

- Gastroscope findings

- Adverse events

- Pathology

3.11 Data collection

Main outcome

- Test results of re-used Pronto Dry

- Test results of new Pronto Dry

- Results of histology with immunohistochemistry

Administrative variable

- Name

- Address

- Identification number

Demographic and base line characteristics

- Age

- Gender

- Provisional diagnosis

Outcome variable

- Gastroscope findings

- Adverse events

- Pathology

3.12 Data analysis

3.12.1 Basic and demographic variables:

Variable	Type of variable	Statistics
Age (years)	continuous	Mean, SD Min,Max
Sex (male, female)	categorical	Percentage N
Provisional diagnosis	categorical	Percentage N

3.12.2 Outcome variables

Variable	Type of variable	Presentation	Statistics
Scope finding	Categorical	Percentage	Percentage
Pathology	Categorical	Percentage	Percentage
Adverse effect	Categorical	Percentage	Percentage
Immunohistochem	Categorical	proportion	percentage
New Pronto Dry	Categorical	proportion	percentage
Re-used Pronto Dry	Categorical	proportion	Kappa agreement Sensitivity Specificity Accuracy Positive predictive value Negative predictive value Likelihood ratio

Sensitivity = a/ a+c 95% CI = (a/a+c) $\pm$ 1.96 $\sqrt{\frac{(a/a+c)(c/a+c)}{a+c}}$

Specificity=d/d+b 95% CI = d/d+b ± 1.96 $\sqrt{\frac{(d/b+d)(b/b+d)}{b+d}}$

Positive predictive value =a/a+b 95% CI = a/a+b ± 1.96 $\sqrt{\frac{(a/a+b)(b/a+b)}{a+b}}$

Negative predictive value =d/d+c 95% CI=d/d+c ± 1.96 $\sqrt{\frac{(d/d+c)(c/d+c)}{d+c}}$

accuracy $= \frac{a+d}{a+b+c+d}$ 95%

CI= $\frac{a+d}{a+b+c+d} \pm 1.96 \sqrt{\frac{(a+d/a+b+c+d)(b+c/a+b+c+d)}{a+b+c+d}}$

Likelihood ratio for positive test=(LR^+) = $\frac{a/a+c}{b/b+d}$

Likelihood ratio for negative test=(LR^-) = $\frac{d/d+b}{a/a+c}$

95% CI likelihood = $e^w - e^x$

w = $\log_e$ LR - [1.96 x SE ($\log_e$ LR)] x = $\log_e$ LR + [1.96 x SE ($\log_e$ LR)]

SE ($\log_e$ LR^+) = $\sqrt{\frac{1}{a}-\frac{1}{a+c}+\frac{1}{b}-\frac{1}{b+d}}$ SE ($\log_e$ LR^-) = $\sqrt{\frac{1}{d}-\frac{1}{b+d}+\frac{1}{c}-\frac{1}{a+c}}$

Kappa statistic

$P_o = \frac{a+d}{a+b+c+d}$

$P_e = \frac{(a+b)(a+c)}{(a+b+c+d)^2} + \frac{(c+d)(b+d)}{(a+b+c+d)^2}$

$K = \frac{Po-Pe}{1-Pe}$

$$SE\ (K) = \sqrt{\frac{P_O(1-P_O)}{n(1-Pe)^2}}$$

$$95\%\ CI = K \pm 1.96SE(K)$$

3.14 Ethical consideration

All eligible patients were enrolled in to the study and then obtained inform consent from these patients, and this study was also approved by Ethical committee review board Rajavithi Hospital.

3.15 Limitation

202 re-used Pronto Dry tests are required for this study that may not have enough re-used tests in stock .

3.16 Implication

New Pronto Dry test is not very expensive. Its cost 200-300 baths/test, but if we can use the re-used one to test H. pylori infection with high sensitivity and specificity, this may be very useful in the patients and health care provider to save a lot of money per year. However health economic analysis in terms of cost-effectiveness have to be done before generalized implication.

CHAPTER IV

RESULTS OF THE STUDY

After being recruited in accordance with the study exclusion and inclusion criteria, totally 202 patients underwent gastroscopy for diagnosis of upper GI tract pathologies , during March 2004 through January 2005. Among 202 patients, 103 were male and 99 were female. Age of patients ranged between 17 to 84 years (as indicated in Table1).

Table 1 Baseline and demographic data

	Range(year)	Mean ± SD
Age	17-84	51.92 ± 14.31
Male (51%)(n=103)	19-83	51.74 ± 14.08
Female (49%)(n=99)	17-84	52.10 ± 14.63

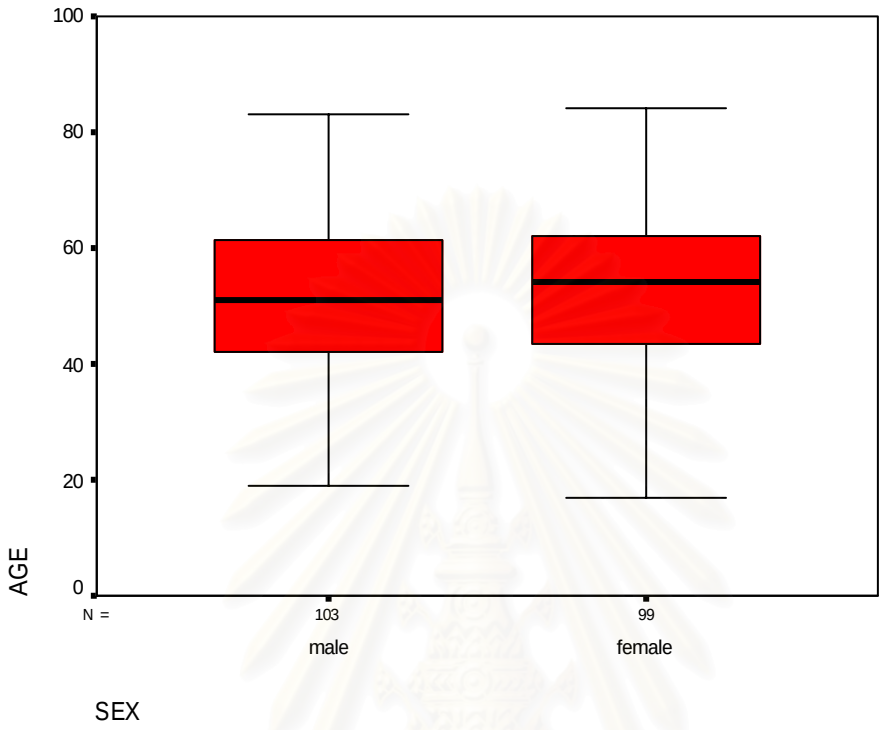


Figure 1. Box and Whisker plot showing demographic data of the patients enrolled in the study.

Provisional diagnosis according to chief complaint, gastroscope finding and the pathology were categorized in detail as shown in Table 2

Table 2 Provisional diagnosis, Scope finding, Pathology

	Provisional diagnosis	Scope finding	Pathology
Gastritis	34 (16.8%)	137(67.8%)	184 (91.1%)
Duodenitis	4 (2%)	11 (5.4%)	3(1.5%)
GU	7 (3.5%)	17 (8.4%)	1 (0.5%)
DU	8 (4%)	4 (2.0%)	0 (0%)
ESV	1 (0.5%)	8 (4%)	0 (0%)
Dyspepsia	108 (53.5%)	-	-
Normal	-	16(7.9%)	13(6.4%)
CA	3(1.5%)	2(1 %)	0(0%)
Other	37(18.3%)	7(3.5%)	1(0.5%)

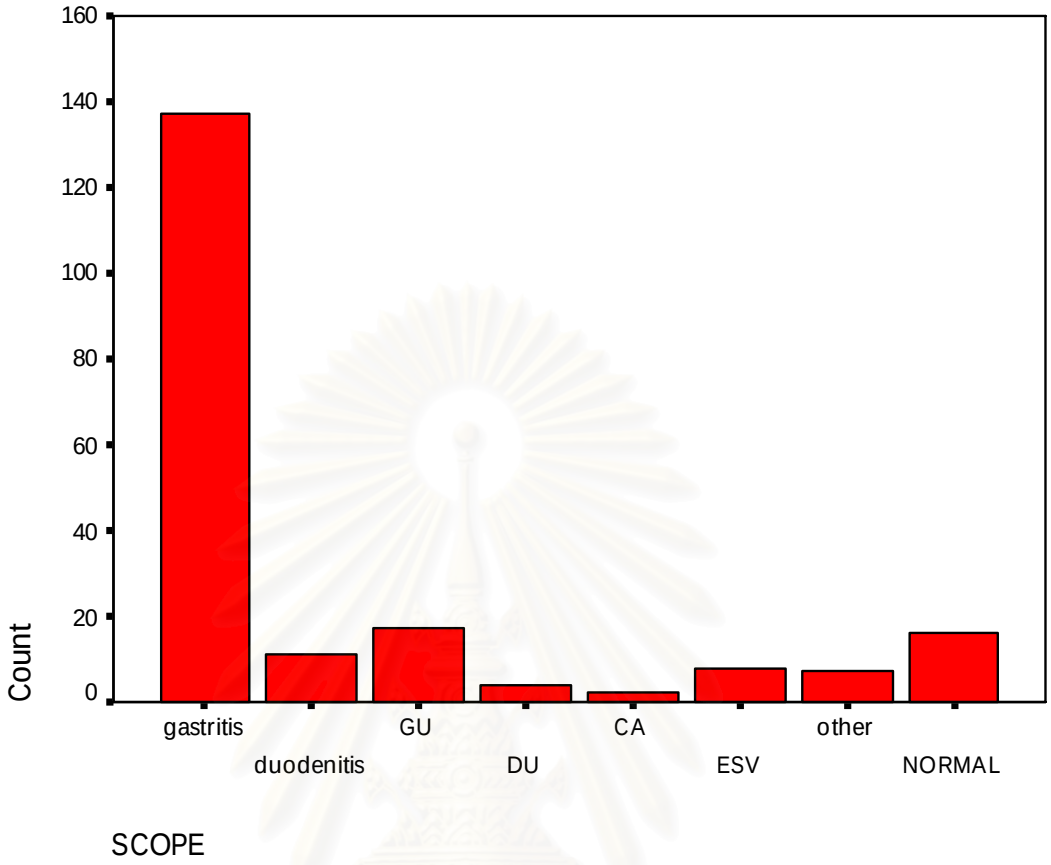


Figure 2.

The data above demonstrated that the prevalence of gastritis was very high as 67.8% while the second most commonly found GI pathology was GU, with the incidence of 8.4%. Regarding some patients who had dyspeptic symptom, however, categorized in the normal rank after gastroscope finding, they can be classified as “functional dyspepsia”. The overall prevalence in this group was 7.9%. There was one missing data in pathology result.

Two patients should have been excluded from the study owing to their GI bleeding symptom. However, they completed the study because of the fact that the GI Bleeding they

had occurred several days before they underwent gastroscopic procedure. Those patients were classified as “others” including cholangiocarcinoma, hepatocellular carcinoma and hiatal hernia.

Table 3 Results of urease test (at 1 hour.)

	New Pronto Dry test +ve	New Pronto Dry test –ve
Re-used Pronto Dry test+ve	34	0
Re-used Pronto Dry test –ve	26	142

Table 4 Results of urease test (at 24 hours.)

	New Pronto Dry test +ve	New Pronto Dry test –ve
Re-used Pronto Dry test +ve	83	2
Re-used Pronto Dry test –ve	34	83

Table 3, 4 2x2 table showing the results of new Pronto Dry test and re-used Pronto Dry test for diagnosis H. pylori infection at 1 hour. and 24 hours.

The concordance results of new and re-used Pronto dry test at 1 hour, 24 hours was 176/202, 166/202 respectively. When these data were analyzed as follow:

Kappa statistic a=34,b=0,c=26,d=142 at1hour. a=83,b=2,c=34,d=83 at24 hours.

$$P_o = \frac{a+d}{a+b+c+d}$$

$$P_e = \frac{(a+b)(a+c)}{(a+b+c+d)^2} + \frac{(c+d)(b+d)}{(a+b+c+d)^2}$$

$$K = \frac{P_O - Pe}{1-Pe}$$

$$SE\ (K) = \sqrt{\frac{P_O(1-P_O)}{n(1-Pe)^2}}$$

$$95\%\ CI = K \pm 1.96SE(K)$$

The Kappa agreement was 0.63 (95% CI 0.51-0.74), 0.65 95% CI (0.55-0.74) at 1 hour, 24 hours respectively.

Table 5 Results of immunohistochemistry, new Pronto Dry test

	immuno histochemistry +ve	Immuno histochemistry –ve
New Pronto Dry test +ve	47	13
New Pronto Dry test -ve	19	123

The data above showed that concordance rate of positive Pronto Dry test and immuno histochemistry was 47/202. This indicated that H. pylori infection was 0.23. In other words, prevalence of H. pylori infection was 23% on the basis of the criteria of gold standard to detect H. pylori infection in this study. When different methods were used to detect this infection, the different prevalence we obtained. When the new Pronto Dry test or histology was used , the prevalence was 32.7%.When we interpreted the results of new Pronto Dry at 24 hours like CLO test ,the prevalence would be 57.9%.As shown detail in table 3,4,5.

Table 6 Results of re-used Pronto Dry test to detect H. pylori infection

	H. pylori +ve	H.pylori –ve
Re-used Pronto Dry test +ve	29	5
Re-used Pronto Dry test -ve	18	150

This table showed the results of H. pylori infection based on criteria as immunohistochemistry and new Pronto Dry must either be positive. When the re-used Pronto Dry test was applied to detect H.pylori infection, the sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and likelihood ratio could be calculated as follow:

a=29 ,b=5,c=18,d=150

Sensitivity =a/ a+c 95% CI = (a/a+c) $\pm$ 1.96 $\sqrt{\frac{(a/a+c)(c/a+c)}{a+c}}$

=61.70% (95% CI 47.29-74.70)

Specificity=d/d+b 95% CI = d/d+b ± 1.96 $\sqrt{\frac{(d/b+d)(b/b+d)}{b+d}}$

=96.77% (95% CI92.99-98.81)

Positive predictive value =a/a+b 95% CI = a/a+b ± 1.96 $\sqrt{\frac{(a/a+b)(b/a+b)}{a+b}}$

=85.3% (95% CI 70.37-94.41),

Negative predictive value =d/d+c 95% CI=d/d+c ± 1.96 $\sqrt{\frac{(d/d+c)(c/d+c)}{d+c}}$

=89.29% (95% CI 83.91-93.32)

accuracy = $\frac{a+d}{a+b+c+d}$ 95%

CI= $\frac{a+d}{a+b+c+d} \pm 1.96 \sqrt{\frac{(a+d/a+b+c+d)(b+c/a+b+c+d)}{a+b+c+d}}$

=88.61% (95% CI 83.66-92.46)

Likelihood ratio for positive=(LR^+) = $\frac{a/a+c}{b/b+d}$ = 19.12 (95%CI 7.91-46.19)

Likelihood ratio for negative test=(LR^-) = $\frac{d/d+b}{a/a+c}$ = 1.56 (95%CI 1.08-2.27)

95% CI = $e^w - e^x$

w = $\log_e$ LR - [1.96 x SE ($\log_e$ LR)] x = $\log_e$ LR + [1.96 x SE ($\log_e$ LR)]

SE ($\log_e LR^+$) = $\sqrt{\frac{1}{a} - \frac{1}{a+c} + \frac{1}{b} - \frac{1}{b+d}}$ SE ($\log_e LR^-$) = $\sqrt{\frac{1}{d} - \frac{1}{b+d} + \frac{1}{c} - \frac{1}{a+c}}$

CHAPTER V

DISCUSSION

Nowadays, there are numerous invasive and noninvasive tests available for the diagnosis of H. pylori infection .When endoscopy is clinically indicated. Nevertheless, it has been recommended that invasive techniques should be used. In clinical practice, many endoscopists prefer one certain invasive method of diagnosis, i.e. the rapid urease test (RUTs). The RUTs gives a relatively quick diagnosis, and is less expensive than other invasive diagnostic techniques (histology, microbiology). Urea breath test yield 90% sensitivity and 96% specificity *(44)*. However, the use of breath test is limited by the high cost of equipment, delayed result reading, and exposure to radioactive emissions (C^{14} urea breath test). Single serological test for anti-helicobacter immuno globulin G anti bodies may produce false positive results when test soon after eradication and may be unreliable in the elderly and in those with suppressed immune function *(45)*.

The search for an ideal test to diagnose H. pylori infection has met with only partial success until recently. it has been reported that H. pylori antigen can be measured in

human stool with an enzyme immune assay (EIA). This method may prove to be a valuable non-invasive diagnostic tool *(46)*.However, there were many tests to diagnose H. pylori infection, some false positive and false negative results were also found. Until now, there was no real gold standard to diagnose H. pylori infection. Theoretical ,culture should be 100% specific if the procedure was performed properly. Use of culture alone as the gold standard may yield false negative results due to inherent difficulties of culture. Combination of different tests may reduce false positive as well as false negative results and may be employed as gold standard in many studies. As shown in study of Laheij et al *(47)* (Table 7)

Table 7

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) percentages for culture, histology and CLO test as a single test, and in combination

	Sensitivity (%)	Sensitivity (%)	PPV (%)	NPV (%)
Culture	91.4	96.3	94.2	94.4
Histology	90.3	97.8	96.4	93.8
CLO test	94.9	96.7	95.0	96.6
Culture or histology	99.2	94.2	91.9	99.4
Culture of CLO test	99.6	93.1	90.6	99.7
Histology or CLO test	99.5	94.6	92.4	99.7

	Sensitivity (%)	Sensitivity (%)	PPV (%)	NPV (%)
Culture and histology	82.5	99.9	99.9	89.5
Culture and CLO test	86.7	99.9	99.8	91.9
Histology and CLO test	85.7	99.9	99.9	91.3
Concordance between 2 of the 3 tests	98.3	99.7	99.6	98.8

The previous rapid urease test (CLO test) had some disadvantages due to the need of incubation period up to 24 hours of refrigeration for storage and to be warmed to room temperature before use. On the contrary ,the Pronto Dry test, new urease test, can be kept at room temperature and interpreted within 1 hour. It is therefore, convenient for patients to receive treatment before leaving endoscopic room.

This study clearly showed that prevalence of H. pylori infection when using urease test (Pronto Dry) together with immunohistochemistry as gold standard was 23%. This figure was less than those of many studies that the prevalence varied from 31% to 71.7% *(48, 49)*, that because of difference in tests of diagnosis and the prevalence that varied in each geographic region,and this figure was also less than the previous study by Arnantapunpong *(43)* that we used to calculate the sample size.In that study , only CLO test was used to

determine prevalence. When new Pronto Dry test was interpreted as same as CLO test at 24 hours, the prevalence would be 59.7%. H. pylori infection was found as 22.5% in gastritis, duodenitis GU, PU,and was found as 25% in those cases that organic cause was not found that classified as functional dyspepsia. The reported prevalence of H. pylori patient with functional dyspepsia range from 39% *(50)* to 87% *(51)* .Nevertheless, the pathogenesis of functional dyspepsia is unknown. It remained unclear whether H. pylori infection actually caused symptoms or it was just an associated finding .

The present study excluded the cases of GI Bleeding because many studies showed that the prevalence of H. pylori in patients with GI bleeding was less than that in patients with uncomplicated ulcer *(52)*.The other reason was that acid suppressing agents were known to show anti-urease activity to be associated with the reduction in the density of bacteria. According to environmental and cost saving problems some investigator tried to bring previous used urease test (CIO test) back to use again if no reaction or color change in the previous used one, especially in the developing country. The promising result was demonstrated with high sensitivity and specificity for re-used CIO test. There was no study confirm the promising result in re-used Pronto Dry test. However, the same principal chemical substances were found in these two urease tests with some differences in preservatives or antibiotics might result in different outcomes when reused Pronto Dry test

employed. This study was hence aimed to evaluate whether the reused Pronto Dry Test is appropriate to be brought back once again.

The sensitivity, specificity of re-used Pronto Dry test in this study was 61.70% (95% CI 47.29-74.70), 96.77% (95% CI92.99-98.81), respectively,compared with the previous study reported by Chialong Lee et al. *(4),* which found that re-used ClO test could be used to diagnose H. pylori infection with 98.6% sensitivity and 98.2% specificity. Moreover, in such study, considering the concordance results that obtained from 312 in 317 cases; 204 pairs were positive and another 102 pairs were negative. While in this study concordance results showed 34 pairs as positive and 142 pairs as negative. This difference may result from 1)Difference in reference gold standard .In Lee C*(4)* study, only CLO test was used as gold standard 2) The interval to final use of urease test that can be brought back to use again was 2-15 days. However , in this study the time interval detected was ranging from 7 days-to 6months. When the re-used test was kept for a long time, it could cause chemical change in test kit and consequently delay chemical reaction time of the test.

Although re-used Pronto Dry test showed high specificity , the new test had higher sensitivity, specificity and its cost is only 200-300 baht/test. Therefore, importantly ,the question is" Is it worth using the re-used Pronto Dry test?". Cost- effectiveness study was required to answer this concern. Based on the results of the present study, the kappa agreement of new against re-used Pronto Dry test at 1 hour was 0.63 (95% CI 0.51-0.74),

that indicated intermediate agreement. Therefore it is not appropriate to recommend to replace the new Pronto Dry test with the re-used Pronto Dry test.

In addition to 61% sensitivity of the re-used Pronto Dry test for diagnosis H.pylori infection , it is not appropriate to use in general diagnostic test for screening. Explanation of some false negative results and the intermediate kappa agreement were:

1)Less media were left for implantation tissue specimen in to test kit because the previous tissue had not been removed properly. So chemical reaction did not occur in the test kit.

2) H.Pylori did not uniformly colonize the lining of the stomach and varied in density.

3) Problem association with histological examination, the results depended on the competence of pathologist and the validity of staining technique.

This study, we extended the interpretation the results of Pronto Dry test up to 24 hours in order to compare with the results of Pronto Dry test at one hour. We found that kappa agreement at 24 hours was 0.65 (95% CI 0.55-0.74),that did not show obvious difference from the kappa agreement of this test at one hour and still indicated intermediate agreement .

Bacterial contamination such as Proteus or Pseudomonas that may produce urease even at low level and the presence of Helicobacter heilmannii (H. heilmannii) (formerly known as Gastro spirillum haminis) which is found in up to 0.2% of patients undergoing gastroscopy *(53)* could play a major role in false positive results in this study.

CHAPTER VI

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Re-used Pronto Dry test is not appropriate to use again because of the intermediate sensitivity even high specificity. Overall prevalence of H. pylori infection detected by urease test together with immunohistochemistry in this study is 23%. The prevalence of functional dyspepsia is 7.9%.

6.2 Recommendations

1)Re-used Pronto Dry should not recommended to be used in screening for diagnosis H. pylori infection.

2)Prevalence of H.pylori infection should be reported and specified in details what method of detection.

3). Further research should focus on appropriate time interval to keep the re-used Pronto Dry test from the time final use that could be brought back to use again with higher sensitivity, specificity, and accuracy.

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APPENDICES

APPENDIX A

Selection of patients

Each patients must be fulfill of the following criteria before enter to this study.

	Yes	No
1) Age 15-75	☐	☐
2) Patients with dyspepsia and have any one of the following:	☐	☐
2.1 New onset dyspepsia in individuals over age 50 years old.	☐	☐
2.2 Dyspepsia associated with dysphagia and/or weight loss.	☐	☐
2.3 Those who have not responded to an appropriate trial of empiric therapy.	☐	☐
2.4 Those patients using NSAIDS or other ulcerogenic agents.	☐	☐
2.5 Those with signs or symptoms of UGI tract obstruction.	☐	☐
2.6 Those whose ethnic and/or racial background is associated with increased risk for UGI malignancies or other significant disease states.	☐	☐
2.7 Patients with dyspepsia in whom symptoms recur after therapy of H.pylori is completed.	☐	☐

If patients have any one of the following criteria will be excluded from this study.

	Yes	No
1) Patients recently taken antibiotic, bismuth, proton pump inhibitor within 2 weeks.	☐	☐
2) Pregnancy.	☐	☐
3) GI bleeding.	☐	☐
4) Refuse to participate or continue study.	☐	☐
5) Poor medical condition could not tolerate endoscopic procedure.	☐	☐

APPENDIX B

Case record form

Topic: Diagnosis Helicobacter pylori by re-used Pronto Dry test

Investigator: Dr. Somboon Subwongcharoen MD. Contact 02-2456078

ID no……

Date of entry……

Patient name……HN……☐Ward…....☐ OPD

Address……

Contact number……

Base line characteristics

1) age……
2) sex ☐male ☐ female

Variable outcomes

1) provisional diagnosis……..
2) results of ultrasound upper abdomen
   ☐ gall stone ☐ chronic pancreatitis ☐ gall stone with CBD stone ☐ liver mass
3) gastro scope findings
   ☐ gastritis ☐duodenitis ☐GU ☐ DU ☐Ca stomach ☐esophageal varices
   ☐ gastric varices ☐ others……
4) pathological reports
   ☐ gastritis ☐ duodenitis ☐ GU ☐ DU ☐ Ca stomach ☐ esophageal varices
   ☐ gastric varices ☐others……
5) if this is not the first time of gastroscope, what is the previous H. pylori test result?
   ☐ positive ☐ negative ☐ not done

Main outcomes

1) result of detection H. pylori by re-used Pronto Dry
   ☐ positive ☐ negative
2) result of detection H.pylori by new Pronto Dry
   ☐ positive ☐ negative
3) result of histology with immunohistochemistry
   ☐ positive ☐ negative

Adverse events

1) adverse events occur

☐ yes ☐ no

2) details of adverse events ……

……..……..

APPENDIX C

ข้อมูลผู้ป่วย เพื่อการพิจารณาตัดสินใจเข้าร่วมในโครงการการศึกษาวิจัย

การศึกษา การตรวจเชื้อ H.pylori ด้วยชุดตรวจ Pronto Dry ที่เคยใช้แล้วมาใช้ตรวจซ้ำ

บทนำ

อาการจุกเสียดแน่น บริเวณลิ้นปี่เป็นอาการที่พบได้บ่อยในคนไข้ที่มาพบแพทย์ สาเหตุสำคัญที่เป็นต้นเหตุ อาจเกิดแผลในกระเพาะอาหาร กระเพาะอาหารอักเสบ ลำไส้เล็กส่วนต้นอักเสบ กรดไหลย้อนกลับทำให้หลอดอาหารอักเสบ เนื้องอกกระเพาะอาหาร นิ่วในถุงน้ำดี การวินิจฉัยแยกโรคส่วนหนึ่งสามารถทำได้จากการซักประวัติ และตรวจร่างกาย หรือการให้ยาลดการหลั่งกรดไปรับประทานดูสักระยะหนึ่ง ในบางครั้งจำเป็นที่จะต้องได้รับการตรวจพิเศษ เช่น อัลตร้าซาวด์ กลืนแป้งและเอ็กซเรย์ หรือการส่องกล้องตรวจทางเดินอาหาร การพิจารณาชนิดการตรวจพิเศษขึ้นอยู่กับแพทย์ผู้รักษา ในรายที่แพทย์ผู้รักษาพิจารณาว่าผู้ป่วยจำเป็นต้องได้รับการส่องตรวจด้วยกล้องตรวจทางเดินอาหาร ซึ่งส่วนใหญ่ต้องมีข้อบ่งชี้ดังนี้

1. จุกแน่น อายุมากกว่า 50 ปี
2. ให้ยารักษาด้วยยาลดกรดไม่ดีขึ้น
3. อาการจุกแน่นร่วมกับน้ำหนักลด กลืนลำบาก
4. ใช้ยากลุ่ม NSAID รักษาโรคปวดข้อปวดกระดูกร่วมอยู่
5. มีแนวโน้มทางเชื้อชาติ หรือกรรมพันธุ์สัมพันธ์กับมะเร็งทางเดินอาหาร
6. คนไข้ที่กลับมาเป็นใหม่หลังจากให้การรักษาเชื้อ H. pylori แล้ว

ในปัจจุบันมีการศึกษายืนยันแล้วว่า เชื้อแบคทีเรียในกระเพาะอาหารที่เรียก Helicobacter pylori เป็นสาเหตุสำคัญที่ทำให้เกิดอาการแน่นจุก (dyspepsia),แผลในการเพาะอาหาร, กระเพาะอาหารอักเสบ, ลำไส้เล็กส่วนต้นอักเสบ,มะเร็งกระเพาะอาหาร ส่วนใหญ่ผู้ป่วยที่ได้รับการส่องตรวจทางเดินอาหาร จึงมักได้รับการตรวจเชื้อแบคทีเรีย H. pylori โดยการตัดชิ้นเนื้อเล็กๆ ขนาด 2-3 mm 2-3 ชิ้น ใส่ลงในชุดตรวจที่เรียกว่า Pronto Dry ไปด้วยในขณะเดียวกันซึ่งสามารถอ่านผลได้ใน 1 ชม. ซึ่งหากพบว่ามีเชื้อ Helicobacter pylori แพทย์ก็จะให้ยาฆ่าเชื้อนี้ ซึ่งจะทำให้อาการต่าง ๆ เหล่านี้หายได้

รายละเอียดการศึกษาวิจัย

การศึกษานี้เป็นการศึกษาชุดตรวจสำเร็จรูป Pronto Dry ที่เคยใช้ไปแล้วว่าจะสามารถนำกลับมาใช้ใหม่ โดยมีความไวและความจำเพาะไม่น้อยกว่า 90% เปรียบเทียบกับชุดตรวจ Pronto Dry ที่ยังไม่เคยใช้ (ของใหม่) และเทียบกับผลพิสูจน์ทางชิ้นเนื้อโดยการย้อมพิเศษ โดยผู้ป่วยที่เข้าสู่การศึกษาวิจัยครั้งนี้ เป็นผู้ป่วยที่จำเป็นต้องได้รับการตรวจวินิจฉัยส่องกล้องทางเดินอาหารตามข้อพิจารณาของแพทย์ผู้รักษาอยู่แล้ว โดยผู้ป่วยที่เข้าร่วมการศึกษาทั้งหมด จะได้รับการส่องตรวจตามขั้นตอน วิธีการ เหมือนผู้ป่วยที่ต้องส่องกล้องตรวจที่ไม่เข้าโครงการวิจัยนี้ทุกอย่าง ยกเว้นเพียงแต่ชิ้นเนื้อที่ตัดออกมาเพิ่มอีก 1-2 ชิ้น โดยทั้งหมดจะถูกแบ่งเป็น 3 ส่วน ดังนี้

(1) ตรวจกับชุดตรวจ Pronto Dry ที่เคยใช้แล้ว

(2) ตรวจกับชุดตรวจ Pronto Dry อันใหม่

(3) ส่งตรวจเนื้อเยื่อโดยวิธีย้อมพิเศษ ซึ่งจะแตกต่างกับผู้ไม่ร่วมโครงการวิจัยนี้ คือชิ้นเนื้อทั้งหมดจะส่งตรวจกับชุดตรวจ Pronto Dry ของใหม่เท่านั้น

การประเมินผล

- การประเมินผลชุดตรวจจะทำใน 1 ชั่วโมง หลังชิ้นเนื้อสัมผัสกับชุดตรวจ ทั้งชุดตรวจของใหม่ และชุดตรวจที่เคยใช้แล้วนำกลับมาใช้ใหม่ ส่วนผลตรวจทางเนื้อเยื่อโดยวิธีย้อมพิเศษ จะใช้เวลาประมาณ 7 วัน
- การให้การรักษาจะยึดตามผลของชุดตรวจ Pronto Dry ของใหม่ และผลทางเนื้อเยื่อเป็นหลักในการให้การรักษาผู้ป่วยต่อจากนั้น
- ผลข้างเคียงที่เกิดขึ้นจากการทำวิจัยนี้ ไม่แตกต่างกับกลุ่มผู้ป่วยที่ไม่เข้าร่วมโครงการวิจัย เพราะขั้นตอนวิธีการ ส่องกล้อง เหมือนกัน ส่วนการแบ่งชิ้นเนื้อ เป็นส่วนๆ ทำภายหลังจากได้ชิ้นเนื้อออกมาแล้ว ทำภายนอกตัวผู้ป่วย ซึ่งอย่างไรก็ตามผลข้างเคียงที่อาจเกิดขึ้นจะได้รับการเฝ้าระวังและแก้ไขทันที ถ้าเกิดมีปัญหา

ประโยชน์ที่ได้รับ

การยืนยันผลการตรวจเชื้อ H. pylori โดยใช้ ชุด Pronto Dry ชุดใหม่ร่วมกับผลทาง

ชิ้นเนื้อเยื่อโดยวิธีย้อมพิเศษ (โดยปกติไม่ได้ส่งตรวจชิ้นเนื้อเยื่อโดยวิธีย้อมพิเศษ) จะให้ความแม่นยำสูงมาก ในการวินิจฉัยเชื้อ H. pylori ทำให้ท่านได้รับการตรวจวินิจฉัย และรักษาได้ถูกต้องมากกว่า โดยท่านไม่ต้องเสียค่าใช้จ่ายจากการยืนยันการตรวจชิ้นเนื้อเยื่อโดยวิธีย้อมพิเศษนี้

ข้อมูลที่ได้รับ

จะเป็นประโยชน์อย่างยิ่งกับประเทศชาติในการหมุนเวียนทรัพยากรกลับมาใช้ใหม่ โดยนำชุด Pronto Dry ที่เคยถูกใช้ตรวจไปแล้วกลับมาใช้ใหม่ในกรณีที่ผลการวิจัยยืนยันว่าใช้ได้ดีมีความแม่นยำสูง

จำนวนผู้เข้าร่วมโครงการ

ผู้เข้าร่วมโครงการ 202 ราย

คุณสมบัติผู้เข้าร่วมโครงการ

- อายุ 15-85 ปี ไม่จำกัดเพศ
- ไม่ได้ตั้งครรภ์
- จุกแน่นห้อง มีข้อบ่งชี้ที่จำเป็นต้องได้รับการตรวจโดยการส่องกล้องทางเดินอาหารส่วนต้น ในแผนกศัลยกรรม โรงพยาบาลราชวิถี
- ไม่ได้มีภาวะทางอายุรกรรม ซึ่งการส่องกล้องตรวจไปทำให้เกิดภาวะเครียดหรือชักนำให้ภาวะโรคประจำตัวมีอาการหนักขึ้น
- ไม่มีภาวะเลือดออกในทางเดินอาหาร
- ไม่ได้รับยากลุ่ม Bismuth ปฏิชีวนะ proton pump inhibitor ภายใน 2 สัปดาห์

การรักษา ความลับ

ผลการศึกษานี้จะใช้สำหรับวัตถุประสงค์ทางวิชาการเท่านั้น ข้อมูลต่าง ๆ เกี่ยวกับตัวท่านจะถูกเก็บเป็นความลับ การตีพิมพ์เผยแพร่ผลงานทางวิชาการจะไม่มีการระบุรายชื่อผู้ร่วมโครงการ คณะกรรมการจริยธรรมของการศึกษานี้และหน่วยงานที่รับผิดชอบอาจทบทวนบันทึกข้อมูลทางการแพทย์ของท่าน โดยมีวัตถุประสงค์เพื่อตรวจดูความถูกต้องของข้อมูลและวิธีการต่าง ๆ ของการศึกษาวิจัย และข้อมูลทางการแพทย์ของท่านอาจถูกนำไปจัดเก็บไว้ในคอมพิวเตอร์

จากการลงนามในเอกสารยินยอมเข้าร่วมโครงการวิจัย

ท่านอนุญาตให้ดูแลบันทึก เก็บข้อมูล และโอนย้ายข้อมูลดังกล่าวข้างต้นได้

สิทธิผู้ป่วย

การเข้าร่วมการศึกษานี้เป็นไปโดยสมัครใจ ท่านอาจปฏิเสธที่จะเข้าร่วมโครงการ หรือถอนตัวจากการศึกษาได้ตลอดเวลา โดยไม่กระทบต่อการดูแลรักษาที่จะได้รับจากแพทย์

การลงนาม

เพื่อเข้าร่วมโครงการศึกษาวิจัย ท่านหรือผู้แทนโดยชอบด้วยกฎหมายต้องลงนามพร้อมวันที่ในใบยินยอมเข้าร่วมโครงการวิจัยที่แนบด้วยกันนี้ หากท่านมีปัญหา หรือข้อสงสัยกรุณาติดต่อนายแพทย์สมบูรณ์ ทรัพย์วงศ์เจริญ กลุ่มงานศัลยศาสตร์ ชั้น 11 ตึกสิรินธร โรงพยาบาลราชวิถี 01-618 4873

ใบยินยอมเข้าร่วมการศึกษาวิจัย (Consent form)

เลขที่คนไข้..... ชื่อและนามสกุล.....

ข้าพเจ้าได้รับทราบจากแพทย์ผู้รักษา ซึ่งได้ลงนามด้านท้ายของหนังสือนี้ถึงวัตถุประสงค์ ลักษณะ และแนวทางการศึกษาเรื่องการตรวจเชื้อ H. pylori ด้วยชุดตรวจ Pronto Dry ที่เคยใช้แล้วนำกลับมาใช้ใหม่ รวมทั้งทราบถึงผลดีที่จะได้รับผลข้างเคียง และความเสี่ยงที่อาจเกิดขึ้น ข้าพเจ้าได้ซักถาม ทำความเข้าใจเกี่ยวกับการศึกษาดังกล่าวนี้ เป็นที่เรียบร้อยแล้ว

ข้าพเจ้ายินดีเข้าร่วมการศึกษาวิจัยนี้โดยสมัครใจ และอาจถอนตัวจากการเข้าร่วมการศึกษานี้เมื่อใดก็ได้ โดยไม่จำเป็นต้องแจ้งเหตุผล และยอมรับสิ่งไม่พึงประสงค์ที่อาจเกิดขึ้น และจะปฏิบัติตัวตามคำแนะนำของแพทย์ทุกประการ

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็น ด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้ายินดีให้ข้อมูลของข้าพเจ้าแก่คณะแพทย์ผู้รักษา เพื่อประโยชน์ในการศึกษาวิจัยครั้งนี้

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้วและมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

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(ชื่อผู้ป่วย)	(ลายเซ็น)	(วันที่)
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(ชื่อแพทย์)	(ลายเซ็น)	(วันที่)

VITAE

Mr. Somboon Subwongcharoen was born on August 15th, 1962 in Bangkok, Thailand. He graduated as medical doctor from the faculty of medicine Siriraj Hospital, Mahidol University in 1987. After he graduated, he worked as surgeon in Chaingrai provincial hospital from 1987-1990. He took Thai board of general surgeon in 1993 after he completed resident training in department of surgery Rajavithi Hospital. He undertook fellowship in liver transplantation at Austin Hospital Australia from 1994-1995. Now he is working as general surgeon, hepatobiliary and liver transplant consultant in department of surgery Rajavithi Hospital.