Non-Invasive

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Non-invasive *Helicobacter pylori* diagnostic methods in Indonesia

Running head: Non-invasive *H. pylori* test in Indonesia

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Abstract

Although the prevalence of *Helicobacter pylori* infection in Indonesia is relatively lower compared to other countries, *H. pylori* is still an important pathogen associated with severe gastric mucosal damage and dyspeptic symptoms. Invasive is not an ideal method due to the lack of endoscopic center and remain expensive without full covering by social incurance. Among non-invasive methods, urea breath test (UBT) is widely available in indonesia, suggesting become the primary option especially to ensure successful *H. pylori* eradication. However, there was no local validation both for ^{13}C- and ^{14}C-UBT. Although for some experts, stool antigen test (SAT) is cheaper and suitable for use in active infections before and after eradication; the custom and habits are two obstacles for delivering the stool with on time and fresh. Additionally, only polyclonal antibodies and qualitative SAT kit are available with a low sensitivity. Serology is a widely validated method and shows a good accuracy although cannot distinguish the active or inactive infection. In our observation, its also as the main choice of experts and patients due to simple, inexpensive and widely known. Urine test is alternative for saving costs and reduce endoscopic workload and have a high accuracy although lower sensitivity than other countries. Next studies are necessary to prove its validity to be used throughout Indonesia especially in areas with low prevalence of *H. pylori* infection. Finally, a validated UBT and SAT are considered be a non-invasive practical approaches for detection of *H. pylori* infection in Indonesia with serology and urine test as an alternative strategy.

Keywords: Non-invasive, *Helicobacter pylori*, Urea Breath Test, Stool Antigen Test, Serology

Introduction

Some invasive diagnostic methods such as rapid urease test, histopathology and culture have developed to detect *H. pylori*, a gram negative bacteria which are the primary cause of chronic gastritis, gastric atrophy and gastric cancer [1]. The accuracy of invasive test is sufficient and commonly use in daily practice. However, due to an inexpensive, simple, convenient, and user friendly; the indirect tests, such as urea breath test (UBT), stool antigen test (SAT) and serology have been introduced to diagnose *H. pylori* infection [2].

Indonesia is a country consists of 18,108 islands inhabited 267,842,292 people thus include as the fourth largest population of the world. The prevalence of *H. pylori* infection in Indonesia is relatively low which is 22.1% [3] compared to neighbors countries such as Malaysia, Thailand and Philippines (24.3 to 49%, 54.1 to 76.1% and 60 %, respectively) [4-6]. Water sources, age, religion are risk factors for *H. pylori* infection in several ethnic groups in Indonesia [3]. The East-Asian-type-*cagA* with 6-bp deletion type and EPIYT motif, high proportion of m2, *dupA* negative or short type *dupA*, and double positive of *jhp0562/β-(1,3)galT* are the predominant virulence factors which may associated with less gastric cancer incidence [7]. We also found the complete integrating conjugative elements TFSS 4b type was less predominant in and tended to have higher severity of gastric mucosa [8]. The prevalence of metronidazole and levofloxacin resistance strains is high but the resistance of amoxicillin and tetracycline is low in Indonesia. We suggested that in some regions in Indonesia; clarithromycin- or metronidazole-based triple therapy needs to be carefully considered for eradicating *H. pylori* [9]. To counter a high metronidazole and clarithromycin resistance rates, furazolidone-, rifabutin-, and sitafloxacin-based therapies might become an alternative regimens, whilst sitafloxacin should be considered for eradication of levofloxacin-resistant strains [10].

Importantly, dyspepsia was sixth and fifth of the 10 most prevalent outpatient and

inpatient diseases in Indonesia, respectively [11]. In addition, a relative high prevalence of GERD was found in an area with low prevalence of *H. pylori* infection of Indonesia with several risk factors including smoking, history of proton-pump inhibitor use, and higher economic group [12]. However GI endoscopist in Indonesia is still limited, in 2013 between 252 million population, there were only 515 GI endoscopist (ratio 1:489 320) that had accredited competencies [11, 13]. Those number were lacking compared to USA or England with ratio 1:37 037 and 1:49 000, respectively [11]. Moreover, hospitals that are able to provide GI endoscopy services are also limited, out of 33 provinces in Indonesia there are only 313 hospitals, most of which provide services in Java Island [11]. Therefore, the utility on invasive diagnosis in Indonesia had many obstacles due to the availability endoscopy. In this review, we summarized the current condition of non-invasive diagnosis in Indonesia and proposed some recommendation.

UBT

H. pylori has the ability to produce high active urease in the stomach during infection, an enzyme that converts urea to ammonium and labeled CO2 [14, 15]. In this test, isotope labeled urea will be eaten by the patient, then *H. pylori* produces a breakdown of urease enzyme product in the stomach [16]. The labeled CO2 will diffuses into epithelial cells, after passing a reaction that is catalyzed by the reaction, then be absorbed in the blood following the bloodstream and excreted through the lungs [14]. Finally, it is detected and recorded when in exerted through the lungs and exhaled breath after 10 min and can be measured as an indicator the presence or absence of *H. pylori*, suggesting UBT can detect current infection [16, 17]. With the sensitivity and specificity more than 90%, UBT is the best non-invasive methods although a less reliable for patient with history gastric resection or PPI consumption [14].

Two methods were used for labelling the urea including a stable heavy isotope ^{13}C and the radioactive isotope ^{14}C [18]. The ^{13}C-UBT is non-invasive, accurate however relatively expensive due to a requirement of mass spectrometric analysis which may remains restricted in large cities. In pediatrics and pregnancy, ^{13}C is also safer because it is not contain radiation hazards. In fact, across 34 province of Indonesia, only a total 10 of ^{13}C-UBT was available in Indonesia in the four main cities; 3 centers in Jakarta and 2 centers in Surabaya of Java Island, 3 centers in Medan of sumatera Island, and 2 centers in Makassar of Sulawesi Island. In addition of limited resources, all of the cost is lack of regular reimbursement by Indonesian social insurance. With the cost IDR 1,200,000 (USD 85 estimated July 2019), this method is relatively expensive, and might can not become a common method to use for *H. pylori* detection. Recently, ^{13}C-UBT could be performed using a more simple infrared spectrophotometer because that is more compact, which is less expensive and easier use than mass spectrometry [12]. Practically, most of Indonesian gastroenterologist used this method to evaluate *H. pylori* positivity after eradication beside of SAT [19, 20]. According to the Asia-Pasific consensus [21] to improve the accuracy of the test, stop taking medications such as bismuth salts or antibiotics for 4 weeks and PPI for 2 weeks, and fasted for a minimum of 4 hours [18, 21]. For most of patients, these preparations are not convenience especially if they had a severe symptoms. Because UBiT®-IR300 infrared spectrophotometers recently are not available, most of the Indonesian centers used a new version of infrared spectral analyzer (POCone FT-IR®, Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan) which was claimed more simple, easy maintenance, faster, and accurate. We also used 75 mg, not 100 mg tablet of ^{13}C-urea as previously described [22]. In contrast with a recommendation of gurgling to avoid catalytic positive bacteria in the oral cavity and oropharynx [23], the utility of a film-coated tablet-based UBT (UBIT, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) are given without gargling.

The validation of United States [16] and Europe [24] suggested a lower dose of ^{13}C-UBT (75-125 mg) than the original (350 mg) but not lower than 75 to avoid poor results [25]. Ho, a dose of 50 mg in children was found in several studies that have been used to diagnose of *H. pylori* infection [22, 26]. At the same dose of ^{13}C-urea, low production of endogenous CO2 in younger children has a relatively high isotope ratio of ^{13}CO2/^{12}CO2 [26]. However, in Indonesia we use a similar dose among adult and children. From beginning we also do not administering free of citric acid based on the manufacture instruction. Previous study suggested an additional citric acid to increase sensitivity and specificity [27], especially with the long term of PPI utility. In addition, when citric acid pre-treatment was not included it will decreased the accuracy [28]. We also do not have a data about modification of lateral recombinant position for patients with partial gastrectomy [44]. Collected breath samples were analyzed with a ^{13}C-UBT with cut-off value 2.5% as recommended by the manufacturer. Unfortunately, the cut-off has not been validated for both adults and children and we are struggling to actualize it. The calculated optimal cut-off points of UBT are important in populations that have a low prevalence because they are able to express higher delta over baseline (DOB) value (e.g., healthy volunteers). In contrast, dyspepsia patients whom the prevalence of infection is higher than normal population, low DOB values must be considered [29].

The ^{14}C is not usually recommended during pregnancy due to little risk of radiation hazards [18], although it has been published that in children with ^{14}C-UBT, a lower radiation can be used safely [26]. In Indonesia, a total 16 centers have ^{14}C-UBT including 9 centers in North Sumatera of Sumatera Island; 1 center in Jakarta, 2 centers in West Java, 2 centers in East Java and 1 center in Yogyakarta of Java Island, and 1 center in Bali Island. All the centers using HUBT-20A1 analyzer (Headway, Shenzhen, China) from similar company with ^{14}C-urea capsule contain 27.8 kilo-becquerel (kBq). We used cut-off points 50% to

discriminate *H. pylori* infection as manufacture instruction. Recently, we are validating the ^{14}C-UBT in difference rate of *H. pylori* infection. In the area with a lack endoscopy such as Indonesia, UBT could significantly reduce the number of endoscopies associated with costs to the health-care system and distress to patient caused by discomfort and travel. The modifications of 14C-urea dose and breath-collection times may solve problem of ^{14}C-UBT utility among pregnancy and children, but it is still not accepted in Indonesia.

SAT

SAT is noninvasive, inexpensive and do not show age dependence for the diagnostic accuracy [30]. Immunoassay enzyme (EIA) based on polyclonal antibodies is the initial method of SAT and demonstrated a high accuracy [31]. However, most of the results were inconsistent and the application of a monoclonal antibody-based approach was developed which have been shown to be able reduce false-positive findings and increase specificity [32]. The pre-treatment monoclonal antigen technique was better than the polyclonal technique with a sensitivity of 96% vs. 90%, specificity of 97% vs. 94%, positive predictive value of 96% vs. 91% and negative predictive value of 97% vs. 85%, respectively [33]. The 4-8 weeks anti-secretory therapy also showed that monoclonal antigen was better than polyclonal [33, 34]. In Indonesia, SAT is not require expensive special equipment and chemicals and will be cheaper compared to UBT, thus widely use troughout the country with the cost IDR 300,000 (USD 20 estimated July 2019). In addition, SAT does not require fasting, and with novel monoclonal antibodies, it is not require discontinuation of PPI [35].

Enzyme immunoassay (EIA) and immunochromatographic (ICA) are both SAT methods. EIA has better accuracy than ICA even though the latter uses monoclonal antibodies [36, 37]. EIA-based such as a commercial kit Primier platinum HpSA (Meridian Diagnostic, Cincinnati, OH, USA) may applicable in Indonesia. The composition of the

sample was mixed with 200 µL of the sample diluents. One drop of enzyme conjugates was added to the microwells, which were incubated for 1 hours at room temperature and washed five times. The results will be read by spectrophotometry after one drop of the stop solution to end the reaction. Manufacturer's recommendations assume a positive result if absorbance (450/630) ≥0.160 [38]. A value of 0.300 is reported provide the best diagnostic value with sensitivity, specificity and accuracy were 93.9%, 95.7% and 94.8% respectively and a a cut-off value of 0.130 was a lesser sensitivity (89.5%) and specificity (83.3%) [39, 40]. However most of commercial lab in Indonesia are not interested to use the method due to an increasing cost, thus reducing potensial profit.

ICA-based has the advantage of being able to rapid diagnoses of *H. pylori*. Thus, ICA may useful in developing countries with many remote areas such as Indonesia. A proper accuracy of ICA-based SAT can be in stock in many hospitals in Indonesia, thus the examination can be carried out in small laboratory considering this test does not require special equipment and special experts. It was suggested in the first time applied in clinical practice, an acceptable number was revealed with a sensitivity of 88% and a specificity of 94% [41]. Most of our center and commercial laboratory use a rapid SAT methods using monoclonal antibodies based on lateral flow ICA which were recently developed such as On-Site *H. pylori* Ag Rapid Test-cassette (CTK Biotech Inc., San Diego, CA, USA) [18, 32]. In daily practice is very suitable because this test has more practical steps [26]. As the manufacture instruction, Feces was taken as much as 5-10 cc for antigen test examination. The device contains an antibody to *H. pylori*, if stool *H. pylori* antigen, a reaction between the antigen-antibodies and the coloring agent will appear as a red (stem line) line in the instrument test zone. Antigen on specimen will be detected in 15 minutes. Result is reported positive if two red lines in the control zone (C) and test zone (T) were appeared, while it is reported negative if a visible red line in the control zone was appeared and it is invalid if

there is no red line that was appeared in the test zone or control while the control zone is not red. If the result is invalid, then the examination must be repeated using a new tape. Our study revealed a low diagnostic value with sensitivity, specificity, positive predictive value and negative predictive value were 38%, 94%, 55% and 88% respectively [42]. In addition the *H. pylori* strain used is different from that in Indonesia [42], several factors influence the results of SAT. Low antigens due to low colonization in the stool and low ability to react can produce false-negative results [20]. In a low prevalence of *H. pylori* country such as Indonesia, *H. pylori* density number was also low suggesting high risk of low sensitivity. Incubation time also has an important factor, the sensitivity of readings at 30 minutes and 60 can reach 76.9% and 78.6% respectively compared to 20 minutes reaching 59.1% [26]. Formless or watery stools can reduce accuracy due to diluted antigens [37]. If the sampel not tested in a short time (less than seven days), it needs to be keep stored at low temperatures (-5 to -25) °C. Stool samples that stored -80 °C for 225 days still have good sensitivity and specificity [37]. For Indonesian, collecting stools is more difficult than blood samples. They cannot predict well when the defecation time and the most may not feel comfortable for the delivery process.

We have to concern about *H. pylori* test accuracy. A validation study used Pronto Dry (Medical Instruments Corporation, Solothurn, Switzerland) at Cipto Mangunkusumo Hospital in Jakarta reported the sensitivity and specificity were only 66.7% and 78.9 %, respectively with 0.274 as a cut-off value [43]. In addition, among 54 (85.7%) of 63 dyspeptic patients were positive based on several methods, 42 were positive by only stool antigen test, which suggests the potential for false-positive results. Therefore local validation test is a very important factor, because differences in the antigenicity of *H. pylori* strains affect the result of SAT [32].

Serology

In general, detection of specific-antibody following the exposure to the various *H. pylori* antigens can be a useful method in clinical practices due to can be accepted by patients, cheap and fast [18]. An important study [44] reviewed 36 commercial kits used in 26,812 patients in different populations, the sensitivity range was 57% to 100 and the specificity was 31% to 100% [44]. Thus, a validated serology is useful as for initial screening especially in a country with lack of endoscopic centers, before histology or culture was confirmed [45], however it must be noted that test and treat strategy is not recommended in low prevalence of *H. pylori* area. Our group revealed that an ELISA kit (Eiken, Co. Ltd., Tokyo, Japan) had low sensitivity by using the cut-off value from the manufacturer's instructions (positive if ≥10 U/mL, sensitivity and specificity were 66.7% and 97.2%, respectively). Then, we suggested the best cut-off values of ≥5.5 U/mL to increase sensitivity become 86.7% [13]. The use of serology tests in screening dyspepsia patients can save costs and reduce endoscopic workload by up to 30% [33]. Nevertheless, serology test are not recommended for children since the problem of *H. pylori* specific antibodies level [30].

Antibody preparations for each kit are closely related to diagnostic accuracy [46]. The accuracy of kits made by eastern countries will be more accurate for detecting *H. pylori* strains in eastern countries compared to kits made in western countries. Tha accuracy of the diagnostic kits made from western countries was low when applied in the Japanese patients [47, 48]. It was reported in a study that comparing diagnostic accuracy of ELISA kits between western and eastern for detection the of IgG *H. pylori* in Japan, western ELISA kit the accuracy was 86.8 % and eastern ELISA kit the accuracy was 92.3 % [49]. Therefore, the use of antigens of local *H. pylori* strain will affect the success of serological tests in Indonesia.

Serological tests use blood samples to detect IgG antibodies through ELISA method. Similar like SAT, the accuracy of EIA-based serological tests is better than ICA-based. A study comparing 29 commercial serology tests, 17 EIA-based and 12 ICA-based tests showing the accuracy 9 of 17 EIA-based tests of more than 90% therefore, only 1 in 12 ICA-based tests that have an accuracy of more than 90% [2]. Assay immunoblot has better specificity, but sensitivity is worse than EIA, this method involves high expertise and special costs so it is not used in clinical laboratories [17]. ELISA is the most common method used in Indonesia. After *H. pylori* was successfully treated, *H. pylori* IgG antibodies will still last for several months [50]. In addition, serological tests could lead false-negatives. It may occur for new infection when the antibody levels are not sufficiently elevated because IgG antibody appears approximately 21 days after *H. pylori* infection [51]. Recently, we are validating an ICA-based kit (The MP Diagnostics ASSURE®, MP Biomedicals, USA) against with histopathology as golden standard. They proposed a recombinant current infection marker (CIM) as indication for current infection for covering the lackness of serology.

Urine Test

Several tests for detection *H.pylori* antibody using urine and saliva samples have shown high sensitivity and specificity [52-54]. The sampling method easily without special skills, tools and is cheaper than that of serum, however the concentration of *H. pylori* antibodies in saliva and urin are lower than in serum, which is a big problem [17]. False negative results can occur in urine-based ELISA *H.pylori* specific IgG has low concentration in the urine. A study in Indonesia, a commercial kit urine test (RAPIRUN® stick, Otsuka Pharmaceutical Co., Tokyo, Japan) to detect *H.pylori* antibodies in urine proved to be reliable for detecting *H. pylori* infection in Indonesia. [55]. Mixing 0.3 ml of fresh urine and 0.3 ml of dilute solution to make an approximately 2-fold dilution was the first step of the test and after that, standing

a test stick in the mixture of urine and dilute solution. A colloidal gold-conjugated anti-human IgG (Fc) polyclonal antibody (goat) was enclosed inside the test stick. *H. pylori* antigen was used to immobilized the the test line of evaluation section and the anti-human IgG polyclonal antibody was used control line [56]. If the two red bands appears on the test line after applying the sample within 15 min at 25 °C-30 °C it was considered positive. The sample was counted as negative when the red band showed on the control line only. Invalid result due to error in the assay steps or overly diluted urine was considered if the red band absent in the control line. RAPIRUN test validation result in Indonesia showed 83.3%, 94.7%, 71.4% 97.3% and 93.2% for sensitivity, specificity, positive predictive value, negative predictive, respectively. In Japan and Vietnam, it was also reported the use of urine rapid tests had sensitivity of 93.1%, specificity 92.3% and accuracy of 92.0% [14]. Our group also used RAPIRUN in minor ethnic groups in remote areas of North Sulawesi and found an identical results with serological test findings [57]. When urine test showed a positive result, we used the disposable gastric brush to obtain gastric juice and small gastric tissues for *H. pylori* culture. However in our experience, RAPIRUN showed a lesser accuracy in low prevalence area of *H. pylori* in Indonesia [58], and requires more time to interprete rather than manual instruction.

5. Conclusions

The use non-invasive *H pylori* testing in Indonesia may reduce overall endoscopic workload and financial savings generated for Indonesian social insurance. A validated UBT and SAT are considered be a practical approaches for detection of *H. pylori* infection in Indonesia with serology and urine test as an alternative strategy.

1 **Acknowledgements**

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