Identification of Helicobacter

by Muhammad Miftahussurur

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Identification of *Helicobacter pylori* infection in symptomatic patients in Surabaya, Indonesia, using five diagnostic tests

M. MIFTAHUSSURUR[1,2,3], S. SHIOTA[1], R. SUZUKI[1], M. MATSUDA[1], T. UCHIDA[4], Y. KIDO[1], F. KAWAMOTO[1], U. MAIMUNAH[2,3], P. ADI[2,3], Y. REZKITHA[3], NASRONUDIN[3], I. NUSI[2] AND Y. YAMAOKA[1,5]*

[1] *Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Yufu, Japan*
[2] *Gastroentero-Hepatology Division, Department of Internal Medicine, Airlangga University Faculty of Medicine, Surabaya, Indonesia*
[3] *Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia*
[4] *Department of Molecular Pathology, Oita University Faculty of Medicine, Yufu, Japan*
[5] *Department of Gastroenterology and Hepatology, Baylor College of Medicine and Michael DeBakey Veterans Affairs Medical Center, Houston, TX, USA*

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SUMMARY

The prevalence of *Helicobacter pylori* infection in Indonesia is controversial. We examined the *H. pylori* infection rate in 78 patients in a hospital in Surabaya using five different tests, including culture, histology, immunohistochemistry, rapid urease test, and urine antibody test. Furthermore, we analysed virulence factors in *H. pylori* strains from Indonesia. The *H. pylori* infection rate was only 11·5% in all patients studied, and 2·3% of Javanese patients and 18·0% of Chinese patients were infected ($P=0·01$). Although severe gastritis was not observed, activity and inflammation were significantly higher in patients positive for *H. pylori* than in patients negative for *H. pylori*. Among genotypes identified from five isolated strains, *cagA* was found in four; two were *vacA* s1m1. All *cagA*-positive strains were *oipA* 'on' and *iceA1* positive. We confirmed both a low *H. pylori* infection rate and a low prevalence of precancerous lesions in dyspeptic patients in a Surabaya hospital, which may contribute to the low incidence of gastric cancer in Indonesia.

Key words: *Helicobacter pylori*, Indonesia, virulence factors.

INTRODUCTION

Helicobacter pylori is a spiral-shaped, Gram-negative bacterium that establishes chronic colonization in the human stomach and is a causative pathogen of various gastroduodenal diseases, including gastritis, peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue lymphoma [1]. *H. pylori* infection generally results in chronic gastritis, but a small proportion of infected patients develop more severe diseases such as peptic ulcers and gastric cancer [2]. In Asia, gastric cancer is a significant health problem with a greatly variable geographical incidence. Based on the age-standardized incidence rate of gastric cancer, Asian countries are categorized as high risk (e.g. Japan, Korea, China), intermediate risk (e.g. Vietnam), or low risk (e.g. Thailand and Indonesia) for gastric cancer [3].

Indonesia is a developing country at the south-eastern tip of mainland Asia and Oceania; it is an archipelago of more than 13600 islands with

* Author for correspondence: Y. Yamaoka, MD, PhD, Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu-City, Oita 879-5593, Japan.
(Email: yyamaoka@oita-u.ac.jp)

Fig. 1. Geographical map of Surabaya.

a multi-ethnic society with more than 1000 ethnic and sub-ethnic groups delineated by the Wallace Line, a faunal boundary that separates the ecozones and organisms of Asia and Australia. The age-standardized incidence rate of gastric cancer in Indonesia was reported to be 2·8/100000, which is relatively low among Asian countries (International Agency for Research on Cancer; GLOBOCAN2012, http://globocan.iarc.fr/). Although the prevalence of *H. pylori* infections in Indonesia has been investigated, the reports are controversial and contradictory (0–68%) [4, 5]. In addition, to our knowledge, no report has examined *H. pylori* virulence factors in Indonesian strains. Therefore, it remains unclear whether the low incidence of gastric cancer in Indonesia is due to low infection rates or low *H. pylori* pathogenicity. In this study, we examined the *H. pylori* infection rate in a Surabaya hospital using five different tests. We also identified and analysed virulence factors in Indonesian *H. pylori* strains.

METHODS

Study population

From August 9 to 20 November 2012, 103 consecutive patients with dyspepsia underwent endoscopy at the endoscopic clinic in Dr Soetomo Teaching Hospital, Surabaya, Java island (Fig. 1). Twenty-five patients, including 19 with bleeding related to oesophageal varices and six with history of partial gastric resection, were excluded from this study. Finally, a total of 78 patients with dyspepsia (41 women and 37 men, mean age 49·1 ± 12·4 years, range 14–77 years) were included. The final study population consisted of 43 Javanese, 27 Chinese, four Flores, two Madurese, one Sundanese, and one Batak patient. Experienced endoscopists (U.M. and I.N.) collected four gastric biopsy specimens during each endoscopy session: three samples from the lesser curvature of the antrum about 3 cm from the pyloric ring and one sample from the greater curvature of the corpus. Biopsy specimens for culture were immediately placed under refrigeration at −20 °C, and stored at −80 °C within a day of collection until used for culture testing. Three antrum specimens were used for *H. pylori* culture, rapid urease test (CLO test), and histological examination. One corpus specimen was used for histological examination. Peptic ulcers and erosive gastritis were identified by endoscopy. Normal stomach mucosa was defined as the absence of any activity and inflammation in both the antrum and corpus upon histological examination. Patients with evidence of activity or inflammation in the antrum or corpus upon histological examination were considered positive for gastritis. Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of Dr Soetomo Teaching Hospital (Surabaya, Indonesia) and Oita University Faculty of Medicine (Yufu, Japan).

Ethical standards

We declare that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

H. pylori infection status

To maximize diagnostic accuracy, *H. pylori* infections were diagnosed based on the combined results of five different methods, including culture, histology, immunohistochemistry, rapid urease, and urinary antibody tests. For *H. pylori* culture, one antrum biopsy specimen was homogenized in saline and inoculated onto Mueller–Hinton II agar medium (Becton Dickinson, USA) supplemented with 7% horse blood without antibiotics. The plates were incubated for up to 10 days at 37 °C under microaerophilic conditions

(10% O_2, 5% CO_2, 85% N_2). *H. pylori* bacteria were identified on the basis of colony morphology, Gram staining results, and positive reactions for oxidase, catalase, and urease. Isolated strains were stored at −80 °C in Brucella broth (Difco, USA) containing 10% dimethyl sulfoxide and 10% horse serum.

All biopsy materials for histological testing were fixed in 10% buffered formalin and embedded in paraffin. Serial sections were stained with haematoxylin and eosin as well as Giemsa stain. Gastric mucosa were evaluated based on the updated Sydney system [6]. The bacterial load was classified into four grades: 0, 'normal'; 1, 'mild'; 2, 'moderate'; and 3, 'marked' [6]. Samples with bacterial loads greater than or equal to grade 1 were considered positive for *H. pylori*.

Immunohistochemistry was performed as previously described [7]. Briefly, after antigen retrieval and inactivation of endogenous peroxidase activity, tissue sections were incubated with *α-H. pylori* antibody (Dako, Denmark) overnight at 4 °C. After washing, the sections were incubated with biotinylated goat anti-rabbit IgG (Nichirei Co., Japan), followed by incubation with an avidin-conjugated horseradish peroxidase solution (Vectastain Elite ABC kit; Vector Laboratories Inc., USA). Peroxidase activity was detected using an H_2O_2/diaminobenzidine substrate solution.

Urinary *H. pylori* status was evaluated with a rapid urine test (RAPIRUN® *H. pylori* antibody, Otsuka Pharmaceutical Co., Japan) according to the manufacturer's instructions. The reported sensitivity, specificity, and accuracy of the kit in the Japanese population have been reported to be 92·0%, 93·1%, and 92·3%, respectively [8]. Immediately after collection, patients' urine samples were tested for *H. pylori* antibodies. A skilled technician blinded to patients' information measured and analysed all urine samples.

Patients were considered to be negative for *H. pylori* infection when all five test results were negative, whereas patients with at least one positive test result were considered positive for *H. pylori* infection.

Determination of gastritis stage

The degree of inflammation, neutrophil activity, atrophy, intestinal metaplasia, and bacterial density were classified into four grades according to the updated Sydney system: 0, 'normal'; 1, 'mild'; 2, 'moderate'; and 3, 'marked' [6]. Samples with grade 1 or more atrophy were considered atrophy-positive [9]. In addition, gastritis stage was assessed based on topographic locations (antrum and corpus), according to the Operative Link on Gastritis Assessment (OLGA) system [10].

H. pylori isolation and genotyping

H. pylori colonies were cultured from antral biopsy specimens using standard methods [11]. *H. pylori* DNA was extracted from these colonies for *H. pylori* genotyping using the QIAamp DNA Mini kit (Qiagen, USA) according to the manufacturer's directions. *CagA* status was determined by polymerase chain reaction (PCR) amplification and direct sequencing of a conserved region of *cagA* using the previously reported primers cagOMF and cagOMR [12]. *VacA* genotyping (s1 or s2, and m1 or m2) was also performed as described previously [13, 14]. The presence of *jhp0562*, and *β-(1,3)galT* were determined based on PCR product size as described previously [15]. *OipA* status ('on' or 'off') was determined by PCR and sequencing [16]. *IceA* genotype (*iceA1* or *iceA2*), and *dupA* prevalence were determined by PCR as described previously [17, 18]. The amplified fragment was detected by 1·5% agarose gel electrophoresis and ultraviolet transilluminator. DNA sequencing was performed using an ABI 3130 Genetic Analyzer (Applied Biosystems, USA) according to the manufacturer's instructions.

Statistical analysis

Data were analysed using SPSS, version 19 (SPSS Inc., USA). Discrete variables were tested using the χ^2 test; continuous variables were tested using Mann–Whitney U and t tests. A two-tailed P value <0·05 was considered statistically significant.

RESULTS

H. pylori infection rate in dyspeptic patients in Surabaya

The total study population of 78 patients with dyspepsia consisted of four patients aged ⩽29 years, 11 patients aged 30–39 years, 30 patients aged 40–49 years, 17 patients aged 50–59 years, and 16 patients aged ⩾60 years. Table 1 shows *H. pylori*-positive rates for each test. Histology and immunohistochemistry test results were completely concordant. However, rapid urease test results, had the highest positivity rate in this study population (9·0%, 7/78). Three patients

Table 1. Helicobacter pylori *infection rate by diagnostic test*

	Age group (years)					
	⩽29	30–39	40–49	50–59	⩾60	Total
n	4	11	30	17	16	78
Urinary test	0 (0·0%)	1 (9·1%)	1 (3·3%)	2 (11·8%)	0 (0·0%)	4 (5·1%)
RUT	0 (0·0%)	1 (9·1%)	3 (10·0%)	1 (5·9%)	2 (12·5%)	7 (9·0%)
Culture	0 (0·0%)	2 (18·2%)	1 (3·3%)	1 (5·9%)	1 (6·3%)	5 (6·4%)
Histology	0 (0·0%)	2 (18·2%)	2 (6·7%)	1 (5·9%)	1 (6·3%)	6 (7·7%)
IHC	0 (0·0%)	2 (18·2%)	2 (6·7%)	1 (5·9%)	1 (6·3%)	6 (7·7%)
Final	0 (0·0%)	2 (18·2%)	3 (10·0%)	2 (11·8%)	2 (12·5%)	9 (11·5%)

RUT, Rapid urease test; IHC, immunohistochemistry.

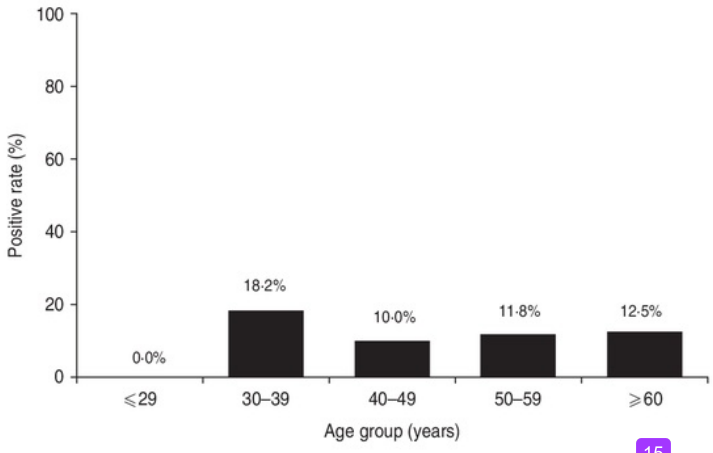

Fig. 2. *Helicobacter pylori* infection rate in Surabaya by age group. Five different methods were used to test for *H. pylori* infection, including culture, histology, immunohistochemistry, rapid urease test, and *H. pylori* urine antibody. Patients were considered negative for *H. pylori* when all test results were negative; *H. pylori*-positive status required at least one positive test result.

were positive for *H. pylori* by all five tests. Two patients were positive by four tests and negative by the urinary antibody test. Two patients were positive only by the rapid urease test. One patient was positive by histology and another was positive only by the urinary antibody test. When patients were categorized as positive for *H. pylori* with at least one positive test result, the overall of *H. pylori* infection rate was 11·5% (9/78). The infection rate by age group was 0% (0/4) for patients aged ⩽29 years, 18·2% (2/11) for patients aged 30–39 years, 10·0% (3/30) for patients aged 40–49 years, 11·8% (2/17) for patients aged 50–59 years, and 12·5% (2/16) for patients aged ⩾60 years. Figure 2 shows the *H. pylori* infection rate according to age group. There was no statistically significant relationship between *H. pylori* infection rate and age ($P = 0·89$).

H. pylori infection rate according to endoscopic diagnosis

Among 78 patients, 29 showed no activity or inflammation in either the antrum or the corpus by histological examination; these patients were considered the

Table 2. *Histological scores according to* Helicobacter pylori *infection status*

	H. pylori (+)	*H. pylori* (–)	*P*
n	9	69	
Age, years	53·2 ± 14·7	48·5 ± 12·1	0·29
Male	7	30	0·05
Antrum			
Activity	0·56 (1)	0·07 (0)	<0·0001
Inflammation	0·89 (1)	0·42 (0)	0·02
Atrophy	0·44 (0)	0·35 (0)	0·53
Intestinal metaplasia	0·00 (0)	0·00 (0)	1·00
Bacterial density	0·44 (0)	0·00 (0)	<0·0001
Corpus			
Activity	0·67 (1)	0·06 (0)	<0·0001
Inflammation	0·56 (0)	0·14 (0)	0·02
Atrophy	0·00 (0)	0·03 (0)	0·60
Intestinal metaplasia	0·00 (0)	0·00 (0)	1·00
Bacterial density	0·78 (1)	0·00 (0)	<0·0001
OLGA score	0·44 (0)	0·35 (0)	0·53

OLGA, Operative Link on Gastritis Assessment.
Age is presented as mean age (±standard deviation), and histology data are presented as mean (median).

normal group. One of 29 subjects (3·4%) in the normal group was positive for *H. pylori* infection. However, among 31 patients with gastritis, seven (22·6%) were positive for *H. pylori*, a significantly higher rate than that in the normal group ($P = 0·02$). Peptic ulcers were found in eight patients (seven gastric ulcers and one duodenal ulcer). Interestingly, none were infected with *H. pylori*. The *H. pylori* infection rate in subjects with erosive gastritis was 10·0% (1/10). No gastric cancer was detected in our study.

H. pylori infection rate according to ethnic group

Among 43 Javanese patients, only one (2·3%) was positive for *H. pylori*. *H. pylori* infections were found in 5/27 Chinese (18·0%) patients, a significantly higher rate than that in the Javanese study population ($P = 0·01$). Two of four Flores patients were positive for *H. pylori*. One Batak patient was positive for *H. pylori* infection. Both Madurese and Sundanese patients were negative for *H. pylori* infection.

Gastric mucosa status

Histological findings showed that 51 patients had grade 0 antrum atrophy; 26 had grade 1, only one had grade 2, and none had grade 3 atrophy. In the corpus, 76 had grade 0 and only two had grade 1 atrophy. Because samples with a grade 1 or more score were considered atrophy-positive, 27 patients (34·6%) had mucosal atrophy in the antrum, and two (2·5%) patients also had corpus mucosal atrophy. Gastritis stage was assessed according to the OLGA system; 65·3% (51/78) had stage 0. Stage I was found in 33·3% (26/78) of patients. One patient (1·2%) had stage II gastritis. Stages III and IV were not found in this study population. Histological scores according to *H. pylori* infection status are shown in Table 2. The percentage of men tended to be higher in the group positive for *H. pylori* infection ($P = 0·05$). However, there were no statistically significant differences in histological scores between men and women (all $P > 0·05$). Activity in both the antrum and corpus was significantly higher in patients positive for *H. pylori* than in patients negative for *H. pylori* [0·56 (1) *vs.* 0·07 (0) in the antrum, 0·67 (1) *vs.* 0·06 (0) in the corpus, all $P < 0·0001$]. In addition, inflammation both in the antrum and corpus was significantly higher in patients positive for *H. pylori* than in patients negative for *H. pylori* [0·89 (1) *vs.* 0·42 (0) in the antrum, 0·56 (0) *vs.* 0·14 (0) in the corpus, all $P = 0·02$]. Scores for atrophy in both the antrum and corpus were not significantly different between patients positive and negative for *H. pylori* infection [0·44 (0) *vs.* 0·35 (0), $P = 0·53$ for the antrum, 0·00 (0) *vs.* 0·03 (0), $P = 0·60$ for the corpus, respectively]. Overall, only one patient showed moderate gastritis in the antrum. However, she was not infected with

Table 3. Helicobacter pylori *genotypes and patient ethnic groups in strains isolated in Indonesia*

ID strain	Ethnic group	*cagA*	*cagA* genotype	*vacA*	*iceA1*	*iceA2*	*jhp0562*	*β-(1,3)galT*	*oipA*
Ind-7	Javanese	Positive	ABB (East Asian)	s1m1	Positive	Negative	Positive	Negative	On
Ind-47	Flores	Negative	—	s1 m2	Negative	Positive	Positive	Positive	On
Ind-69	Chinese	Positive	ABD (East Asian)	s1 m2	Positive	Negative	Negative	Positive	Off
Ind-71	Chinese	Positive	ABD (East Asian)	s1m1	Positive	Negative	Positive	Positive	On
Ind-79	Flores	Positive	ABD (East Asian)	s1s2 m2	Positive	Positive	Positive	Negative	On

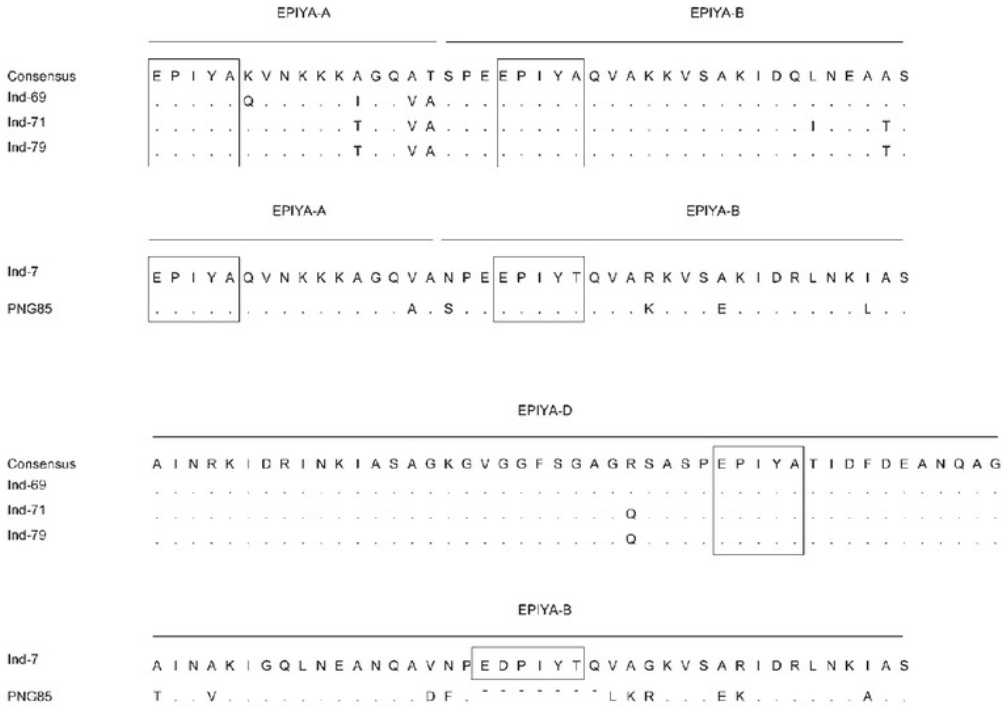

Fig. 3. Sequence analysis of CagA structural polymorphisms in Indonesian strains. Three strains were ABD type. The ABB type was similar to that of strain was classified as hpSahul type by multi-locus sequence typing using seven housekeeping genes.

H. pylori. Only two patients showed mild atrophy in the corpus; however, they were also negative for *H. pylori*. No patient positive for *H. pylori* infection showed corpus atrophy. No patients had intestinal metaplasia irrespective of *H. pylori* infection. OLGA scores were not statistically different between patients positive and negative for *H. pylori* infection [0·44 (0) *vs.* 0·35 (0), $P = 0·53$].

H. pylori genotypes in Indonesian strains

Five strains were successfully isolated and analysed for *H. pylori* virulence factors. Table 3 shows *H. pylori* genotypes by ethnic group in these Indonesian strains; *cagA* was found in four of five strains. Sequence analysis revealed that three strains and one strain possessed East Asian ABD and ABB types, respectively. Figure 3 shows sequence analysis of CagA structural polymorphisms in Indonesian strains. Strains with East Asian-type CagA were isolated from two Chinese patients and one Flores patient. The strain with ABB genotype was isolated from a single Javanese patient. The *cagA*-negative strain was isolated from a Floresian patient. All *cagA*-positive strains were *oipA* 'on'. *VacA* analysis showed two s1m1 strains, two s1 m2, and one s1s2 m2 genotypes.

Table 4. *Summary of previous* Helicobacter pylori *prevalence studies in Indonesia*

First-named Author [ref.]	Study period	Area	*n*	Average age, years (range)	Test	Positive rate
Syam [21]	2001	Jakarta	63	42·4 (16–73)	Stool antigen	66·7% (42/63)
					Rapid urease test	4·8% (3/63)
					Histology	11·1% (7/63)
Tokudome [19]	2003	Yogyakarta	91	48·0 for men	Urea breath test	4% in men and 0% in women
				46·6 for women	Serum antibody	5% in men and 4% in women
Tokudome [4]	2005	Semarang	171	57·4 for men	Urea breath test	0% in men and 0% in women
				49·2 for women	Serum antibody	2% in men and 2% in women
Syam [22]	2003–2004	6 cities	550	44·98 (15–82)	Histology	10·2% (56/550)
Saragih [23]	1998–2005	Jakarta	2903	No information	Histology	12·8% (52/407) in 1998 2·9% (50/403) in 2005
Aulia [20]	2007	Jakarta	70	47·6 (18–79)	Histology	5·7% (4/70)
Abdullah [5]	1998–1999	Jakarta	125	50·3 (18–82)	Rapid urease test *	68% (85/125) in the antrum 4% (5/125) in the corpus
					Culture *	
					Histology *	
Arinton [24]	2005	Purwokerto	81	56·8 (45–75)	PCR	41·9% (34/81)
Zhao [25]	2007	Mataram	294	34·0 (6–74)	Urea breath test	11·2% (33/294)

UBT, Urea breath test; PCR, polymerase chain reaction.
* This study tested for *H. pylori* by histology, culture, and rapid urease test.

IceA1 single positive (*iceA2*-negative) status was identified in four strains; one strain was positive for both *iceA1* and *iceA2*. One *cagA*-negative strain was *iceA2* single positive. Two strains were *jhp0562*-positive/*β-(1,3)galT*-negative. Two strains were double positive for *jhp0562* and *β-(1,3)galT*. One *cagA*-negative strain had a *jhp0562*-negative/*β-(1,3)galT*-positive genotype. Two strains were positive for short-type *dupA*; no strains were identified with intact long-type *dupA* genotypes.

Nucleotide sequencing

Nucleotide sequence data for three strains with ABD type and one with ABB type are available under DDBJ accession numbers AB921015 to AB921018.

DISCUSSION

Although we included only patients with dyspepsia in this study, we found a low of *H. pylori* infection rate in Surabaya, Indonesia, consistent with previous reports [4, 19, 20]. In addition, we found severe gastritis and intestinal metaplasia to be rare in in patients from a hospital in Surabaya. This supports suggestions that the low incidence of gastric cancer in Indonesia may be associated with the low *H. pylori* infection rate and the low prevalence of precancerous legions.

Several studies have examined the prevalence of *H. pylori* in Indonesia (Table 4). However, the reported prevalence ranged from 0% to 68% [4, 5]. These differences might be attributed to the different study populations and different tests for *H. pylori* infection. Six studies included patients with dyspepsia [5, 20–24], whereas three other studies included study participants from the general population [4, 19, 25]. In nine studies, five used histological examination for diagnosis [5, 20–23]. Four studies reported low infection rates (5·7–12·8%) [20–23]. One study reported a high infection rate (68%) [5]; however, these authors did not include their definition for *H. pylori*-positive status, although they stated that they used a rapid urease test, culture, and histology for diagnosis. The *H. pylori* infection rate examined by the urea breath test was low in multiple reports (0–11·2%) [4, 19, 25]. One study used PCR methods to detect *H. pylori ureC* [24] and found a high *H. pylori*-positive rate (41·9%).

Therefore, it is necessary to recognize differences in *H. pylori* test accuracy. For example, 54 (85·7%) of 63 dyspeptic patients were positive based on rapid urease testing and microscopic detection of *H. pylori* [21]. However, among these patients, 42 were positive by only stool antigen test, which suggests the potential for false-positive results. In addition, differences in the results by histological examination might be due to the different evaluation criteria adopted by different studies. This could be overcome by the

standardization of biopsy location and instrument, sample size, and using the same pathologists to read results. Different kit types may also contribute to different results. Tokudome *et al.* examined patients' serum for *H. pylori* antibodies using an enzyme-linked immunoassay (ELISA) kit (Kyowa Medex Co., Japan) produced and tested in Japan [19]. Unfortunately, the authors did not mention the accuracy of the ELISA test kit was determined using antigens extracted from Japanese strains. It is important to develop ELISA kits using *H. pylori* strains native to the study population.

In the present study, we used five different *H. pylori* tests to increase diagnostic accuracy as well as to compare results among tests. We found that the *H. pylori* infection rate was very low, irrespective of the test. We previously reported the prevalence of *H. pylori* infection in Bhutan using the same criteria [26], although we substituted urinary testing for serological testing in this study. The *H. pylori* prevalence was quite high (73·4%) in Bhutan; the concordance between different tests was also very high [26]. Importantly, the same pathologist (T.U.) and microbiologist (M.M.) performed the experiments in both studies, which suggests a very small potential for bias. Furthermore, our preliminary study showed complete concordance between serology and urinary test results in Manado, Indonesia (M. Miftahussurur and Y. Yamaoka, unpublished data). These results suggest that our *H. pylori* infection criteria are reliable. Even when patients with a single positive test result were considered positive for *H. pylori* infection, the *H. pylori* infection rate in our patients from a Surabaya hospital was only 11·5% (9/78). The rapid urease test showed the highest positive rate (9·0%). Among nine patients, only three were positive by all five tests. Our data confirmed that the *H. pylori* infection prevalence is quite low in patients from a Surabaya hospital. In our study, severe gastritis and intestinal metaplasia were also rare in Indonesia. Consistent with this observation, Abdullah *et al.* found that the grade and activity of gastritis and mucosal atrophy was higher in Japanese than in Indonesian patients positive for *H. pylori* [5]. That difference may explain the disparity in the incidence of gastric cancer between Indonesia and Japan. However, activity and inflammation in both the antrum and corpus were significantly higher in patients positive for *H. pylori* than in negative patients in Indonesia. Furthermore, although no patient had intestinal metaplasia irrespective of *H. pylori* infection in this study, other research in the Malay ethnic group has found intestinal metaplasia and dysplasia to be significantly associated with *H. pylori* infection even in regions with low *H. pylori* prevalence [27]. These results suggest that *H. pylori* should be eradicated even in areas with low *H. pylori* prevalence.

The low *H. pylori* infection rate in Indonesia is a different trend compared to other developing countries. In general, environmental factors, such as poor living conditions, are associated with higher *H. pylori* infection rates. However, sanitary conditions (food hygiene and drinking water) alone cannot explain the low *H. pylori* infection prevalence in Indonesia, because approximately 50% of the population in Indonesia still use basic environmental conditions for sanitation (UNICEF, http://www.unicef.org/). A low *H. pylori* infection rate was also reported in the neighbouring country of Malaysia. Similarly, the incidence of gastric cancer is also low in Malaysia. Host genetic factors might contribute to a reduced susceptibility to *H. pylori* infection, a possibility suggested in the ethnic Malaysian population [28, 29]. Other environmental factors such as the frequent use of 'budu' or local anchovy sauce, and 'pegaga' or centenella asiatica have also been reported to be associated with the low prevalence of *H. pylori* in Malaysia [30]. Further studies of host and environmental factors in Indonesia are necessary to better elucidate reasons for the low *H. pylori* infection prevalence in Indonesia and Malaysia.

Previous studies used *H. pylori* strains isolated from Javanese patients. Although the number of subjects was small, to our knowledge, this is the first study to compare the *H. pylori* infection rate in different ethnic groups. Interestingly, the highest *H. pylori* rate was found in patients from the Chinese Indonesian population instead of patients from the Javanese population. However, the prevalence of *H. pylori* infection in Indonesians of Chinese descent was lower than that of Chinese non-immigrants [31]. Environmental factors might contribute to the lower *H. pylori* infection rate in Chinese Indonesians. The transmission routes of *H. pylori* are still not entirely understood, but human-to-human spread through oral–oral or faecal–oral routes are considered the most plausible routes for infection [32]. Therefore, intra-racial or intra-community spread such as transmission from mother to child might contribute to these racial differences in *H. pylori* infection rates. Although only one isolate was isolated from a Javanese patient, it had an ABB type. Interestingly, sequence analysis showed that the *cagA* repeat region

of this strain was similar (homology 90·5%) to that of strain PNGhigh85 (Fig. 3), which was isolated in Papua (New Guinea) and classified as hpSahul type by multi-locus sequence typing using seven housekeeping genes [33]. The eastern sections of Indonesia, especially Papua, were geographically connected to Australia as a single continent (Sahul) about 60 000 years ago; the Javanese isolate might have some historical connection with the Sahul-type strain. A larger sample size is necessary to elucidate the origin of *H. pylori* strains in Indonesia.

Although the number of samples was not sufficient for statistically significant conclusions, we also examined *H. pylori* virulence factors in Indonesian strains in detail. In general, *cagA* positive (especially East Asian-type *cagA*), *vacA* s1m1, *oipA* 'on', *iceA1* positive, *jhp0562*-positive/*β*-(*1,3*)*galT*-negative, and intact long-type *dupA* positive are considered to be more virulent [34]. Our study revealed that some strains had this more virulent genotype. Further studies with increased sample numbers are necessary to better elucidate the virulence of Indonesian *H. pylori* strains. Furthermore, an increased number of samples might be useful to clarify the association between *H. pylori* genotype and ethnic groups in Indonesia.

Our study has several limitations. First, we could not obtain information about medications used by the study participants. Therefore, it is possible that we included patients who had been administered antibiotics, histamine-2 receptor antagonists (H2 blockers), or proton pump inhibitors, which can influence *H. pylori* infection prevalence. However, a previous report found that the prevalence of *H. pylori* infection in Indonesia was quite low (10·2%) even when patients taking proton pump inhibitors were excluded from the study population [22]. Interestingly, none of the eight patients diagnosed with peptic ulcers were positive for *H. pylori* in our study. A recent report in an elderly population found that the absence of *H. pylori* infection did not reduce the risk of bleeding peptic ulcers in patients with other risk factors, especially those who were receiving drug treatments [35]. Unfortunately, we did not obtain information on the usage of non-steroidal anti-inflammatory drugs that are also an important factor for the development of peptic ulcers [36]. Further information is necessary to elucidate the mechanisms of peptic ulcer development in Indonesia. Second, we obtained samples from a hospital in Surabaya, which located in the eastern part Java island and the second largest city in Indonesia. Sanitary conditions vary by area in Indonesia, although they are generally better in western regions than in eastern areas. Therefore, our results cannot be generalized across Java or Indonesia. In addition, we included only patients with dyspepsia in our study population, and not members of the general population. In Indonesia, many patients with dyspepsia are not covered by the Indonesian health insurance system. Therefore, it is difficult for them to undergo endoscopy. Further investigation from all regions of Indonesia is necessary to elucidate the reasons for the low rate of gastric cancer.

In conclusion, we found a low of *H. pylori* infection rate in dyspeptic patients in Surabaya. In addition, we found severe gastritis and intestinal metaplasia to be rare even in patients positive for *H. pylori* infection. Our findings support previous reports of low incidence rate of gastric cancer in Indonesia and may be attributed to the low *H. pylori* infection rate and low prevalence of precancerous legions. However, activity and inflammation in both the antrum and corpus were significantly higher in patients with *H. pylori* than that in those without. Some *H. pylori* strains were of more virulent genotypes. Therefore, early diagnosis and treatment of *H. pylori* infection is necessary for symptomatic patients in Surabaya to reduce chronic complications risk.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Peek Jr. RM, Blaser MJ.** *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nature Reviews Cancer* 2002; **2**: 28–37.

2. **Fox JG, Wang TC.** Inflammation, atrophy, and gastric cancer. *Journal of Clinical Investigation* 2007; **117**: 60–69.
3. **Ferlay J, *et al.*** Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer* 2010; **127**: 2893–2917.
4. **Tokudome S, *et al.*** *Helicobacter pylori* infection appears essential for stomach carcinogenesis: observations in Semarang, Indonesia. *Cancer Science* 2005; **96**: 873–875.
5. **Abdullah M, *et al.*** *Helicobacter pylori* infection and gastropathy: a comparison between Indonesian and Japanese patients. *World Journal of Gastroenterology* 2009; **15**: 4928–4931.
6. **Dixon M, *et al.*** Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *American Journal of Surgical Pathology* 1996; **20**: 1161–1181.
7. **Uchida T, *et al.*** Immunohistochemical diagnosis of the *cagA*-gene genotype of *Helicobacter pylori* with anti-East Asian CagA-specific antibody. *Cancer Science* 2007; **98**: 521–528.
8. **Yamamoto S, *et al.*** A new rapid test for detecting anti-*Helicobacter pylori* antibody excreted into urine. *Helicobacter* 2000; **5**: 160–164.
9. **Bornschein J, *et al.*** Serological assessment of gastric mucosal atrophy in gastric cancer. *BMC Gastroenterology* 2012; **12**: 10.
10. **Rugge M, *et al.*** Gastritis staging in clinical practice: the OLGA staging system. *Gut* 2007; **56**: 631–636.
11. **Yamaoka Y, *et al.*** Relationship of *vacA* genotypes of *Helicobacter pylori* to *cagA* status, cytotoxin production, and clinical outcome. *Helicobacter* 1998; **3**: 241–253.
12. **Matsunari O, *et al.*** Association between *Helicobacter pylori* virulence factors and gastroduodenal diseases in Okinawa, Japan. *Journal of Clinical Microbiology* 2012; **50**: 876–883.
13. **Yamaoka Y, *et al.*** Relationship between the *cagA* 3′ repeat region of *Helicobacter pylori*, gastric histology, and susceptibility to low pH. *Gastroenterology* 1999; **117**: 342–349.
14. **Atherton J, *et al.*** Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. *Journal of Biological Chemistry* 1995; **270**: 17771–17777.
15. **Oleastro M, *et al.*** Identification of markers for Helicobacter pylori strains isolated from children with peptic ulcer disease by suppressive subtractive hybridization. *Infection and Immunity* 2006; **74**: 4064–4074.
16. **Yamaoka Y, *et al.*** Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. *Gastroenterology* 2002; **123**: 414–424.
17. **Yamaoka Y, *et al.*** Relationship between *Helicobacter pylori iceA, cagA*, and *vacA* status and clinical outcome: studies in four different countries. *Journal of Clinical Microbiology* 1999; **37**: 2274–2279.
18. **Takahashi A, *et al.*** Intact long-type *dupa* as a marker for gastroduodenal diseases in Okinawan subpopulation, Japan. *Helicobacter* 2013; **18**: 66–72.
19. **Tokudome S, *et al.*** Rare *Helicobacter pylori* infection as a factor for the very low stomach cancer incidence in Yogyakarta, Indonesia. *Cancer Letters* 2005; **219**: 57–61.
20. **Aulia D, Manz GO, Simadibrata M.** Pepsinogen I concentration in organic dyspepsia patients at Gastroenterology Division, Department of Internal Medicine, Cipto Mangunkusumo Hospital. *Acta Medica Indonesia* 2009; **41**: 107–114.
21. **Syam AF, *et al.*** Accuracy of *Helicobacter pylori* stool antigen for the detection of *Helicobacter pylori* infection in dyspeptic patients. *World Journal of Gastroenterology* 2005; **11**: 386–388.
22. **Syam AF, *et al.*** Evaluation of the use of rapid urease test: Pronto Dry to detect *H pylori* in patients with dyspepsia in several cities in Indonesia. *World Journal of Gastroenterology* 2006; **12**: 6216–6218.
23. **Saragih JB, *et al.*** Incidence of *helicobacter pylori* infection and gastric cancer : an 8-year hospital based study. *Acta Medica Indonesia* 2007; **39**: 79–81.
24. **Arinton IG.** Adjustment of cut-off values in ELISA for detection of *Helicobacter pylori* infection. *Acta Medica Indonesia* 2011; **43**: 88–91.
25. **Zhao Y, *et al.*** Association between HLA-DQ genotypes and haplotypes vs *Helicobacter pylori* infection in an Indonesian population. *Asian Pacific Journal of Cancer Prevention* 2012; **13**: 1247–1251.
26. **Vilaichone RK, *et al.*** Extremely high prevalence of *Helicobacter pylori* infection in Bhutan. *World Journal of Gastroenterology* 2013; **19**: 2806–2810.
27. **Yeh LY, *et al.*** Chronic atrophic antral gastritis and risk of metaplasia and dysplasia in an area with low prevalence of *Helicobacter pylori*. *Indian Journal of Gastroenterology* 2009; **28**: 49–52.
28. **Maran S, *et al.*** Gastric precancerous lesions are associated with gene variants in *Helicobacter pylori*-susceptible ethnic Malays. *World Journal of Gastroenterology* 2013; **19**: 3615–3622.
29. **Lee YY, Mahendra Raj S, Graham DY.** *Helicobacter pylori* Infection – a boon or a bane: lessons from studies in a low-prevalence population. *Helicobacter* 2013; **18**: 338–346.
30. **Lee YY, *et al.*** Sociocultural and dietary practices among Malay subjects in the north-eastern region of Peninsular Malaysia: a region of low prevalence of *Helicobacter pylori* infection. *Helicobacter* 2012; **17**: 54–61.
31. **Shi R, *et al.*** Prevalence and risk factors for *Helicobacter pylori* infection in Chinese populations. *Helicobacter* 2008; **13**: 157–165.
32. **Goh KL, *et al.*** Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter* 2011; **16** (Suppl. 1): 1–9.
33. **Olbermann P, *et al.*** A global overview of the genetic and functional diversity in the *Helicobacter pylori cag* pathogenicity island. *PLoS Genetics* 2010; **6**: e1001069.
34. **Shiota S, Suzuki R, Yamaoka Y.** The significance of

virulence factors in *Helicobacter pylori*. *Journal of Digestive Disease* 2013; **14**: 341–9.

35. **Lee, *et al.*** Absence of *Helicobacter pylori* is not protective against peptic ulcer bleeding in elderly on offending agents: lessons from an exceptionally low prevalence population. *Peer Journal*. Published online: 11 February 2014. doi:10.7717/peerj.257.
36. **Malfertheiner P, *et al.*** Management of *Helicobacter pylori* infection – the Maastricht IV/Florence Consensus Report. *Gut* 2012; **61**: 646–664.

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