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Original Article

Prevalence and virulence factors of *Helicobacter pylori* isolated from oral cavity of non-disease, gastritis, and gastric cancer patients

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Gastric cancer

Abstract *Background/purpose:* The oral cavity is considered a reservoir of *Helicobacter pylori* associated with gastric infection. It aimed to examine the prevalence of *H. pylori* strains from the oral cavity and gastric tissue of patients with different stage of gastric-diseases. Strains were further characterized for virulence genes, adhesion ability, and inflammation responses.
Materials and methods: 11 non-disease, 15 gastritis, and 15 gastric cancer participated in the study. After clinical examination, gastric biopsies, saliva and plaque samples were collected and *H. pylori* levels were examined by real-time PCR and cultivation. The *cag*A and *vac*A genes were investigated from the culture strains. Adhesion ability and pro-inflammatory responses were analyzed in comparison between the presence of virulent genes and disease status.
Results: Relatively poor periodontal condition was found among gastric cancer patients. Prevalence of *H. pylori*-positive was 84.8% and 19.5% by real-time PCR and cultivation, respectively.

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The *cag*A and *vac*A gene-positive strains were 52.6% and 5.3%, respectively, which were found more in gastric cancer patients. The *cag*A gene-positive strains were found to be higher in gastric cancer patients, and strains had significantly higher adhesion ability and pro-inflammation expressions than the *cag*A gene-negative strains.
Conclusion: Colonization by *H. pylori* in oral cavity was confirmed, and the *cag*A gene-positive strains play a crucial role in both adhesion and inflammatory responses. The presence of *H. pylori* and its virulence gene in oral cavity should be received attention. An eradication of such strains from oral cavity may help to prevent the transmission and recolonization to gastric organs.

Introduction

Helicobacter pylori (*H. pylori*) is found worldwide, that colonizes more than 50% of the global population.[1,2] Its colonization was observed mainly in the human gastric epithelium, which is associated with a risk factor for peptic and duodenal ulcers, mucosa-associated lymphoid tissue (MALT), and gastric cancer.[3,4] This organism can be detected in saliva and dental plaque, which indicates that the oral cavity may be a potential reservoir for *H. pylori* or a possible route of transmission to other sites. The human oral cavity is the initial part of the body that connects to the digestive tract, and studies have currently found that the oral microbiome plays a crucial role in both oral and systemic health. Some reports showed that oral *H. pylori* and stomach infection presented a high recurrence rate of gastritis (13.2%–18.4%).[5] Failure to eliminate *H. pylori* from the mouth could lead to recolonization in the stomach. Pathogenicity of *H. pylori* comprises various virulence genes including *cag*A and *vac*A genes, which involve in colonization, chronic inflammation, and carcinogenic processes.[6] However, there is a variation of prevalence and virulence genes of *H. pylori* in different geographic areas and clinical stages showing that gastric cancer subjects have a higher *Bab*A expression compared to normal subjects.[7,8]

Our previous study found that clinical *H. pylori* strains isolated from patients with different clinical stages including non-disease, gastritis, and gastric cancer, and *H. pylori* strains isolated from the patients with gastric cancer had relatively higher adhesion ability than the others.[9] However, only a few strains have been included and virulent genes have not been investigated yet. Therefore, this study aimed to examine the prevalence of *H. pylori* strains from the oral cavity and gastric tissue of patients with different clinical stages including non-disease, gastritis, and gastric cancer. And the strains were further characterized for virulence genes, adhesion ability, and inflammation responses.

Materials and methods

Patients and collection of biopsies

The study has been approved by the Human Ethics Committee, Faculty of Medicine, Prince of Songkla University (REC.63-540-10-1). The study took place at the Songklanagarind hospital belonging to the Faculty of Medicine, Prince of Songkla University. Patients were recruited from those who had a provision diagnosis for the requirement of the gastroscopy and gave consent to participate in the study. The sample size was determined to be 15 participants in each group (non-diseased, gastritis, and gastric cancer) based on the study of Gunathilake et al.[10] The exclusion criteria were as follow: receiving antibiotics within 2 weeks, under chemotherapy, or having any systemic diseases. After the gastroscopy, patients had been categorized into groups according to the final diagnosis.

Two pieces of gastric biopsies, one from the body and one from the antrum of the stomach, were obtained by the consultant gastroenterologists (SS and AK), and transferred into a vial containing transport medium for *H. pylori*. After collection, the samples were transferred for processing and cultured within 2 h.

Oral examination and sample collection

Before the oral examination, 3 mL of unstimulated whole saliva was collected in a sterile bottle. Individual supragingival- and subgingival-pool plaques were collected from the mesio-buccal of 6 teeth (16, 11, 26, 36, 31, and 46) into a microcentrifuge tube and were measured for the weight of plaques. A 1500 μL of PBS buffer (pH 7.0) was added and mixed thoroughly. All samples were transferred for sample processing.

Oral examinations were then performed for all subjects by WM and RP including teeth present, probing depth, bleeding on probing depth, and clinical attachment of whole mouth at six sites per tooth (mesiobuccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual), plaque index (PI) and gingival index (GI) according to Quigley and Hein[11] and Loe and Silness,[12] respectively.

Samples processing

Biopsy samples were cut and homogenized, and then were suspended in 1500 μL of PBS buffer (pH 7.0). Each 100 μL of tissue- and oral samples (saliva, supragingival- and subgingival plaques) was cultured on the blood agar and selective medium for *H. pylori* (Himedia Laboratories, Kennett Square, PA, USA), and the remaining samples were kept in −80 °C until used for the culture, PCR, and real-time PCR.

Detection and identification of *H. pylori* isolates using cultivation and PCR

All clinical isolates were identified based on their typical morphology on a selective medium for *H. pylori* and provided the positive reaction for catalase, oxidase, and urease test. The DNA of all isolates was then extracted using a DNA extraction kit (Bio-helix, New Taipei City, Taiwan), and amounts of DNA were measured using the μdrop™ plate (Thermo Fisher Scientific, Singapore, Singapore). The extracted DNA was confirmed as *H. pylori* using specific primers (5-GCCAATGGTAAATTAGTT-3′ and 5′-CTCCTTAATT-GTTTTTAC-3′),[13] and the reaction comprised of 10 ng DNA extracted, 2.5 mM dNTP, distilled water, 10× buffer, 2.5 mM $MgCl_2$, 5 pM of each specific primer and 0.5 unit Taq polymerase. PCR condition was run at 95 °C for 5 min followed by 35 cycles of 95 °C for 2 min, 44 °C for 1 min, 72 °C for 2 min, and final extension at 72 °C for 8 min. The PCR product was checked on agarose gel giving a PCR product of 411 bp under UV light, and *H. pylori* ATCC43504 was used as a positive control.

Detection of *H. pylori* presence and quantification in samples using PCR and real-time PCR

A 500 μL of gastric tissues and oral samples were extracted for DNA using a DNA extraction kit (Bio-helix), and the extracted DNA was used to determine the *H. pylori* presence and quantification in samples using PCR and real-time PCR, respectively. The extracted DNA was examined for the presence of *H. pylori* in samples using the specific primers mentioned above.

For detection of the *H. pylori* quantity using real-time PCR, a 20 μL of reaction mixture consisted of 100 ng DNA extracted, 10 μL real-time PCR solution (Meridian Bioscience, Memphis, TN, USA), 5 pM of each specific primer for *H. pylori*[13] and distilled water. The reaction was run at 52 °C for 10 min and 95 °C for 2 min followed by 40 cycles of 95 °C for 20s, 44 °C for 25 s, and 72 °C for 25 s. The *H. pylori* quantification was calculated by a standard curve constructed from the Cq value and log CFU/mL, which was reported as log CFU/mL.

Examination of *cag*A and *vac*A genes with specific primer using PCR

The extracted DNA of *H. pylori* strains was examined for the presence of *cag*A and *vac*A genes using PCR with a specific primer for *cag*A (5′-ATAATGCTAAATTAGACAACTTGAGCGA-3′ and 5′-AGAAACAAAAGCAATACGATCATTC-3′)[14] and *vac*A (5′-ACACCGCAAAATCAATCGCC-3′ and 5′-CCCCAACAATGGCTG-GAATG-3′).[15] A reaction contained 10 ng extracted DNA, 2.5 mM dNTP, 5 pM of each primer, 2.5 mM $MgCl_2$, and 0.5 Unit Taq polymerase. The conditions were run at 95 °C for 5 min followed by 35 cycles of 95 °C for 2 min, 60 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 8 min. The PCR product was run using agarose gel and it showed PCR product at 100 bp for both genes under UV light. *H. pylori* ATCC43504 was used as a positive control.

Determination of mRNA expression of *cag*A gene using real-time PCR

H. pylori strains were cultured on a blood agar plate and were incubated at 37 °C for 24 h under anaerobic conditions. Then, all strains were extracted total RNA using a Purelink RNA mini kit (Thermo Fisher Scientific, Carlsbad, CA, USA), and the qualification and quantification were detected using the μdrop™ plate (Thermo Fisher Scientific). A 100 ng/mL of total RNA was used to measure the mRNA expression for *cag*A gene using real-time PCR which was normalized with the *ure*A gene (housekeeping gene). *H. pylori* ATCC43504 was used as a control and the expression was set to 1.0.

Determination of adhesion ability of *H. pylori* strains to various epithelial cells

Human adenocarcinoma gastric cell line (AGS), human oral squamous cell carcinoma (H357), human colorectal adenocarcinoma cells (Caco-2), and the human normal intestinal epithelial cell line-6 (HIEC-6) were included in this study. All cells (10^5 cells/mL) were seeded in 24-well cell plates with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and incubated at 37 °C with 5% CO_2 until appropriate for use.

Adhesion ability of 19 *H. pylori* strains was investigated using the modified method of Sophatha et al.[16] An individually tested strain (10^8 CFU/mL) was added into each well of cells for 1 h. The cells were washed to remove unbound bacteria and trypsin—EDTA was added for releasing the bacteria cells. Adhered bacteria were counted using the plate count agar method and adhesion ability was expressed as percentages according to Sophatha et al.[16]

Determination of pro-inflammatory cytokines expression in human adenocarcinoma gastric cell line (AGS) and human periodontal ligament (PDL) cells

Human periodontal ligament cells (PDL) were isolated from the roots of freshly extracted teeth (EC6201-01-P-LR) and human adenocarcinoma gastric cell line (AGS) was thawed from a freezer. The cells were incubated at 37 °C and 5% CO_2 for 3—5 days. Then, both cells were sub-cultured using the trypsinization method, and 1×10^6 cells/mL of the cells were seeded into 6-well plates and incubated at 37 °C, 5% CO_2 for 3 days.

To investigate the cytokine expression, AGS or PDL cells were treated with 100 μg/mL of CWEs[17] of individual bacterial strains and left untreated as a negative control for 24 h. The treated cells were examined for cytokine mRNA expressions using a real-time PCR. A total RNA was extracted using a Purelink RNA mini kit (Thermo Fisher Scientific) following the manufacturer's instructions. The cDNA was synthesized by the Superscript™ first-strand cDNA system kit (Thermo Fisher Scientific, Waltham, MA, USA). The mRNA levels of interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α were performed in a

20 μL mixture consisting of 5% of cDNA (v/v), the Sensifast™SYBR No-ROX reagent (Meridian Bioscience), and 5 pM of each primer. The primer sequences were shown in a previous study,[18] and the mixture was then run with the CFX96 Touch™ Real-Time PCR detection system (BioRad, Foster, CA, USA). The real-time PCR condition was 40 cycles for all markers with the denaturing temperature at 95 °C for 20s, annealing temperatures at 60 °C for 20s, and the polymerizing temperature at 72 °C for 25s. This experiment used glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as housekeeping genes for comparing gene expression. The average induction of each cytokine was expressed from these separate experiments.

Statistical analysis

The descriptive analysis was used as follows: general characteristic data were shown as the number of subjects for sex, mean ± SD for age, and percentage of *H. pylori* found in samples by PCR. Teeth presence, gingival recession, pocket depth, clinical attachment loss, and bleeding on probing and *H. pylori* levels are shown as median (min–max) while the mRNA expressions and adhesion ability are revealed as 25, 50, and 75 percentiles. The significant difference between stages of diseases (non-disease, gastritis, and gastric cancer) or type of samples (tissues and oral samples) was calculated by the Kruskall–Wallis test followed by the Mann–Whitney *U* test. The significant difference in mRNA expression and adhesion ability between *cag*A-positive and *cag*A-negative was calculated by the Mann–Whitney *U* test. A *P*-value less than 0.05 is considered statistically significant.

Results

Among 41 patients including, 11 non-disease, 15 gastritis, and 15 gastric cancer, the overall baseline between the groups was not statistically significant, details in Table 1. However, age and clinical periodontal condition (gingival recession, pocket depth, clinical attachment loss, and bleeding on probing) in gastric cancer patients were slightly higher compared to other groups. The number of total teeth and teeth per subject was lower than the others.

The prevalence of *H. pylori*-positive in all samples was 84.8% by the PCR method (Table 2). The amount of *H. pylori* by the real-time PCR demonstrated that there was a wide range of levels between the patients and type of samples. *H. pylori* levels among patients with gastric cancer tend to be higher than the others. The supra- and sub-gingival samples had a higher *H. pylori* level than saliva and tissue biopsy samples and the body tissue samples had a higher level than the antrum (Table 2).

The recovery of *H. pylori* strains from patients was 19.5% (8 of 41) by cultivation. A total of 19 strains was found from different diseased status and the presence of virulent genes (*cag*A and *vac*A) is presented in Table 3. *H. pylori* strains were found more among gastric cancer patients (52.6%) than non-disease (26.3%) and gastritis (21.1%). They were also more prevalent in periodontal

Table 1 Characteristic data and oral examination of subjects.

Subjects	All subjects (n = 41)	Non-disease (n = 11)	Gastritis (n = 15)	Gastric cancer (n = 15)
Age: (years)	61.3 ± 14.1	52.8 ± 14.5	61.2 ± 14.2	67.6 ± 11.0
Sex: (male/female)	20/21	3/8	5/10	12/15
Oral examination:	(n = 30)	(n = 11)	(n = 14)	(n = 5)
Teeth:				
- Total teeth	651	278[a]	271[a]	102[a]
- per subjects [median, (min–max)]	26 (2–32)	26 (16–30)	25 (2–32)	22 (5–30)
Gingival recession: (%)				
- median, (min–max)	54.2, (0.0–100.0)	40.0, (0.0–72.0)[b]	48.5, (9.4–100.0)[ab]	88.3, (60.0–100.0)[a]
- mean ± SD	57.6 ± 35.8	37.3 ± 27.6	62.3 ± 39.5	83.9 ± 18.4
Pocket depth: (mm)				
- median, (min–max)	2.7, (0.0–9.3)	3.0, (0.0–7.0)[a]	2.0, (0.0–9.0)[b]	3.0, (0.0–12.0)[a]
- mean ± SD	2.3 ± 1.9	2.4 ± 1.5	2.0 ± 2.0	2.5 ± 2.3
Clinical attachment loss: (mm)				
- median, (min–max)	2.7, (0.0–17.0)	3.0, (0.0–9.0)[a]	2.0, (0.0–17.0)[b]	3.0, (0.0–11.0)[a]
- mean ± SD	2.8 ± 2.6	2.6 ± 1.8	2.5 ± 2.8	3.4 ± 3.3
Bleeding on probing: (%)				
- median, (min–max)	43.9, (8.0–100.0)	38.5, (8.0–100.0)[a]	48.0, (17.9–100.0)[a]	72.2, (27.8–100.0)[a]
- mean ± SD	54.6 ± 29.7	45.6 ± 29.7	57.9 ± 30.3	63.4 ± 29.3

The lowercase letters showed significant differences between disease status.

Table 2 The levels of *Helicobacter pylori* in various samples using PCR and real-time PCR.

Type of samples	*H. pylori* found by PCR (%) and real-time PCR [median (min, max)]							
	All subjects (n = 41)		Non-disease (n = 11)		Gastritis (n = 15)		Gastric cancer (n = 15)	
	PCR (%)	real-time PCR	PCR (%)	real-time PCR	PCR (%)	real-time PCR	PCR (%)	real-time PCR
Antrum (Log CFU/mg)	82.9	1.6 (0.0, 4.0)	63.6	1.3 (0.0, 3.0)^{b,B}	93.3	1.6 (1.0, 2.9)^{ab,C}	86.7	1.9 (1.0, 4.0)^{a,C}
Body (Log CFU/mg)	85.4	2.3 (0.0, 6.3)	63.6	1.6 (0.4, 3.8)^{b,A}	100.0	2.6 (0.9, 6.0)^{a,B}	86.7	2.9 (1.0, 6.3)^{a,B}
Saliva (Log CFU/mg)	85.4	2.9 (1.1, 3.9)	63.6	2.4 (1.1, 3.1)^{b,A}	100.0	2.9 (1.7, 3.7)^{a,AB}	86.7	3.1 (2.1, 3.7)^{a,B}
Supragingival plaque (Log CFU/mg)[a]	86.7	3.2 (1.5, 4.9)	72.7	2.4 (1.5, 3.5)^{c,A}	100.0	3.2 (1.5, 4.5)^{b,A}	80.0	4.4 (3.9, 4.9)^{a,A}
Subgingival plaque (Log CFU/mg)[a]	83.9	3.1 (1.2, 5.1)	72.7	2.2 (1.2, 4.0)^{c,A}	93.3	3.1 (2.0, 4.5)^{b,A}	80.0	4.6 (3.9, 5.1)^{a,A}

The lowercase letters showed significant differences in the same row.
The capital letters showed significant differences in the same column.
[a] Oral samples in the cancer group were collected from 5 patients.

Table 3 Number of *H. pylori* strains and their genes (*cag*A$^+$ and *vac*A$^+$) found in subjects with different disease status.

Number of *H. pylori*	All	Non-disease	Gastritis	Gastric cancer	Pocket depth >5 mm.	clinical attachment >5 mm.
Found in subject (%)	8 of 41 (19.5)	2 of 11 (18.8)	2 of 15 (13.3)	4 of 15 (26.6)	4 of 7 (57.1%)	6 of 7 (85.7%)
Strains found (%)	19	5 of 19 (26.3)	4 of 19 (21.1)	10 of 19 (52.6)	10 of 17 (58.8)	16 of 17 (94.1%)
Strains with *cag*A$^+$ gene (%)	10 of 19 (52.6)	3 of 19 (15.8)	3 of 19 (15.8)	4 of 19 (21.0)	6 of 10 (60%)	10 of 10 (100%)
Found in saliva (*cag*A$^+$ gene)	10 (3)	2 (0)	3 (2)	5 (1)	10 (3)	10 (4)
Found in supragingival plaque (*cag*A$^+$ gene)	7 (5)	3 (3)	0	4 (2)	5 (2)	5 (5)
Found in subgingival plaque (*cag*A$^+$ gene)	2 (2)	0	1 (1)	1 (1)	1 (1)	1 (1)
Strains with *vac*A$^+$ gene (%)	1	0	0	1 (5.3)	recession	1 (1)

conditions; pocket depths > 5 (58.8%) and clinical attachment loss > 5 (94.1%). The strains were recovered in the saliva samples (52.6%) than supragingival plaque (36.9%) and supragingival plaque (10.5%), no strain was received from tissue samples (Table 3).

The *cag*A gene-positive strains were 52.6%, which were found more in gastric cancer patients (21.0%) than non-disease (15.8%) and gastritis (15.8%). The presence of *vac*A gene positive could be detected in only one strain (5.3%) in a gastric cancer patient (Table 3). It showed that the *cag*A gene-positive strains could provide expression more than the *cag*A gene-negative strains. The higher expression was observed among the *cag*A gene-positive strains derived from the gastric cancer patients (Fig. 1).

The adhesion ability of all 19 clinical *H. pylori* strains to various cell lines including human adenocarcinoma gastric cell line (AGS), human oral squamous cell carcinoma (H357 cells), human colorectal adenocarcinoma cells (Caco-2), and human normal intestinal epithelial cell line-6 (HIEC-6) was examined. Results showed that there was a great variation of adhesion ability among the strains, and the significantly higher adhesion ability of strains was found in AGS cells compared to other cell lines (Fig. 2A). The *cag*A gene positive strains had the significantly higher adhesion

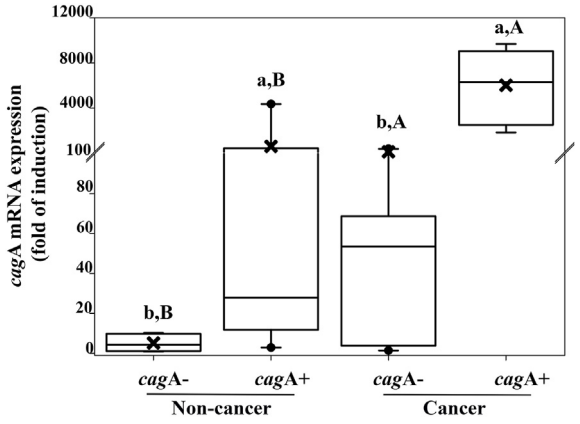

Figure 1 The expression of the *cag*A gene-positive and *cag*A gene-positive strains among the strains derived from non-cancer and cancer patients were shown as a boxplot (exhibiting five values from the bottom to the top: the minimum values, the first quartile, the median is marked by a horizontal line in the box, the third quartile, and the maximum values, respectively). Lowercase letters showed significant difference of mRNA expression in the same disease status. Capital letters showed significant difference of mRNA expression in the same *cag*A positive or *cag*A negative strains in different disease status ($P < 0.05$).

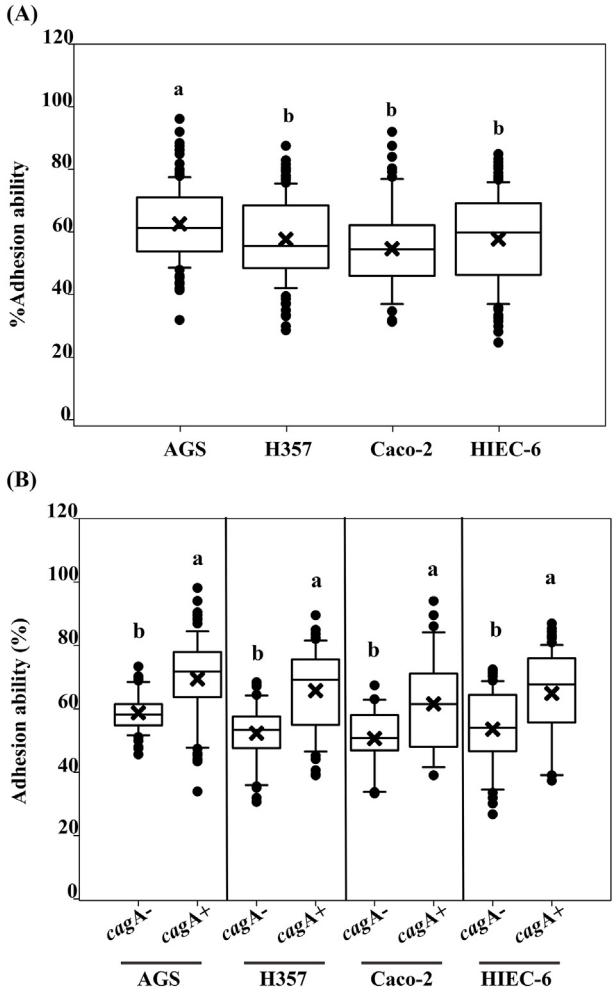

Figure 2 Adhesion abilities of all clinical *H. pylori* strains to various cell lines (A), and compared between *cag*A gene-positive and *cag*A gene-positive strains (B). Results showed as a boxplot (exhibiting five values from the bottom to the top: the minimum values, the first quartile, the median is marked by a horizontal line in the box, the third quartile, and the maximum values, respectively). Lowercase letters showed significant difference of adhesion ability between the different tested cell lines and in difference between the *cag*A positive or *cag*A negative strains in the same cell line ($P < 0.05$).

ability than the *cag*A gene negative strains in all tested cell lines (Fig. 2B). When the adhesion ability was considered among the strains with the presence of *cag*A gene together with the disease status (non-gastric- and gastric-cancer), the *cag*A gene-positive strains in gastric cancer patients had the significantly higher adhesion ability to AGS and H357 cells than the *cag*A gene negative strains. There was no significant difference in the *cag*A gene negative strains between non-gastric cancer and gastric cancer (Fig. 3).

The pro-inflammation expressions (IL-1β, IL-6, IL-8, and TNF-α) and *cag*A gene were investigated in periodontal fibroblast PDL and AGS cells, it demonstrated that in general the expressions of all pro-inflammation were observed more in AGS cells than PDL cells. The *cag*A gene-positive strains gave significantly higher pro-inflammation expressions than *cag*A gene-negative strains, and the highest expression was found among the strains from gastric cancer patients (Fig. 4A and B).

Discussion

H. pylori is classified as a class I carcinogen due to its association with the development of gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma.[18] The presence of oral *H. pylori* has been much received attention since the oral cavity is the first entry connected to the gastrointestinal tract. Consequently, oral *H. pylori* has been considered a potential source of gastric infection[5] or oral–oral transmission route.[19] A number of techniques including molecular methods, immunological or biochemical approaches, and traditional culture method, have been employed for the investigation of *H. pylori* in oral samples e.g., saliva, and plaques. However, the findings of prevalence rate among those samples were found to be a great variation.[5] In this study, the prevalence of *H. pylori* in general was found 84.8% and 19.5% by PCR and culture methods, respectively, and it was accepted that PCR method was more sensitive than culture.[20] Moreover, *H. pylori* strains could not be recovered from biopsy tissues, it is noted that most samples gave negative culture showing lower 10^3 CFU/mL by the real-time PCR. This may explain by that because the low number of *H. pylori* found using real-time PCR in samples, which may lead to undetectable by cultivation. It is similar to the previous reports.[20] Both PCR and culture methods revealed that *H. pylori* was found more among gastric cancer patients. This may result from the higher clinical periodontal conditions (gingival recession, pocket depth, and clinical attachment loss) found in gastric patients. It was reported that poor periodontal health

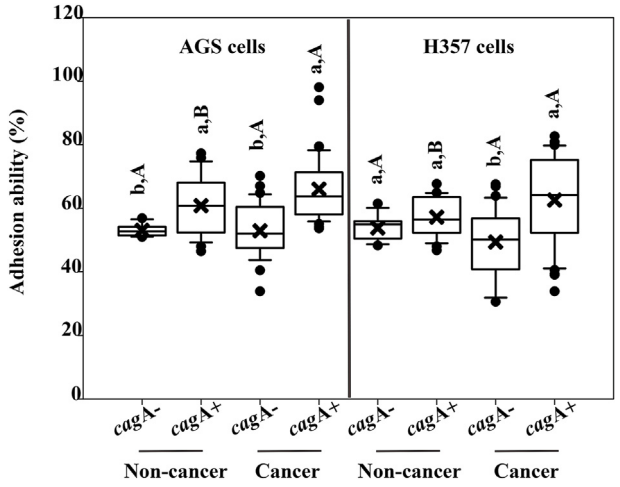

Figure 3 Adhesion abilities *H. pylori* strains to AGS and H357 cell lines showed as a boxplot (exhibiting five values from the bottom to the top: the minimum values, the first quartile, the median is marked by a horizontal line in the box, the third quartile, and the maximum values, respectively). Lowercase letters showed significant difference between the *cag*A positive and *cag*A negative strains in the same disease status. Capital letters showed significant difference in the same *cag*A positive or *cag*A negative strains in different disease status ($P < 0.05$).

having pocket depths > 5 mm. related to *H. pylori* infection of adults in the U.S. population.[21] In addition, there are reviews have shown a close relationship between the presence of oral *H. pylori* and the severity of periodontal diseases.[5,22]

Oral cavity has been considered an important potential reservoir for *H. pylori* recolonization of the gastric infection, however, the existing information on the oral *H. pylori* and their virulence factors is few. This may be the first conducting of adhesion ability of *H. pylori* strains with human oral keratinocytes, while most previous studies have been reported in gastric mucosa cells.[23] It was found that adhesion ability of *H. pylori* strains to oral keratinocytes (H357), especially *cag*A positive strains, was high, although it was lower than AGS cells. Results supported the finding of *H. pylori* strains could exist in oral cavity.

Among the *H. pylori* virulence factors, *cag*A and *vac*A are the most pathogenic oncoproteins involved in colonization and damage to gastric epithelium, increased cell proliferation, chronic inflammation, and carcinogenic processes.[24,25] During the adhesion of *cagA*-positive *H. pylori* strains to gastric cells, intense inflammatory responses, especially IL-8, is secreted by gastric epithelial cells.[26–28] This study provided information on oral *H. pylori* strains and their virulence gene function of *cag*A gene. In this study, it could not examine the function of *vac*A gene due to only one strain has been obtained. Results confirmed that there were stimulated immune responses, especially IL-8, in PDL cells same as in gastric cells. It also reveals a possible association between the presence of the major virulence factors *cag*A gene and diseases outcome (gastric- and periodontal diseases). A meta-analysis has

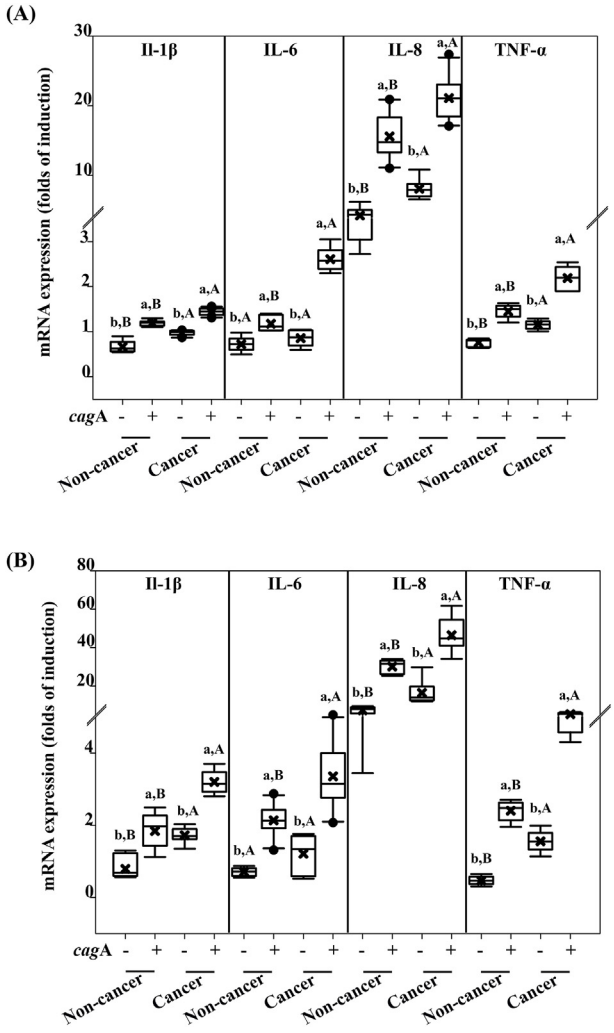

Figure 4 Pro-inflammation expressions (IL-1β, IL-6, IL-8, and TNF-α) and presence of *cag*A gene in periodontal fibroblast PDL (A) and AGS (B) cells, showed as a boxplot (exhibiting five values from the bottom to the top: the minimum values, the first quartile, the median is marked by a horizontal line in the box, the third quartile, and the maximum values, respectively). Lowercase letters showed significant difference between the *cag*A positive and *cag*A negative strains in the same disease status. Capital letters showed significant difference in the same *cag*A positive or *cag*A negative strains in different disease status ($P < 0.05$).

shown that there is a possible association of periodontal diseases with gastric infection, although it is not statistical significance.[22]

In conclusion, it is an endeavor to understand the prevalence of *H. pylori* and its function in oral cavity. The colonization by *H. pylori* in oral cavity has been confirmed, and the *cag*A gene-positive strains play a crucial role in both adhesion and inflammatory responses. According to the findings, the presence of *H. pylori* and its virulence gene in oral cavity should be received attention. An eradication of such strains from oral cavity may help to prevent the transmission and recolonization to gastric organs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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