

Predominant mucosal *IL-8* mRNA expression in non-*cagA* Thais is risk for gastric cancer

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Abstract

AIM: To study gastric mucosal interleukine-8 (*IL-8*) mRNA expression, the cytotoxin-associated gene A (*cagA*) mutation, and serum pepsinogen (PG) I / II ratio related risk in Thai gastric cancer.

METHODS: There were consent 134 Thai non-cancer volunteers who underwent endoscopic narrow band imaging examination, and 86 Thais advance gastric cancer patients who underwent endoscopic mucosal biopsies and gastric surgery. Tissue samples were taken by endoscopy with 3 points biopsies. The serum PG I, II, and *Helicobacter pylori* (*H. pylori*) immunoglobulin G (IgG) antibody for *H. pylori* were tested by enzyme-linked immunosorbent assay technique. The histopathology description of gastric cancer and non-cancer with *H. pylori* detection was defined with modified Sydney Score System. Gastric mucosal tissue *H. pylori* DNA was extracted and genotyped for *cagA* mutation. Tissue *IL-8* and cyclooxygenase-2 (COX-2) mRNA expression were conducted by real time relative quantitation polymerase chain reaction. From 17 Japanese advance gastric cancer and 12 benign gastric tissue samples, all were tested for genetic expression with same methods as well as Thai gastric mucosal tissue samples. The multivariate analysis was used for the risk study. Correlation and standardized *t*-test were done for quantitative data, *P* value < 0.05 was considered as a statistically significant.

RESULTS: There is a high non *cagA* gene of 86.8 per cent in Thai gastric cancer although there are high yields of the East Asian type in the positive *cagA*. The *H. pylori* infection prevalence in this study is reported by combined histopathology and *H. pylori* IgG antibody test with 77.1% and 97.4% of sensitivity and specificity, respectively. The serum PG I / II ratio in gastric cancer is significantly lower than in the non-cancer group, *P* = 0.045. The serum PG I / II ratio of less

than 3.0 and *IL-8* mRNA expression ≥ 100 or $\log_{10} \geq 2$ are significant cut off risk differences between Thai cancer and non-cancer, $P = 0.03$ and $P < 0.001$, respectively. There is a significantly lower PGI/II ratio in Japanese than that in Thai gastric cancer, $P = 0.026$. Serum PG I / II ratio at cut off less than 3.0 and *IL-8* mRNA expression Raw RQ > 100 or $\log_{10} > 2$ are significantly difference between Thai cancer group when compared to non-cancer group, $P = 0.013$ and $P < 0.001$, respectively. In the correlation study, low PG I / II ratio does not associate with chronic atrophic gastritis severity score in Thais non-cancer cases. However, there is a trend, but not significant convert correlation between *IL-8* mRNA expression level and low PG I / II ratio in Thai positive *H. pylori* infection. The high expression of *IL-8* gene demonstrates a poorer prognosis by stage and histology.

CONCLUSION: Predominant gastric mucosal *IL-8* mRNA expression level, *H. pylori* infection, and low PG I / II ratio are relative risks for Thai gastric cancer without correlation with *cagA* mutation.

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Key words: Gastric cancer; *CagA* mutation; Interleukine-8 mRNA expression; *Helicobacter pylori*; Pepsinogen I / II ratio

Core tip: A high level of interleukine-8 (*IL-8*) mRNA expression was detected in more than eighty percent of Thai gastric cancer patients and nearly two fold in the normal Thai population. The majority of northern Thai gastric cancer patients who had negative *cagA* gene *Helicobacter pylori* infection even with or without its mutation, still have a high *IL-8* mRNA expression level. The pathogenesis of Thai gastric cancer may primarily involve another gate-way besides the bacterial factor. The results show that there is a predominantly cancer inflammation state regulated by *IL-8* mRNA expression level that can be detected in Thai gastric cancer patients.

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INTRODUCTION

Gastric cancer pathogenesis is a well-known worldwide multifactorial condition. The gastric cancer incidence rate in Thailand ranks ninth by 4.1:100000 in males and 2.1:100000 in females. Despite being a low incidence country, northern Thailand has a higher gastric cancer in-

cidence rate with 6.6:100000 in males that ranks fifth of overall cancer in the northern Thai region, and 4.5:100000 in females^[1]. The author was interested in the carcinogenesis of gastric cancer in Thais, and why the incidence in Thais is much different from other East Asian countries. The interleukin-8 (*IL-8*) gene is one of the principal mediators for the inflammatory response gate way that was first reported in 1970s, and it is one of factors that are possible to affect gastric cancer carcinogenesis^[2]. A recent case-controlled surveillance study in northern Thailand on cytokine gene *IL-1b-511* mutations in three East Asian populations showed no predominantly correlated specific causative factor responsible for differences among ethnics and histologic types^[3,4]. Therefore, the author proposed the study on other gate-ways of cytokine expression in the human gastric mucosal cell.

Recently, an *in vitro* study showed the association of the mucosal tissue *IL-8* mRNA expression related to the *Helicobacter pylori* (*H. pylori*), positivity cytotoxin-associated gene A (*cagA*) gene. The East Asian genotype was reported in Japanese gastric cancer in about 85% of the cases. Many *in vitro* studies showed this toxicity gene related to gastric mucosal cell injury, inflammation, and oncogenic potential^[5-7]. The *cagA*, East Asian genotype is commonly detected in chronic gastritis and gastric cancer of the Japanese^[8,9]. There is reported data that a low serum pepsinogen (PG) I / II ratio of less than 3.0 with a PG I level of less than 70 ng/dL was considered as a high risk factor for Japanese gastric cancer^[10,11]. There is no recent *in vivo* study reporting a correlation among these above factors, especially *IL-8* and cyclooxygenase-2 (COX-2) mRNA expression level in Thais.

The author hypothesized that gastric mucosal tissue *IL-8* mRNA expression may be different among ethnicities, and it may correlate to other reference pathogenesis factors. This study aimed to look for the risk and correlation of these factors in Thai gastric cancer. The level of *IL-8*, COX-2 mRNA expression, and *cagA* gene mutation distribution were also to be the first report in Thai gastric cancer.

MATERIALS AND METHODS

Research methodology was considered and permitted by Thai and Japanese local ethical committees, the NRCT and Japan Society for the Promotion of Science code ID-NRCT 10726.

Patient characteristics and volunteer selection

An experimental based cross-sectional study was conducted in the Gastrointestinal Surgery and Endoscopy Unit, Chiang Mai University Hospital from 2007 through 2010. Informed consents were obtained from 86 Thai gastric cancer patients who underwent narrow band imaging (NBI) endoscopy and gastric surgery during year 2007-2010, and 134 Thai non-cancer volunteers who underwent NBI endoscopic examination from 2006 to 2008. All gastric cancer patients in this study had locally

advanced gastric cancer, and underwent examinations by endoscopy before curative gastric resection. Seventeen advanced stage Japanese gastric cancer and 12 non-cancer surveillance patients were recruited. Peptic ulcer disease was excluded in this study. Gastric mucosal tissue samples were taken by endoscopy with three biopsy sites for pathology and bimolecular genetic tests before surgical treatment. In cancer cases, biopsy points were specified from non-necrotic areas of the tumor. The histopathology description of the tumor and histologic type were defined. For pathological examination in both groups, chronic gastritis and metaplasia with *H. pylori* detection were classified with a modified Sydney Score System.

Serum PG I and II level, and *H. pylori* immunoglobulin G antibody test

A 5 cc sample of venous blood was collected from each study participant. The red blood cell and serum separation was done, and preserved at -20 °C. The serum PG I, II, and immunoglobulin G (IgG) antibody for *H. pylori* were tested by the standard enzyme-linked immunosorbent assay technique. The standard cut off value used was a PG I level of more than 70 ng/mL or PG I / II ratio more than 3.0 for no atrophy or positive Grade 1, PG I < 70 ng/mL and PG I / II ratio < 3.0 excluding severe atrophy for moderate atrophy or positive Grade 2, and PG I < 30 ng/mL and PG I / II ratio < 2.0 for severe atrophy or positive Grade 3, respectively^[10,11]. All samples were tested twice for reliability confirmation (Toyobo, co, Ltd., Japan)

Tissue *H. pylori* DNA extraction and *cagA* genotyping method

The tissue *H. pylori* DNA extracted from the lower antral position in the stomach was examined by the polymerase chain reaction method, and genotyped for *cagA* mutation in all samples by the author (Samples were also examined by double blinded test by Toyobo, co, Ltd). The *H. pylori* positive control of *cagA* positive strain number 11638 (Western), 26695 (Western), and F57 (East Asian) were provided by the collaborative institute. The bacterial tissue DNA and genotyping method with primers used in this study were conducted as recently described. The specific oligonucleotide primers forward (5'-AAAAGC-GACCTTGAAAAATTC-3'; nucleotides 2299-2319), reverse-1 (5'-CTTCATTTTTTTGAGCTTGTTGAGC-3'; nucleotides 2488-2463) and reverse-2 (5'-ATTAAT-GCGTATGTGGCTGTTAGTAGC-3'; nucleotides 3222-3195, were originally described by Azuma *et al*^[12].

Cell line culture and gastric mucosal total mRNA extraction with reverse transcriptase reaction for cDNA synthesis

The AGS cell line was grown before cell collection for mRNA extraction at a cell count of 2×10^6 - 4×10^6 . They underwent a total mRNA extraction protocol. The technique followed was a reverse transcriptase reaction using a commercial high capacity RNA-to-cDNA kit (Applied Biosystems)^[13].

Gastric mucosal *IL-8* and *COX-2* mRNA expression by relative quantification real time reverse transcription-polymerase chain reaction

We conducted the experiment from three positions of gastric mucosal biopsies in all Thais and Japanese study participants. All of gastric mucosal tissue samples were transformed to cDNA after total mRNA extraction. The analysis was substantially correctable by analysis both in raw relative quantitation (RQ) and log₁₀ value for adjusted normal distribution curve. All Human TaqMan probe primer express that was used in this study had 81-base pairs (bp) *IL-8* specific human primer assay ID number Hs99999034_m1, 111- base pairs (bp) *COX-2* assay ID number Hs01573471_m1, and 121- base pairs (bp) specific human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Hs99999905_m1 those designed and supplied by Applied Biosystems, United States. The internal control was performed by GAPDH of a matched number template. The real-time relative quantitation value was measured by comparing to the base line value of AGS cell line subject control before making the analysis.

Statistical analysis

A student *t*-test was used for quantitative data, *IL-8* and *COX-2* mRNA expression level, and PG level. The χ^2 test was used for qualitative data. The correlation study for pair factors was done in subgroup analysis for defined groups of ethnic, cancer and non-cancer populations. The multivariate analysis was used for risk study for both non-normal distribution and normal distribution curve data bases. STATA 11.0, United States and SPSS 16, United States were used for statistical analysis, and the *P* value of less than 0.05 was considered statistically significant.

RESULTS

There were 86 cases of advanced gastric cancer and 45 (33.8%) normal control cases, 46 (34.6%) non-peptic disease benign lesions without recent history of any treatment, and 42 (31.6%) chronic gastritis cases among 134 non-cancer control cases who were included in the genetic expression experiment. Thai male and female cancer incidences are 60.5% (52/86) and 34.0% (39/86), respectively. Males are also the predominant gender in Japanese. Both nations have significantly high incidence of gastric cancer at age 40 years old or above.

The *H. pylori* infection prevalence is reported by combined histopathology, *H. pylori* IgG antibody level, and 23S rDNA results that have 77.1% and 97.4% of sensitivity and specificity, respectively. Among Thai cancer patients and non-cancer volunteers, *H. pylori* prevalence was 72.1% and 71.6%, respectively. Meanwhile, Thai gastric cancer cases had a *cagA* genotype demonstrated in only 7/62 (12.3%) in positive *H. pylori* infection cases by 23S rDNA that yields six cases of East Asian type and one case of Western type. In non-cancer volunteers, there were 62/98 (63.9%) of positive *cagA* and 34/98 (36.1%) of negative *cagA* genotyping in positive *H. pylori* infec-

Table 1 Characteristics of 220 Thais examined for interleukine-8 mRNA expression *n* (%)

Variable	Cancer (<i>n</i> = 86)	Benign (<i>n</i> = 134)
Sex		
Male	52 (60.5)	41 (30.6)
Female	34 (39.5)	93 (69.4)
Age (yr)		
< 40	5 (5.8)	28 (20.9)
≥ 40	81 (94.2)	106 (79.1)
mean ± SD	56 ± 11.3	48.5 ± 11.2
Alcohol drinking		
No	50 (58.1)	80 (59.7)
Yes	36 (41.9)	54 (40.3)
Smoking		
No	62 (72.1)	122 (91.0)
Yes	24 (27.9)	12 (9.0)
Diseases		
Normal	-	45 (33.8)
Benign lesion (polyps, erosion, mild superficial gastritis)	-	46 (34.6)
Chronic active gastritis	-	42 (31.6)
Cancer	86 (100.0)	-

tion cases that yielded 47.7% of East Asian, 27.4% of Western, and 24.9% of Mixed genotype. For the six year follow up of 18 cases of high grade chronic atrophic gastritis (CAG group) in non-cancer Thais who had long term *H. pylori cagA* East Asian type infection, no one has developed gastric cancer.

The enzyme PG results, showed a significantly lower PG I / II ratio with a mean of 3.3 ± 1.7 in gastric cancer patients than one in non-cancer volunteers, $P = 0.045$, and of other CAG, $P = 0.002$. There is a significantly lower PG I / II ratio in Japanese gastric cancer than in Thai gastric cancer, $P = 0.026$.

For *IL-8* and *COX-2* mRNA expression results, 86 Thai gastric cancers were tested successfully in comparison with 134 Thai non-cancer volunteers. The detection rates of *IL-8* mRNA expression were 77/86 (89.5%) in Thai gastric cancer and 102/134 (74.6%) in Thai non-cancer volunteers. Thai population characteristic data that was examined for *IL-8* mRNA expression is demonstrated in Table 1. Serum enzyme PG I, II level, and *H. pylori* infection status are demonstrated in the cancer population and non-cancer volunteers in Thais is demonstrated in Table 2. We found a remarkable number of Thai gastric cancers with a negative *cagA*; therefore, *IL-8* mRNA expression was examined and the cut-off point of expression value difference is demonstrated in Table 3. Serum PG I / II ratio at cut-off point of less than 3.0 and raw RQ ≥ 100 or $\log_{10} \geq 2$ of *IL-8* mRNA expression level showed the significantly different between the Thai gastric cancer group and the non-cancer group, $P = 0.045$ and $P < 0.001$, respectively. In the multivariate analysis application, the four co-factors related to gastric cancer risk including *IL-8* mRNA expression in Thais are shown in Table 4.

At the same stage of advanced gastric cancer, the mean levels of *IL-8* mRNA expression in Thai cancer

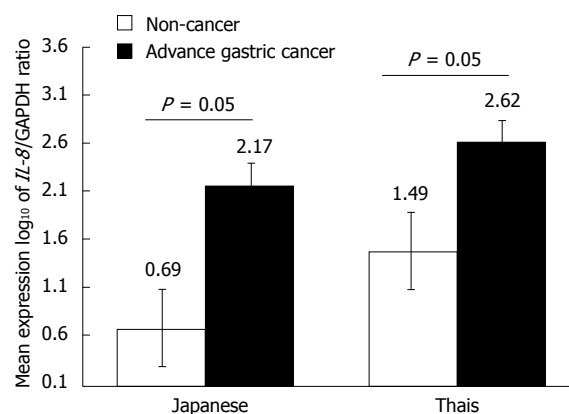


Figure 1 Mean interleukine-8 mRNA expression level measurement of relative quantitation by real-time reverse transcription-polymerase chain reaction study in gastric cancer comparing with non-cancer population both Japanese and Thais. In Japanese and Thais, gastric mucosal interleukine-8 (*IL-8*) mRNA expression in cancer is higher than in non-cancer with $P = 0.05$. The mean level of *IL-8* mRNA expressions in Thai cancer and Japanese cancer were 9615.65 ($\log_{10} = 2.62$) and 1509.11 ($\log_{10} = 2.17$), respectively, $P = 0.014$. The mean level of *IL-8* mRNA expression in non-cancer Thais is 2262 ($\log_{10} = 1.49$) while that in non-cancer Japanese is 10.79 ($\log_{10} = 0.69$), $P < 0.001$.

and Japanese cancer were 9615.65 ($\log_{10} = 2.62$) and 1509.11 ($\log_{10} = 2.17$), respectively, $P = 0.014$. For gastric cancer risk at cut-off *IL-8* expression level by \log_{10} greater than two in Thais and Japanese, odds ratio (OR) = 7.97 (95%CI: 3.75-16.97, $P < 0.001$) and OR = 4 (95%CI: 1.29-12.40), respectively. In the non-cancer group, we found that the *IL-8* mRNA expression level was lower than cancer population with a significant difference, $P < 0.001$. The total mean *IL-8* mRNA expression in non-cancer Thais was 2262 ($\log_{10} = 1.49$) while that in Japanese non-cancer was 10.79 ($\log_{10} = 0.69$), $P < 0.001$. In comparison within the same ethnic group, the mean levels of *IL-8* mRNA expression in Thai and Japanese cancer were higher than those in non-cancer, $P = 0.05$ as showed in Figure 1.

The *COX-2* mRNA expression did not indicate significant rising level with detection rate of 65% in Thai and Japanese gastric cancer. In comparison with *IL-8* mRNA expression, although the level of *COX-2* mRNA expression was slightly higher in gastric cancer than normal gastric mucosal tissue, there were much lower levels than those of *IL-8* mRNA expression.

In the correlation study, low PG I / II ratio was not associated with the CAG severity score in Thai non-cancer cases because of a few number of CAG in both Thai gastric cancer and non-cancer populations in this study. There was no significant difference for the *IL-8* mRNA expression level in cancer between positive and negative *H. pylori* infection. There was no direct correlation of *IL-8* mRNA expression level and serum IgG levels. In subgroup analysis, there was a significant difference of higher levels in groups of poorly differentiated histopathology in comparing both nations. For the diffuse histologic type, the *IL-8* mRNA expression level is about 1.5 times higher than that of intestinal histologic type with a statistically significant difference in Japanese.

Table 2 Serum enzyme pepsinogen I, II level and *Helicobacter pylori* infection detection results in Thais *n* (%)

Variable	Cancer (<i>n</i> = 86)	Benign (<i>n</i> = 134)	<i>P</i> value
PG I / II, (ng/μL), mean ± SD			
I	57.39 ± 46	54.86 ± 68.5	0.780
II	19.42 ± 21	15.42 ± 11.7	0.090
PG I / II ratio			
≤ 3	28 (39.4)	34 (27.9)	0.045 ¹
> 3	43 (60.6)	88 (70.1)	
<i>H. pylori</i> pathology			
Negative	31 (36.8)	60 (44.8)	0.001
Positive	55 (63.2)	74 (55.2)	
Serum IgG			
Negative	31 (46.3)	57 (44.2)	0.820 ¹
Positive	36 (53.7)	72 (55.8)	
CagA genotyping in positive 23S rDNA			
Negative	55 (88.7)	62 (64.6)	< 0.001 ¹
Positive	7 (12.3)	34 (35.4)	
<i>H. pylori</i> Infection status			
True negative	20 (24.4)	37 (36.0)	0.120 ¹
True positive	62 (75.6)	96 (64.0)	

¹Some numbers are not included in the analysis due to missing laboratory entries. The statistical analysis was performed by χ^2 of each factor. IgG: Immunoglobulin G; CagA: Cytotoxin-associated gene A; PG: Pepsinogen; *H. pylori*: *Helicobacter pylori*.

Table 4 Multivariate risk analysis for Thai gastric cancer

Variable	OR	95%CI	<i>P</i> value
Male	4.32	2.06-9.04	< 0.001
<i>H. pylori</i> infection status	0.98	0.96-0.99	0.020
PG II / I ratio ≤ 3	2.06	0.94-4.47	0.060
<i>IL-8</i> mRNA expression	7.97	3.75-16.97	< 0.001

OR: Odds ratio; *H. pylori*: *Helicobacter pylori*; PG: Pepsinogen; *IL-8*: Interleukin-8.

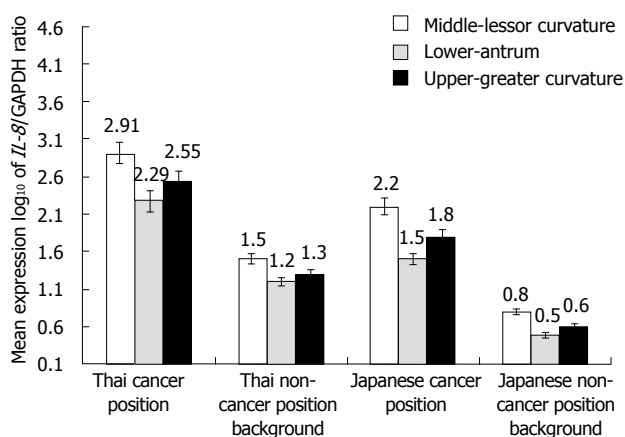


Figure 2 The mean interleukine-8 mRNA expression in Thais divided by histology and cancer position. Middle is the lessor curvature and non-cancer position, lower is the antrum, and Upper is the greater curvature. *IL-8*: Interleukine-8.

High *IL-8* mRNA expression was primarily found in the non-*cagA* Thai gastric cancer population. There was a significantly different mean *IL-8* mRNA expression level between groups of negative *cagA* by $\log_{10} = 2.46 (\pm 1.04)$

Table 3 Molecular genetic results of cyclooxygenase-2 and interleukine-8 mRNA expression in Thais *n* (%)

Variable	Cancer (<i>n</i> = 86)	Benign (<i>n</i> = 134)	<i>P</i> value
COX2 raw RQ			
No expression detection	30 (34.90)	43 (53.10)	< 0.001 ¹
Expression detection	56 (65.10)	38 (46.90)	
COX2 raw RQ mean ± SD	41.69 ± 4.90	5.37 ± 4.20	< 0.001 ¹
COX2 \log_{10} (N, %)			
mean ± SD	1.62 ± 0.96	0.73 ± 0.62	< 0.001 ¹
<i>IL-8</i> raw RQ			
No expression detection	9 (10.47)	32 (25.37)	< 0.001
Expression detection	77 (89.53)	102 (74.63)	
<i>IL-8</i> raw RQ			
≤ 100 or undetected	33 (38.37)	105 (78.36)	< 0.001
> 100	53 (61.63)	29 (21.64)	
mean ± SD	9615.64 ± 49715.00	2262.29 ± 10454.60	< 0.010
<i>IL-8</i> \log_{10}			
≤ 2 or undetected	32 (37.21)	105 (78.36)	< 0.001
> 2	54 (62.79)	29 (21.64)	
mean ± SD	2.62 ± 1.10	1.49 ± 1.20	< 0.010

¹Some numbers are not included in the analysis due to missing laboratory data. RQ: Relative quantity; COX2: Cyclooxygenase-2; *IL-8*: Interleukin-8.

Table 5 Comparative means interleukine-8 mRNA expression level detection between Thai and Japanese Cancer populations divided by histopathology *n* (%)

Histopathology	Thai (<i>n</i> = 77)	Japanese (<i>n</i> = 17)	<i>P</i> value
Diffuse type	55 (71.4)	4 (23.5)	0.01
mean ± SD	2.85 ± 1.10	2.55 ± 0.52	0.95
Intestinal type	22 (28.6)	13 (76.5)	
mean ± SD	2.52 ± 1.11	1.56 ± 1.06	0.01

Statistical difference between groups (*P* = 0.04). *IL-8*: Interleukin-8.

and positive *cagA* by $\log_{10} = 3.29 (\pm 1.68)$ in the Thai gastric cancer group. However, there were few numbers of Thai gastric cancers with positive *cagA*. In other subgroup analysis of 18 Thais who had high grade CAG, some level of *IL-8* mRNA expression in the 12 Japanese non-cancer patients appeared which an equivalently lower level.

Gastric cancer mucosal tissue *IL-8* mRNA expression in the cancer position had a significantly higher mean level than its level at the non-cancer background position in both Thai and Japanese shown in Figure 2. There was significantly different *IL-8* mRNA expression level between intestinal (favorable) and diffuse (unfavorable) histologic cell types. In Thai gastric cancer, the poorly differentiated gastric adenocarcinoma and signet ring cell were predominantly found in this study. The \log_{10} *IL-8* mRNA mean expressions in unfavorable cell type were 2.55 and 2.85 in Japanese and Thais, respectively, as shown in Table 5. There is a significantly higher level of *IL-8* mRNA expression in diffuse cell type than that in a differentiated histologic cell type, *P* = 0.04. The differentiated histologic cell type in Thais has higher expression level than that in Japanese with a statistically significant difference, *P* = 0.013. The RT-PCR results of gastric mucosal tissue and AGS

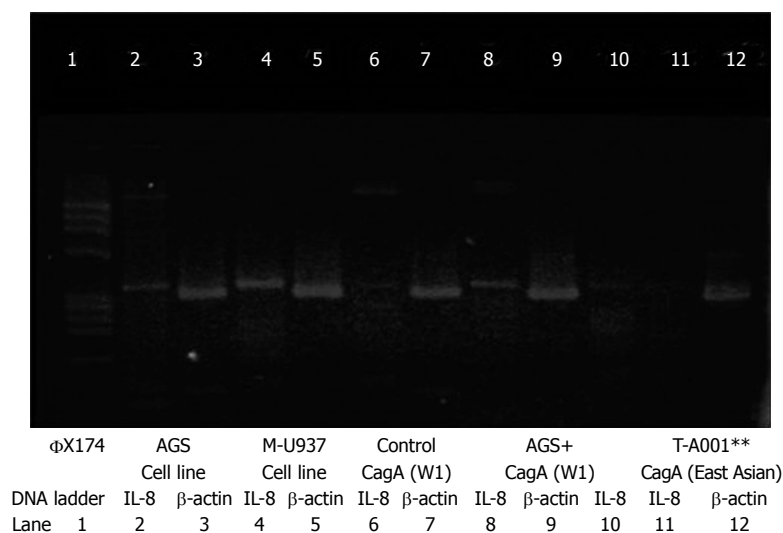


Figure 3 Basic experiment result on real-time reverse transcription-polymerase chain reaction of interleukine-8 mRNA expression with AGS, macrophage cell line, normal gastric mucosal cell, AGS cancer cell line co-culture with two strains of *cagA Helicobacter pylori*, and positive *cagA*, Thai non-cancer samples sequences showed on 12 lanes. M: Marker.

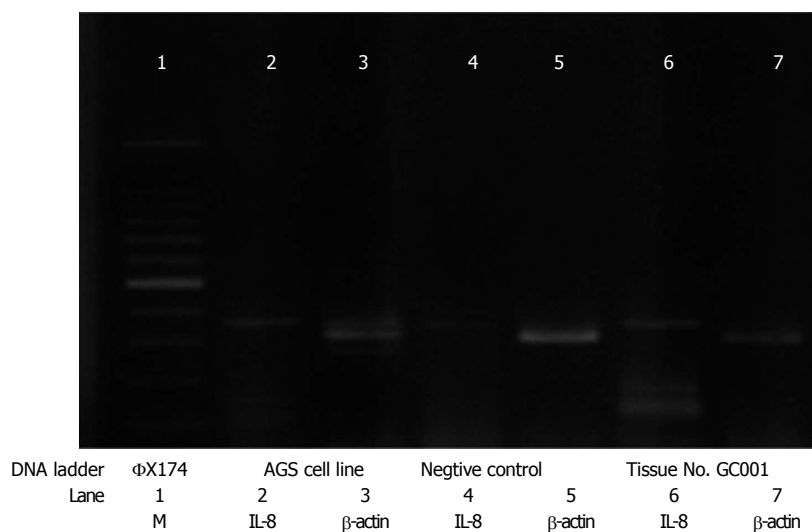


Figure 4 Real-time reverse transcription-polymerase chain reaction result of interleukine-8 mRNA expression from AGS, negative control, and Thai gastric cancer mucosal tissues. The positive results of interleukine-8 (*IL-8*) mRNA expression appeared at 320 bp band comparing with 300 bp band of marker (M) in lane 1.

cell line *IL-8* mRNA expression were demonstrated in Figure 3. and Figure 4. However, there was no significant difference of *IL-8* mRNA expression level between positive and negative *H. pylori* infection in subgroup analysis of non-cancer background positions.

In summary, *IL-8* mRNA expression level is predominantly found, and trend toward an inverted correlation to PG I / II low ratio in Thai gastric cancer patients. There is no direct correlation of *IL-8* mRNA expression level with the *cagA* gene mutation in Thai gastric cancer.

DISCUSSION

In the present *in vivo* study, there is a significant risk of *IL-8* mRNA expression level predominantly found rising up more than 80% of northern-Thai gastric cancer. There is significantly higher level of *IL-8* mRNA expression in poorly differentiated than in well differentiated carcinoma in both Thailand and Japan. There is a trend of converted independent correlation with the very low PG I / II ratio in gastric cancer as well as a few numbers of Thai severe CAG, but no direct correlation with positive *H. pylori* infection or *cagA* genotypes.

Higher level *IL-8* mRNA expression in non-cancer Thais comparing with non-cancer Japanese demonstrated the difference of gastric mucosal defense and genetic expression between the two nations. Thai gastric cancer has less background of CAG. In this study, there is no evidence that showed a direct correlation of *IL-8* mRNA expression with *cagA* mutation genotype in *H. pylori* positive cases. Predominant *IL-8* mRNA expression level resulted in non-atrophic mucosa of both gastric cancer and non-cancer Thais. The result is different from previous *in vitro* or some *in vivo* studies in high incidence gastric cancer countries, such as Japan, China, and South Korea.

In Thai gastric cancer patients, *IL-8* mRNA expression level at the lesser curvature is the most represented location. Its predominantly high level may represent relatively vascular invasion as well as major gastric mucosal inflammation. However, the area of gastric antrum in Thai gastric cancer patients has less activity than the lesser curvature because atrophy occurs more frequently.

Also, a high level of *IL-8* mRNA expression is matched with the poor prognosis by histopathology cell type and tumor stage. Long-term bacterial infection has less effect to change the gastric mucosa into CAG. In our

recent six year followed up study in non-cancer cases controlled with positive *H. pylori* infection and without eradication of 500 new non-cancer cases, a few people developed severe gastritis and still have a high PG I / II ratio. In this study, the number of high PG I / II ratio in Thai cancer is still about 45% which is nearly the same percentage in our recently published study^[4].

A few gastric cancer preventive models on natural Thai products were reported^[14]. There is one study on diet consumption in Thais showing a linkage of gastric cancer risk. The factors which were found to be a higher risk but not statistically significant were low intake of vegetables and fruits (OR = 1.2, 95%CI: 0.74-1.96) and Jeaw prik (mainly chilly with Plara broth or pickled fish), a kind of preserved food in North and North-eastern regions of Thailand (OR = 1.2, 95%CI: 0.76-2.01)^[15]. The consensus of the Asian Pacific guideline on gastric cancer prevention is still debated in some experts' opinions^[16]. *H. pylori* infection screening in a low risk gastric cancer population is not recommended, but serum PG may be helpful to screen the high risk population in northern Thailand. We reported its different characteristics that rely on *H. pylori* IgG antibody and PG I / II ratio in our cancer populations comparing to the data in a Japanese report^[17].

The Japanese study primarily reported the relation of *cagA* genotype and a low PG I / II ratio. Nevertheless, approximately half of the Thai cancer population demonstrates a low ratio of PG I / II similar to the Japanese. There are still a small number of Thais who had a severe atrophy score related to *H. pylori* infection though in the positive *cagA* Thai population.

In this study, the negative *cagA* gene is found in the majority of Thai gastric cancer unlike the Japanese. In Thais, the poorly differentiated cell type gastric adenocarcinoma occurred mainly in the negative *cagA* gene *H. pylori* infection, not in Western type *cagA*.

There is a small number of the Thai population who had a severe atrophy score of gastric mucosa found in our recent and present collaborative study^[18]. Reduction of the fundic gland in chronic gastritis was also related to the low level of PG I / II ratio in Japanese^[19,20]. In our recent study of subgroup analysis on the PG I / II ratios, there was no significant difference between CAG and gastric cancer group, $P = 0.12$. However, the low PG I / II ratio was significantly related to gastric cancer when compared to normal population in a recent match-case control study by OR of 2.3 (95%CI: 1.10-4.80), $P = 0.025$ ^[4]. The tumor location demonstrated locations mainly at the upper portion and corpus in both of our studies. In the present study, the author found risk for cancer by OR 2.06 (95%CI: 0.94-4.47), $P = 0.059$ that seems to be close to the result of our recent study. The low PG I / II ratio was not found to be a high percentage in Thais unlike in Japanese gastric cancer^[9].

For other genetic host factors, in one interesting study, two of the four gastric carcinoma cell lines expressed vascular endothelial growth factor (VEGFR-3) mRNA. In 17 of 36 gastric carcinoma specimens, VEGFR-3-specific

immune activity was detected in tumor cells. These angiogenesis and lymphangiogenesis were also detected in VEGF-C-transfected tumors than in control tumors^[21]. *IL-8* mRNA expression is found to be the gate way mechanism of the vascular epithelial growth factor. For *IL-1* gene, it is a pro-inflammatory cytokine, and the T/T genotype of *IL-1β-511* is suspected as the risk factor of both hypochlorhydria related *H. pylori* infection and gastric cancer in a case-control population in the United States^[22]. The author reported that *c/c* genotype was a risk in Japanese, and a lower number of *c/c* genotype was found as a minor risk related to Thai gastric cancer^[4].

Recent *in vivo* animal models studies showed the expressions of *IL-8* and COX-2 had linkage to the epithelial cell which was co-infected with *H. pylori*. However, our preliminary study reported that there was no difference of *IL-8* mRNA expression level between a cell line which was co-infected with *H. pylori* and the Thai gastric mucosa tissues which had positive or negative *H. pylori*^[23,24]. The toleration, remarkable host response to cancer inflammatory process, healing of stomach mucosal turnover rate, and re-healing process of ulcer in Thais may be different and caused by host susceptible differences to the virulence bacteria.

The theory regarding inflammatory cytokine's influence on cancer development was first contributed by Rudolph Virchow around 150 years ago^[25]. Many studies regarding *IL-8* gene expression remarkably found significant relation with *H. pylori* infection and many *cag* pathogenicity island both *in vitro*^[26,27] and in some number in *in vivo* study of Japanese cancer^[28,29]. However, no study has demonstrated differences of expression level in the individualized host^[30].

In this study, Japanese gastric cancer has a lower *IL-8* mRNA expression on average than that in Thai gastric cancer patients at the same stage of disease and *H. pylori* infection status. However, Japanese gastric adenocarcinoma cases are mostly an intestinal type and infected by positive *cagA* strain *H. pylori*. In contrast with northern Thai gastric adenocarcinoma cases, they are mostly diffuse histologic cell type, and infected by negative *cagA* strain *H. pylori*. Therefore, the authors speculate that the predominant level of *IL-8* mRNA expression found in non-*cagA* gene *H. pylori* infection is not directly related to atrophic gastritis mucosa in Thai gastric cancer.

Some results in this study are unexpected outcomes and different from our recent knowledge of *in vitro* and *in vivo* study in Japanese atrophic gastric mucosa which almost easily infected by positive *cagA* *H. pylori* infection. The *cagA* was also the suspected cause of the *IL-8* gene expression rising.

This result in Thais showed that gastric mucosal tissue *IL-8* mRNA expression has a higher level in the advanced stage and poorer differentiated cell type than in favorable histology or differentiated cell type. The author was suspicious that the less atrophic background of Thai stomach cancer and non-cancer gastric mucosa may be caused by non-*cagA* *H. pylori* infection. However, the high level of mRNA *IL-8* gene expression in Thai gastric can-

cer cases may be explained by the cancer inflammation carcinogenesis that may not be directly related to only *H. pylori* infection in Thais.

The level of *IL-8* mRNA expression in Thai gastric cancer or poorly differentiated gastric carcinoma may be regulated by other factors besides of *H. pylori* infection. Also, unknowns remain regarding how long *IL-8* mRNA expression has been high before the occurrence of gastric cancer or after becoming a more advanced stage. Although the author analyzed the level of *IL-8* and *COX-2* mRNA expression level in normal mucosa and of advanced gastric cancer, this occurrence could not be shown in early gastric cancer. The environmental factors and bacterial virulence effect cannot be excluded.

In this study, the signet ring cell type is predominantly found in Thai gastric cancer population. The poor prognostic histological cell type may have different disease carcinogenesis related to gastric mucosal tissue *IL-8* mRNA high expression level and severity of the disease. The *COX-2* mRNA expression level is directly correlated with only the *H. pylori* infection, and tended to be suppressed unlike *IL-8* mRNA expression. The author supposed that extremely high gastric mucosal *IL-8* expression level may relate to other factors, such as VEGF that could not be demonstrated in this study and should be explored further.

In conclusion, this present *in vivo* study shows results of new factual data on predominant gastric mucosal *IL-8* mRNA expression level in Thai gastric mucosal biopsy tissues in both non-cancer and gastric cancer volunteers. In the present study, the northern Thai gastric cancer population has a high incidence of signet ring cell by the nature of histologic type. The positive *H. pylori* may be one of the co-factors, though the host is infected with non-*cagA* gene and still has an extremely high *IL-8* mRNA expression level. This cytokine expression may represent the individual host defense in both high and low incidence gastric cancer ethnics. The factual results in an experimental based study demonstrated how prevalence of the northern Thai gastric cancer host had active co-infection or had been recently infected by non-*cagA* gene *H. pylori* infection. The *IL-8* mRNA expression level does not directly correlate to non-*cagA* *H. pylori* infection in Thai gastric cancer. However, there is a trend of converted correlation between *IL-8* mRNA expression and low ratio PG I / II without statistical significance, and it seems to be an independent correlation. By these preliminary results, the author expected to do further study on *IL-8* mRNA expression that may act as one of prognostic genetic biomarkers in clinical practice and for chemotherapy application in the nearby future. A study on the current cancer chemotherapy with northern Thai gastric cancer population is on-going.

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COMMENTS

Background

The incidence of gastric carcinoma is very low although the incidence of *Helicobacter pylori* (*H. pylori*) seemed not to be low in Thais. However, the northern Thai population still has the highest gastric cancer incidence in Thailand. Currently, the disease incidence is rising faster than in the past and still the second cause of cancer death worldwide. The cause and risk factors for gastric cancer carcinogenesis in Thais is unclear especially the risk related with *H. pylori* infection or other cofactors.

Research frontiers

Interleukine-8 (*IL-8*) mRNA expression is a common event found in some epithelial malignancies and in gastric adenocarcinoma either due to *H. pylori* caused chronic inflammation or other causes by unrelated carcinogens. It is not clear how the level of this expression related to gastric adenocarcinoma. The authors demonstrated that the predominant overexpression of *IL-8* mRNA could be a potential relative risk for gastric adenocarcinoma in Thais and demonstrated the difference of its level in relationship with the histologic type of gastric cancer.

Innovations and breakthroughs

Recent reports have highlighted the importance of cytotoxin-associated gene A (*cagA*) *H. pylori* infection and its mutation type that is shown predominantly in Japanese gastric cancer carcinogenesis. Particularly in the well differentiated histologic type, the *IL-8* mRNA expression level in the Japanese seems to be much lower than in Thais. However, its level in poorly differentiated cell type of Thai gastric adenocarcinoma has less atrophic background and a higher level of expression than in well differentiated gastric adenocarcinoma. This is the first study to report how measurement of *IL-8* mRNA expression level demonstrates risk in non-*cagA* *H. pylori* infection of Thai gastric cancer and the trend of differences in carcinogenesis related to *H. pylori* infection between high and low incidence ethnics. Furthermore, their *in vivo* studies would suggest that the *IL-8* mRNA expression level yields high prevalence detection in gastric adenocarcinoma and may be a useful tool for gastric cancer prognostic or therapeutic study.

Applications

By understanding different *IL-8* mRNA expression levels, this study may represent a future study with a tissue molecular biomarker for gastric cancer.

Terminology

IL-8 mRNA expression is pro-inflammatory cytokines that is detected in gastric epithelial mucosa and gastric cancer cell lines, such as Kato III and AGS cells.

Peer review

The authors examined the expression of *IL-8* and cyclooxygenase-2 in AGS cell line, normal gastric mucosa, gastritis, and gastric adenocarcinoma tissues. It revealed that *IL-8* mRNA expression predominantly increased in poorly differentiated or signet ring cell gastric adenocarcinoma that showed the trend of a poorer prognosis. The expression was not directly correlated to *cagA* *H. pylori* infection and its mutation type. The results are interesting and may represent a different carcinogenesis of Thai gastric cancer in comparison to recent Japanese studies.

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