Retrospective Cohort Study

Correlation of *Helicobacter pylori* and interleukin-8 mRNA expression in high risk gastric cancer population prediction

Waiwan Chongruksut, Sirikan Limpakan (Yamada), Bandhuphat Chakrabandhu, Chidchanok Ruengorn, Sirisak Nanta

AIM: To evaluate (1) the association of the *Helicobacter pylori* (H. pylori) test and interleukin-8 (IL-8) mRNA expression alone and the severity of gastric cancer (GC); (2) the association of both tests were added to patients' characteristics to identify Thai suspected patients of gastric cancer who would receive the most benefit; and (3) diagnostic value of levels of IL-8 mRNA expression for gastric cancer.

METHODS: A cross-sectional analytical study was completed with 220 patients with 86 GC patients who underwent endoscopy with gastric surgery divided into non-metastasis and metastasis groups, and 134 patients with benign lesions who underwent endoscopic examination, at the Gastrointestinal Surgery and Endoscopy Unit, Chiang Mai University Hospital between 2006 and 2010. Of 220 patients, 86 cases of diagnosed gastric adenocarcinoma were in an advanced stage and 134 cases were non-cancer patients.

RESULTS: The IL-8 mRNA expression showed predominant association with advanced GC when compared to *H. pylori* infection alone [OR (95%CI); 0.86 (0.49-1.53) vs 5.44 (3.08-9.62)] when including the patients' characteristics the highest of the area under the receiver operating characteristic curves (AuROC) of the model were males older than 40 years of age [AuROC (95%CI); 0.81 (0.75-0.86)]. However, preliminary testing for diagnostic indices of four cut-off points of IL-8 mRNA expression to predict the severity of GC cases found an increasing suboptimal trend from the likelihood ratio of positive to differentiate the severity in the GC group. The IL-8 mRNA expression showed a predominant association with GC when compared to *H. pylori* infection, especially in males older than 40 years of age who may benefit most from this test.

CONCLUSION: The future research of IL-8 mRNA expression to predict severity in the gastric cancer group should be warranted.
expression; Gastric cancer

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: The author reviewed updated basic research studies regarding linkages of inflammatory cytokine genetic expression level and gastric cancer (GC) risk prediction in the Thai population. The review focused on interleukin-8 (IL-8) mRNA expression and Helicobacter pylori (H. pylori) infection in which are found an increasing risk for GC and aggressive histologic types. We performed the epidemiologic data in-depth analysis, and make the various cut off points to discern which level of IL-8 mRNA expression has remarkable predictive risk in comparison with H. pylori infection to predict GC occurrence.

INTRODUCTION

Gastric cancer (GC) is one of the most common cancers and continues to remain a major public health problem in the world, with a varying prevalence from 11% to 56% in different areas. The prevalence of GC in the United States was an estimated 74035 people[1] and estimated 934000 cases, 56% of new cases from Eastern Asia, 41% from China, and 11% from Japan[2]. In Thailand, the incidence rate of GC was 4.1:100000 for males and 2:100000 for females, especially in the northern part of Thailand which has a higher GC incidence rate with 6.6:100000 in males, and 4.5:100000 in females[3].

GC is one of the most common causes of death from cancer worldwide, and most of the cases occur in developing countries[4]. A gender difference of mortality of GC was reported; 14.3 per 100000 in men and 6.9 per 100000 in women worldwide[5], as well as geographic counties; 20% mortality rate in Western countries vs up to 60% in Asia[6].

Early diagnosis is crucial because of the possibility of early metastasis to other organs such as, liver, pancreas, omentum, esophagus, bile ducts, and lymph nodes[7]. If GC was detected at an early stage, the five year survival was approximately 90%[8]. Thus, in developing countries, early detection is most needed. The standard method or diagnosing GC is through the upper digestive endoscopy combined with biopsy and histopathological evaluation of the biopsy samples[9]. This method has a high diagnostic accuracy of 95% to 99%[10].

Helicobacter pylori (H. pylori) infection is widely regarded as the most important risk factor in the development of GC[10] with from 0.5% to 2.0% developing gastric adenocarcinoma[10]. A meta-analysis of 34 cohort and case-control studied patients found that H. pylori carried a relative risk of GC of 3.02 (95%CI: 1.92-4.74) in high risk settings (China, Japan and Korea) and 2.56 (95%CI: 1.99-3.29) in low risk settings (Western Europe, Australia, and the United States)[11]. Epidemiologic data indicates that GC occurs more frequently in populations with higher rates of H. pylori infection, and the World Health Organization has classified this bacterium as a Class 1 carcinogen for GC[9]. H. pylori infection was important in the process of tissue remodeling, angiogenesis, tumor invasion and metastasis[12] and induces a number of genes in host cells that are potential determinants of inflammation, angiogenesis, and metastasis including interleukin-8 (IL-8) gene expression[13]. However, it remains unclear how H. pylori infection activates specific transcription factors and induces gene expression. Yamada et al[14] indicated that the H. pylori infection in Thai GC patients was reported by combined histopathology and H. pylori IgG antibody test with 77.1% and 97.4% of sensitivity and specificity, respectively.

Moreover, IL-8 mRNA expression is one of the factors that were possible influences which affect GC[15]. Yamada et al[14,16] reported that GCs were detected in more than 80% of Thai patients with high levels of IL-8 mRNA expression, while H. pylori infection and IL-8 mRNA expression were relative risks for Thai GC, therefore IL-8 mRNA expression may be a useful diagnostic and prognostic risk marker for GC. Similarly, Macri et al[17] indicated that the level of serum IL-8 mRNA expression may act as marker of GC. The high expression of IL-8 mRNA expression was directly demonstrated with a poor prognostic histologic type in GC[14,16].

Although, the association of H. pylori infection and IL-8 mRNA expression and GC were demonstrated from several studies, most studies did not evaluate the association of these biomarkers and the GC severity, i.e., no cancer, non-metastasis, and metastasis stage. There might be an increasing possibility of GC by gradient of IL-8 mRNA expression. Moreover, some studies showed an association with independent factors such as advanced age, sex, and alcohol drinking. Therefore, this present study aimed to evaluate (1) the association of H. pylori test and IL-8 mRNA expression alone and the severity of GC; (2) the association of both tests added to patients’ characteristics to identify Thai suspected patient risk of GC who will receive the greatest benefit for follow up endoscopy; and (3) diagnostic value of four different levels of IL-8 mRNA expression for GC cases.

MATERIALS AND METHODS

A cross-sectional analytical study was conducted in patients over 18 years of age. Eighty-six patients who underwent endoscopy were diagnosed with GC, and 134 patients who underwent endoscopic examination were diagnosed as non-GC, at the Gastrointestinal Surgery and Endoscopy Unit, Chiang Mai University Hospital between 2006 and 2010. All patients were comprehensively examined.
by a gastrointestinal pathologist for H. pylori infection and combined histopathological diagnostic results. The outcomes of the study were divided into non-GC, and GC. In GC patients, those who were categorized in cancer Stages I, II, III and IV were in the GC group.

Tissue samples were taken by endoscopy with tissue IL-8 mRNA expression conducted by real time relative quantitation polymerase chain reaction. Additionally, baseline characteristics; gender, age, alcohol drinking, smoking, stages of cancer, histological pathology were obtained by a physician and nurse, using a case record form. All enrolled patients were examined by endoscopy with a pathology result for H. pylori infection and received biopsy of tissues with IL-8 mRNA expression. This study excluded all patients without results of pathology or tissue IL-8 mRNA expression. The present study was approved by the Institutional Review Boards of the Faculty of Medicine, Chiang Mai University.

**Statistical analysis**

The demographic data were analyzed using $\chi^2$ to test between groups, and the test for trend was used to test for proportion. An ordinal logistic regression, both univariable and multi-variable models, were performed to determine association of H. pylori and IL-8 mRNA expression and severity of GC with or without patients’ characteristics presented with crude and adjusted odds ratio with 95%CI. The area under the receiver operating characteristic curves (AuROC) was calculated and compared using a standard method. IL-8 mRNA expression level was divided into four different cut-off points, and AuROC was compared to select the best cut-off point. Performance of each IL-8 mRNA expression cut-off point was then evaluated for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LHR+), and negative likelihood ratio (LHR-) in only GC cases. A statistical significance level or alpha of 0.05 was selected for Type I error.

**RESULTS**

Of the 220 patients enrolled in this study, 86 cases were diagnosed with GC and underwent endoscopy with gastric surgery, and 134 non-cancer patients underwent endoscopic examination. Among those diagnosed with a non-GC, 45 cases were normal, 46 had benign lesions (polyps, erosion, mild superficial gastritis), and 42 cases had chronic active gastritis. When categorized by staging, 41 cases (47.67%), and 34 cases (39.53%) were in Stage III and Stage IV, respectively.

Two groups of patients were therefore assigned by order of severity to non-cancer, and advanced GC. Patients’ characteristics found statistically significant when testing for trend were sex, age, and smoking status. The majority of GC patients were male, aged $\geq 40$ years, and had a history of smoking (Table 1).

According to H. pylori active infection status and pathology, no statistical differences were found among the groups. While, IL-8 mRNA expression had the highest level in the metastatic GC group (median 325) and non-cancer group (median 19.72), respectively. An IL-8 mRNA expression was transformed to log$_{10}$ and divided into five cut-off points. The higher the cut-off point, the higher proportion of the severity of GC was demonstrated (Table 2).

The value of H. pylori, and IL-8 mRNA expression as biomarkers, alone or combined with patients’ characteristics, were determined. The results showed the predominant performance of IL-8 mRNA expression over H. pylori pathology and serum IgG results (Model 1 vs Model 2). H. pylori pathology results in accordance with significant demographic characteristics, i.e., sex and age showed lower performance compared to IL-8 mRNA expression alone (Model 3 vs Model 2). Adding IL-8 mRNA expression in a model with sex and age, the AuROC of probability to predict severity occurrence of GC was increased (Model 4 vs Model 2). However, adding H. pylori pathology in the last model did not enhance a predictability of GC severity in the last model (Model 5 vs Model 4). Therefore, IL-8 mRNA expression is useful to differentiate severity of GC especially when combined with sex and age (Table 3). The IL-8 mRNA expression used was the best cut-off point of two to predict the severity of GC; AuROC of cut-off-point one through four was 0.64, 0.71, 0.60, and 0.53, respectively (data not shown).

We further analyzed the prediction ability of the model containing IL-8 mRNA expression across age group and sex. The likelihood positive ratio of all models

| Characteristics | Gastric cancer $n = 86$ | Non-gastric cancer $n = 134$ | $P$ value |
|-----------------|------------------------|-----------------------------|-----------|
| Sex             |                        |                             | $<0.001$  |
| Male            | 52 (60.47)             | 41 (30.60)                  |           |
| Female          | 34 (39.53)             | 93 (69.40)                  |           |
| Age             |                        |                             | 0.005     |
| $\geq 40$       | 5 (5.81)               | 28 (20.90)                  |           |
| $< 40$          | 81 (94.19)             | 106 (79.10)                 |           |
| Mean ± SD       | 56 ± 11.29             | 48.5 ± 11.21                |           |
| Alcohol drinking | 36 (41.87)             | 51 (38.06)                  | 0.679     |
| Smoking         | 24 (27.91)             | 12 (8.96)                   | $<0.001$  |
| Diseases        |                        |                             |           |
| Normal          | 0                      | 45 (33.83)                  |           |
| Benign lesion   | 0                      | 46 (34.59)                  |           |
| Chronic active gastritis | 0 | 42 (31.58) |           |
| Gastric cancer  | 86 (100)               | 0                           |           |
| Stage           |                        |                             |           |
| I a             | -                      | -                           |           |
| I b             | -                      | -                           |           |
| II a            | 1 (1.16)               | -                           |           |
| II b            | 2 (2.33)               | -                           |           |
| IIIa            | 8 (9.30)               | -                           |           |
| III b           | 41 (47.67)             | -                           |           |
| IV              | 34 (39.53)             | -                           |           |
| Histological grade |                  |                             |           |
| Poorly differentiated | 25 (29.76) | -       |           |
| Signet ring cell | 36 (42.82)             | -                           |           |
| Moderate differentiated | 16 (19.05) | -       |           |
| Well differentiated | 7 (8.33)               | -                           |           |
was statistically significant. The largest yield of the LHR+ was the model with all variables. Apparently, IL-8 mRNA express has the highest yield of the LHR+ in males who are older than 40 years old compared to the younger age or in the female group (LHR+ 14.54 vs 3.38) due to acceptable LHR+ (more than 5.0, theoretical suggested LHR+). Therefore, IL-8 mRNA expression may be most useful in the Thai male with the age older than 40 years (Table 4).

Because the trend of prediction in the severity of GC of IL-8 mRNA expression was observed, diagnostic indices were determined only in 86 GC patients who were categorized by metastatic status although it was still localized. Under four different cut-off points of IL-8 mRNA expression, the sensitivity was highest in the IL-8 mRNA expression Level one (96.8%) and continuously declined to 12.9% in the cut-off point of Level four. In the opposite, specificity for GC metastasis increased from 8.7% in IL-8 mRNA expression level one to 93.5% in Level four. The LHR+ increased from 1.06 to 1.98 of the Level one to Level four (Table 5). The AuROC of all of the cut-off points were not statistically significant (P-value of difference = 0.832) with less than a 60% range in all groups. There might have been a lack of ample sample size so the IL-8 mRNA expression level could differentiate severity in the diagnosed GC group (Table 5).

The AuROC of H. pylori alone and AuROC of IL-8 mRNA expression alone in prediction of GC occurrence is shown in Figures 1 and 2. Comparable AuROC of IL-8 mRNA expression in an adjusted model by sex, age, and H.pylori, and additional AuROC of both IL-8 mRNA expression and H.pylori infection in prediction of GC are shown in Figure 3.

**DISCUSSION**

In this study, performances of H. pylori infection and IL-8 mRNA expression were determined as to whether there was an association with GC which was divided into two groups: Non-GC, and GC. The IL-8 mRNA expression showed a predominant association with GC when compared to H. pylori infection, especially in males older than 40 years of age. In addition, there was a trend of the probability of GC with increasing levels of IL-8 mRNA expression. Further, preliminary testing for diagnostic indices of four cut-off points of IL-8 mRNA expression to predict severity of GC cases was performed. However, we found an increasing suboptimal trend from the likelihood ratio of positive (less than five times) which may be due to the small sample size to differentiate severity in GC groups.

GC has a high incidence rate in the northern part of Thailand. Epidemiological studies have shown that H. pylori is associated closely with the development of GC and it is widely regarded as the most important modifiable risk factor for GC. However, when categorizing GC...
by severity, *H. pylori* lost its association in our findings. Unlike, *IL-8* mRNA expression, the results from our study found the superiority of prediction of GC severity over *H. pylori* infection. *IL-8* mRNA expression is one of factors that possibly affects GC[15]. Thai people in the advanced stage of GC showed that gastric mucosal tissue *IL-8* mRNA expression has a higher level value and percentage of poorer differentiated cell type more than in favorable histology or differentiated cell type[14]. This finding was consistent with the results of Yamada et al[14,16] which showed that a high level of *IL-8* mRNA expression was detected more than 80% in Thai advanced GC patients, of cases and they demonstrated that gastric mucosal *IL-8* mRNA expression was a relative risk for Thai GC. Thus *IL-8* mRNA expression may also be a useful diagnostic risk marker for GC. It is possible to use *IL-8* mRNA expression as a good indicator for advanced GC or aggressive types of cancer treatment selection especially in poor prognostic cell type.

Moreover, this study demonstrated the AuROC of *IL-8* mRNA expression when comparing gender with age found that males more than 40 years of age predicted the severity of GC with LHR+ 14.5 times. This may explain recent indications that men have a higher incidence rate and may have a poorer prognosis than women[19]. The increased incidence rate of males could be due to the difference in the lifestyles and habits from females; such as smoking and alcohol consumption[20]. A previous study on sex differences in GC incidence based on the study of etiological hypothesis indicated that the predominance of GC in men was a global phenomenon, and was related to a 10 to 15 year delay in the appearance and onset of GC of intestinal subtype in women compared with men[21,22]. Our data showed that *IL-8* mRNA expression may be a helpful tool to identify advanced risk of GC in Thai patients especially males with an age older than 40 years.

We further investigated diagnostic performances...
The association of Helicobacter pylori infection and interleukin-8 (IL-8) mRNA expression and gastric cancer (GC) were demonstrated from several studies. However, there was a lack of evidence of the association of these biomarkers and GC severity.

Innovations and breakthroughs
The IL-8 mRNA expression showed predominant association with GC when compared to *H. pylori* infection, especially in males with age older than 40 years who may benefit the most from this test. The preliminary testing for diagnostic indices of four cut-off points of IL-8 mRNA expression showed a suboptimal trend to differentiate severity in the GC group.

Peer-review
It might be interesting to analyze IL-8 expression level in different stage and different histological grade, in order to demonstrate whether IL-8 is more sensitive than *H. pylori* in different stage and grade GC.

**REFERENCES**

1. Surveillance, Epidemiology, and End Results Program [Internet]. Available from: URL: http://seer.cancer.gov/
2. Iinoue M, Tsugane S. Epidemiology of gastric cancer in Japan. *Postgrad Med J* 2005; 81: 419-424 [PMID: 15998815 DOI: 10.1136/pgmj.2004.029330]
3. Khuhaprema T, Srivatanakul P. Cancer in Thailand Vol. IV. Bangkok: Bangkok Medical Publisher, 2007: 32-33
4. Broza YY, Kremer R, Tisch U, Gevorkyan A, Shiban A, Best LA, Haick H. A nanomaterial-based breath test for short-term follow-up after lung tumor resection. *Nanomedicine* 2013; 9: 15-21 [PMID: 22967910 DOI: 10.1016/j.nano.2012.07.009]
5. Thrumurthy SG, Chaudry MA, Hochhauser D, Mugal M. The diagnosis and management of gastric cancer. *BMJ* 2013; 347: f6367 [PMID: 24191271 DOI: 10.1136/bmj.f6367]
6. Lee KE, Khoi PN, Xia Y, Park JS, Joo YE, Kim KK, Choi SY, Jung YD. Helicobacter pylori and interleukin-8 in gastric cancer. *World J Gastroenterol* 2013; 19: 8192-8202 [PMID: 24363509 DOI: 10.3748/wjg.v19.i45.8192]
7. Coupland VH, Allum W, Blazey JM, Mendall MA, Hardwick RH, Linklater KM, Moller H, Davies EA. Incidence and survival of oesophageal and gastric cancer in England between 1998 and 2007, a population-based study. *BMC Cancer* 2012; 12: 11 [PMID: 22239598 DOI: 10.1186/1471-2407-12-11]
8. Cooke CL, Torres J, Solnick JV. Biomarkers of Helicobacter pylori-associated gastric cancer. *Gut Microbes* 2013; 4: 532-540 [PMID: 23851317 DOI: 10.4161/gmic.25720]
9. Dooley CP, Larson AW, Stace NH, Remmer IG, Valenzuela JE, Eliasoph J, Colletti PM, Halls JM, Weiner JM. Double-contrast barium meal and upper gastrointestinal endoscopy. A comparative study. *Ann Intern Med* 1984; 101: 538-545 [PMID: 6383166 DOI: 10.7326/0003-4819-101-4-538]
10. Atherton JC. The pathogenesis of Helicobacter pylori-induced...
gastro-duodenal diseases. *Annu Rev Pathol* 2006; 1: 63-96 [PMID: 18039108 DOI: 10.1146/annurev.pathol.1.110304.100125]

11 **Cavaleiro-Pinto M**, Peleteiro B, Lунет N, Баррос H. Helicobacter pylori infection and gastric cardia cancer: systematic review and meta-analysis. *Cancer Causes Control* 2011; 22: 375-387 [PMID: 21184266 DOI: 10.1007/s10552-010-9707-2]

12 **Iwamoto J**, Miozaki Y, Nakajima K, Ohtsubo T, Miura S, Narasaka T, Takeyama H, Omata T, Shimokobe K, Ito M, Takehara H, Matsuoka T. Expressions of urokinase-type plasminogen activator, its receptor and plasminogen activator inhibitor-1 in gastric cancer cells and effects of Helicobacter pylori. *Scand J Gastroenterol* 2005; 40: 783-793 [PMID: 16109653 DOI: 10.1080/00365520510015665]

13 **Futagami S**, Hiratsuka T, Tatsuguchi A, Suzuki K, Kusunoki M, Shinji Y, Shinoki K, Iizumi T, Akamatsu T, Nishigaki H, Wada K, Miyake K, Gudis K, Tsukui T, Sakamoto C. Monocyte chemotactic protein 1 (MCP-1) released from Helicobacter pylori stimulated gastric epithelial cells induces cyclooxygenase 2 expression and activation in T cells. *Gut* 2003; 52: 1257-1264 [PMID: 12912855 DOI: 10.1136/gut.52.9.1257]

14 **Yamada S**, Kato S, Matsuhisa T, Makonkawkeyoon L, Yoshida M, Chakrabandhu T, Lertprasertsuk N, Suttharat P, Chakrabandhu B, Nishiumi S, Chongruksut W, Azuma T. Predominant mucosal IL-8 mRNA expression in non-cagA Thais is risk for gastric cancer. *World J Gastroenterol* 2013; 19: 2941-2949 [PMID: 23704827]

15 **Kozlov SV**. Inflammation and cancer. Methods and protocols. Volume 1: Experimental models and practical approaches. Preface. *Methods Mol Biol* 2009; 511: v-viii [PMID: 19415881 DOI: 10.1007/978-1-59745-447-6]

16 **Yamada S**. Predominant gastric mucosal tissue IL-8 mRNA expression level is non-cagA gene H. pylori infection, and low pepsinogen I/II ratio are relative risk for Thai gastric cancer. Graduate School Kobe, University School of Medicine: JSPS Ronpakku Dissertation PhD in Health Science, 2013

17 **Macri A**, Versaci A, Lodolo S, Scuderì G, Travaglini M, Trimarchi G, Teti D, Fanulli C. Serum levels of interleukin 1 beta, interleukin 8 and tumour necrosis factor alpha as markers of gastric cancer. *Biomarkers* 2006; 11: 184-193 [PMID: 16766394 DOI: 10.1080/1354750600565677]

18 **Guggenheim DE**, Shah MA. Gastric cancer epidemiology and risk factors. *J Surg Oncol* 2013; 107: 230-236 [PMID: 23129495 DOI: 10.1002/jso.23262]

19 **Kato S**, Matsukura N, Togushi A, Masuda G, Matsuda N, Yamada N, Naito Z, Matsuhisa T, Tajiri T. Sex differences in mucosal response to Helicobacter pylori infection in the stomach and variations in interleukin-8, COX-2 and trofoll factor family 1 gene expression. *Aliment Pharmacol Ther* 2004; 20 Suppl 1: 17-24 [PMID: 15298601 DOI: 10.1111/j.1365-2036.2004.01985.x]

20 **Sasidharan S**, Lachumy SJ, Ravichandran M, Latha LY, Gugu A. Epidemiology of Helicobacter pylori among multiracial community in Northern Peninsular, Malaysia: effect of age across sex and race. *Asian Pac J Trop Med* 2011; 4: 72-75 [PMID: 21771421 DOI: 10.1016/S1995-7645(11)60037-0]

21 **Sipponen P**, Correa P. Delayed rise in incidence of gastric cancer in females results in unique sex ratio (M/F) pattern: etiologic hypothesis. *Gastric Cancer* 2002; 5: 213-219 [PMID: 12491079 DOI: 10.1007/s101200200037]

22 **Yao JC**, Schnirer II, Reddy S, Chiang S, Najam A, Yu C, Giacco G, Hess K, Rashid A, Xie K, Lynch P, Ajami JA. Effects of sex and racial/ethnic group on the pattern of gastric cancer localization. *Gastric Cancer* 2002; 5: 208-212 [PMID: 12491078 DOI: 10.1007/s101200200036]

**P- Reviewer:** Lin JY, Yao HR  **S- Editor:** Qi Y  **L- Editor:** A  **E- Editor:** Lu YJ
