JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2018. Vol. **12**(2), p. 459-466

Synergistic Effects of *Helicobacter pylori* Outer Inflammatory Protein A (*oip*A) and cag Pathogenicity Island (*cag* PAI) on Interleukin-1β and Interleukin-8 Gene Expression Levels in Gastric Tissues of Thai Gastroduodenal Patients

Wongwarut Boonyanugomol[1]*, Kamolchanok Rukseree[1], Prasit Palittapongarnpim[2], Worrarat Kongkasem[3] and Seung-Chul Baik[4]

[1]Department of Sciences and Liberal Arts, Mahidol University, Amnat Charoen Campus, Amnat Charoen, 37000, Thailand.
[2]Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand.
[3]Unit of Endoscopy Medicine, Supprasittiprasong Hospital, Ubon Ratchathani, 34000, Thailand.
[4]Department of Microbiology, Gyeongsang National Institute of Health Sciences, Gyeongsang National University School of Medicine, Jinju, Korea.

http://dx.doi.org/10.22207/JPAM.12.2.03

(Received: 20 February 2018; accepted: 19 April 2018)

Helicobacter pylori **outer inflammatory protein A (OipA) has been found to associate with inflammation that is similar to cag pathogenicity island (*cag*PAI). However, the roles of the presence of *oip*A gene involving inflammatory responses *in vivo* need to be clarified. We investigated the association of *oip*A and *cag*PAI on the expression of pro-inflammatory cyotkine genes (*IL-1*β and *IL-8*) in gastric tissues of Thai gastroduodenal patients. We detected the *oip*A and *cag*PAI genes in 35.56% and 68.89%, respectively. The *oip*A "on" status was mostly found (93.75%) in *oip*A-positive samples. We observed higher levels of *IL-1*β and *IL-8* gene expression in *oip*A-positive tissues (with "on" status), similar to those with *cag*PAI-positive tissues. Interestingly, samples positive for both *oip*A and *cag*PAI genes showed significantly higher levels of *IL-1*β *and IL-8* gene expression, when compared with tissues single-positive for either *oip*A or *cag*PAI, or double-negative for these two genes. We conclude that *H. pylori* induces *IL-1*β *and IL-8* gene expression via *oip*A-dependent mechanisms. Furthermore, synergy in the presence of both *oip*A and *cag*PAI genes associated with increased *IL-1*β *and IL-8* gene expression levels in gastric tissues, which suggested that *oip*A plays a critical role in the *H. pylori* pathogenesis.**

Keywords: *H. pylori*, *oip*A, *cag* PAI, IL-1β, IL-8, inflammation.

Helicobacter pylori (*H. pylori*) is a Gram-negative microaerophilic bacterium and a causative agent in various gastroduodenal diseases in humans. The association between *H. pylori* infection and gastric cancer led to its classification as a class I carcinogen [1]. Infection with this bacterium has been found to stimulate of various pro-inflammatory cytokines from gastric cells such as interleukin-1 (IL-1), IL-6 and IL-8, that is play important roles in gastric inflammation [2]. The most important *H. pylori* virulence factor is the *cag* pathogenicity island (*cag*PAI) which encodes a type 4 secretory system (T4SS) and immunodominant antigen of CagA. Strains expressing the *cag*PAI have been associated with a more severe inflammatory response than that induced by *cag*PAI-negative strains. *H. pylori* has been reported to depend upon both T4SS- and CagA-dependent mechanisms to induce IL-8 production in gastric cells, resulting in increased

* To whom all correspondence should be addressed.
Tel/Fax: +66 45 523211;
E-mail: wongwarut.boo@mahidol.edu

inflammation [3,4]. It has also been shown that studies of gastric biopsies from patients infected with *H. pylori cag*-positive strains induce significantly more IL-1α and IL-1β than do *cag*-negative strains [5]. Therefore, several studies have suggested that the *H. pylori cag*PAI is a putative marker for virulent strains of *H. pylori* that are associated with severe gastroduodenal diseases, especially gastric adenocarcinoma [6,7]. Interestingly, however, some IL-1β and IL-8 responses have also been found to be independent of the *cag*PAI mechanisms [8,9]. Therefore, we speculated that several *H. pylori* virulence factors may be involved together in promoting inflammation and disease severity.

Bacterial adherence to gastric epithelial cells is a critical step in *H. pylori* pathogenesis. *H. pylori* contains a large family of outer membrane proteins (OMPs), comprising 32 members involved in adhesion, immunostimulatory functions and micropore formation [10,11]. The outer inflammatory protein A (OipA), or HopH, encodes one of the outer membrane proteins, with the *oip*A gene located approximately 100 kb from the *cag* PAI in the *H. pylori* genome [12]. Aside from promoting bacterial adherence to gastric epithelial cells, OipA (status "on") increases inflammatory responses by inducing IL-8 production and regulating its secretion through the PI3/Akt pathway in these cells [13]. Using purified OipA protein was shown to cause cell cytotoxicity and apoptotic cell death by modulation of Bax/Bcl-2 levels in the AGS human gastric epithelial cell line, indicating that *H. pylori* OipA may play a pathogenic role by damaging host cells [14,15]. However, previous studies on the role of OipA-induced inflammation have been reported and revealed that mutagenesis of the *H. pylori oip*A gene did not alter IL-8 production in gastric cells *in vitro* nor in animal model studies [16,17]. Currently, the effect of *H. pylori* OipA on inflammatory responses of IL-8 is still controversial, in particular its role *in vivo*. Additionally, the association of OipA with the expression of IL-1β has not yet been examined. We hypothesized that *H. pylori* OipA may associate with pro-inflammatory responses by regulating the expression of IL-1β and IL-8 in the human gastric cells *in vivo*. To investigate this question, the purpose of the current study was to determine the association between the *oip*A and *cag*PAI gene status of *H. pylori* infections with *IL-1*β and *IL-8* gene expression levels in the gastric tissue samples from Thai subjects with gastroduodenal disease (gastritis and peptic ulcer disease).

MATERIALS AND METHODS

Patients and Sample Collection

Gastric tissues were obtained from subjects undergoing gastroduodenal examination at the unit of endoscopy medicine, Supprasittiprasong Hospital, Ubon Ratchathani, Thailand. *H. pylori* infection status of the subjects was determined by the Rapid Urease Test kit (RUT). A total of 45 gastric tissues were collected for this study from subjects with confirmed gastritis (GT=35) and peptic ulcer disease (PUD=10). Ethic committee approval was received for this study from The Institutional Review Board of Mahidol University (COA.NO. 2016/157.0912). Written inform consent was obtained from patients who participated in this study.

Genomic DNA Extraction and PCR

Genomic DNA was extracted from RUT positive gastric tissues using DNA purification kits (RBC Real Genome, RBCBioscience), according to the manufacturer's protocol. Isolated DNA was solubilized by Tris-EDTA (TE) buffer and kept at -20°C until polymerase chain reaction (PCR) was performed.

PCR was used to detect *H. pylori* using primers specific for *16SrRNA*, *oip*A, and *cag*PAI. All primer sequences were selected from published data with slight modifications, as shown in Table 1 [18-20]. The PCR were prepared in a volume of 25 µl using a ready-to-use PCR master mix (OnePCR Ultra®, Bio-Helix Co., LTD. Taiwan), containing 0.5 µM of each primer. PCR amplifications were performed in an automated thermal cycler (BioRad T100™, USA) with the following cycle conditions: *16SrRNA*: 35 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C; *cag*PAI: 35 cycles of 1 min at 94 °C, 1 min at 57 °C, and 1 min at 72 °C; and *oip*A: 35 cycles of 1 min at 94 °C, 1 min at 57 °C, and 1 min at 72 °C. PCR products were analyzed on 1.5% agarose gels and visualized under a UV illuminator. *H. pylori oip*A amplicons were subsequently sequenced to determine their functional status by observing the stop codons of a signal-peptide coding region [12].

RNA Extraction and mRNA Quantification

Total RNA was extracted from RUT positive tissues using RiboZol™ (Amresco, VWR Company, USA), according to the manufacturer's protocol. RNA pellets were solubilized with RNAse-free distilled water. The RNA yield and quality were measured by spectrophotometer. RNA (1 µg) was reverse transcribed using SuperScript™ III Reverse Transcriptase (Invitrogen), as described by the manufacturer. *IL-1*β and *IL-8* gene expression were quantified by the SYBR Green assay on a ThermalCycler96® Real-time PCR (Roche Molecular Systems, Inc.) with an initial denaturation at 95°C, followed by 40 cycles of 95°C for 30 s and 60°C for 30 sec, and normalized to *18SrRNA*. The primer sequences for *IL*-1β, *IL-8* and *18SrRNA* genes are listed in Table 1 [21,22]. Gene expression levels were quantified using the ΔΔCT-method.

Table 1. Primers used in the study

Genes	Primer sequences
16SrRNA	F: GCGACCTGCTGGAACATTAC
	R: CGTTAGCTGCATTACTGGAGA
*oip*A	F: GTTTTTGATGCATGGGATTT
	R: GTGCATCTCTTATGGCTTT
*cag*PAI	F: ACATTTTGGCTAAATAAACGCTG
	R: TCTCCATGTTGCCATTATGCT
IL-1β	F: GCACGATGCACCTGTACGAT
	R: CACCAAGCTTTTTTGCTGTGAGT
IL-8	F: ACTGAGAGTGATTGAGAGTGGAC
	R: AACCCTCTGCACCCAGTTTTC
18SrRNA	F: CGGCGACGACCCATTCGAAC
	R: GAATCGAACCCTGATTCCCCGTC

Data Analysis

All data were performed using GraphPad Prism software (version 5.0) and significance evaluated with the Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

As shown in table 2, 45 RUT positive samples were confirmed to harbor *H. pylori* infection by PCR amplification. A 401-bp PCR product, indicating the presence of the *oip*A gene, was obtained in 16 (35.56%) of these subjects. No significant differences between the gastritis and PUD were detected. Subsequently, the *oip*A functional status ("on" / "off") was determined by PCR-based sequencing. We found that in 15 of 16 (93.75%) subjects, the *oip*A status was "on".

The *cag*PAI-positive samples was found 68.89% (31/45) of gastric tissues of Thai gastroduodenal subjects (Table 2). The vital components of *cag*PAI, *cag*A and *cag*E, were also found in the *cag*PAI positive samples (data not shown). There were no significant differences between the gastritis and PUD. The frequency

Table 2. The prevalence of *oip*A and *cag*PAI genes in Thai subjects with gastroduodenal disease

Genes	Patient groups		Total (n=45)
	Gastritis(n=35)	PUD(n=10)	
*oip*A$^+$	13 (37.14%)	3 (30%)	16 (35.56%)
*oip*A$^+$ status on	12 (92.31%)	3 (100%)	15 (93.75%)
cag PAI$^+$	22 (62.86%)	9 (90%)	31 (68.89%)

Table 3. Distribution of *H. pylori cag*PAI and *oip*A combined genotypes in Thai subjects with gastroduodenal disease

Gene combinations	Patient groups		Total (n=45)
	Gastritis(n=35)	PUD(n=10)	
*cag*PAI$^-$ / *oipA*$^-$	10 (28.57%)	0 (0%)	10 (22.22%)
*cag*PAI$^+$ / *oipA*$^-$	12 (34.29%)	7 (70%)	19 (42.22%)
*cag*PAI$^-$ / *oipA*$^+$	3 (8.57%)	1 (10%)	4 (8.89%)
*cag*PAI$^+$ / *oipA*$^+$	10 (28.57%)	2 (20%)	12 (26.67%)

distributions of the combined genotypes of *H. pylori cag*PAI and *oip*A in the 45 samples of Thai subjects are presented in table 3. We clustered a total 4 different *cag*PAI / *oip*A genotype combinations, out of which the most prevalent genotypes was *cag*PAI^{+} / *oip*A^{-}, but the genotype of *cag*PAI^{-}/*oip*A^{-} was not found in PUD.

In order to study the association of *H. pylori oip*A and *cag*PAI genes with inflammatory responses, we determined *IL-1*β *and IL-8* gene

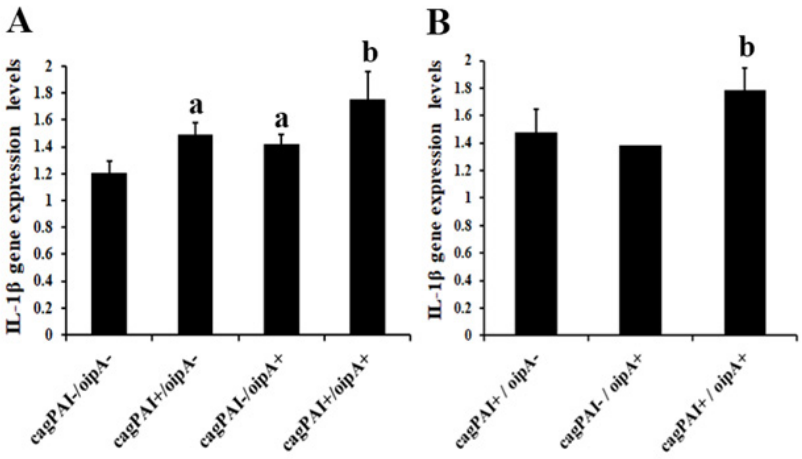

Fig. 1. *IL-1*β gene expression levels in the presence/absence of *oip*A and *cag*PAI genes in gastric tissues of subjects with gastritis (A) and PUD (B).
[a] significant differences when compared with double-negative *cag*PAI / *oip*A gene status (p<0.05).
[b]significant differences when compared with single-positive *oip*A, *cag*PAI gene status, or double-negative for these two genes (p<0.05).

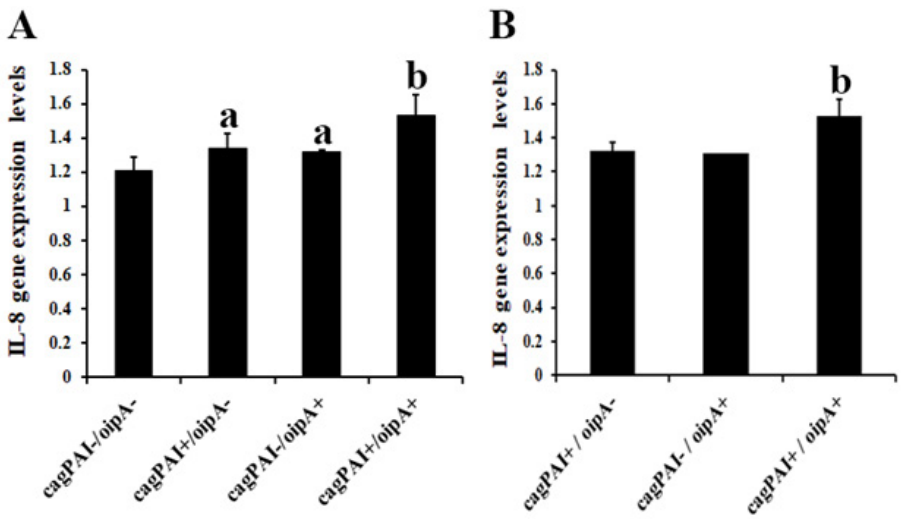

Fig. 2. *IL-8* gene expression levels in the presence/absence of *oip*A and *cag*PAI genes in gastric tissues of subjects with gastritis (A) and PUD (B).
[a]significant differences when compared with double-negative *cag*PAI / *oip*A gene status (p<0.05).
[b]significant differences when compared with single-positive *oip*A, *cag*PAI gene status, or double-negative for these two genes (p<0.05)

expression levels in the gastric tissues of Thai subjects with gastroduodenal disease. As shown in fig. 1 and 2, *IL-1*β *and IL-8* gene expression levels in samples containing single-positive gene status of *cag*PAI (*cag*PAI$^+$/*oip*A$^-$) were significantly higher than in those containing double-negative genes (*cag*PAI$^-$/*oip*A$^-$) (p<0.05). Similarly, samples containing single-positive *oip*A gene status (*cag*PAI$^-$/*oip*A$^+$) were found to have significantly increased *IL-1*β and *IL-8* gene expression levels when compared with those with a double-negative gene status (*cag*PAI$^-$/*oip*A$^-$) (p<0.05), however, the levels were slightly lower than for the *cag*PAI$^+$ samples (*cag*PAI$^+$/*oip*A$^-$). Our study, only one sample carrying *oip*A-functional "off" status was combined with the *cag*PAI-negative samples that showed the decreasing of *IL-1*β and *IL-8* gene expression. Interestingly, we found that samples with double-positive gene status for *oip*A and *cag*PAI (*cag* PAI$^+$/*oip*A$^+$), had higher *IL-1*β *and IL-8* levels compared with single-positive and double-negative samples. These results were consistent for subjects with gastritis and PUD.

DISCUSSION

H. pylori has been shown to stimulate the production of various pro-inflammatory cytokines from gastric epithelial cells [23]. IL-1β is a pro-inflammatory cytokine that has been involved in pain, inflammation and autoimmune condition [24]. The biological effects of IL-1β is involved to induce expression of many cytokine genes by either initiating their transcription or stabilizing their mRNA, including tumor necrosis factor α (TNF-α), IL-2, IL-6, IL-12, interferon α, β, and γ, granulocyte-colony stimulating factor and macrophage-colony stimulating factor [25]. The *in vitro* study revealed that *H. pylori cag*A and *cag*E mutant strains showed a marked decrease in the induction of IL-1β production of human peripheral blood mononuclear cells. It was suggested that the components of *cag*PAI more frequently associated with the production of IL-1β [26] , especially *H. pylori* requires the expression of *cag*E for the full induction of IL-1, IL-12, and TNF- α in the immature dendritic cells [27]. However, the molecular mechanisms of *H. pylori* lipopolysaccharide (LPS) and vacuolating cytotoxin A (VacA) induced IL-1β have been determined [9,28]. Thus, based on the previous data, we assumed that other components of *H. pylori* might be associated with the production of IL-1β.

IL-8 is also a pro-inflammatory cytokine and is well known to be a chemoattractant for neutrophils and an activator of macrophages that plays important role in pro-inflammatory responses. This chemokine is also associated with cell proliferation, migration and survival of endothelial cells, thereby potentiating the epithelial-mesenchymal transition and survival of cancer cells, required for cancer progression [29]. IL-8 has been found to play a dominant role in gastric epithelial cells exposed to *H. pylori* infection and in pathological processes leading to gastroduodenal disease [30]. It was shown that *H. pylori* can also stimulate gastric epithelial cells to produce IL-8 through a CagA-dependent mechanism. In addition to the CagA protein, bacterial cell wall components, such as peptidoglycan and heptose-1,7-bisphosphate, are also translocated via the T4SS into host epithelial cells and recognized by the nucleotide-binding oligomerization domain receptor (NOD) and the TRAF-interacting protein with forkhead-associated domain (TIFA), respectively, to inducs NF-κB activation and up-regulation of pro-inflammatory immune responses [31,32]. Although previous studies reported that *H. pylori cag*PAI mutant strains induced reduced levels of IL-8 secretion *in vitro*, other *H. pylori* components may also be involved [33]. This suggests that although the *cag*PAI may promote clinical disease, other factors important for *H. pylori* pathogenicity remain to be investigated [34].

Approximately 4% of the *H. pylori* genome is composed of genes encoding OMPs. Among them, OipA has emerged as another virulence factor involved in promoting PUD and gastric cancer [12]. Previous *in vitro* study showed that IL-8 production was significantly reduced in epithelial cells stimulated with *H. pylori oip*A mutant strains on a *cag*-positive background, indicating the important role of OipA in IL-8 production[12] Conversely, OipA-positive strains on a *cag*-negative background, did not induce higher IL-8 amounts than OipA-negative strains. These results suggest that the OipA protein alone is not able to stimulate IL-8 secretion in AGS cells, but that OipA expression seems to support

Cag-induced cytokine secretion [35]. However, the effect of OipA on IL-8 secretion *in vitro* might not be sufficient to explain the exact role of OipA under physiological conditions *in vivo*. Additionally, the association of OipA on the expression of IL-1β has not been extensively studied. Thus, we evaluated the association of the *oip*A gene, in the presence or absence of the *cag*PAI, on *IL-1*β and *IL-8* gene expression levels in gastric tissues of subjects with gastroduodenal disease. We found that *IL-1*β *and IL-8* gene expression levels in gastric tissues was dependent on the *cag*PAI gene (*cag*PAI$^+$/oipA$^-$). We also found that tissue samples positive for the *oip*A gene (*cag*PAI$^-$/*oip*A$^+$) also exhibited higher levels of *IL-1*β *and IL-8* gene expression, when compared with those double-negative for the *oip*A and *cag*PAI genes. Our data indicated that increasing levels of *IL-1*β *and IL-8* gene expression might be associated with the *oip*A gene. Additionally, the highest levels of *IL-1*β *and IL-8* gene expression were found in the tissue samples that were positive for both *oip*A and *cag*PAI, when compared to single-positive gene and double-negative genes of *oip*A and/or *cag*PAI. It is possible that the association of *oip*A and *cag*PAI might be associated with heightened levels of *IL-1*β *and IL-8* gene expression in gastric tissues. The results of the present study are consistent with the findings of previous *in vitro* studiy which showed that *H. pylori oip*A mutant bacteria displayed reduced levels of cell adherence and induced less IL-8 secretion in the AGS cell line. Furthermore, these mutant bacteria were unable to translocate CagA into gastric epithelial cells [36]. Taken together, these data suggest that *H. pylori* OipA might involve in bacterial adherence leading to *IL-1*β *and IL-8* production through direct signaling and that, moreover, this OMP might co-operate with the *cag*PAI to induce inflammation.

CONCLUSIONS

Our study investigate that *H. pylori oip*A gene associates with increased IL-1β expression in gastric tissues of subjects with gastroduodenal disease, as well as IL-8 expression. Our findings conclude that *IL-1*β and *IL-8* gene expression levels were dependent on the *H. pylori oip*A gene. In addition, synergistic effects of *oip*A combined with *cag*PAI have been found to associate with increased levels of *IL-1*β and *IL-8* gene expression. These data suggest that OipA might be an important *H. pylori* virulence factor associated with inflammation. However, the molecular mechanisms of *oip*A-induced *IL-1*β expression should be further determined.

ACKNOWLEDGEMENTS

This study was supported by the grant of Thailand research Fund (MRG5980069). We kindly thank the staff at Endoscopy Medicine, Sapprasittiprasong Hospital, for gastric tissue collection. We acknowledge Dr. Richard L. Ferrero (Hudson Institute of Medical Research) for review and English editing. Finally, we would like to thank Central Laboratory, Amnat Charoen Campus, Mahidol University, for the instruments used in this study.

REFERENCES

1. NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. *JAMA* 1994;**272**(1):65-69.
2. Noach LA, Bosma NB, Jansen J, Hoek FJ, van Deventer SJ, Tytgat GN. Mucosal tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8 production in patients with *Helicobacter pylori* infection. *Scand J Gastroenterol* 1994;**29**(5):425-429.
3. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev* 2006;**19**(3):449-490.
4. Tegtmeyer N, Lind J, Schmid B, Backert S. *Helicobacter pylori* CagL Y58/E59 mutation turns-off type IV secretion-dependent delivery of CagA into host cells. *PLoS One* 2014;**9**(6):e97782.
5. Peek RM, Jr., Miller GG, Tham KT, Perez-Perez GI, Zhao X, Atherton JC, *et al*. Heightened inflammatory response and cytokine expression *in vivo* to *cag*A$^+$ *Helicobacter pylori* strains. *Lab Invest* 1995;**73**(6):760-770.
6. Figura N, Guglielmetti P, Rossolini A, Barberi A, Cusi G, Musmanno RA, *et al*. Cytotoxin production by *Campylobacter pylori* strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. *J Clin Microbiol* 1989;**27**(1):225-226.
7. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, *et al*. Infection with *Helicobacter pylori* strains possessing *cag*A is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res*

1995;**55**(10):2111-2115.
8. Beswick EJ, Bland DA, Suarez G, Barrera CA, Fan X, Reyes VE. *Helicobacter pylori* binds to CD74 on gastric epithelial cells and stimulates interleukin-8 production. *Infect Immun* 2005;**73**(5):2736-2743.
9. Luo JJ, Li CY, Liu S, Yu W, Tang SY, Cai HL, *et al*. Overexpression of *Helicobacter pylori* VacA N-terminal fragment induces proinflammatory cytokine expression and apoptosis in human monocytic cell line through activation of NF-kappaB. *Can J Microbiol* 2013;**59**(8):523-533.
10. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, *et al*. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* 1997;**388**(6642):539-547.
11. Alm RA, Bina J, Andrews BM, Doig P, Hancock RE, Trust TJ. Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. *Infect Immun* 2000;**68**(7):4155-4168.
12. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of *Helicobacter pylori*. *Proc Natl Acad Sci U S A* 2000;**97**(13):7533-7538.
13. Tabassam FH, Graham DY, Yamaoka Y. *Helicobacter pylori*-associated regulation of forkhead transcription factors FoxO1/3a in human gastric cells. *Helicobacter* 2012;**17**(3):193-202.
14. Teymournejad O, Mobarez AM, Hassan ZM, Noori S, Moazzeni SM, Khoramabadi N. Cloning, Expression, Purification and Toxicity Evaluation of *Helicobacter pylori* Outer Inflammatory Protein A. *Indian J Microbiol* 2013;**53**(4):391-394.
15. Teymournejad O, Mobarez AM, Hassan ZM, Talebi Bezmin Abadi A. Binding of the *Helicobacter pylori* OipA causes apoptosis of host cells via modulation of Bax/Bcl-2 levels. *Sci Rep* 2017;**7**(1):8036.
16. Dossumbekova A, Prinz C, Mages J, Lang R, Kusters JG, Van Vliet AH, *et al*. *Helicobacter pylori* HopH (OipA) and bacterial pathogenicity: genetic and functional genomic analysis of hopH gene polymorphisms. *J Infect Dis* 2006;**194**(10):1346-1355.
17. Franco AT, Johnston E, Krishna U, Yamaoka Y, Israel DA, Nagy TA, *et al*. Regulation of gastric carcinogenesis by **Helicobacter pylori** virulence factors. *Cancer Res* 2008;**68**(2):379-87.
18. Gramley WA, Asghar A, Frierson HF, Jr., Powell SM. Detection of *Helicobacter pylori* DNA in fecal samples from infected individuals. *J Clin Microbiol* 1999;**37**(7):2236-2240.
19. Kauser F, Hussain MA, Ahmed I, Ahmad N, Habeeb A, Khan AA, *et al*. Comparing genomes of *Helicobacter pylori* strains from the high-altitude desert of Ladakh, India. *J Clin Microbiol* 2005;**43**(4):1538-1545.
20. Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE, Reece CA, *et al*. Analyses of the cag pathogenicity island of *Helicobacter pylori*. *Mol Microbiol* 1998;**28**(1):37-53.
21. Tsai KW, Lai HT, Tsai TC, Wu YC, Yang YT, Chen KY, *et al*. Difference in the regulation of IL-8 expression induced by uropathogenic *E. coli* between two kinds of urinary tract epithelial cells. *J Biomed Sci* 2009;**16**:91.
22. Bhat IA, Naykoo NA, Qasim I, Ganie FA, Yousuf Q, Bhat BA, *et al*. Association of interleukin 1 beta (IL-1beta) polymorphism with mRNA expression and risk of non small cell lung cancer. *Meta Gene* 2014;**2**:123-133.
23. Maekawa T, Kinoshita Y, Matsushima Y, Okada A, Fukui H, Waki S, *et al*. *Helicobacter pylori* induces proinflammatory cytokines and major histocompatibility complex class II antigen in mouse gastric epithelial cells. *J Lab Clin Med* 1997;**130**(4):442-449.
24. Ren K, Torres R. Role of interleukin-1beta during pain and inflammation. *Brain Res Rev* 2009;**60**(1):57-64.
25. El-Omar EM. The importance of interleukin 1beta in *Helicobacter pylori* associated disease. *Gut* 2001;**48**(6):743-747.
26. Khamri W, Walker MM, Clark P, Atherton JC, Thursz MR, Bamford KB, *et al*. *Helicobacter pylori* stimulates dendritic cells to induce interleukin-17 expression from CD4+ T lymphocytes. *Infect Immun* 2010;**78**(2):845-853.
27. Galgani M, Busiello I, Censini S, Zappacosta S, Racioppi L, Zarrilli R. *Helicobacter pylori* induces apoptosis of human monocytes but not monocyte-derived dendritic cells: role of the *cag* pathogenicity island. *Infect Immun* 2004;**72**(8):4480-4485.
28. Basak C, Pathak SK, Bhattacharyya A, Mandal D, Pathak S, Kundu M. NF-kappaB- and C/EBPbeta-driven interleukin-1beta gene expression and PAK1-mediated caspase-1 activation play essential roles in interleukin-1beta release from *Helicobacter pylori* lipopolysaccharide-stimulated macrophages. *J Biol Chem* 2005;**280**(6):4279-4288.
29. Yuan A, Chen JJ, Yao PL, Yang PC. The role of interleukin-8 in cancer cells and microenvironment interaction. *Front Biosci* 2005;**10**:853-865.
30. Crabtree JE, Wyatt JI, Trejdosiewicz LK, Peichl P, Nichols PH, Ramsay N, *et al*. Interleukin-8 expression in *Helicobacter pylori* infected,

normal, and neoplastic gastroduodenal mucosa. *J Clin Pathol* 1994;**47**(1):61-66.

31. Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, *et al*. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori cag* pathogenicity island. *Nat Immunol* 2004;**5**(11):1166-1174.
32. Stein SC, Faber E, Bats SH, Murillo T, Speidel Y, Coombs N, *et al*. *Helicobacter pylori* modulates host cell responses by CagT4SS-dependent translocation of an intermediate metabolite of LPS inner core heptose biosynthesis. *PLoS Pathog* 2017;**13**(7):e1006514.
33. Boonyanugomol W, Chomvarin C, Hahnvajanawong C, Sripa B, Kaparakis-Liaskos M, Ferrero RL. *Helicobacter pylori cag* pathogenicity island (*cag*PAI) involved in bacterial internalization and IL-8 induced responses via NOD1- and MyD88-dependent mechanisms in human biliary epithelial cells. *PLoS One* 2013;**8**(10):e77358.
34. Jenks PJ, Megraud F, Labigne A. Clinical outcome after infection with *Helicobacter pylori* does not appear to be reliably predicted by the presence of any of the genes of the cag pathogenicity island. *Gut* 1998;**43**(6):752-758.
35. Odenbreit S, Swoboda K, Barwig I, Ruhl S, Boren T, Koletzko S, *et al*. Outer membrane protein expression profile in *Helicobacter pylori* clinical isolates. *Infect Immun* 2009;**77**(9):3782-3790.
36. Horridge DN, Begley AA, Kim J, Aravindan N, Fan K, Forsyth MH. Outer inflammatory protein a (OipA) of *Helicobacter pylori* is regulated by host cell contact and mediates CagA translocation and interleukin-8 response only in the presence of a functional *cag* pathogenicity island type IV secretion system. *Pathog Dis* 2017;**75**(8).