**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) is a human pathogen that colonizes the stomach epithelium. Under normal circumstances, the host is unable to clear the infection, which may lead to life-long chronic inflammation. According to previous studies, *H. pylori* is associated with gastric diseases such as peptic ulceration and gastric adenocarcinoma and with exogastric diseases, such as cardiovascular and cerebrovascular diseases and type 2 diabetes mellitus. Cytotoxin-associated gene A (*CagA*) seropositivity in particular is associated with increased risks of certain diseases involving the lower stomach and duodenum and decreased risks of gastroesophageal reflux disease (GERD) and its sequelae. According to 1 study, gastric cancer (GC) patients have higher *H. pylori* and *CagA* seroprevalence than the matched controls, confirming that *CagA*-positive (*CagA*+) *H. pylori* infection is associated with a higher risk of GC. Additionally, *CagA+* *H. pylori* strains may contribute to the pathogenesis of early atherosclerosis by aggravating immunoinflammatory reactions. Coronary artery disease is also significantly associated with *H. pylori* infection, particularly with strains expressing *CagA* proteins. In addition, *CagA+* *H. pylori* strains may act as a trigger and play a role in the pathogenesis of cardiac syndrome X (CSX) via chronic inflammation. Conversely, colonization with *CagA+* *H. pylori* is not an independent risk factor for severe coronary heart disease. Therefore, the relationships of *CagA+* and *CagA−* strains with their hosts and with diseases remain unknown. Generally, chronic alcohol consumption is associated with enhanced susceptibility to both systemic and mucosal infections. Different quantities of alcohol consumption have different effects on

**Abstract:** The aim of this study was to evaluate the effect of *H. pylori* (*H. pylori*) cytotoxin-associated gene A (*CagA*) coupled with chronic alcohol ingestion on cytokine profiles. A total of 215 male subjects were divided into the following four groups: 130 alcohol *H. pylori* *CagA*-negative consumers (*CagA−*) (group A), 50 alcohol *H. pylori* *CagA*-positive consumers (*CagA+*) (group B), 24 nonalcohol *H. pylori* *CagA*-negative consumers (group C), and 11 nonalcohol *H. pylori* *CagA*-positive consumers (group D). The serum CagA, C-reactive protein (CRP), interleukin (IL)-6, IL-10, E-selectin, adiponectin (ADP), and tumor necrosis factor-α (TNF-α) levels were measured through enzyme-linked immunosorbent assays (ELISAs).

After adjusting for age and mean alcohol drinking history, a multivariable linear regression analysis revealed that the mean daily alcohol consumption, IL-6, TNF-α, and ADP levels were significantly increased with increases in the serum CagA concentrations (*P* = 0.008, *P* = 0.000, *P* = 0.000, and *P* = 0.006, respectively). The serum IL-6 and IL-10 levels of group A were significantly lower than those of group B (all *P* = 0.000). Furthermore, the serum IL-6 and IL-10 levels of groups A and C were significantly lower than those of group D (all *P* = 0.000), and the serum IL-6 and IL-10 levels of group C were significantly lower than those of group B (all *P* = 0.000). The serum ADP and E-selectin levels of groups B and D were significantly higher than those of group A (*P* = 0.000). The serum ADP levels of group B were significantly higher than those of group C (*P* = 0.000), and the serum ADP and E-selectin levels of group C were significantly lower than those of group D (*P* = 0.000 and *P* = 0.005, respectively). Finally, the serum TNF-α levels of groups B, C, and D were significantly higher than those of group A (all *P* = 0.000), and the serum TNF-α levels of group C were significantly higher than those of group D (*P* = 0.005).

In conclusion, *H. pylori* *CagA* may result in significantly higher levels of several inflammatory markers in both alcohol consumers and non-alcohol consumers. However, chronic alcohol ingestion coupled with *H. pylori* *CagA* positivity does not result in significant changes in cytokine profiles.
the human body. A previous study showed that modest alcohol intake, from 10 to 20 g of ethanol per day, may have decreased cardiovascular mortality. Another study revealed that a significant decrease in carotid intima-media thickness (CIMT) occurred with increased alcohol consumption of daily intake levels of up to 80 g in men. A recent study revealed that heavy and binge alcohol consumption is an important risk factor related to an increasing incidence of gastric cancer in a population not infected with *H pylori*. Additionally, as shown in several studies, chronic inflammation-associated cytokine profiles are present during the development of certain diseases. However, the associations between CagA-positive *H pylori* coupled with alcohol intake and inflammation remain controversial. Therefore, the objective of the present study was to explore the effect of *H pylori* CagA coupled with chronic alcohol ingestion on cytokine profiles by measuring the serum levels of inflammatory and anti-inflammatory cytokines.

**MATERIALS AND METHODS**

**Ethics Statement**

This protocol was approved by the Clinical Research Ethics Committee of Taishan Hospital of Shandong Province, and written informed consent was obtained from all of the enrollees.

**Subject Selection**

A total of 215 male subjects undergoing health examination were enrolled between January 2012 and May 2015. All of the subjects were evaluated based on whether they chronically ingested alcohol by a questionnaire on alcohol consumption. *H pylori* CagA positivity was diagnosed based on the serum *H pylori* CagA levels at Taishan Hospital, Shandong Province. The subjects enrolled in this study included 180 subjects that chronically ingested alcohol and 35 control subjects. The diagnosis of chronic alcohol ingestion was defined as a daily ethanol intake >40 g in men for a period >5 years. Additionally, a serum *H pylori* CagA level of at least 80 pg/mL was considered positive (CagA+), whereas a *H pylori* CagA level >80 pg/mL was considered negative (CagA–). The subjects were divided into the 4 following groups according to whether they exhibited *H pylori* CagA positivity: 130 alcohol *H pylori* CagA-negative consumers (group A), mean age of 46.84 ± 7.08 years, mean alcohol drinking history of 5.77 ± 1.81 years, and mean daily alcohol consumption of 66.23 ± 21.68 g; 50 alcohol *H pylori* CagA-positive consumers (group B), mean age of 46.23 ± 7.04 years, mean alcohol drinking history of 5.63 ± 1.59 years, and mean daily alcohol consumption of 67.38 ± 19.58 g; 24 nonalcohol *H pylori* CagA-negative consumers (group C), mean age of 48.04 ± 6.53 years; and 11 nonalcohol *H pylori* CagA-positive consumers (group D), mean age of 46.00 ± 5.64 years. A cross-sectional study was conducted to evaluate the correlations between *H pylori* CagA and the profiles of cytokines associated with alcohol consumption based on the serum levels of *H pylori* CagA, C-reactive protein (CRP), interleukin (IL)-6, IL-10, adiponectin (ADP), E-selectin, and tumor necrosis factor-α (TNF-α) measured through enzyme-linked immunosorbent assays (ELISAs).

The exclusion criteria included the following: smoking, fever, infectious diseases, anti-inflammatory drugs, antibiotics, medication for killing *H pylori*, and suffering from other diseases.

**Experimental Setup and Reagents**

The experiment equipment included an Enzyme Standard Instrument (Type ANTHOS 2010, Austria), and the reagents used in this study included *H pylori* CagA, CRP, IL-6, IL-10, ADP, E-selectin, and TNF-α ELISA kits (Shanghai Enzyme-Linked Immune Co. Ltd, produced by R&D Systems).

**Blood Collection and Handling**

On the same day as the general health examination, peripheral venous blood samples were collected after overnight fasting for at least 10 hours. For measurements of serum *H pylori* CagA, CRP, IL-6, IL-10, ADP, E-selectin, and TNF-α, the samples were collected into ice-cold tubes containing EDTA (1 mg/mL) and centrifuged at 3000 rpm/minute for 10 minutes. The plasma was then stored at −70°C until being assayed. To ensure the reliability of the experimental results, all of the serum samples were carefully preserved and not subjected to repeated freeze-thaw cycles. Finally, all of the parameters were measured by ELISA according to the manufacturer’s instructions.

**Statistical Analysis**

The SPSS statistical package (version 19.0 for Windows; SPSS Inc., Chicago, IL) was used for all of the statistical analyses. For the 4 groups, the normality of the distribution and the homogeneity of variances were assessed using the Kolmogorov test before the statistical analysis. To allow for covariance and confounders, multivariable linear regression analysis was performed to investigate the associations of the serum CagA concentrations with cytokine profiles after adjusting for age, mean alcohol drinking history, and mean daily alcohol consumption. If the variables followed a normal distribution, the variables are expressed using the means ± standard deviations. One-way analysis of variance (ANOVA) was used to analyze multiple sample means, and the Bonferroni post-hoc test was used to analyze differences between 2 groups. However, if variables without a normal distribution were expressed as medians and ranges, Kruskal–Wallis ANOVA was used for comparisons among the 4 groups, and the Mann–Whitney *U* test was used for comparisons between 2 groups. Differences with *P* < 0.05 were considered statistically significant. However, when the Bonferroni post-hoc test was used, statistical significance was accepted at *P* ≤ 0.008.

**RESULTS**

No differences in age were found among the 4 groups or between 2 groups, and no differences in the mean alcohol drinking history and mean daily alcohol consumption were found between group A and group B (all *P* > 0.05). After adjusting for age and the mean alcohol drinking history, a multivariable linear regression analysis showed that the mean daily alcohol consumption and the IL-6, TNF-α, and ADP levels were significantly increased with increases in the serum CagA concentration (all *P* > 0.05). The serum CRP, IL-6, and IL-10 concentrations in the 4 groups are listed in Table 1. The differences in the serum IL-6 and IL-10 levels were significant (*H* = 69.53, *P* = 0.000, and *H* = 84.560, *P* = 0.000, respectively). The serum IL-6 and IL-10 levels in group A were significantly lower than those in group B (all *P* = 0.000). Furthermore, the serum IL-6 and IL-10 levels in...
groups A and C were significantly lower than those in group D (all $P = 0.000$), and the serum IL-6 and IL-10 levels in group C were significantly lower than those in group B (all $P = 0.000$). However, no differences in the serum CRP levels were found among the 4 groups or between 2 groups (all $P > 0.008$).

The serum ADP, E-selectin, and TNF-α concentrations in the 4 groups are listed in Table 2. In the differences in the serum ADP, E-selectin, and TNF-α levels were significant ($H = 74.50, P = 0.000$; $H = 44.81, P = 0.000$; and $H = 57.22, P = 0.000$, respectively). The serum ADP and E-selectin levels in groups B and D were significantly higher than those in group A ($P = 0.000$). Additionally, the serum ADP level in group B was significantly higher than that in group C ($P = 0.000$), and the serum ADP and E-selectin levels in group C were significantly lower than those in group D ($P = 0.000$ and $P = 0.005$, respectively). Finally, the serum TNF-α levels in groups B, C, and D were significantly higher than those in group A (all $P = 0.000$), and the serum TNF-α level in group C was significantly higher than that in group D ($P = 0.005$).

**DISCUSSION**

*H pylori* may induce strong mucosal inflammation and local and systemic immune responses, which manifest as significant changes in both inflammatory cytokines, including TNF-α, E-selectin, IL-8, and IL-6, and anti-inflammatory cytokines, such as IL-10, IL-17, and IL-23. For example, infection of gastric epithelial cells by *H pylori* stimulates the activation of nuclear factor-kB (NF-kB) and the upregulation of interleukin-8 (IL-8) expression. Moreover, both IL-6 and IL-11 are strongly upregulated in gastric cancer biopsies, and it has been shown that IL-6 may increase STAT3 and ERK1/2 activation in *H pylori*-dependent gastritis. Moreover, IL-11 expression is associated with adenocarcinoma development. These lines of evidence suggest that IL-6 and IL-11 may serve as potent inducers of human gastric cancer. Additionally, *H pylori* CagA may activate the STAT3 signaling pathway in vitro and in vivo, suggesting that chronic *H pylori* infection may promote the development of gastric cancer. However, in a contradictory study, *H pylori* did not cause changes in the systemic secretion of certain cytokines. Therefore, the relationship between *H pylori* and inflammation remains unclear. Nevertheless, *H pylori* CagA is known to play a critical role in various diseases via changes in inflammatory cytokines. According to a previous study, the *H pylori* CagA+ strain may induce severe gastritis, and the CagA pathogenicity island provides a growth advantage to *H pylori* strains and is associated with an increased inflammatory response at the gastric mucosal level. In duodenal ulcer (DU) patients, the serum IL-12 but not IL-13 concentrations were influenced by

**TABLE 1.** Comparison of Serum CRP, IL-6, and IL-10 Levels Among the 3 Groups (ng/L, Medians With Ranges)

| Groups | N  | CRP       | IL-6       | IL-10       |
|--------|----|-----------|------------|-------------|
| A      | 130| 3.16 (2.13–8.22) | 12.40 (7.00–91.00)* | 319.55 (14.00–1112.00)* |
| B      | 50 | 3.26 (2.23–9.94) | 31.50 (9.00–109.00)* | 699.05 (10.00–2921.00)* |
| C      | 24 | 2.87 (1.28–7.55) | 14.00 (7.00–47.00)* | 224.10 (126.00–825.00)* |
| D      | 11 | 2.28 (1.18–9.63) | 51.10 (21.00–100.00)§ | 642.90 (45.00–1773.00)§ |
| H      |    | 7.247 | 69.53 | 84.560 |
| P      |    | 0.064 | 0.000 | 0.000 |

CRP = C-reactive protein, IL-6 = interleukin-6, IL-10 = interleukin-10.

$^a P = 0.000$ (IL-6: group A vs group C).

$^b P = 0.000$ (IL-6: groups A and C vs group D).

$^c P = 0.000$ (IL-6: group B vs group C).

$^d P = 0.000$ (IL-6: group B vs group C).

$^e P = 0.000$ (IL-10: group A vs group B).

$^f P = 0.000$ (IL-10: group A and C vs group D).

$^g P = 0.000$ (IL-10: group A and C vs group D).

$^h P = 0.000$ (IL-10: group B vs group C).

**TABLE 2.** Comparison of Serum ADP, E-Selectin, and TNF-α Levels Among the 3 Groups (ng/L, Medians With Ranges)

| Groups | N  | ADP       | E-Selectin | TNF-α       |
|--------|----|-----------|------------|-------------|
| A      | 130| 1192.00 (96.00–4789.00) | 25.40 (4.00–194.00) | 212.20 (29.00–1518.00) |
| B      | 50 | 2491.00 (860.00–10412.00) | 72.70 (6.00–265.00)§ | 601.80 (1.00–2078.00)§ |
| C      | 24 | 1107.00 (110.00–3498.00) | 75.00 (15.00–265.00)§ | 350.60 (169.00–1284.00)§ |
| D      | 11 | 3032.00 (1582.00–6354.00) | 108.95 (35.00–213.00)§ | 973.50 (427.00–1676.00)§ |
| H      |    | 74.50 | 44.81 | 57.22 |
| P      |    | 0.000 | 0.000 | 0.000 |

ADP = adiponectin, TNF-α = tumor necrosis factor-α.

$^a P = 0.000$ (ADP: groups B and D vs group A).

$^b P = 0.000$ (ADP: group B vs group C).

$^c P = 0.000$ (ADP: group C vs group D).

$^d P = 0.000$ (E-selectin: groups B and D vs group A).

$^e P = 0.005$ (E-selectin: group C vs group D).

$^f P = 0.000$ (TNF-α: groups B, C, and D vs group A).

$^g P = 0.005$ (TNF-α: group C vs group D).
bacterial CagA, independent of the vaculating cytotoxin (VacA) status, suggesting that high IL-12 levels may contribute to the susceptibility to DU in CagA+ individuals. Furthermore, high IL-18 levels in CagA+ subjects predispose these individuals to susceptibility to digestive ulcers. And, serum IL-8 and nitric oxide (NO) levels are significantly correlated with CagA+ H pylori strain infection. A recent study indicated that the upregulation of miR-155 and miR-146b decreases. In addition, H pylori CagA+ (HpCagA+) results in IL-6 overexpression, which may weaken the eradication of HpCagA+ and contribute to ulcer development. Moreover, the significantly increased activation of signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinase (ERK1/2) in H pylori-dependent gastritis was found to be further enhanced in the presence of CagA+ H pylori strains. As a result, the combined detection of the serum IL-8, NO, and H pylori CagA levels will contribute to the early diagnosis of precancerous lesions in the stomach. In the present study, after adjusting for age and the mean alcohol drinking history, a multivariable linear regression analysis showed that the serum CagA concentrations are associated with the mean daily alcohol consumption and the levels of certain inflammatory cytokines, such as IL-6, TNF-α, and ADP, supporting the viewpoint that CagA is involved in the inflammatory response. Simultaneously, our present subgroup study have determined that in H pylori CagA+ subjects without chronic alcohol ingestion, the levels of several inflammatory markers such as IL-6, ADP, E-selectin and TNF-α, and anti-inflammatory markers such as IL-10 were significantly increased compared with their levels in patients of the control group, who were neither chronic alcohol consumers nor H pylori CagA+. Thus, H pylori CagA may enhance the levels of inflammatory and anti-inflammatory markers, consequently causing an inflammatory response. However, contradictory results suggest that CagA is not required for the H pylori-induced activation of NF-κB and upregulation of IL-8 expression in gastric epithelial cells and that host inflammatory responses in the gastric mucosa are not correlated with the expression of the babA2, cagA, and vacAs1 genes. To date, the conclusions reached by studies on the relationship between H pylori CagA and the profile of inflammatory cytokines are incongruous and thus require further investigations.

Changes in the cytokine balance are responsible for several of the systemic and hepatic manifestations of alcoholism. Ethyl alcohol consumption in humans and experimental animals is also associated with an increased incidence and severity of infections, which is attributed to the immunosuppression that is associated with ethyl alcohol consumption. Several studies have demonstrated that alcohol consumption may result in certain cytokine alterations such as altered IL-6, IL-8, IL-10, IL-12, and TNF-α levels as well as ICAM-1 and E-selectin levels. BMI is the only predictor of high-sensitivity CRP levels and TNF-α levels because obesity induces increases in certain serum inflammatory markers. Additionally, alcohol consumption increases insulin sensitivity, and obesity-related alterations in insulin sensitivity are coupled with alterations in inflammatory genes. Alcohol may specifically improve insulin sensitivity by increasing the expression of anti-inflammatory genes. Studies have demonstrated that the sources and functions of cytokines, including cytokines abnormally secreted by natural killer cells from patients with chronic alcoholism, depend on both the existence of liver disease and the status of ethyl alcohol intake. Chronic ethanol exposure can improve the responsiveness of lipopolysaccharide (LPS)-stimulated macrophage IL-6 and TNF-α production, indicating that this effect may result from ethanol-induced alterations in intracellular signaling through nuclear factor (NF)-κB. Toll-like receptors (TLRs) also play an important role in the innate immune response and link the innate and adaptive responses. Thus, the elimination of TLR4 abolishes the effects of ethanol on the innate and adaptive inflammatory responses induced by ethanol treatment in macrophages. Although a few studies have examined the interaction between H pylori infection and chronic alcohol ingestion and found that alcohol consumption appears to be associated with H pylori infection, alcohol consumption, particularly wine consumption, may reduce the risk and facilitate the elimination of H pylori infection, suggesting a protective mechanism of adequate alcohol consumption that is mediated by "adaptive eye contact." However, studies on the association between ethanol ingestion and both H pylori CagA and cytokine profiles are lacking. According to our previous study, H pylori infection may result in significant changes in the cytokine profiles of the inflammatory marker E-selectin and the anti-inflammatory marker IL-10 in chronic alcohol consumers because of the interaction between H pylori infection and chronically ingested alcohol. Our present study demonstrates that H pylori CagA is correlated with IL-6, IL-10, ADP, E-selectin, and TNF-α levels in chronic alcohol consumers. Furthermore, regardless of chronic alcohol ingestion, the levels of inflammatory markers such as CRP, IL-6, E-selectin, ADP, E-selectin and TNF-α and the anti-inflammatory marker IL-10 were significantly higher in H pylori CagA+ subjects than in H pylori CagA-subjects. However, a comparison of H pylori CagA+ subjects with and without chronic alcohol ingestion and H pylori CagA-subjects with and without chronic alcohol ingestion demonstrated that their cytokine profiles did not differ. Therefore, chronic alcohol ingestion does not result in significant changes in the cytokine profiles of subjects who are H pylori CagA+. The combination of the results described in the literature and the findings of the present study may lead to the conclusion that although the consumption of alcohol may appear to reduce H pylori infection, it does not significantly reduce the inflammatory response.

In conclusion, H pylori CagA may result in significantly higher levels of several inflammatory markers in subjects with and without chronic alcohol consumption. However, chronic alcohol consumption does not result in significant changes in the cytokine profiles of subjects who are H pylori CagA+. The effect of chronic alcohol consumption on serum cytokine levels requires additional studies with larger sample sizes.

Our study has several limitations. First, the sample size used in this study was small, and the sample was biased in that it only included male subjects. Second, the potential impact of other factors, such as overweight, other drugs, and potentially influential variables, was not controlled. Third, our conclusions are only based on a prospective observational study, which has certain limitations, rather than on a completely randomized controlled study. Finally, the effects of different types and amounts of alcohol in H pylori CagA+ subjects were not examined in our study.

ACKNOWLEDGMENTS

The authors also acknowledge the technical assistance of Yisheng Sun and Hong Wang.

REFERENCES

1. Svensson H, Hansson M, Kilhamn J, et al. Selective upregulation of endothelial E-selectin in response to Helicobacter pylori-induced gastritis. Infect Immun. 2009;77:3109–3116.
2. Calvino Fernández M, Parra Cid T. *H. pylori* and mitochondrial changes in epithelial cells. The role of oxidative stress. *Rev Esp Enferm Dig*. 2010;102:41–50.

3. Perez-Perez GI, Peek RM, Legath AJ, et al. The role of CagA status in gastric and extragastric complications of *Helicobacter pylori*. *J Physiol Pharmacol*. 1999;50:833–845.

4. Konturek SJ, Konturek PC, Bielanski W, et al. Serum progastatin and its products, gastric acid secretion and serum pepsinogen I in gastric cancer. *Digestion*. 2003;68:169–177.

5. Mayr S, Kiechl MA, Mendall J, et al. Increased risk of atherosclerosis is confined to CagA-positive *Helicobacter pylori* strains: prospective results from the Bruneck study. *Stroke*. 2003;34:610–615.

6. Kowalski M. *Helicobacter pylori* (H. pylori) infection in coronary artery disease: influence of *H. pylori* eradication on coronary artery lumen after percutaneous transluminal coronary angioplasty. The detection of *H. pylori* specific DNA in human coronary atherosclerotic plaque. *J Physiol Pharmacol*. 2001;52(1 Suppl 1):3–31.

7. Rasm Y, Raesi S, Seyyed Mohammadzad MH. Association of inflammation and cytotoxin-associated gene a positive strains of *Helicobacter pylori* in cardiac syndrome X. *Helicobacter*. 2012;17:116–120.

8. Mehran Rogha, Davood Dadkhah, Zahra Pourmoghaddas, et al. Association of *Helicobacter pylori* infection with severity of coronary heart disease. *ARYA Atheroscler J*. 2012;7:138–141.

9. Mandrekar P, Catalano D, White B, et al. Moderate alcohol intake in humans attenuates monocyte inflammatory responses: inhibition of nuclear regulatory factor kappa B and induction of interleukin 10. *Alcohol Clin Exp Res*. 2006;30:135–139.

10. Schminke U, Luedemann J, Berger K, et al. Association between alcohol consumption and subclinical carotid atherosclerosis. *Stroke*. 2005;36:1746–1752.

11. Ma SH, Jung W, Weiderpass E, et al. Impact of alcohol drinking on gastric cancer development according to *Helicobacter pylori* infection status. *Br J Cancer*. 2015;113:1381–1388 doi: 10.1038/bjc.2015.333. Epub 2015 Sep 17.

12. Galustian C, Elviss N, Chart H, et al. Interactions of the gastrotropic bacterium *Helicobacter pylori* with the leukocyte-endothelium adhesion molecules, the selectins—a preliminary report. *FEMS Immunol Med Microbiol*. 2003;36:127–134.

13. Qu B, Su J, Wang Z, et al. Effect of *H. pylori* infection on cytokine profiles and oxidative balance in subjects with chronic alcohol ingestion. *PLoS ONE*. 2015;10:e0129352 doi:10.1371/journal.pone.0129352.

14. Innocenti M, Thoreson AC, Ferrero RL, et al. *Helicobacter pylori*-induced activation of human endothelial cells. * Infect Immun.* 2002;70:4581–4590.

15. Cheng SF, Li L, Wang LM. miR-155 and miR-146b negatively regulates IL-6 in *Helicobacter pylori* (caga+) infected gastroduodenal ulcer. *Eur Rev Med Pharmacol Sci*. 2015;19:607–613.

16. Jackson CB, Judd LM, Menheniott TR, et al. Augmented gp130-mediated cytokine signalling accompanies human gastric cancer progression. *J Pathol*. 2007;213:140–151.

17. Jafarzadeh A, Mirzaee V, Ahmad-Beygi H, et al. Association of the CagA status of *Helicobacter pylori* and serum levels of interleukin (IL)-17 and IL-23 in duodenal ulcer patients. *J Dig Dis*. 2009;10:107–112.

18. Gil JH, Seo JW, Choi HS, et al. Role of Treg and TH17 cells of the gastric mucosa in children with *Helicobacter pylori* gastritis. *J Peadiatr Gastroenterol Nutr*. 2014;58:245–251.

19. Abbas Z, Yakoob J, Usman MW, et al. Effect of *Helicobacter pylori* and its virulence factors on portal hypertensive gastropathy and interleukin (IL)-8, IL-10, and tumor necrosis factor-alpha levels. *Saudi J Gastroenterol*. 2014;20:120–127 doi: 10.4103/1319-3767.129477.

20. Peng YC, Ho SP, Shyu CL, et al. Clarithromycin modulates *Helicobacter pylori*-induced activation of nuclear factor-(B through classical and alternative pathways in gastric epithelial cells. *Clin Exp Med*. 2014;14:53–59.

21. Bronte-Tinkew DM, Terebiznik M, Franco A, et al. *Helicobacter pylori* cytotoxin-associated gene A activates the signal transducer and activator of transcription 3 pathway in vitro and in vivo. *Cancer Res*. 2009;69:632–639 doi: 10.1158/0008-5472.CAN-08-1191.

22. Reshetnikov OV, Kurilovich SA, Varaksin NA, et al. The level of serum cytokines in children infected with various strains of *Helicobacter pylori*. *Ekm Klin Gastroenterol*. 2010;46:52–54.

23. Wang J, Court M, Jeremy AH, et al. Infection of Mongolian gerbils with Chinese *Helicobacter pylori* strains. *FEMS Immunol Med Microbiol*. 2003;36:207–213.

24. Saruç M, Goksel G, Ozkaya S, et al. The effect of CagA status on response to *Helicobacter pylori* eradication therapy in Western Turkey. *Br J Med Biol Res*. 2001;34:1435–1439.

25. Eskandari-Nasab E, Sepanjnia A, Moghaddam Pour M, et al. Circulating levels of interleukin (IL)-12 and IL-13 in *Helicobacter pylori*-infected patients, and their associations with bacterial CagA and VacA virulence factors. *Scand J Infect Dis*. 2013;45:342–349.

26. Rezaeifar A, Eskandari-Nasab E, Moghaddam Pour M, et al. The association of interleukin-18 promoter polymorphisms and serum levels with duodenal ulcer, and their correlations with bacterial CagA and VacA virulence factors. *Scand J Infect Dis*. 2013;45:584–592.

27. Song CF, Sun LP, Dai WY, et al. Significance of serum level of NO and IL-8 in *Helicobacter pylori* associated gastric diseases. *Zhonghua Zhong Liu Za Zhi*. 2003;25:25–260.

28. Audibert C, Janvier B, Grignon B, et al. Correlation between IL-8 induction, cagA status and vacA genotypes in 153 French *Helico- bacter pylori* isolates. *Res Microbiol*. 2000;151:191–200.

29. Sibley DA, Osna N, Kusynski C, et al. Alcohol consumption is associated with alterations in macrophase responses to interferon-gamma and infection by Salmonella typhimurium. *FEMS Immunol Med Microbiol*. 2001;32:73–83.

30. Song CF, Sun LP, Dai WY, et al. Significance of serum level of NO and IL-8 in *Helicobacter pylori* associated gastric diseases. *Zhonghua Zhong Liu Za Zhi*. 2003;25:258–260.

31. Wen S, Velin D, Felley CP, et al. Expression of *Helicobacter pylori* virulence factors and associated expression profiles of inflammatory genes in the human gastric mucosa. *Infect Immun*. 2007;75:5118–5126.

32. González-Quintela AI, Domínguez-Santalla MJ, Pérez LF, et al. Influence of acute alcohol intake and alcohol withdrawal on circulating levels of IL-6, IL-8, IL-10 and IL-12. *Cytokine*. 2000;12:1437–1440.

33. Heberlein A, Käser M, Lichtinghagen R, et al. TNF-α and IL-6 serum levels: neurobiological markers of alcohol consumption in alcohol-dependent patients? *Alcohol*. 2014;48:671–676.

34. Amedei A, Cappon A, Codolo G, et al. The neurophil-activating protein of *Helicobacter pylori* promotes Th1 immune responses. *J Clin Invest*. 2006;116:1092–1101.

35. Samy N, Hashim M, Sayed M, et al. Clinical significance of inflammatory markers in polycystic ovary syndrome: their relationship to insulin resistance and body mass index. *Dis Markers*. 2009;26:163–170.

36. Paulson QX, Hong J, Holcomb VB, et al. Effects of body weight and alcohol consumption on insulin sensitivity. *Natu J*. 2010;9:14.
37. Laso FJ, Lapeña P, Madruga JI, et al. Alterations in tumor necrosis factor-alpha, interferon-gamma, and interleukin-6 production by natural killer cell-enriched peripheral blood mononuclear cells in chronic alcoholism: relationship with liver disease and ethanol intake. *Alcohol Clin Exp Res.* 1997;21:1226–1231.

38. Maraslioglu M, Oppermann E, Blattner C, et al. Chronic ethanol feeding modulates inflammatory mediators, activation of nuclear factor-κB, and responsiveness to endotoxin in murine Kupffer cells and circulating leukocytes. *Mediators Inflamm.* 2014;2014:808695.

39. Pascual M, Fernández-Lizarbe S, Guerri C. Role of TLR4 in ethanol effects on innate and adaptive immune responses in peritoneal macrophages. *Immunol Cell Biol.* 2011;89:716–727.

40. Zhang L, Eslick GD, Xia HH, et al. Relationship between alcohol consumption and active *Helicobacter pylori* infection. *Alcohol Alcohol.* 2010;45:89–94.

41. Tursi A, Cammarota G, Papa A, et al. Effect of adequate alcohol intake, with or without cigarette smoking, on the risk of *Helicobacter pylori* infection. *Hepatogastroenterology.* 1998;45:1892–1895.

42. Brenner H, Rothenbacher D, Bode G, et al. Inverse graded relation between alcohol consumption and active infection with *Helicobacter pylori*. *Am J Epidemiol.* 1999;149:571–576.

43. Kuepper-Nybelen J, Rothenbacher D, Brenner H. Relationship between lifetime alcohol consumption and *Helicobacter pylori* infection. *Ann Epidemiol.* 2005;15:607–613.