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Research paper

Up-regulated Th17 cell function is associated with increased peptic ulcer disease in *Helicobacter pylori*-infection

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ABSTRACT

Background: During *Helicobacter pylori* (*H. pylori*) infection $CD4^+$ T cells in the gastric lamina propria are hyporesponsive and polarized by Th1/Th17 cell responses controlled by Treg cells. The objective of this study was to determine the number of Th17 cells in gastric mucosa of patients with gastritis and peptic ulcer and determined the relationship between main virulence factor of *H. pylori* and Th17 cells.

Methods and materials: A total of 89 *H. pylori*-infected gastritis patients, 63 *H. pylori*-infected peptic ulcer patients and 48 *H. pylori*-negative non-ulcer dysplasia patients were enrolled in this study. The number of Th17 was determined by immunohistochemistry. IL-8 and IL-17A expressions were determined by real-time polymerase chain reaction (qPCR). Also, the grade of chronic and active inflammation was investigated for involvement according to the density of neutrophils and mononuclear in gastric mucosal crypts, from one to all crypts.

Results: The number of Th17 cells and the expression of IL-8 and IL-17A in infected patients were significantly higher than uninfected subjects. The number of Th17 cells and the expression of IL-8 and IL-17A in infected patients with peptic ulcer were significantly higher than patients with gastritis. Additionally, the numbers of Th17 cells as well as the expression of IL-8 and IL-17A were positively correlated with the degree of *H. pylori* density in infected patients with peptic ulcer, while this correlation was negative in infected patients with gastritis. The numbers of Th17 cells as well as the expression of IL-8 and IL-17A were positively correlated with the degree of chronic inflammation.

Conclusion: The predominant Th17 cell responses may play a role in the pathogenesis of peptic ulcers disease in infected patients.

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative gastroduodenal pathogen. It is one of the highly abundant human pathogens that infects approximately 50% of the world population and is linked to chronic gastritis, peptic ulcer disease and gastric cancer (Bagheri et al., 2016a; Phan et al., 2017; Suzuki et al., 2012). Infection with *H. pylori* is usually acquired in early childhood but in the absence of treatment persists for life (Mendoza-Elizalde et al., 2015; Shirzad et al., 2015). These different clinical outcomes have been attributed to the interplay of several factors, including virulence factors of *H. pylori*, host genetic susceptibility, local innate and adaptive immune responses, as well as environmental conditions (e.g. smoking, malnutrition, high salt intake, vitamin and antioxidants deficiency) (Alarcon-Millan et al., 2016; Armitano et al., 2013; Shahi et al., 2015; Tang et al., 2015). *H. pylori* infection causes severe local inflammation in the gastric mucosa (Bagheri et al., 2016c; Bagheri et al., 2013). For instance, increased $CD3^+CD4^+$ T cells in gastric lamina propria (LP) of patients infected with *H. pylori* may play an important role in the pathogenesis of persistent infection (Eaton et al., 2001). $CD4^+$ Th cells are major effector cells in the immune responses to *H. pylori*. Although the numbers of $CD4^+$ T cells in the gastric lamina propria with a memory phenotype increase during *H. pylori* infection, these T cells are hyporesponsive (Lina et al., 2015). Because this hyporesponsiveness contributes to chronic infection, there

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have been targeted efforts to understand the mechanisms employed by *H. pylori* to downregulate T cell responses. A novel subset of effector T cells, identified by secretion of interleukin (IL)-17, has been defined as Th17 cells. Th17 cells are distinct from Th1 and Th2 cells in their differentiation and function (Asadi-Samani et al., 2017; Park et al., 2005). Retinoic acid-related orphan receptor γT (RORγT) is the orphan nuclear receptor that regulates the development of Th17 cells and the expression of IL-17 (Ratajewski et al., 2012). Recent studies also have shown that the levels of IL-17 are increased in the gastric mucosa of human infected with *H. pylori* (Bagheri et al., 2016b). Indeed, IL-17 is one of the earliest cytokines detected in the gastric mucosa of *H. pylori*-infected mice (Algood et al., 2007). Moreover, IL-17 has the ability to stimulate IL-8 production in both gastric epithelial cells and lamina propria antigen presenting cells, raising the possibility that this cytokine may play an important role in the recruitment of inflammatory cells during *H. pylori* infection (Bagheri et al., 2015a). However, the relationship between Th17 cells and the pathogenesis of peptic ulcer remain unclear. Therefore, the aim of this study was to investigate the relationship between the frequency of Th17 cells and *H. pylori* virulence factors in a cohort of Iranian adult patients. In addition, we examined Th17 cells frequency with the disease clinical outcomes in this population.

2. Materials and methods

2.1. Study population and patient sampling

A total of 200 subjects, undergoing endoscopy at Hajar University Hospital, were included in the study. The study was approved by the human research ethics committee at Tehran University of Medial Sciences and Shahrekord University of Medical Sciences and informed consent was obtained from each volunteer before participation. The groups were characterized as follows: the *H. pylori*-positive group with gastritis ($n = 89$: 35 males, 54 females; mean age: 50.18 ± 15.02 years); *H. pylori*-positive group with peptic ulcer diseases ($n = 63$: 39 males, 24 females; mean age: 50.16 ± 15.3 years); *H. pylori*-negative group with non-ulcer dysplasia ($n = 48$: 19 males, 29 females; mean age: 50.96 ± 19.77 years). Biopsy specimens were obtained from the antral region of the stomach. Four gastric mucosal biopsy specimens were obtained from each patient. Two specimens were used for DNA and RNA extraction and one specimen was used for histopathological studies. One antral specimen was directly placed into a medium containing urea to perform the rapid urease test (RUT). *H. pylori*-infection was determined by the rapid urease test, PCR (16srRNA and glmM) and histological examination of biopsies taken from the corpus. Patients were classified as *H. pylori*-infected only if the four tests were positive. Patients were classified as *H. pylori*-negative only if the four tests were negative. After medical chart review, patients with chronic heart, kidney, lung or liver diseases, having history of gastric surgery or anti-*H. pylori* eradication therapy, as well as patients taking non-steroidal anti-inflammatory drugs within one week prior to endoscopy were excluded from this study.

2.2. Microscopic examination and immunohistochemistry

Two biopsy specimens were taken from the antrum for histopathological evaluation. Sections of biopsy specimens were embedded in 10% buffered formalin, and then embedded in paraffin, cut in sequential 4 μm sections and subsequently stained with hematoxylin and eosin (H&E) for grading and evaluating the severity of gastritis. The specimens were also subjected to modified Giemsa staining for visualization of *H. pylori* using light microscopy. The colonization of *H. pylori* on the gastric epithelium was graded on a 3-point scale of severe colonization (the presence of large groups of organisms on the surface and upper pits of more than two-thirds of the mucosal surface), mild colonization (individual organisms, covering less than one-third of the mucosal surface) and moderate colonization (between these two). We defined an ulcer as a circumscribed mucosal break (> 5 mm in diameter, with apparent depth) in the stomach or duodenum, covered with exudates. The histological severity of gastritis was blindly graded from normal to severe based on the degree of mononuclear cell (MNC) and polymorphonuclear leukocyte (PMN) infiltration according to the Updated Sydney system (Dixon et al., 1996) on a four-point scale: 0, no; 1, mild; 2, moderate; and 3, severe changes. For immunohistochemical analysis, 4-μm serial sections were made and spread on poly-L-lysine-coated slides. Paraffin sections were dried in a 70 °C oven for overnight, deparaffinized in three changes of xylene and hydrated using a series of alcohols (100%, 100%, 80% and 70%). Antigen retrieval was performed routinely by immersing the sections in citrate buffer (10 mM Sodium Citrate, 0.05% Tween 20, pH 6.0) in a pressure cooker by autoclaving for 20 min. The sections were then incubated with protein block (Abcam, England) for 60 min to block nonspecific background staining. Subsequently, Rabbit anti-human CD4 antibody (ab133616, Abcam, UK) at a 1:450 dilution and Rabbit anti-human RORγ antibody (ab219106, Abcam, UK) at a 1:350 dilution were applied, respectively, to the sections that were latter incubated overnight in a humidified chamber at 4 °C. On the second day, endogenous peroxidase activity was blocked with 3% H2O2 in TBS for 15 min. Afterwards, Biotinylated goat anti-rabbit and mouse IgG (ab93697, Abcam, UK) were applied and the sections were incubated for 1 h at room temperature. Then, applying Streptavidin Peroxidase Plus, the sections were incubated for 10 min at room temperature. Afterwards Applying DAB (ab94665, Abcam, UK) to tissue, the sections were incubated for 10 min. Sections were counterstained for 1 min with Meyer's hematoxylin and then mounted. Human hodgkin's lymphoma tissue was used as a positive control for Foxp3. Additional sections were processed without primary antibody as a negative control. The number of $CD4^+$ and Th17 was calculated by counting positive lymphocytes throughout the entire area of tissue section at 10 high power fields. Results were expressed as the mean value and interquartile range of all tested patients in each group.

2.3. H. pylori detection and virulence genotyping

Detection of *H. pylori* and virulence genotyping was performed by polymerase chain reaction (PCR). Primer sequences and PCR conditions reported by Salimzadeh et al. (Salimzadeh et al., 2015).

2.4. Real-time PCR

Total RNA from the biopsy samples was extracted using a TRIzol® Plus RNA Purification Kit according to the supplier's instructions. Complementary DNA (cDNA) was synthesized using reverse transcriptase (RT) using the First Strand cDNA Synthesis Kit (Fermentas Life Sciences, cat- K1622). For cDNA synthesis 2.5 microgram of pure RNA was used as template. Using the TaqMan RT-PCR system, amplification of IL-8, IL-17A and β-actin cDNA was performed in a Rotorgene 3000 (Corbett Research). The real time-PCR reactions were performed in a total volume of 20 μl containing 5.75 μl of nuclease-free H2O, 3 μl of synthesized cDNA solution, 10 μl of 2× Rotor-Gene Probe PCR Master Mix (Qiagen, Germany), 0.5 μl of each primer (10 pM) and 0.25 μl of the TaqMan probe (10 pM). Negative controls for real time-PCR amplification were prepared by omitting the cDNA sample from the reaction mixture. Thermal cycling was initiated with a first denaturation step at 95 °C for 10 min and followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. The primer and probe sequences for IL-8, IL-17A and β-actin cDNA have been previously published (Bagheri et al., 2016b; Tal et al., 2010). Expression of IL-8 and IL-17A mRNA relative to β-actin mRNA were determined using the $2^{-\Delta\Delta Ct}$ method.

2.5. Statistical analysis

Experiments were performed in duplicate, and all data were shown

Table 1
Demographic characterization of PUD and G subjects.

Group [n (%)]	Gender		Age
	Males [n (%)]	Females [n (%)]	
Gastritis 89 (44.5)	35 (39.3)	54 (60.7)	50.18 ± 15.02
Peptic Ulcer Diseases 63 (31.5)	39 (61.9)	24 (38.1)	50.16 ± 15.3
Uninfected 48 (24)	19 (39.6)	29 (60.4)	50.96 ± 19.77
Total 200 (100)	93 (46.4)	107 (53.5)	50.22 ± 16.2

as mean ± SEM. The normal distribution of data was confirmed by Shapiro-Wilk normality test. Quantitative data was evaluated by Student *t*-test (for comparison between two samples) or by ANOVA (for multiple comparisons) and Turkey post hoc analysis, which compares infected groups to a specific control group by GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA, USA). Categorical data (between virulence factors and type of disease) were presented as frequencies. Pearson's correlation analysis for parametric data was used to assess the relationship between variables. Statistical significance was assumed if a *P*-value was < 0.05.

3. Results

3.1. Demographic characterization of study population

The groups were characterized as follows: the *H. pylori*-positive group with gastritis ($n = 89$: 35 males, 54 females; mean age: 50.18 ± 15.02 years); the *H. pylori*-positive group with peptic ulcer diseases ($n = 63$: 39 males, 24 females; mean age: 50.16 ± 15.3 years); the *H. pylori*-negative group with non-ulcer dysplasia ($n = 48$: 19 males, 29 females; mean age: 50.96 ± 19.77 years) (Table 1). According to the Table 2, there was no significant relationship between gender and frequency of Th17 cells in our study population.

3.2. Relation of Th17 cells and cytokines expression with gastroduodenal diseases

The presence of Th17 cells in the gastric mucosa of gastroduodenal diseases was determined in two consecutive sections. The expression of RORγt was seen in the nucleus of lymphocytes (Fig. 1A-B). We observed infected patients with peptic ulcer had significantly higher number of Th17 cells than infected patients with gastritis and uninfected patients (Fig. 2A). The fold changes in the frequency of Th17 cells in the infected patients with peptic ulcer compared to infected patients with gastritis and uninfected individuals were 1.51 and 3.24 respectively. To determine the immunomodulatory effects of *H. pylori* infection at the mucosal surfaces, we analyzed expression of cytokines (e.g. IL-8 and IL-17A) in the gastric tissues by real time-PCR. The relative mRNA levels of IL-8 and IL-17A were significantly elevated in infected patients with peptic ulcer compared to infected patients with gastritis and uninfected patients. The fold changes in IL-8 and IL-17A expression levels in the infected patients with peptic ulcer compared to uninfected individuals were 5.37 and 4.04 respectively. In addition, we noted higher expression of IL-8 and IL-17A mRNA in mucosal tissues obtained from gastritis patients compared to uninfected individuals however their expression levels were lower compared with peptic ulcer patients. Infected patients with peptic ulcer had higher expression of mRNA for IL-8 and IL-17A compared with infected patients with gastritis which enumerated by 2.42 and 1.9 folds respectively (Fig. 2B-C).

Table 2
Relationship between gender and frequency of Th17 cells in our study population.

Gender	The frequency of Th17 cells (Mean ± SD)			
	Uninfected group	Infected group	Gastritis group	PUD group
Males	4.211 ± 3.06	8.841 ± 6.38	7.842 ± 5.52	8.840 ± 6.23
Females	4.621 ± 3.45	7.649 ± 6.52	8.000 ± 6.07	8.429 ± 8.79
P value	0.672	0.409	0.930	0.865

3.3. Relation of Th17 cells and cytokines expression with cagA virulence factor

We also analyzed immunological changes at the mucosal sites with the cytotoxin-associated gene A (cagA) of *H. pylori*. No significant correlation in the number of Th17 cells was noted between *H. pylori*-infected patients with *cagA*-positive and *H. pylori*-infected patients with *cagA*-negative (Fig. 3A; $P = 0.823$). We found that *H. pylori*-infected patients with *cagA*-positive had a significantly higher expression of IL-8 mRNA levels in comparison to *H. pylori*-infected patients with *cagA*-negative (Fig. 3B; $P = 0.025$). In addition, no significant difference was found in the expression of IL-17A mRNA levels between *H. pylori*-infected patients with *cagA*-positive and *H. pylori*-infected patients with *cagA*-negative (Fig. 3C). The fold changes in IL-8 and IL-17A expression levels in the infected patients with *cagA*-positive compared to infected patients with *cagA*-negative was 2.33 and 1.44 respectively.

3.4. Relation Th17 cells and cytokines expression with oipA virulence factor

We also investigated whether the *H. pylori* outer inflammatory protein (OipA), which is an important virulence factors is associated with observed changes in mucosal biopsies. *H. pylori*-infected patients with *oipA*-positive had a significantly higher number of Th17 cells compared with the *H. pylori*-infected patients with *oipA*-negative (Fig. 4A; $P = 0.012$). The fold changes in the number of Th17 cells in the infected patients with *oipA*-positive compared to infected patients with *oipA*-negative was 1.55. In addition, we found that *H. pylori*-infected patients with *oipA*-positive had a significantly higher expression of IL-8 and IL-17A mRNA levels in comparison to *H. pylori*-infected patients with *oipA*-negative (Fig. 4B-C: IL-8, $P < 0.0001$, IL-17A, $P = 0.018$). The fold changes in IL-8 and IL-17A expression levels in the infected patients with *oipA*-positive compared to infected patients with *oipA*-negative was 4.24 and 1.88 respectively.

3.5. Relation Th17 cells and cytokines expression with the degree of H. pylori density

The numbers of Th17 cells as well as the expression of IL-8 and IL-17A were positively correlated with the degree of *H. pylori* density in infected patients with peptic ulcer disease (Fig. 5A-C: Th17, $P < 0.0001$, IL-8, $P < 0.0001$ and IL-17A, $P = 0.0002$). The fold changes in the number of Th17 cells and expression of IL-8 and IL-17A in the infected patients with marked *H. pylori* density compared to infected patients with moderate *H. pylori* density was 1.76, 1.75 and 1.7 respectively. Also, the fold changes in the number of Th17 cells and expression of IL-8 and IL-17A in the infected patients with marked *H. pylori* density compared to infected patients with mild *H. pylori* density was 2.55, 4.59 and 4.08 respectively. While, the numbers of Th17 cells as well as the expression of IL-8 and IL-17A were negatively correlated with the degree of *H. pylori* density in infected patients with gastritis disease (Fig. 5D-F: Th17, $P = 0.005$, IL-8, $P < 0.0001$and IL-17A $P < 0.0001$). The fold changes in the number of Th17 cells and expression of IL-8 and IL-17A in the infected patients with mild *H. pylori* density compared to infected patients with moderate *H. pylori* density was 1.5, 1.55 and 1.9 respectively. Also, the fold changes in the number of Th17 cells and expression of IL-8 and IL-17A in the infected patients with mild *H. pylori* density compared to infected patients with marked *H. pylori* density was 2.66, 4.43 and 3.95 respectively.

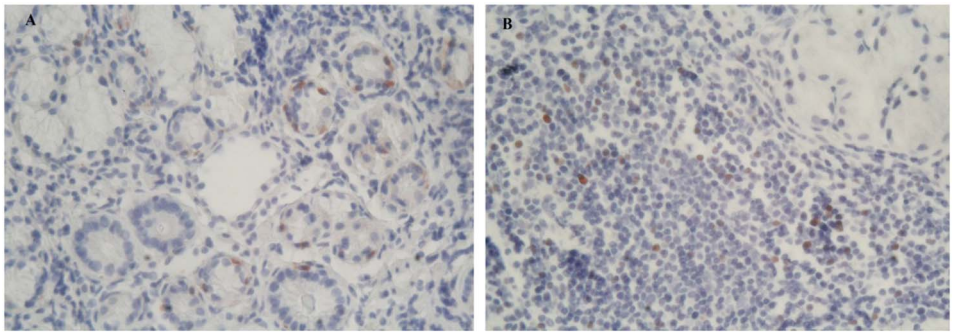

Fig. 1. Immunohistochemical staining of Th17 cells in *H. pylori*-negative *H. pylori*-positive biopsies. (original magnification, 400×). Representative immunohistochemical staining of RORγt in gastric mucosa from A) *H. pylori*-negative individual, B) *H. pylori*-positive patient, RORγt staining was located in the nucleus of T lymphocytes.

3.6. Relation Th17 cells and cytokines expression with the degree of chronic inflammation

The numbers of Th17 cells as well as the expression of IL-8 and IL-17A were positively correlated with the degree of chronic inflammation (Fig. 6A-C: Th17, $P = 0.017$, IL-8, $P = 0.001$ and IL-17A, $P = 0.01$).

3.7. Correlation between the number of Th17 cells and different parameters in infected patients with H. pylori

The number of Th17 cells was found to be positively correlated with IL-8 and IL-17A mRNA level in infected patients with peptic ulcer disease (Fig. 7A-B: IL-8, $r = 0.729$, $P < 0.0001$ and IL-17A, $r = 0.903$, $P < 0.0001$). Moreover, the number of Treg cells was inversely related to the number of Th17 cells in infected patients with gastritis disease (Fig. 7C: $r = -0.823$, $P < 0.0001$). Also, our results showed that the number of Th17 cells was inversely related to the number of Treg cells in infected patients with peptic ulcer disease (Fig. 7D: $r = -0.902$, $P < 0.0001$). The results showed that the ratio of Th17/Treg cells in infected patients with gastritis disease was inversely related to *H. pylori* density score (Fig. 7E: $r = -0.739$, $P < 0.0001$). In contrast, the ratio of Th17/Treg cells in infected patients with peptic ulcer disease positively correlated with *H. pylori* density score (Fig. 7F: $r = 0.880$, $P < 0.0001$). Levels of IL-8 expression was positively related to the acute inflammatory score ($r = 0.788$, $P < 0.0001$) in infected patients with *H. pylori* (Fig. 7G).

4. Discussion

IL-17 is the signature cytokine produced by Th17 cells and has a mediator role in the inflammation associated with certain autoimmune diseases and host defense against bacterial and fungal pathogens, particularly at mucosal surfaces (Bagheri et al., 2016b). *H. pylori* infection is the main cause of gastric inflammation (Azadegan-Dehkordi et al., 2015; Bagheri et al., 2015b). We first confirmed that the number of Th17 cells in infected patients was significantly higher, compared to uninfected patients. Additionally, the mucosal IL-8 and IL-17A levels were significantly higher in *H. pylori*-positive patients compared with *H. pylori*-negative patients. Our results are in agreement with recent studies which confirmed that the number of Th17 has been shown to be increased in the gastric mucosa of *H. pylori*-infected patients (Serrano et al., 2013; Shamsdin et al., 2015). The number of Th17 cells as well as the expression of IL-17A was independent on *cagA*, but the expression of IL-8 was dependent on *cagA* virulence factor in patients infected with

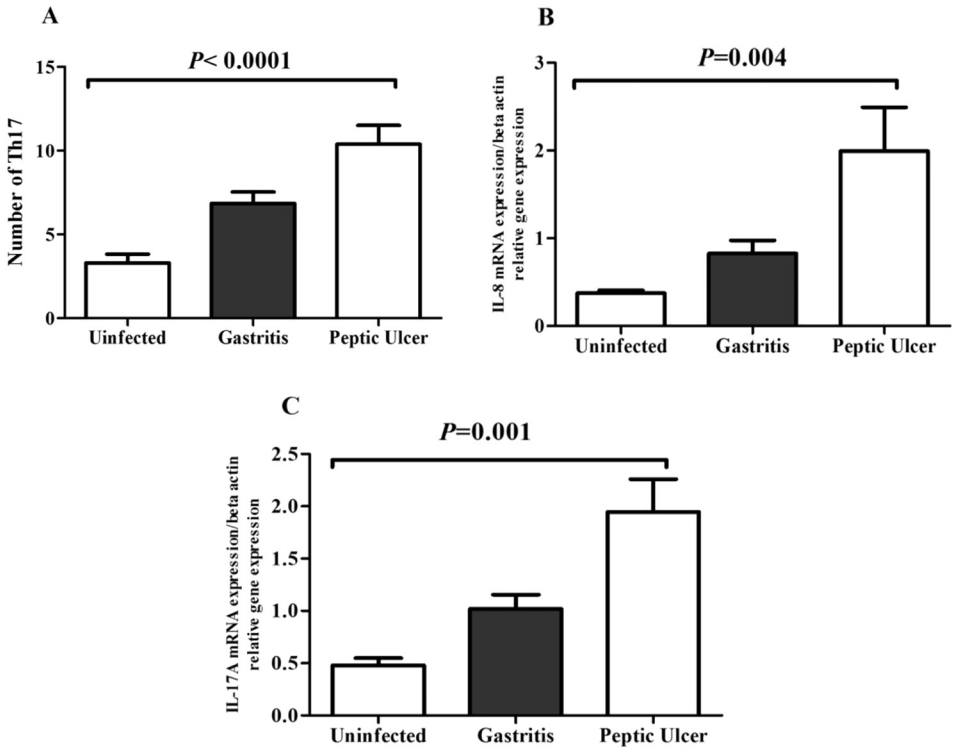

Fig. 2. Column bar graph for the number of Th17 cells and the expression of IL-8 and IL-17A mRNA levels in gastric biopsies of *H. pylori*-uninfected individuals compared with *H. pylori*-infected patients with peptic ulcer or gastritis disease. A) The number of Th17 cells in areas of antral gastric mucosa. RNA was extracted from gastric biopsies of 48 *H. pylori*-uninfected individuals, 63 *H. pylori*-infected patients with peptic ulcer and 89 *H. pylori*-infected patients with gastritis and analyzed for B) IL-8 and G) IL-17A mRNA level by real time-PCR. Levels were normalized to β-actin. *P* values < 0.05 was considered statistically significant using unpaired One-way ANOVA.

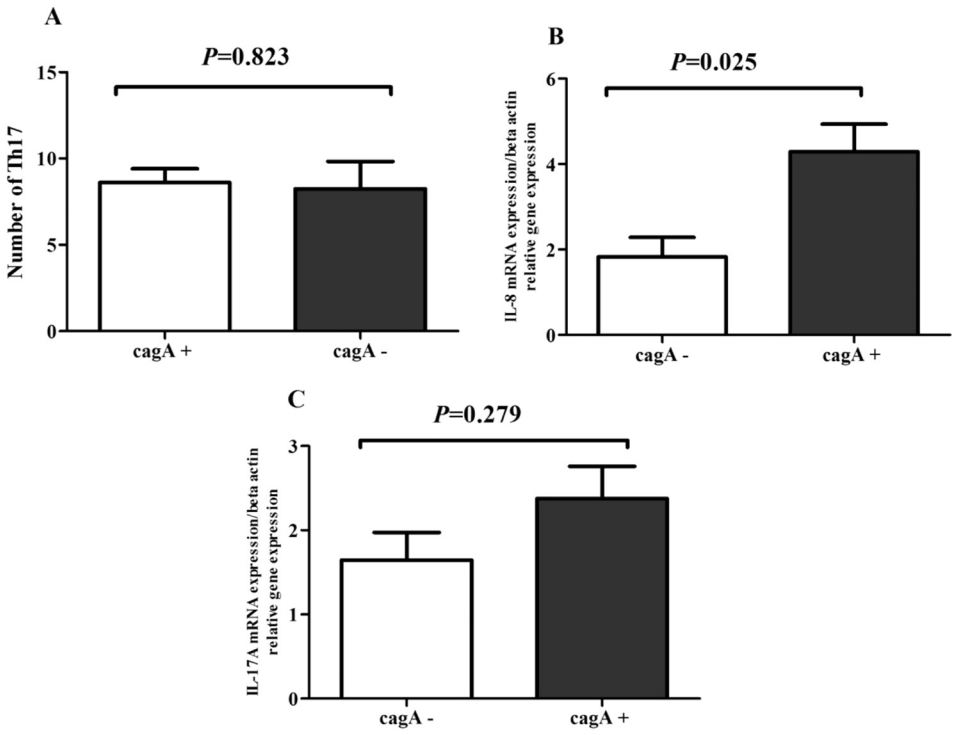

Fig. 3. Column bar graph for the number of Th17 cells and the expression of IL-8 and IL-17A mRNA level according to cagA virulence factor in patients infected with *H. pylori*. Expression of Th17 cells was determined by immunohistochemical staining in two consecutive sections per sample. A) The number of Th17 cells in areas of antral gastric mucosa from patients with *H. pylori* infection. RNA was extracted from gastric biopsies of 106 *H. pylori*-infected patients with *cagA*-positive and 46 *H. pylori*-infected patients with *cagA*-negative and analyzed for B) IL-8 and C) IL-17A mRNA level by real time-PCR. Levels were normalized to β-actin. *P* values ≤ 0.025 was considered statistically significant using Student *t*-test.

H. pylori. The number of Th17 cells as well as the expression of IL-8 and IL-17A was dependent on *oipA*. The presence of cagA and OipA has been linked to enhanced gastric inflammation (Ferreira et al., 2016; Salimzadeh et al., 2015), which is attributed to its potent induction of innate immune responses in epithelial cells. Several studies demonstrated that OipA and cagA positive isolates induced higher IL-8 secretion from gastric epithelial cells than OipA and cagA negative isolates (Ferreira et al., 2016; Salih et al., 2014). In humans, it is well established that cagA positive *H. pylori* is more potent than cagA negative *H. pylori* in developing chronic gastritis with higher risks for peptic ulcer disease and gastric cancer (Chiba et al., 2006; Khatoon et al., 2017). In contrast to our findings, jafarzadeh et al. reported that IL-17 and IL-23 serum levels were increased in *H. pylori*- infected patients. In the duodenal ulcer disease group the mean serum levels of IL-17 in patients testing positive for the anti-cagA antibody was significantly higher than that observed in patients testing negative for this antibody. Accordingly, in the duodenal ulcer group the expression of IL-17 was influenced by the cagA factor. However, the IL-23 serum levels were not influenced by the expression of the cagA factor (Jafarzadeh et al., 2009). Also, Sugimoto et al. demonstrated that IL-1β, IL-17, IL-

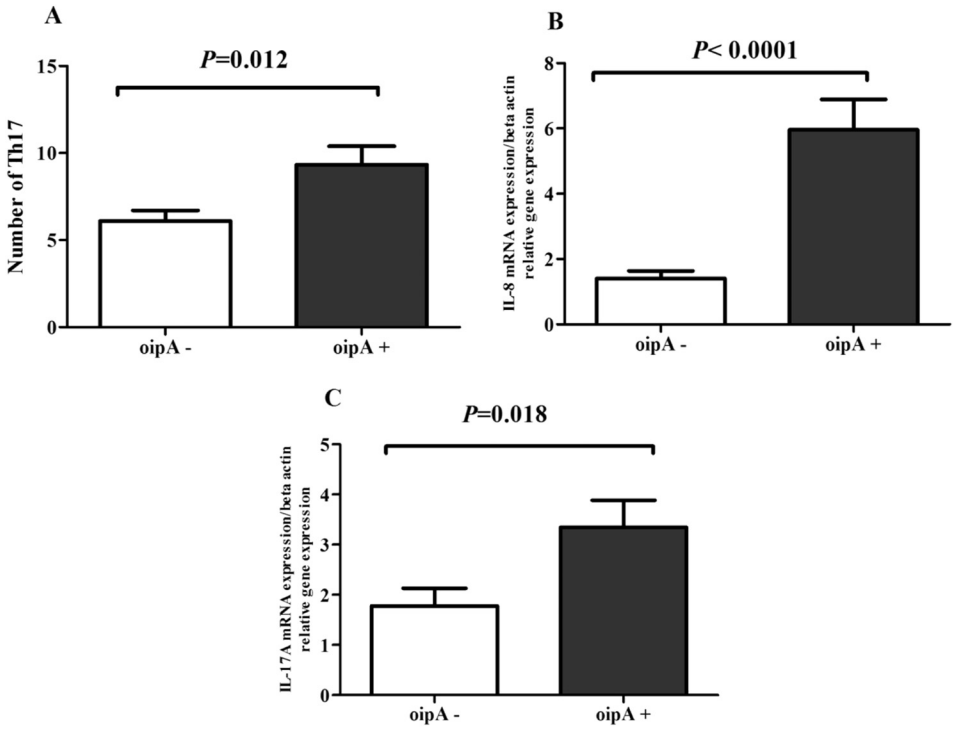

Fig. 4. Column bar graph for the number of Th17 cells and the expression of IL-8 and IL-17A mRNA levels according to the *oipA* virulence factor in patients with *H. pylori* infection. Expression of Th17 cells was determined by immunohistochemical staining in two consecutive sections per sample. A) The number of Th17 cells in areas of antral gastric mucosa from patients with *H. pylori* infection. RNA was extracted from gastric biopsies of 78 *H. pylori*-infected patients with oipA-positive and 126 *H. pylori*-infected patients with oipA-negative; and analyzed for B) IL-8 and C) IL-17A mRNA levels by real time-PCR. Levels were normalized to β-actin. *P* values ≤ 0.025 was considered statistically significant using Student t-test.

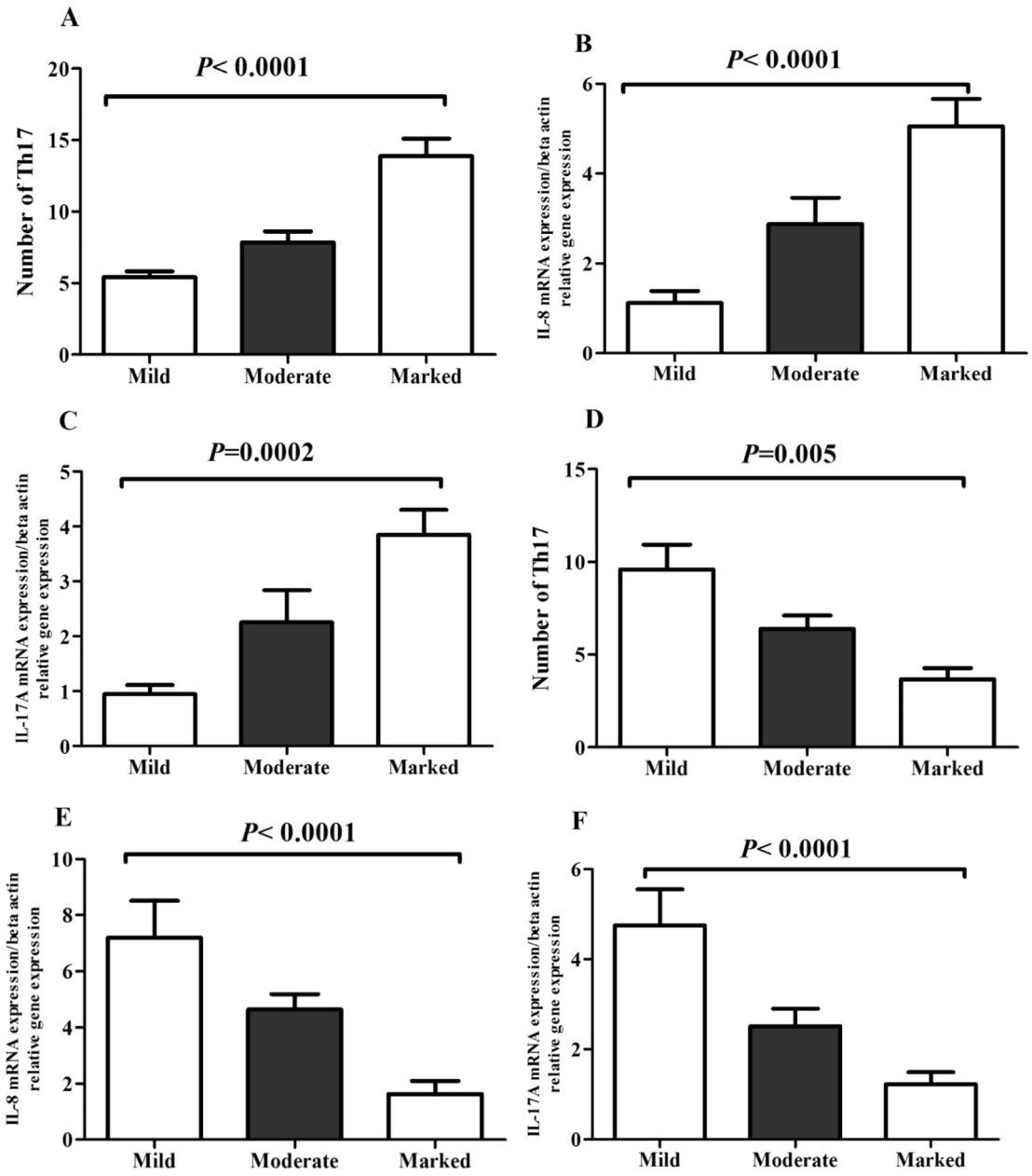

Fig. 5. Column bar graph for the number of Th17 cells and the expression of IL-8 and IL-17A mRNA levels according to *H. pylori* density in infected patients with peptic ulcer disease (graphs A-C) and gastritis disease (graphs D-F). Expression Th17 cells was determined by immunohistochemical staining in two consecutive sections per sample. A and D) The number of Th17 cells in areas of antral gastric mucosa from patients with *H. pylori* infection. RNA was extracted from gastric biopsies of *H. pylori*-infected patients; and analyzed for IL-8 and IL-17A mRNA level by real time-PCR. Levels were normalized to β-actin. *P* values < 0.05 was considered statistically significant using One-way ANOVA.

18, and TNF-α mRNA levels in oipA mutant-infected Mongolian gerbils were significantly lower than in gerbils infected with wild-type strains; the status of oipA determined the gastric inflammation related inflammatory cytokine levels, resulting in peptic ulcer disease and gastric cancer development (Sugimoto et al., 2009). In contrast to the above mentioned findings, Teymournejad et al. has claimed that oipA of *H. pylori* is a DC maturation suppression factor (Teymournejad et al., 2014). In a similar experiment, Kido et al. (Kido et al., 2011) showed that cagA has dual roles in the pathophysiology of *H. pylori*-induced chronic gastritis. The colonization of $cagA^+$ *H. pylori* in the host gastric mucosa is critical for the migration of *H. pylori*-primed $CD4^+$ T cells to the gastric mucosa. In addition, cagA-dependent T-cell priming evokes differentiation of Treg cells. A recently published study indicated an important role for cagA in inducing tolerance (Kaebisch et al., 2014). As a result, *H. pylori* shifts the DC response toward Tregs and away from Th17, which permits persistent infection. In support of these findings, Kao et al. (Kao et al., 2010) and Zhang et al. (Zhang et al., 2010) demonstrated that *H. pylori*-induced dendritic cells (DCs) skew the Th17/Treg balance toward a Treg-biased response that suppresses Th17 immunity through a cagA and vacA independent, TGF-β and IL-10 dependent mechanism. The number of Th17 cells and the expression of IL-8 and IL-17A in infected patients with peptic ulcer were significantly higher than the ones in infected patients with gastritis. Additionally, the numbers of Th17 cells as well as the expression of IL-8 and IL-17A were positively correlated with the degree of *H. pylori* density in infected patients with peptic ulcer. While, the numbers of Th17 cells as well as the expression of IL-8 and IL-17A were negatively correlated with the degree of *H. pylori* density in infected patients with gastritis. Our previous study demonstrated that the number of Treg cells and the expression of IL-10 and TGF-β1 in infected patients with gastritis were

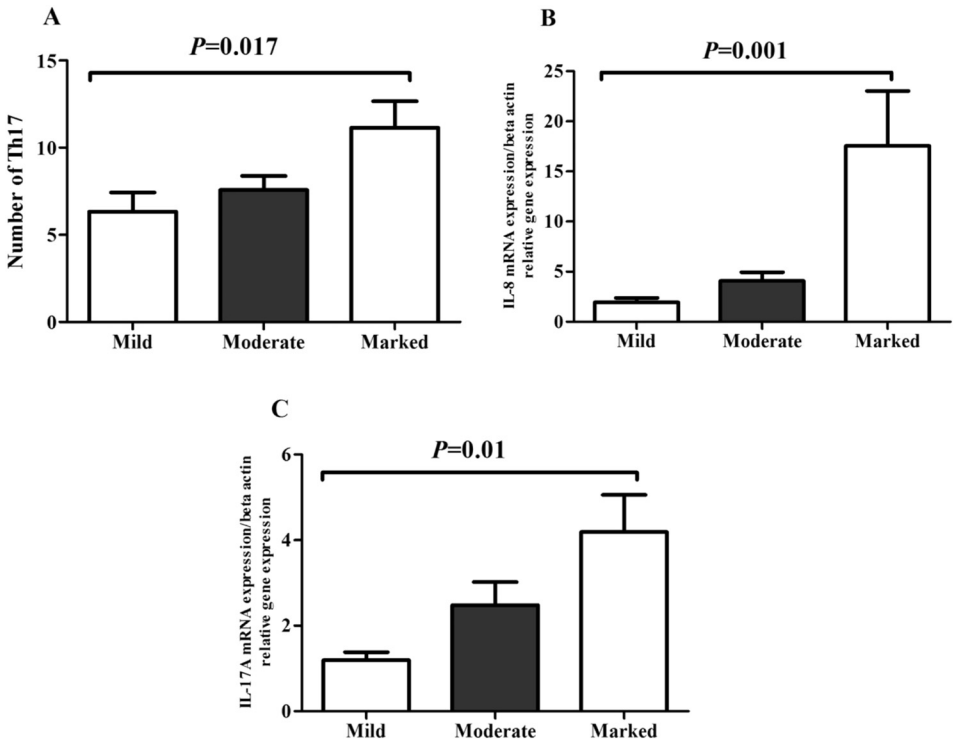

Fig. 6. Column bar graph for the number of Th17 cells and the expression of IL-8 and IL-17A mRNA levels according to the degree of chronic inflammation in patients with *H. pylori* infection: Expression Th17 cell was determined by immunohistochemical staining in two consecutive sections per sample. A) The number of Th17 cells in areas of antral gastric mucosa from patients with *H. pylori* infection. RNA was extracted from gastric biopsies of *H. pylori*-infected patients; and analyzed for IL-8 and IL-17A mRNA level by real time-PCR. Levels were normalized to β-actin. *P* values < 0.05 was considered statistically significant using One-way ANOVA.

significantly higher than infected patients with peptic ulcer diseases (Bagheri et al., 2017). The numbers of Th17 cells as well as the expression of IL-8 and IL-17A were positively correlated with the degree of chronic inflammation in infected patients. In agreement with our results, recent studies in infected patients with *H. pylori* have demonstrated that the frequencies of IFN-γ and IL-17A$^+$ cells correlated positively with inflammation but not with reduced bacterial colonization in the antrum of *H. pylori* infected patients. In the antrum, while there was no significant evidence of correlation between IFN-γ and the degree of *H. pylori* density, a positive correlation between the degree of *H. pylori* density and IL-17A$^+$ cells was seen (Adamsson et al., 2017). The results suggest that in the *H. pylori* infected patients with peptic ulcer disease, Th17 cell responses may be promoting inflammation but not anti-bacterial response in the *H. pylori* infected patients, thus contributing to tissue damage and the persistence of the infection. Recent studies in children infected with *H. pylori* have indicated that the mucosal Th17/IL-17 response in children and adults with *H. pylori*-associated gastritis. Children infected with *H. pylori* had significantly fewer gastric Th17 cells and significantly lower levels of gastric IL-17-specific mRNA and protein compared with that of similarly infected adults, indicating a potent reduction in the mucosal Th17 response in the children (Razavi et al., 2015; Serrano et al., 2013), suggesting Treg participation in the reduced Th17-mediated gastritis and ulceration in the children. The number of Th17 cells was found to be positively correlated with IL-8 and IL-17A mRNA level in infected patients with peptic ulcer disease. The results of the present study showed that the ratio of Th17/Treg cells in infected patients with gastritis disease was inversely related to *H. pylori* density score. In contrast, the ratio of Th17/Treg cells in infected patients with peptic ulcer disease positively correlated with *H. pylori* density score. A level of IL-8 expression was positively related to the acute inflammatory score in infected patients with *H. pylori*. Moreover, the number of Treg cells was inversely related to the number of Th17 cells in infected patients with gastritis disease. Also, our results showed that the number of Th17 cells was inversely related to the number of Treg cells in infected patients with peptic ulcer disease. However, transfer of T lymphocytes into these animals then results in a severe gastritis (Eaton et al., 1999), suggesting that T cells play a major role in promoting the recruitment of inflammatory cells during *H. pylori* infection. In support this concept, IL-17 is able to activate ERK1/2 MAP kinase in gastric epithelial cells thereby promoting IL-8 secretion (Sebkova et al., 2004). In support of these findings, Caruso et al. (Caruso et al., 2008) shown that extracellular signal-regulated kinase (ERK)1/2 activity and IL-8 are highly expressed in freshly isolated *H. pylori*-colonized gastric epithelial cells, and that neutralization of endogenous IL-17 in gastric biopsies of *H. pylori*-infected patients down-regulates ERK1/2 activation and IL-8 production. The released IL-8 attracts neutrophils promoting inflammation (Bagheri et al., 2015a). In addition, it has been reported that that IL-8 play important roles in the pathogenesis of peptic ulcer disease (Bagheri et al., 2015a; Siddique et al., 2014). The involvement of IL-17 in *H. pylori* -related peptic ulcer disease is also supported by the demonstration that this cytokine is able to stimulate both immune and non-immune cells to produce multiple inflammatory mediators, such as IL-1, IL-6, TNF-α, and matrix metalloproteinases (MMP) (Azadegan-Dehkordi et al., 2015; Onishi and Gaffen, 2010; Shamsdin et al., 2017). In conclusion, the predominant Th17 cell responses may play a role in the pathogenesis of peptic ulcers disease in infected patients.

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Conflict of interest

The authors have declared that no competing interests exist. All authors have approved this manuscript.

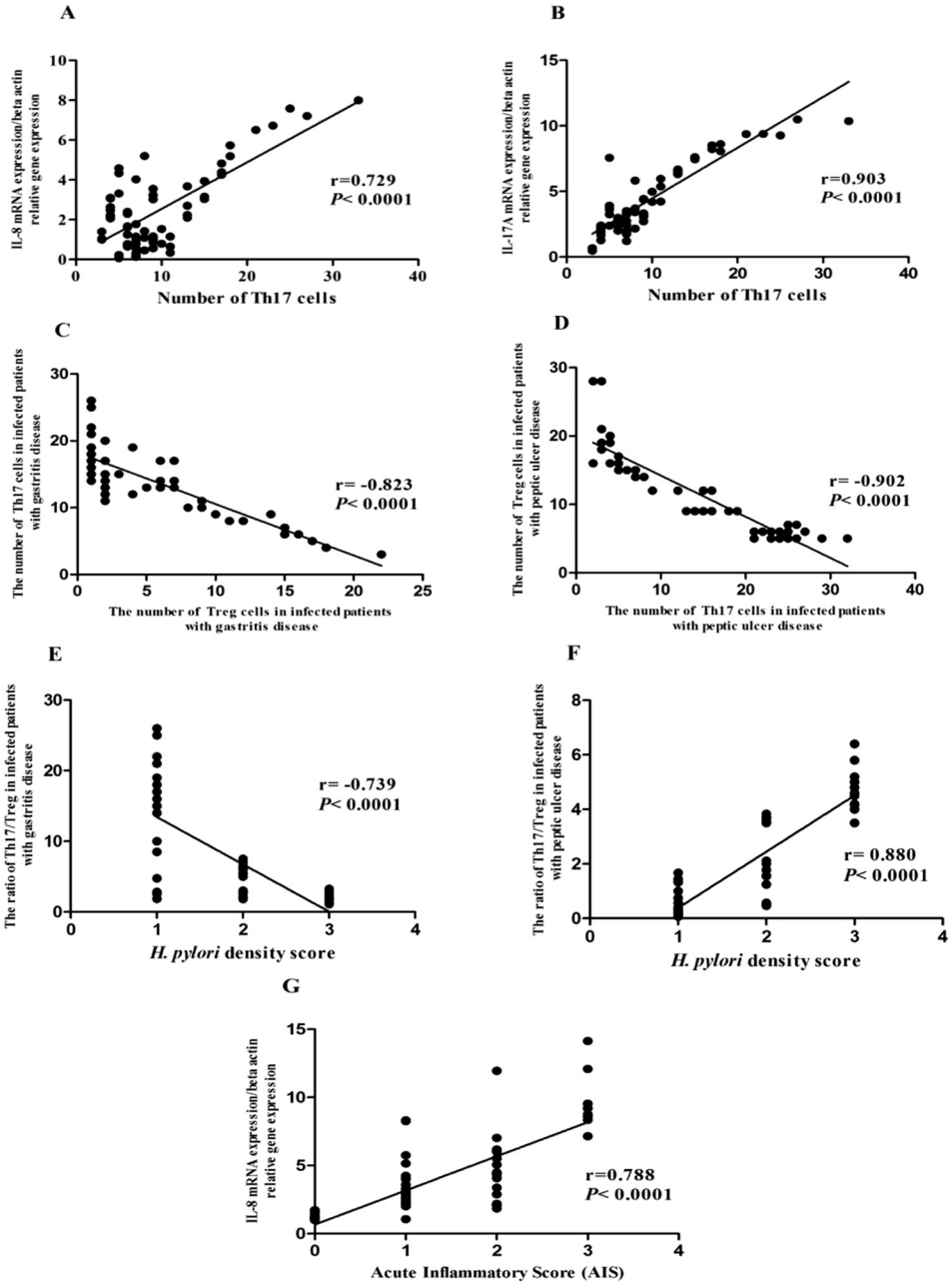

Fig. 7. The correlation between different parameter's in infected patients with *H. pylori*. A) Correlation between Th17 cells and IL-8 expression in infected patients with peptic ulcer disease. B) Correlation between Th17 cells and IL-17A expression from infected patients with peptic ulcer disease. C) Correlation between Th17 cells and Treg cells from infected patients with gastritis disease. D) Correlation between Th17 cells and Treg cells from infected patients with peptic ulcer disease. E) Correlation between the ratio of Th17/Treg cells in infected patients with gastritis disease and *H. pylori* density score. F) Correlation between the ratio of Th17/Treg cells in infected patients with peptic ulcer disease and *H. pylori* density score. G) Correlation between IL-8 expression and acute inflammatory score infected patients with *H. pylori*. Data were analyzed by Spearman's rank correlation.

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