Relationship Between Mucosal TNF- α Expression and Th1, Th17, Th22 and Treg Responses in *Helicobacter Pylori* Infection

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) -induced gastric inflammation in the gastric mucosa and significantly increases the risk of developing gastritis and peptic ulcer disease (PUD). The objective of this research is to determine the role of tumor necrosis factor- α (TNF- α) expression in the gastric mucosa of patients with *H. pylori* –associated gastritis and PUD compared to uninfected patients, and we determined the relation between TNF- α expression and Th1/Th17/Th22, and Treg cells.

Methods: Fifty-five patients with *H. pylori* –associated gastritis, 47 patients with *H. pylori* –associated PUD, and 48 uninfected patients were in this research. Antrum biopsy was used to detect *H. pylori*, virulence factors and histopathological assessments.

Results: Expression of TNF- α in the infected group was significantly higher than the uninfected group. Also, *cagA/oipA*-positive infected patients induce significantly more TNF- α expression than do *cagA/oipA*-negative infected patients. Expression of TNF- α was significantly increased in the PUD group than the gastritis group. Notably, TNF- α expression had a significant positive correlation with the frequency of Th1/Th17/Th22 lymphocytes in the PUD group.

Conclusion: These findings indicate the importance of increasing TNF- α with Th1, Th17, Th22 responses increase as an important risk factor for PUD in context of *H. pylori* infection.

Background

Helicobacter pylori (*H. pylori*) is a bacterial infection that causes stomach inflammation leading to a diversity of upper gastrointestinal disorderliness such as peptic ulcer disease (PUD), gastritis, and gastric cancer (GC) and mucosa-associated lymphoid tissue (MALT) lymphoma [1]. This infection is related with histological gastritis and significant penetration of immune cell such as neutrophils, monocytes and lymphocytes (T and B cells) into the human gastric mucosa, which contribute toward maintaining as well as increasing the gastric inflammation[2, 3]. Activation and recruitment of immune cells site of infection is associated with the inflammatory process and the related cytokine production that can lead to gastric inflammation and subsequently causes gastric mucosal damage [4, 5]. The disorder is the outcome of the complex interaction among the host genetic factors, local innate and adaptive immune responses, *H. pylori* virulence factors, and environmental conditions (e.g. high salt intake, malnutrition, vitamin and antioxidants deficiency and smoking) [6, 7]. Recent studies have shown that inflammatory and anti-inflammatory patterns in the gastric tissue samples are related with bacterial virulence factors [8, 9]. Clinical outcome after infection with *H. pylori* has been proposed to be associated with virulence factors of *H. pylori*, including the outer inflammatory protein a (*oipA*) and the cytotoxin-associated gene (*cagA*) [10–12]. The *cagA* protein has been demonstrated to be involved in the up-regulation of inflammatory cytokines and higher grades of gastric inflammation that can lead to the extension of pathological conditions such as PUD and GC [13].

OipA protein has been confirmed to be involved in the induction of a pro-inflammatory response such as IL-8, which is related with more severe neutrophil infiltration and high density of *H. pylori* [14]. Tumor necrosis factor- α (TNF- α) is a multifunctional proinflammatory cytokine that regulates the immune inflammatory reaction [15]. TNF- α is produced primarily by macrophages, neutrophils, dendritic cells and T cells. TNF- α promotes inflammation by triggering the localized accumulation of immune cells and mediators of inflammation [16]. In addition, TNF- α acts an important role in the mobility of inflammatory processes and tissue destruction such as autoimmune diseases [17]. The T helper (Th) cells that recognize as CD4 + cells, a type of T cell that have a major function in the production of various cytokines. Th1 cells, a Th cell subpopulation can produce a set of cytokines; cause the infiltration of pro-inflammatory M1 macrophages into the site of inflammation [18]. Th17 lineage has gained wide acceptance as a distinct subset of Th cell subpopulation [19]. Th17 cells stimulate the epithelial and stromal cells to release proinflammatory cytokines and chemokines which recruit macrophages, lymphocytes, and neutrophils to the infection site [20]. Moreover, Th22, a novel Th cell subpopulation can trigger the secretion of TNF- α and IL-22 [21, 22]. The regulatory T (Treg) lymphocytes are a subpopulation of Th cell that act to suppress immune response, maintain tolerance and prevention of uncontrolled inflammation [23]. Treg lymphocytes usually repress the increase of antigen-stimulated naive T cells [23]. Regarding the significance of Th1, Th17, Th22, and Treg responses and TNF- α cytokine in the context of inflammation and the probable roles in pathogenesis of *H. pylori*-infection, this research was done to study the role of TNF- α expression in the gastric mucosa of *H. pylori*-positive patients with PUD and gastritis compared to uninfected patients, and we determined the relation between TNF- α expression and the numeral of Treg, Th1, Th17, and Th22 cells in *H. pylori*-positive patients with PUD.

Methods

Patients and sampling

The study population consisted of 48 uninfected patients with non-ulcer dysplasia (29 females, 19 males; age, 50.96 ± 19.77 years; range 30–65), 47 *H. pylori*-positive patients with PUD (20 females, 27 males; age, 50.16 ± 15.3 years; range 33–64) and 55 *H. pylori*-positive patients with gastritis (29 females, 26 males; age, 50.18 ± 15.02 years; range 29–67). This research was approved by the Shahrekord University of Medical Sciences Human Research Ethics Committee (Number IR.SKUMS.REC.1395.159), and prior to participation, obtaining informed consent was done from each volunteer. The rapid urease test, polymerase chain reaction (PCR), and histological examination of the biopsies taken from the body confirmed the *H. pylori* infection. So if all four tests were positive, the patients classified as *H. pylori*-infected. 4 biopsies were gathered and used for the histological examination, rapid urease test, cytokine RNA analysis, detection of *H. pylori*, and assessment of the bacterial virulence factors.

Immunohistochemistry and Histological examination

Our previous researches have determined the number of Treg, Th1, Th17, and Th22 cells in the stomach mucosa quantity of gastro duodenal diseases by immunohistochemistry [24–27]. For the

histopathological estimate, a biopsy sample was taken of gastric antral mucosa. The fixed samples in 10 percent buffered formalin for 24 h, paraffin-embedded, tissue sections (4- μ m) stained sequentially with hematoxylin and eosin for grading and assessing gastritis severity and stained with modified Giemsa because *H. pylori* can be visualized by light microscopy. Colonized *H. pylori* in gastric epithelial have been Classified on a scale of four points including mild, moderate, and severe colonization. We described an ulcer as a mucosal break > 3mm in the exudate covered duodenum or stomach. The slides were tested blinded using a pathologist to the specifications of *H. pylori* infection. Polymorph nuclear cell infiltration and mononuclear cells infiltrating was classified and scored according to Sydney's system: normal = 0, mild = 1, moderate = 2 and severe = 3 [28].

PCR amplification

Genomic DNA was extracted by Bio spin Kit (Bio Flux, Japan) for the polymerase chain reaction (PCR). Sequences of primers and PCR reaction conditions we have before explained by Salimzadeh et al [29].

Quantitative analysis of the TNF- α expression using quantitative polymerase chain reaction (qPCR)

The detail of total RNA extraction and first-strand cDNA synthesis previously described [27].

Oligonucleotide sequences of primer and probe used in this research are as follows: TNF- α (Forward, 5-TCTTCTCGAACCCCGAGTGA-3; Reverse, 5-CCTCTGATGGCACCACCAG-3; probe, 5-FAM-TAGCCCATGTTGTAGCAAACCTCAAGCT-TAMRA-3). The $2^{-\Delta\Delta C_t}$ method was the determining approach of the relative quantification of the ratio of cytokine to β -actin [30].

Data analysis

All data provided are expressed as mean $\pm$ SEM. Checking normality tests done using the Shapiro-Wilk. Statistical analysis was done by t-test for independent samples and one-way ANOVA test, chi-squared (χ^2) test, Pearson's correlation and only values with equal *P*-values or less than 0.05 were determined as significant.

Result

Significant relationship of TNF- α mRNA expression with *H. pylori* infection

The TNF- α mRNA expression was significantly higher in *H. pylori*-infected gastric tissue in comparison with uninfected gastric tissue (Figure. 1). TNF- α fold change in infected group compared to non-infected group was 5.84-fold.

Significant relationship of TNF- α mRNA expression with *H. pylori* virulence factor *cagA* and *oipA*

We investigated whether *H. pylori* virulence factors *cagA* and *oipA*, which are the main virulence factors, were related to the changes observed in TNF- α mRNA expression. Interestingly, the TNF- α mRNA expression was significantly higher in patients infected with *cagA*-positive (*cagA*⁺) and *oipA*-positive

(*oipA*⁺) in comparison with in patients infected with *cagA*-negative (*cagA*⁻) and *oipA*-negative (*oipA*⁻) patients (Figure. 2A-B). TNF-α fold change in *cagA*⁺ and *oipA*⁺ groups compared to *cagA*⁻ and *oipA*⁻ groups was 2.91-fold and 4.25-fold, respectively.

Relation of acute inflammatory score and *H. pylori* virulence factor *cagA* and *oipA*

The association between acute inflammatory score and *H. pylori* virulence factor *cagA* and *oipA* is described in Table 1. *H. pylori* virulence factors *cagA*, *oipA* have a significant association to acute inflammatory score. Therefore, *H. pylori* virulence factor *cagA* and *oipA* enhancement acute inflammatory score.

Table 1
Association between score of acute inflammatory with the *oipA* and *cagA* virulence factors in infected patients

Virulence Factors	Acute Inflammatory Score [n (%)]				Total [n (%)]	<i>P</i> values ^a
	Negative	Mild	Moderate	Severe		
oipA-Positive	7 (31.8)	26 (46.4)	12 (75)	6 (75)	51 (50)	0.004
oipA-Negative	15 (68.2)	30 (53.6)	4 (25)	2 (25)	51 (50)	
cagA-Positive	13 (59.1)	39 (69.6)	13 (81.3)	8 (100)	73 (71.6)	0.019
cagA-Negative	9 (40.9)	17 (30.4)	3 (18.7)	0 (0.0)	29 (28.4)	
^a Fisher's exact test						

Table 2
Status of *oipA* and *cagA* gene of *H. pylori* in PUD and gastritis infected individuals.

Virulence Factors	PUD [n (%)]	Gastritis [n (%)]	Total [n (%)]	<i>P</i> values ^a
oipA-Positive	26 (50.3)	25 (45.5)	51 (50)	0.321
oipA-Negative	21 (44.7)	30 (54.5)	51 (50)	
cagA-Positive	34 (72.3)	39 (70.9)	73 (71.6)	0.873
cagA-Negative	13 (27.7)	16 (29.1)	29 (28.4)	
^a Chi-Square test				

Status of *oipA* and *cagA* genes of *H. pylori* from infected patients with PUD and gastritis

In this research, we have checked the association among the presence of *oipA* and *cagA* genes of *H. pylori* and the different gastro duodenal diseases. Also, *oipA* and *cagA* genes were not shown remarkable differences among *H. pylori*-positive subjects with gastritis and PUD ($P > 0.05$) (Table. 2).

Comparison of TNF- α mRNA expression at *H. pylori*-positive subjects with PUD and gastritis

Meaningful expression of TNF- α mRNA increased at *H. pylori*-positive patients with PUD than *H. pylori*-positive patients with gastritis (Figure. 3). TNF- α fold change in *H. pylori*-positive patients with PUD compared to *H. pylori*-positive patients with gastritis was 2.13-fold.

Significant relationship of TNF- α mRNA expression with scoring acute inflammatory, chronic inflammation score and the grade of density of *H. pylori* in infected subjects.

Also, an enhancement in chronic, acute inflammation score and the grade of infection density is significantly related to increased TNF- α mRNA expression in *H. pylori*-infected subjects (Figure. 4A: $P=0.015$, Figure. 4B: $P=0.0002$, and Figure. 4C: $P=0.025$, respectively).

Correlation between mucosal TNF- α mRNA expression and frequency of Th1, Th17, Th22 lymphocytes and Treg lymphocytes in *H. pylori*-positive patients with PUD

The mucosal TNF- α mRNA expression had a significant positive correlation with the frequency of Th1, Th17, Th22 lymphocytes in *H. pylori*-positive patients with PUD (Figure. 5A: $r=0.554$, $P<0.0001$, Figure. 5B: $r=0.553$, $P<0.0001$ and Figure. 5C: $r=0.582$, $P<0.0001$, respectively). But, the mucosal TNF- α mRNA expression had a significant negative correlation with the frequency of Treg lymphocytes in *H. pylori*-positive patients with PUD (Figure. 5E: $r=-0.372$, $P=0.009$).

Discussion

The host immune responses to *H. pylori* in infected patients strongly stimulate the inflammation of the gastric mucosa, which appeared to have played an important role in the various clinical outcomes including gastritis and PUD [24, 25]. It has been indicated that *H. pylori*-infection is one of the main risk factors for PUD that the mucous membrane is disordered and it causes gastric and duodenal inflammation. *H. pylori* cannot be the main reason for PUD; however, *H. pylori*-induced gastric membrane inflammation may lead to various clinical outcomes [24, 25].

Our outcomes indicated that TNF- α mRNA expression in *H. pylori*-infected patients was higher in comparison with that of *H. pylori* negative patients. These findings are in agreement with previous reports that the TNF- α expression has been demonstrated to increase in the gastric mucosa of *H. pylori*-infected patients [31–34]. Several studies have demonstrated that polymorphisms in the *TNF- α* genes result in high transcriptional promoter activity along with increased gastric inflammation, which are related with an increased risk of *H. pylori*-related GC and PUD developments [35, 36]. The expression of the TNF- α in the infected patients with *-cagA*⁺ and *oipA*⁺ were significantly higher than the infected patients with *cagA*⁻ and *oipA*⁻ negative. Also, the attendance of *oipA*, *cagA* virulence factors increments acute inflammatory score. Kumar Pachathundikandi *et al.* have shown that *H. pylori*-infection induced the transcription factor NF- κ B stimulation and release of IL-8 and TNF- α in a *cagPAI*-dependent way in THP-1 monocytes, which was associated with enhanced TLR-2 and TLR-5 expressions [32]. Research in infected

Mongolian gerbils with *H. pylori* showed that the mucosal pattern of inflammatory cytokines such as IL-1 β , TNF- α , IL-17, and IL-18 in infected Mongolian gerbils with oipA mutant were significantly lower than infected with wild-type strains. Also, *H. pylori* virulence factors oipA determined the levels of the gastric inflammation-related inflammatory cytokine outcome in the progress of GC and PUD [37]. TNF- α mRNA expression in the infected patients with PUD was significantly higher than in the patients with gastritis. Also, an increase in acute inflammatory score, chronic inflammation score and the degree of *H. pylori* density is significantly associated with increased TNF- α mRNA expression in infected patients. Our results are in agreement with the results of the studies conducted on mouse models of the *H. pylori* infection, which demonstrated that the TNF- α mRNA expression were significantly greater in the PUD group than in the non-ulcer group [37]. Also, recent study by Tourani *et al.* Showed that the serum TNF- α level in peptic ulcer patients was significantly higher than the control group [34]. In contrast to our detections, Shamsdin *et al.* demonstrated that and noted no significant change in TNF- α serum levels in the infected patients with PUD and gastritis; however, the TNF- α serum levels in combination with IL-6 were found to be significantly higher in the gastritis patients as compared to the PUD patients. In addition, they observed significantly higher levels of TNF- α in the active chronic and chronic gastritis patients as compared to the uninfected groups [33].

The mucosal TNF- α mRNA expression had a significant positive correlation with the frequency of Th1, Th17, Th22 lymphocytes in the PUD group. Our previous studies in association with the pathogenesis of PUD showed that the frequency of Th1, Th17, Th22 lymphocytes in infected patients with PUD were significantly higher than infected patients with gastritis [24–26]. Th1 cells cause the infiltration of pro-inflammatory M1 macrophages into the site of inflammation [18]. M1 macrophages are usually characterized by the secretion of high levels of pro-inflammatory cytokines such as TNF- α and IL-6 [38]. Th17 lymphocytes stimulate epithelial cells and stromal cells to release IL-8, which is a chemo attractant for neutrophils, and Th17 lymphocytes also induces the production of TNF- α from macrophages and neutrophils cells at the site of infection [20]. Also, the pro-inflammatory cytokine TNF- α may promote Th22 differentiation in the site of inflammation [39]. The increased TNF- α mRNA expression may be able to reflect the degree of tissue damage affected by a peptic ulcer in comparison to gastritis patients. The mucosal TNF- α mRNA expression had a significant negative correlation with the frequency of Treg lymphocytes in the PUD group. Our previous study showed that the frequency of Treg and Breg lymphocytes remarkably increased in gastritis patients rather than PUD patients [2, 27]. The findings suggest that increased levels of TNF- α mRNA in *H. pylori*-infected patients may act an axial role in PUD pathogenesis. The conclusions as well as highlighted the significance of TNF- α increment along with Th1, Th17, Th22 responses increase also a significant risk factor for PUD in the context of *H. pylori* infection.

Abbreviations

PUD: peptic ulcer disease; GC: gastric cancer; MALT: mucosa-associated lymphoid tissue; OipA: outer inflammatory protein A; CagA: cytotoxin-associated protein A; PCR: polymerase chain reaction; TNF- α : Tumor necrosis factor- α .

Declarations

Acknowledgment

None.

Authors' contributions

Conceptualisation: GR, RA, MSSA, MS, FA; Methodology: FA, MSSA, GR; Formal

analysis and investigation: GR, RA, MS; Writing (original draft preparation): MSSA,

FA, GR; Writing (review and editing): FA, GR, RA, MS; Resources: MSSA; Supervision: FA, GR. The author(s) read and approved the final manuscript.

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Availability of data and materials

All relevant data are included in the manuscript.

Ethics approval and consent to participate

This study was approved by the ethical board of Shahrekord University of medical sciences with number: IR.SKUMS.REC.1395.159.

Consent for publication

Not applicable.

Competing interests

None of the authors have any competing interests.

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References

1. Lu Y, Giver CR, Sharma A, Li JM, Darlak KA, Owens LM, Roback JD, Galipeau J, Waller EK. IFN- γ and indoleamine 2,3-dioxygenase signaling between donor dendritic cells and T cells regulates graft versus host and graft versus leukemia activity. *Blood*. 2012;119(4):1075–85.
2. Nahid-Samiei M, Rahimian G, Shafigh M, Taheri F, Karami-Hurestani M, Sanaei MJ, Heshmati M, Bagheri N. Enhanced Frequency of CD19(+)IL-10(+)B Cells in Human Gastric Mucosa Infected by *Helicobacter pylori*. *Am J Med Sci*. 2020;359(6):347–53.
3. Azadegan-Dehkordi F, Shirzad H, Ahmadi R, Bashash D, Abdollahpour-Alitappeh M, Luzzza F, Larussa T, Nahid-Samiei M, Rahimian G, Shafigh MH, et al: **Increased Indoleamine 2, 3-Dioxygenase expression modulates Th1/Th17/Th22 and Treg pathway in humans with Helicobacter Pylori-Infected gastric mucosa.** *Human immunology* 2020.
4. Bagheri N, Azadegan-Dehkordi F, Shirzad M, Zamanzad B, Rahimian G, Taghikhani A, Rafieian-Kopaei M, Shirzad H. Mucosal interleukin-21 mRNA expression level is high in patients with *Helicobacter pylori* and is associated with the severity of gastritis. *Central-European journal of immunology*. 2015;40(1):61–7.
5. Sanaei MJ, Shirzad H, Soltani A, Abdollahpour-Alitappeh M, Shafigh MH, Rahimian G, Mirzaei Y, Bagheri N. Up-regulated CCL18, CCL28 and CXCL13 Expression is Associated with the Risk of Gastritis and Peptic Ulcer Disease in *Helicobacter Pylori* infection. *Am J Med Sci*. 2021;361(1):43–54.
6. Yuzhalin A. The role of interleukin DNA polymorphisms in gastric cancer. *Hum Immunol*. 2011;72(11):1128–36.
7. Shirzad H, Bagheri N, Azadegan-Dehkordi F, Zamanzad B, Izadpanah E, Abdi M, Ramazani G, Sanei MH, Ayoubian H, Ahmadi A, et al. New insight to IL-23/IL-17 axis in Iranian infected adult patients with gastritis: effects of genes polymorphisms on expression of cytokines. *Acta Gastroenterol Belg*. 2015;78(2):212–8.
8. Ferreira RM, Pinto-Ribeiro I, Wen X, Marcos-Pinto R, Dinis-Ribeiro M, Carneiro F, Figueiredo C. *Helicobacter pylori* cagA Promoter Region Sequences Influence CagA Expression and Interleukin 8 Secretion. *The Journal of infectious diseases*. 2016;213(4):669–73.
9. Rahimian G, Sanei MH, Shirzad H, Azadegan-Dehkordi F, Taghikhani A, Salimzadeh L, Hashemzadeh-Chaleshtori M, Rafieian-Kopaei M, Bagheri N. Virulence factors of *Helicobacter pylori* vacA increase markedly gastric mucosal TGF- β 1 mRNA expression in gastritis patients. *Microb Pathog*. 2014;67–68:1–7.
10. Bagheri N, Azadegan-Dehkordi F, Rafieian-Kopaei M, Rahimian G, Asadi-Samani M, Shirzad H. Clinical relevance of *Helicobacter pylori* virulence factors in Iranian patients with gastrointestinal diseases. *Microb Pathog*. 2016;100:154–62.
11. El Khadir M, Alaoui Boukhris S, Benajah DA, Ibrahimi SA, Chbani L, Bouguenouch L, El Rhazi K, El Abkari M, Nejari C, Mahmoud M, et al. *Helicobacter pylori* CagA EPIYA-C motifs and gastric diseases

- in Moroccan patients. *Infection genetics evolution: journal of molecular epidemiology evolutionary genetics in infectious diseases*. 2018;66:120–9.
12. Farzi N, Yadegar A, Aghdaei HA, Yamaoka Y, Zali MR. Genetic diversity and functional analysis of oipA gene in association with other virulence factors among *Helicobacter pylori* isolates from Iranian patients with different gastric diseases. *Infection genetics evolution: journal of molecular epidemiology evolutionary genetics in infectious diseases*. 2018;60:26–34.
 13. Yamaoka Y, Soucek J, Odenbreit S, Haas R, Arnqvist A, Borén T, Kodama T, Osato MS, Gutierrez O, Kim JG. Discrimination between cases of duodenal ulcer and gastritis on the basis of putative virulence factors of *Helicobacter pylori*. *J Clin Microbiol*. 2002;40(6):2244–6.
 14. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of *Helicobacter pylori*. *Proc Natl Acad Sci USA*. 2000;97(13):7533–8.
 15. Thalmaier U, Lehn N, Pfeffer K, Stolte M, Vieth M, Schneider-Brachert W: **Role of tumor necrosis factor alpha in *Helicobacter pylori* gastritis in tumor necrosis factor receptor 1-deficient mice.** *Infection and immunity* 2002, **70**(6):3149–3155.
 16. Waters JP, Pober JS, Bradley JR. Tumour necrosis factor in infectious disease. *J Pathol*. 2013;230(2):132–47.
 17. Redlich K, Hayer S, Maier A, Dunstan CR, Tohidast-Akrad M, Lang S, Turk B, Pietschmann P, Woloszczuk W, Haralambous S, et al. Tumor necrosis factor alpha-mediated joint destruction is inhibited by targeting osteoclasts with osteoprotegerin. *Arthritis rheumatism*. 2002;46(3):785–92.
 18. Farrar JD, Asnagli H, Murphy KM. T helper subset development: roles of instruction, selection, and transcription. *J Clin Investig*. 2002;109(4):431–5.
 19. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun rev*. 2014;13(6):668–77.
 20. Bagheri N, Azadegan-Dehkordi F, Shirzad H, Rafieian-Kopaei M, Rahimian G, Razavi A: **The biological functions of IL-17 in different clinical expressions of *Helicobacter pylori*-infection.** *Microbial pathogenesis* 2015, **81**:33–38.
 21. Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, Cianfarani F, Odorisio T, Traidl-Hoffmann C, Behrendt H, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Investig*. 2009;119(12):3573–85.
 22. Shohan M, Dehghani R, Khodadadi A, Dehnavi S, Ahmadi R, Joudaki N, Houshmandfar S, Shamshiri M, Shojapourian S, Bagheri N. Interleukin-22 and intestinal homeostasis: Protective or destructive? *IUBMB Life*. 2020;72(8):1585–602.
 23. Bagheri N, Azadegan-Dehkordi F, Rahimian G, Rafieian-Kopaei M, Shirzad H. Role of Regulatory T-cells in Different Clinical Expressions of *Helicobacter pylori* Infection. *Arch Med Res*. 2016;47(4):245–54.
 24. Bagheri N, Razavi A, Pourgheysari B, Azadegan-Dehkordi F, Rahimian G, Pirayesh A, Shafigh M, Rafieian-Kopaei M, Fereidani R, Tahmasbi K, et al: **Up-regulated Th17 cell function is associated with increased peptic ulcer disease in *Helicobacter pylori*-infection.** *Infection, genetics and evolution*:

journal of molecular epidemiology and evolutionary genetics in infectious diseases 2018, **60**:117–125.

25. Bagheri N, Shirzad H, Mirzaei Y, Nahid-Samiei M, Sanaei M, Rahimian G, Shafigh M, Zandi F, Tahmasbi K, Razavi A. T-bet(+) Cells Polarization in Patients Infected with *Helicobacter pylori* Increase the Risk of Peptic Ulcer Development. *Arch Med Res*. 2019;50(3):113–21.
26. Sanaii A, Shirzad H, Haghighian M, Rahimian G, Soltani A, Shafigh M, Tahmasbi K, Bagheri N. Role of Th22 cells in *Helicobacter pylori*-related gastritis and peptic ulcer diseases. *Molecular biology reports*. 2019;46(6):5703–12.
27. Bagheri N, Shirzad H, Elahi S, Azadegan-Dehkordi F, Rahimian G, Shafigh M, Rashidii R, Sarafnejad A, Rafieian-Kopaei M, Faridani R, et al: **Downregulated regulatory T cell function is associated with increased peptic ulcer in *Helicobacter pylori*-infection.** *Microbial pathogenesis* 2017, **110**:165–175.
28. Dixon MF, Genta RM, Yardley JH, Correa P: **Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994.** *The American journal of surgical pathology* 1996, **20**(10):1161–1181.
29. Salimzadeh L, Bagheri N, Zamanzad B, Azadegan-Dehkordi F, Rahimian G, Hashemzadeh-Chaleshtori M, Rafieian-Kopaei M, Sanei MH, Shirzad H. Frequency of virulence factors in *Helicobacter pylori*-infected patients with gastritis. *Microb Pathog*. 2015;80:67–72.
30. Bagheri N, Azadegan-Dehkordi F, Rahimian G, Hashemzadeh-Chaleshtori M, Rafieian-Kopaei M, Kheiri S, Gholipour A, Shirzad H. Altered Th17 Cytokine Expression in *Helicobacter pylori* Patients with TLR4 (D299G) Polymorphism. *Immunol Investig*. 2016;45(2):161–71.
31. Collodel G, Moretti E, Campagna MS, Capitani S, Lenzi C, Figura N: **Infection by *CagA*-positive *Helicobacter pylori* strains may contribute to alter the sperm quality of men with fertility disorders and increase the systemic levels of TNF- α .** *Digestive diseases and sciences* 2010, **55**(1):94–100.
32. Kumar Pachathundikandi S, Brandt S, Madassery J, Backert S: **Induction of TLR-2 and TLR-5 expression by *Helicobacter pylori* switches *cagPAI*-dependent signalling leading to the secretion of IL-8 and TNF- α .** *PloS one* 2011, **6**(5):e19614.
33. Shamsdin SA, Alborzi A, Rasouli M, Ghaderi A, Lankrani KB, Dehghani SM, Pouladfar GR. **The importance of TH22 and TC22 cells in the pathogenesis of *Helicobacter pylori*-associated gastric diseases.** *Helicobacter* 2017, **22**(3).
34. Tourani M, Habibzadeh M, Karkhah A, Shokri-Shirvani J, Barari L, Nouri HR. Association of TNF- α but not IL-1 β levels with the presence of *Helicobacter pylori* infection increased the risk of peptic ulcer development. *Cytokine*. 2018;110:232–6.
35. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, et al. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology*. 2003;124(5):1193–201.
36. Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M, Sugimura H, Hishida A. Different effects of polymorphisms of tumor necrosis factor- α and interleukin-1 β on development of peptic ulcer and gastric cancer. *Journal of gastroenterology hepatology*. 2007;22(1):51–9.

37. Sugimoto M, Ohno T, Graham DY, Yamaoka Y. Gastric mucosal interleukin-17 and – 18 mRNA expression in Helicobacter pylori-induced Mongolian gerbils. *Cancer Sci.* 2009;100(11):2152–9.

38. Atri C, Guerfali FZ, Laouini D. **Role of Human Macrophage Polarization in Inflammation during Infectious Diseases.** *International journal of molecular sciences* 2018, 19(6).

39. Sugita S, Kawazoe Y, Imai A, Kawaguchi T, Horie S, Keino H, Takahashi M, Mochizuki M. Role of IL-22- and TNF-alpha-producing Th22 cells in uveitis patients with Behcet's disease. *Journal of immunology.* 2013;190(11):5799–808.

Figures

Figure 1

Comparison of TNF- α mRNA expression in patients with or without *H. pylori*-infection. TNF- α expression was significantly increased in *H. pylori*-infected individuals in comparison with uninfected patients. $P < 0.05$ was considered statistically significant by independent samples t-test (N=number and FC=fold change).

Figure 2

Comparison of TNF- α mRNA expression in patients infected with or without the *cagA* and *oipA* virulence factors. TNF- α expression was significantly increased in patients infected with *cagA*-positive in comparison with patients infected with *cagA*-negative. Also, TNF- α expression was significantly increased in patients infected with *oipA*-positive in comparison with patients infected with *oipA*-negative. $P < 0.05$ was considered statistically significant by independent-samples t-test (N=number and FC=fold change).

Figure 3

Comparison of TNF-α mRNA expression in PUD and gastritis H. pylori-infected individuals. TNF-α mRNA expression was significantly increased in PUD H. pylori-infected individuals than gastritis H. pylori-infected individuals. $P < 0.05$ was considered statistically significant by independent-samples t-test (N=number and FC=fold change).

Figure 4

The correlation between TNF- α mRNA expression and acute inflammatory score, chronic inflammatory score and the grad of the density of *H. pylori* infection in patients. Increase in acute inflammatory score, the score of chronic inflammation and the *H. pylori* density grade is significantly related to increase TNF- α mRNA expression in infected patients. $P < 0.05$ was determine statistically significant by one-way ANOVA (N=number).

Figure 5

The association between TNF-α mRNA expression and the numeral of Th1, Th17, Th22, and Treg lymphocytes in PUD *H. pylori*-positive patients. There was a significant positive association between expression of TNF-α and frequency of Th1, Th17, Th22 lymphocytes, whilst a significant negative association was shown between TNF-α expression and Treg lymphocytes in PUD *H. pylori*-positive patients. The data were analyzed using Spearman's rank correlation.