Journal of Shahrekord University of Medical Sciences
doi: 10.34172/jsums.1005 2025;27(4):152-156 http://j.skums.ac.ir

Original Article

# Analysis of Interleukin 22 Gene Expression in *Helicobacter pylori*-Infected Patients With Inflammation and Peptic Ulcer Compared to Uninfected Controls

**Mehrnoosh Haghighian[1], Noosha Zia Jahromi[1*], Ghorbanali Rahimian[2]**

[1]Department of Biology, Science Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[2]Department of Internal Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

***Corresponding Author:** Noosha Zia Jahromi, Email: Nooshazia.59@gmail.com

**Abstract**

**Background and aims:** Inflammation is a key feature of *Helicobacter pylori* infection and its associated complications, gastritis, and gastrointestinal ulcers. This study aimed to determine the level of expression of interleukin (IL)-22 in the gastric mucosa in the control group and patients with gastritis and peptic ulcer.

**Methods:** This case-control study was conducted on patients with gastrointestinal problems referred to the endoscopy unit of Hajar Hospital of Shahrekord (Iran, 2018). Overall, 135 tissue samples were collected from people suspected of gastritis (n=45), people with peptic ulcers infected with *H. pylori* (n=45), and people without *H. pylori* infection (n=45). IL-22 gene expression was measured using a real-time polymerase chain reaction method compared to the beta-actin internal gene. IL-22 expression was calculated by $2^{-\Delta\Delta ct}$ and statistically analyzed by SPSS16 software and t-student test.

**Results:** In this research, the mean age of the patients was 50.17±15.09 and 50.96±19.77 in the infected and non-infected groups, respectively. There was no significant difference between the two studied groups in terms of age and gender ($P$=0.236). IL-22 expression in *H. pylori-infected* individuals (3.21±0.58) was 3.54-fold higher than that in the control group (0.9±0.14, $P$=0.036). Likewise, IL expression in peptic ulcer (2.2±0.43) was significantly higher than that in those with gastritis (1.12±0.87, $P$=0.012).

**Conclusion:** Despite its pro-inflammatory nature, IL-22 can effectively control bacterial infections and strengthen mucosal defenses, particularly in the context of *H. pylori* infection, highlighting its therapeutic potential.

**Keywords:** *Helicobacter pylori*, Gastritis, Interleukin 22

**Received:** August 11, 2024, **Revised:** January 3, 2025, **Accepted:** January 4, 2025, **ePublished:** December 29, 2025

## Introduction

*Helicobacter pylori* is a Gram-negative, microaerophilic, curved, spiral-shaped bacterium with 3–5 flagella and is mobile (1). *H. pylori*, unlike viruses and bacteria, is highly resistant to stomach acid due to its urease enzyme and quickly colonizes the mucous tissue of the stomach (2). It is a pathogenic bacterium found in the stomachs of over half of the world's people and is mainly associated with inflammation (3). Over 80% of *H. pylori* patients are asymptomatic. In comparison, only 20% of chronic gastrointestinal disease patients develop gastritis, stomach ulcers, intestinal metaplasia, adenocarcinoma, and gastric mucosal lymphoma (4). Gastritis is usually present in all patients infected with *H. pylori* (5). In acute gastritis, neutrophils release reactive oxygen species and other neutrophil contents; they cause damage to the stomach tissue (6). When lymphocytes replace neutrophils, acute gastritis turns into chronic gastritis. Chronic gastritis is normally a symptom of diseases such as stomach ulcers and stomach cancer. *H. pylori* causes 70–85% of gastric ulcers and 90–95% of intestinal ulcers (6). Interleukin-22 (IL-22), initially identified in mice during T-cell research, belongs to the interleukin 10 family and binds to the IL-22R1 and IL-22R2 complex (7). IL-22 enhances skin keratinocytes' antimicrobial defense by increasing the expression of beta-defensin 2, beta-defensin 3, psoriasin, calgranulin A, and calgranulin B (8). Furthermore, the amount of IL-22 increases in infection with *Mycobacterium tuberculosis* and *Klebsiella*, indicating the role of IL-22 in defense against bacteria (9). IL-22, a cytokine, may significantly enhance the expression of antimicrobial proteins in bacterial infections, potentially playing a crucial role in host defense against skin and stomach infections. Studying stomach-intestinal issues in Chaharmahal and Bakhtiari province may help identify effective treatment methods for stomach cancer, enhancing knowledge about the pathogenic mechanisms of *H. pylori*.

## Materials and Methods

### *Sample Collection, Study Population, and Groups*

This case-control study was conducted on patients with gastrointestinal problems referred to the endoscopy

unit of Hajar Hospital of Shahrekord, Iran, in 2018. After receiving the approval of the Ethics Committee of Shahrekord University of Medical Sciences (Ethical approval IR.SKUMS.REC.1395.72), the participants were provided with detailed information about the study's aims and methods. After fully understanding the study, the participants gave their informed consent. In general, 135 tissue samples were collected from people suspected of gastritis (n = 45), people with peptic ulcers infected with *H. pylori* (n = 45), and people without *H. pylori* infection (n = 45). Random sampling was used in this study. Four biopsies were taken from people suspected of having *H. pylori* by a gastroenterologist a super specialist from the end part of the stomach (antrum), which was abnormal. At that time, the participants completed a questionnaire containing personal information, including gender, age, history of illness, presence or absence of gastric ulcer or duodenum, smoking history, antacid and anti-inflammatory drugs, and the like. This study did not include people who had taken aspirin or non-steroidal anti-inflammatory drugs, those with malignancies, patients with heart, lung, liver, and kidney problems, or patients with metabolic disorders and immune system suppression. The following formula was used to determine the sample size. In this calculation, p was assumed to be 0.3, representing the prevalence of H. pylori infection in the population. Based on this assumption and an odds ratio (OR) of 3 with a 95% confidence interval and 91% power, the required sample size was estimated as 45 participants in each group (gastritis, peptic ulcer, and control) (10).

$$P1 = P0\frac{OR}{\left[1+P0\left(OR-1\right)\right]} = 0.63$$

$$P = \frac{\left(r\times P0\right)+P1}{r+1} = 0.46$$

$$n = \left(\frac{r+1}{r}\right)\frac{\left(p\right)\left(1-p\right)\left(Z1-\beta+Z1-\frac{\alpha}{2}\right)^2}{\left(P0-P1\right)^2}) = 45$$

### *Determining the Existence or Lack of Helicobacter pylori by Implementing a Rapid Urease Test*

After sampling, a direct rapid urease test was performed in the clinic on one of the biopsies of the referred patients. Biopsies taken from the patients were placed in a urease solution. After about one hour, the solution's color changed from yellow to red. Urease is an enzyme produced by *H. pylori* that hydrolyzes urea into carbon dioxide, water, and ammonia.

### *Determining the Existence or Lack of Helicobacter pylori and the Degree of Severity of Inflammation by Implementing Pathologic Methods*

According to Sidney's guidelines, tissue sections were prepared from patients' biopsies.

Using hematoxylin-eosin and Giemsa staining methods, the degree of acute inflammation (infiltration of polymorphonuclear cells),chronic inflammation (infiltration of mononuclear cells), glandular atrophy, intestinal metaplasia, and the degree of H. pylori colonization were evaluated. The severity of H. pylori colonization was graded on a scale from 0 to 3. In addition, the extent and the number of infiltrations of the neutrophils and mononuclear cells in the lamina propria were counted with a 40-microscope lens in 5 fields. Finally, the cells were assigned to 4 grades from 0 to 3. All pathology tests were performed without knowing whether a person's biopsy was contaminated with bacteria.

### *Ribonucleic Acid Extraction and Complementary DNA Synthesis*

After RNA extraction using Trizol, the nanodrop device was employed to investigate the quantitative and qualitative amounts of RNA. cDNA synthesis was performed according to the kit protocol using the reverse transcriptase enzyme.

### *Real-Time Polymerase Chain Reaction Technique Using TaqMan Probe*

The amount of proliferated DNA can be measured at any moment and in each cycle using the RT-PCR technique. The TaqMan probe method (5 nuclease assays) was developed based on the combination of the RT-PCR method. The oligonucleotide is typically produced by adding a fluorescent dye (e.g., fluorescein amidite or Victoria) to the 5' end and a quencher (e.g., 5-(6)-carboxytetramethylrhodamine) to the 3' end. In the annealing stage, oligonucleotides or probes are specifically attached to the template DNA. However, due to the existence of a light quencher, they do not emit light fluorescence. In the extension stage, the 5'→3 Taq DNA polymerase exonuclease activity digests the probe. As a result, the fluorescence light is separated from the quencher and emits fluorescence light from itself. The amount of amplification of the desired region in the reaction was determined by measuring the amount of emitted fluorescence light. At the same time, as new DNA is made, the TaqMan probe is connected to new strands, and the fluorescence light radiation increases. Thus, by momentary observation, it is feasible to determine in which PCR cycle the amplified product enters the logarithmic phase. The threshold is a place on the graph where all the samples are multiplied logarithmically, and the cycle where the threshold line cuts the graph of each reaction is called the threshold cycle (CT). The DNA polymerase enzyme in the Master Mix is inactive at ambient temperature. It, thus, prevents the production of non-original products and the creation of primer dimers during the reactions of the initial stages and the first stage of denaturation. This enzyme is activated by incubation for 15 minutes at 95°, and this is when the DNA is completely denatured. The relative investigation method was used for quantitative investigation in RT-

PCR tests. In this method, the exact amount of DNA is unimportant, and only relative changes are examined. The basis of comparison is the CT difference between test and control samples. This method compares the CT values of test samples with the CT values of normal or control samples. Similar to the semi-quantitative method in regular PCR, which requires an internal control, in the RT-PCR method, the CT values of the test samples and the normal tissue are corrected and normalized for the internal control gene, which is a housekeeping gene. This standard gene should be equally expressed in all cells. The standard genes *GAPDH*, *28S rRNA*, *β-Actin*, *HPRT*, and *TATA* box binding protein are examples of these genes. In this study, the *β-actin* gene was considered the internal control.

In each experiment, a negative control was considered for each gene. The negative control sample is a sample that contains all components of a reaction, except for cDNA. The temperature conditions of the TaqMan probe PCR Master Mix kit to perform RT-PCR include 1 cycle for 5 minutes at 95° for the initial denaturation and 45 cycles for denaturation and binding of primer and probe, which are 15 and 16 seconds at 95° and 78°, respectively. The β-actin gene was used as an internal control. The forward primer sequence for *β*-actin was 5'-AGCCTCGCCTTTGCCGA-3', the reverse primer was 5'-CTGGTGCCTGGGGGCG-3', and the probe was FAM-CCGCCGCCCGTCCACACCCGC-TAMRA. For the IL-22 gene, the forward primer sequence was 5'-GCAGGCTTGACAAGTCCAACT-3', the reverse primer was 5'-GCCTCCTTAGCCAGCATGAA-3', and the probe was FAM-CCAGCAGCCCTATATCACCAACCGC-TAMRA.

### Data Analysis Method

The collected information was processed and statistically analyzed using the SPSS software, version 22. The Student's t-test was employed to compare between groups, and $P < 0.05$ was considered statistically significant.

## Results

The information about the participants in terms of age and gender is provided in Table 1. Overall, 152 patients infected with *H. pylori* and 48 uninfected patients participated in this study. Of 152 infected patients, 74 were male, and 78 were female. There were 48 patients in the uninfected group, including 19 males and 29 females. The mean age of the patients was 50.17 ± 15.09 and 50.96 ± 19.77 in the infected and non-infected groups, respectively. The statistical results revealed no significant relationship between infected and non-infected people based on age and gender ($P = 0.236$).

### The Results of Determining the Efficiency of Real-Time Polymerase Chain Reactions

To check the efficiency of the RT-PCR and primers and to quantitatively analyze gene expression by the RT-PCR technique, a standard curve was obtained for each of the *IL-22* and *β-actin* genes. For this purpose, the successive dilutions of the cDNA sample obtained from the control cells were prepared and amplified with specific primers for both *IL-22* and *β-actin* genes. The CT diagram (the cycle in which the fluorescent amplification rate crosses the threshold line) was plotted against the concentration. The slope of the graph was used to calculate the amplification efficiency.

It should be noted that to obtain ΔCT, the CT of the *IL-22* gene of each sample was subtracted from that of the *β-actin* gene. Then, ΔΔCT (the difference of the ΔCT of all samples from the average ΔCT of the control samples) and ΔΔCT-2 were calculated.

### The Relationship Between the Expression of Interleukin 22 in People Infected With Helicobacter pylori and the Uninfected Group

According to Figure 1, our statistical analysis demonstrated a significant relationship in the expression of the *IL-22* gene between patients infected with *H. pylori* and the control group. The expression of the *IL-22* gene in people infected with *H. pylori* increased 3.54 times compared to the control group ($P = 0.036$).

### The Relationship Between the Expression of Interleukin 22 and the Type of Disease in People Infected With Helicobacter pylori

Based on our statistical analysis (Figure 2), a significant relationship was observed between the expression of IL-22 and the type of disease in people infected with *H. pylori*. The expression of IL-22 in infected patients with peptic ulcer increased 1.97 times compared to patients with gastritis ($P = 0.012$).

The *IL-22* gene expression in people with peptic ulcers was significantly higher than in people with gastritis ($P = 0.012$).

**Table 1.** Demographic Information of Infected and Non-Infected Patient Groups by Age and Gender

| Variable | | H. pylori Non-Infected Group N (%) | H. pylori-Infected Group N(%) | *P* value |
|---|---|---|---|---|
| Total | | 48 (24) | 152 (76) | - |
| Gender | Male | 19 (39.6) | 74 (48.7) | 0.27 |
| | Female | 29 (60.4) | 78 (51.3) | |
| Age (mean ± SD) | | 50.96 ± 19.77 | 50.17 ± 15.09 | 0.801 |

*Note*. SD: Standard deviation; *H. pylori*: *Helicobacter pylori*.

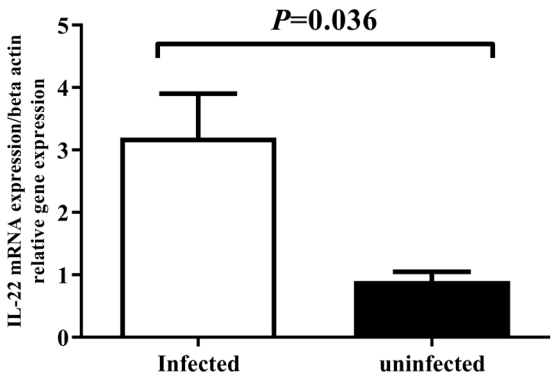


**Figure 1.** *IL-22* Gene Expression Relationship in Infected People Compared to the Control Group
*Note*. IL: Interleukin 22. *IL-22* gene expression significantly increased compared to the non-infected group ($P=0.036$).

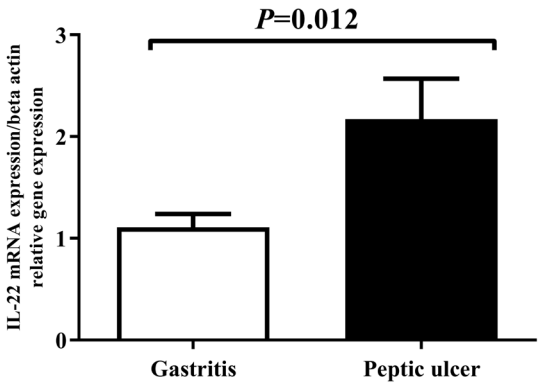


**Figure 2.** Relationship Between the Expression of the *IL-22* Gene and the Type of Disease in Infected People
*Note*. IL: Interleukin 22.

## Discussion

This study investigated the expression of the *IL-22* gene in people infected with *H. pylori*. This bacterium can cause bacterial infections. The results confirmed that the expression of the *IL-22* gene increased in people with *H. pylori*, suggesting that Th22 cells are associated with *H. pylori* infection and precancerous lesions that can lead to cancer. The findings also revealed that IL-22 and IL-17A act together to induce the expression of pro-inflammatory chemokines and antimicrobial peptides in the mucosal epithelial cells of people with gastritis. Dixon showed that IL-22 and IL-17A act together to induce the expression of pro-inflammatory chemokines (e.g., IL-8 and IL-6), as well as antimicrobial peptides (e.g., beta-defensin, lipocalin, and calprotectin), in the mucosal epithelial cells of people with gastritis (11). The results of Zindl et al revealed that IL-22, secreted by Th22 cells, is crucial in controlling infection with certain Gram-negative bacteria in individuals with chronic and active gastritis (12, 13). Zindl reported the antibacterial role of IL-22 produced by T cells in colonic crypts (14). Our results demonstrated that IL-22 promotes inflammation and tissue damage in *H. pylori* infections, raising questions about the paradoxical behavior of Th22 cells and IL-22, which may protect against certain diseases. Zhuang et al found that individuals infected with *H. pylori* have significantly higher levels of IL-22 than those not (16), aligning with our findings. Based on our findings, individuals with peptic ulcers had significantly higher levels of *IL-22* gene expression than those with gastritis. Shamsdin et al observed that the level of IL-22 protein expression in people with peptic ulcers was considerably higher than in people with gastritis (15), which conforms to our study findings. IL-22 functions as both a protective and inflammatory cytokine, with its balance dependent on the presence or absence of IL-17A, which can increase inflammation (16). Shamsdin et al also found that the level of IL-22 in people with peptic ulcer was higher than in people with gastritis.

On the other hand, IL-17A is also higher in people with peptic ulcers (15). Peptic ulcer patients experience higher inflammation and tissue damage compared to gastritis patients. Higher *IL-22* gene expression was justified, and a strong relationship was reported between increased tumor necrosis factor-alpha and IL-6 levels and bacterium infection, with a positive correlation with most infectious diseases (17).

## Conclusion

*H. pylori* infection is a common cause of digestive diseases, such as gastritis and peptic ulcers. A direct relationship was found between IL-22 and *H. pylori* infection, making IL-22 a potential diagnostic biomarker. Our findings revealed that increased IL-22 levels are linked to inflammation, tissue damage, and disease progression. Further research is needed to understand the cells and cytokines involved in *H. pylori* infection and its potential therapeutic targets. Further understanding of the disease's complete mechanism will aid in targeting key cells and cytokines, leading to improved treatment protocols.

### Acknowledgements

This study is taken from the master's dissertation and supported by the Research Vice-Chancellor of Shahrekord Azad University (Approval No. IR.SKUMS.REC.1395.72), with special gratitude to people who helped us to do this research.

### Authors' Contribution

**Conceptualization:** Mehrnoosh Haghighian.
**Data curation:** Mehrnoosh Haghighian.
**Formal analysis:** Mehrnoosh Haghighian.
**Funding acquisition:** Mehrnoosh Haghighian.
**Investigation:** Mehrnoosh Haghighian.
**Methodology:** Noosha Zia Jahromi.
**Project administration:** Noosha Zia Jahromi.
**Resources:** Ghorbanali Rahimian.
**Software:** Mehrnoosh Haghighian.
**Supervision:** Noosha Zia Jahromi.
**Validation:** Noosha Zia Jahromi.
**Visualization:** Mehrnoosh Haghighian.
**Writing–original draft:** Mehrnoosh Haghighian.
**Writing–review & editing:** Mehrnoosh Haghighian.

## Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this work.

## Ethical Approval

Ethical considerations in this study included obtaining permission from the Ethics Committee of Shahrekord University of Medical Science (Ethical No. IR.SKUMS.REC.1395.72) and obtaining written consent from the participants to participate in the study.

## Funding

No specific funding was received for this study.

## References

1. Blanchard TG, Czinn SJ. Identification of *Helicobacter pylori* and the evolution of an efficacious childhood vaccine to protect against gastritis and peptic ulcer disease. Pediatr Res. 2017;81(1-2):170-6. doi: 10.1038/pr.2016.199.
2. Huang JY, Goers Sweeney E, Guillemin K, Amieva MR. Multiple acid sensors control *Helicobacter pylori* colonization of the stomach. PLoS Pathog. 2017;13(1):e1006118. doi: 10.1371/journal.ppat.1006118.
3. Rugge M, Fassan M, Graham DY. Epidemiology of gastric cancer. In: Strong VE, ed. Gastric Cancer: Principles and Practice. Cham: Springer International Publishing; 2015. p. 23-34. doi: 10.1007/978-3-319-15826-6_2.
4. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001;345(11):784-9. doi: 10.1056/NEJMoa001999.
5. Iino C, Shimoyama T. Impact of *Helicobacter pylori* infection on gut microbiota. World J Gastroenterol. 2021;27(37):6224-30. doi: 10.3748/wjg.v27.i37.6224.
6. Gobert AP, Wilson KT. Induction and regulation of the innate immune response in *Helicobacter pylori* infection. Cell Mol Gastroenterol Hepatol. 2022;13(5):1347-63. doi: 10.1016/j.jcmgh.2022.01.022.
7. Shohan M, Dehghani R, Khodadadi A, Dehnavi S, Ahmadi R, Joudaki N, et al. Interleukin-22 and intestinal homeostasis: protective or destructive? IUBMB Life. 2020;72(8):1585-602. doi: 10.1002/iub.2295.
8. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med. 2008;14(3):282-9. doi: 10.1038/nm1720.
9. Lopez DV, Kongsbak-Wismann M. Role of IL-22 in homeostasis and diseases of the skin. APMIS. 2022;130(6):314-22. doi: 10.1111/apm.13221.
10. Sanaii A, Shirzad H, Haghighian M, Rahimian G, Soltani A, Shafigh M, et al. Role of Th22 cells in *Helicobacter pylori*-related gastritis and peptic ulcer diseases. Mol Biol Rep. 2019;46(6):5703-12. doi: 10.1007/s11033-019-05004-1.
11. Dixon BR, Radin JN, Piazuelo MB, Contreras DC, Algood HM. IL-17a and IL-22 induce expression of antimicrobials in gastrointestinal epithelial cells and may contribute to epithelial cell defense against *Helicobacter pylori*. PLoS One. 2016;11(2):e0148514. doi: 10.1371/journal.pone.0148514.
12. Shamsdin SA, Alborzi A, Rasouli M, Ghaderi A, Bagheri Lankrani K, Dehghani SM, et al. The importance of TH22 and TC22 cells in the pathogenesis of *Helicobacter pylori*-associated gastric diseases. Helicobacter. 2017;22(3):e12367. doi: 10.1111/hel.12367.
13. Ota N, Wong K, Valdez PA, Zheng Y, Crellin NK, Diehl L, et al. IL-22 bridges the lymphotoxin pathway with the maintenance of colonic lymphoid structures during infection with *Citrobacter rodentium*. Nat Immunol. 2011;12(10):941-8. doi: 10.1038/ni.2089.
14. Zindl CL, Witte SJ, Laufer VA, Gao M, Yue Z, Janowski KM, et al. A nonredundant role for T cell-derived interleukin 22 in antibacterial defense of colonic crypts. Immunity. 2022;55(3):494-511.e11. doi: 10.1016/j.immuni.2022.02.003.
15. Shamsdin SA, Alborzi A, Rasouli M, Hosseini MK, Bagheri Lankrani K, Kalani M. Alterations in Th17 and the respective cytokine levels in *Helicobacter pylori*-induced stomach diseases. Helicobacter. 2015;20(6):460-75. doi: 10.1111/hel.12224.
16. Zhuang Y, Cheng P, Liu XF, Peng LS, Li BS, Wang TT, et al. A pro-inflammatory role for Th22 cells in *Helicobacter pylori*-associated gastritis. Gut. 2015;64(9):1368-78. doi: 10.1136/gutjnl-2014-307020.
17. Tourani M, Habibzadeh M, Karkhah A, Shokri-Shirvani J, Barari L, Nouri HR. Association of TNF-α but not IL-1β levels with the presence of *Helicobacter pylori* infection increased the risk of peptic ulcer development. Cytokine. 2018;110:232-6. doi: 10.1016/j.cyto.2018.01.003.