OPEN ACCESS

Haya: The Saudi Journal of Life Sciences

Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Detection of Interleukin-27 and Matrix Metalloproteinase-9 Levels in Patients Serum of *Helicobacter pylori*

Khalil Ismail Abid Mohammed[1]*, Wifaq M. Ali[1], Ameen Abdul Hasan AL-Alwany[2], Suha A. AL-Fakhar[1], Jinan M. Mousa[1]

[1]Clinical Communicable Diseases Research Unit /College of Medicine, University of Baghdad, Baghdad, Iraq
[2]Department of Internal Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq

DOI: https://doi.org/10.36348/sjls.2026.v11i07.001 | **Received:** 03.05.2026 | **Accepted:** 25.06.2026 | **Published:** 01.07.2026

***Corresponding author:** Khalil Ismail Abid Mohammed
Clinical Communicable Diseases Research Unit /College of Medicine, University of Baghdad, Baghdad, Iraq
Email: drkhalilmohammed1967@gmail.com

Abstract

The study was carried out to detection of H.pylori in [200] patients who attended two teaching hospitals in Baghdad. The diagnosis done by Immunochromatography methods. Stools and blood Samples was taken from each patient as well as other [30] healthy control matching in their age. The study included detection the Levels of Interleukin-27 and Matrix Metalloproteinase-9in sera of the patients and the control. The result indicated presence of H pylori antigen in 115 cases 69 cases of males and 46 of females, Also, the results indicated increasing levels of Interleukin-27 and Matrix Metalloproteinase-9 in patients' sera in comparison with healthy control.

Keywords: Helicobacter pylori, Interleukin-27, Matrix Metalloproteinase -9.

INTRODUCTION

Helicobacter pylori is a gram-negative, flagellated, microaerophilic bacterium that selectively colonizes the gastric mucosa [1] After entering the stomach, this spiral, Gram-negative, microaerophilic bacterium penetrates the mucus gastric layer [2], but does not traverse the epithelial barrier. Most of H. pylori organisms are free living in the mucus layer, but some organisms attach to the apical surface of gastric epithelial cells [3].

H.pylori uses urease and α-carbonic anhydrase to generate ammonia and HCO32- which mitigate the effects of low pH [4, 5] After established in the inner mucus layer, several outer membrane proteins, including BabA, SabA, AlpA, AlpB and HopZcan mediate bacterial adherence to gastric epithelial cells. Once attached, bacterial effector molecules, both secreted [vacuolating cytotoxin [VacA] and cytotoxin associated gene A [CagA]] or attached [components of the type Ⅳ secretion system [CagL]], modulate gastric epithelial cell behavior leading to loss of cell polarity, release of nutrients and chemokines [e.g., interleukin [IL-8], and regulation of acid secretion via control of gastrin and H +/ K+ ATPase [6, 7] H. pylori infection can be associated with several clinical complications such as gastritis, peptic ulcer disease gastric cancer and mucosa-associated lymphoid tissue [MALT] lymphoma [8].

Infection showed infiltration of lymphocytes and monocytes, along with significantly increased expression ofIL-1, IL-8, and IL-6 in the gastric antrum [7]. Anti-H.pylori immunoglobulin [Ig]M and IgG responses were detected in the serum of infected individuals 4 wk after infection, the numbers of gastric CD4 +and CD8+ T cells were increased compared to pre infection levels [9]. Levels of cytokines [interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], IL-1, IL-6, IL 7, IL-8, IL-10, and IL-18], are increased in the stomach of H. pylori-infected humans compared to uninfected humans [IL-17A is the most widely studied member of the IL-17 family of cytokines [IL-17A-F], and is produced by Th17 CD4 + T cells as well as other subsets of immune cells [10]. H.pylori infection also leads to the generation of regulatory T cells [Treg] [11] The cytokine IL-12 has a key role in the generation of Th1 cells and other cytokines such as IL-27 influence this process [12]. IL-27 is a new member of the IL-6/IL-12 family that is produced by activated antigen presenting cells including monocytes, dendritic cells and endothelial cells.[13]It has been reported that like IL-12, IL-27 induces the

development of naive Th cells into Th1 cells and also has synergistic effects with IL-12 to trigger the synthesis of the Th1 cytokine [13]. These observations suggest that IL-27 may have an important role in H. pylori related pathogenesis [14, 15].

Helicobacter pylori plays an important role in the pathogenesis of chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric mucosa–associated lymphoid tissue lymphoma. [16]. The epithelial cytokine/chemokine response is particularly important in the early stages of H. pylori–induced inflammation. Although most infected individuals develop only superficial gastritis, some patients progress to chronic gastritis, duodenal ulceration, or, rarely, cancer. This variability in clinical manifestation may arise be cause of differences in virulence of individual H. pylori strains. Several virulence factors have been described and include the presence of a vacuolatingcytotoxin [VacA] and acytotoxin-associated antigen A [CagA] [17,18].

Matrix metalloproteinases [MMPs] are a family of enzymes that degrade most of the macromolecules making up the extracellular matrix [ECM]. MMPs have a broad and overlapping substrate specificity and collectively can degrade most, if not all, components of the ECM. Gelatinase A [MMP-2], and gelatinase B [MMP-9] together can cleave gelatins [types I and V], pericellular and minor collagens [types IV, V, VII, and X], elastin, and fibronectin. The ECM of the gastric mucosa is composed of a number of macromolecules, such as collagen, laminin, proteoglycan elastin, fibronectin, and hyaluronic acid. The MMPs are believed to be active in connective tissue remodeling associated with various physiologic processes [including bone resorption as well as uterine or breast involution] and in pathologic conditions such as cancer and inflammatory diseases. It has been suggested that MMP-2 may participate in the physiologic turnover of the gastric ECM, whereas MMP-9 may be important in the early phase of gastric ulcer formation [19]. MMP-9 is considered an important factor in facilitating lymphatic invasion and metastases in early gastric carcinoma.[20]

Thus, although studies have implicated MMPs in matrix remodeling associated with gastric ulcer and cancer, surprisingly few studies have examined the links between H. pylori and MMP expression [21, 22]. The present study was designed to evaluate the effect of H. pylori on the expression of interleukin-27 and MMP-9 in infected patients.

MATERIALS AND METHODS

During a period of eight months from December 2025 to April 2026, a study was conducted at two teaching hospitals in Baghdad on freshly collected stool samples from a total number of 200 cases of gastroenteritis among adult patients. Stool samples were collected from each patient in sterile disposable screw cap containers. These were labeled with number, date, and name of each subject. A questionnaire containing demographic, clinical, and environmental data was obtained from each case. The existence of H. pylori in fresh stool samples was investigated at the microbiology laboratory of the same hospital using an immunochromatographic test.

H. Pylori Antigen Detection

Immunochromatographic assay [Weifang Kanghua Biotech china] for antigenic detection of H.pylori and were done according to instructions of the manufacturers. Allowing the card device test reagents and stool samples to reach to room temperature prior to testing. A separate stool collection tube and device were used for each sample and the assay was done right after collection. To detect H.pylori, approximately 100mg or 100 microliter of stool sample was put and shaken in collection tube containing the diluents. Four drops or 100µl was dispensed in the circular window of the card.

The results [appearance of the colored bands] were read after 10 minutes. This H.pylori KIT is qualitative Immunochromatographic assay for determination of H.pylori in fecal samples. The membrane on the test band region is pre - coated with mouse monoclonal antibodies against H.pylori antigens. During testing, the sample is allowed to react with the colored conjugates [anti-H.pylori mouse monoclonal antibodies-red microspheres] which were pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the colored particles migrate. In the case of positive result, the specific antibodies present on the membrane will capture the colored particles and a red colored line becomes visible.

The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a red colored band always appear. The presence of this red band serves as 1 verification that sufficient volume is added, 2-that proper flow is obtained and 3-as an internal control for the reagents. Insufficient specimen volume, incorrect procedural or deterioration of the reagents are the most likely reasons for control line failure. Negative results were indicated by only one red band [control line]. For positive result, in addition to the control band, a red band also appear on the site of result line. A total absence of the control colored band regardless the appearance or not of the result line was evaluated as an invalid result.

Blood Samples

Three mL of venous blood was obtained from each patients and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in eppendorf tubes and stored in deep freeze at –20c°until the time for using. The current study included Immunological level of interleukin -27 [IL-27] which

estimated by ELISA according to manual procedure of [Elabscience, Biotechnology]. and Matrix Metalloproteinase-9 [MMP-9] which estimated by ELISA according to manual procedure of [Cloud-Clone Corp, USA].

Inclusion criteria: Every person infected with H pylori and confirmed by a doctor through clinical manifestation was included in the present study.

Exclusion criteria: Every person without clear clinical symptoms of H pylori was excluded.

Statistical Analysis:

The results were analyzed using statistical system SPSS version -18 [T-testing]

RESULTS

Gender

Distribution of H. pylori patients according to their gender, were studied, among them 69 were males out of 120 and 46 were females out of 80. In a general H. pylori antigen was revealed in 115 of fecal samples out 200 [Table-1].

Immunological study

Interleukin -27and Matrix Metalloproteinase -9

The level of Interleukin-27 and Matrix Metalloproteinase -9 increased significantly in patients groups in comparison with healthy control [Table-2].

Table 1: Distribution of H. pylori patients according to their gender

***H.pylori* Antigen**	**Total**	**Positive No %**	**Negative No %**
Males	120	69 60	51 60
Females	80	46 40	34 40
Total	200	115 100	85 100

Table 2: The level of Interleukin-27[pg/mL] and Matrix Metalloproteinase -9[ng/ml] in patients with H pylori

Parameters	**Groups**	**Mean**	**SD**	**SE**	**t-test**	**P-value**
IL-27	patients	1.22	0.39	0.07	5.56	0.01
	Control	0.76	0.46	0.16		
MMP9	patients	1.38	0.30	0.05	8.20	0.01
	Control	0.91	0.05	0.01		

SD= Standard Deviation SE = Standard Error

The results of the study indicated statistically non-significant between the mean levels of Interleukin -27 and Matrix Metalloproteinase -9 of males and Females of infected patients [Table-3].

Table 3: Comparative the mean levels of IL-27[pg/mL] [and MMP-9[ng/mL] between the gender of patients with H pylori

Parameters	**Gender**	**Mean**	**SD**	**SE**	**t-test**	**P-value**
IL-27	Males	1.32	0.42	0.10		
	Females	1.12	0.36	0.09	1.43	0.16
MMP-9	Males	1.36	0.33	0.08	0.37	
	Females	1.40	0.27	0.06		0.70

The results show the region under the curve which was 91.0% for IL-27, and, 98.6% for MMP-9 shows the value of the sensitivity, which was 81% for IL-27and 84% forMMP-9 and the specificity, which was 100% for both [Table-4 and Fig-1].

Table 4: ROC measurement to study IL-27[pg/mL] and MMP-9[ng/mL] in patients and controls Area Under the Curve

Parameter	**Area**	**Cutoff**	**SE**	**P-value**	**C.I 95%**		**SN**	**SP**
					Lower Bound	**Upper Bound**		
IL-27	.910	1.20	.046	.000	.820	1.000	81	100
MMP-9	.982	1.06	.019	.000	.944	1.000	84	100

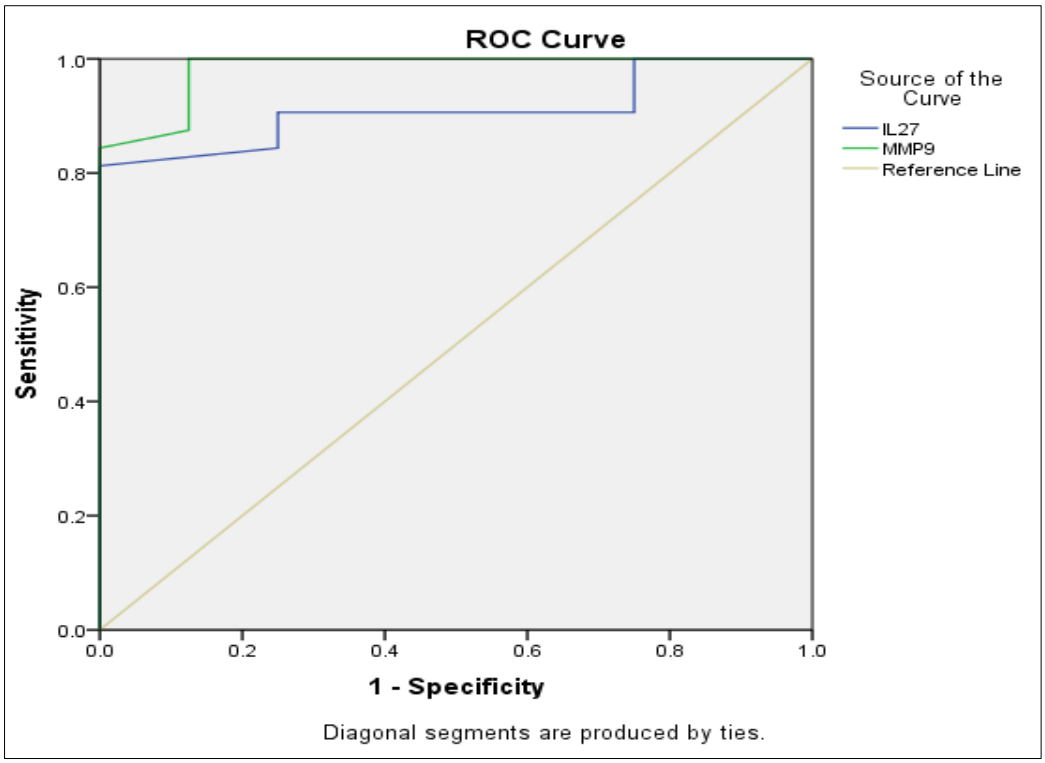

Fig. 1: The Sensitivity and Specificity of the ELISA test for IL-27and MMP-9

DISCUSSION

Helicobacter pylori was identified in 115 stool samples of patients out of 200 samples [Table-1]. The infections may be due to lack of sanitary facilities and poor living condition among the major causes of infection. The rate of infection in males was higher than females. The result indicated that H. Pylori infection in males was higher than in female the results in line with other results were reported by [23, 24]. In a general the main H.pylori transmission routes are fecal–oral and oral–oral transmission. The main H.pylori transmission pattern is person-to-person transmission [25].

The present study shows there are higher serum concentrations of IL-27 in H. pylori-infected patients than in the healthy control groups[Table-2] It has been reported that IL-27 enhances the differentiation of Th1 cells from naive Th cells and has also a synergistic effect with IL-12 to induce the secretion of Th1-type cytokines such as IFN-γ and IL-2[26]Moreover, it has been demonstrated that IL-27R-deficient mice exhibit a reduced IFN-γ response and are susceptible to infection with intracellular pathogens including Listeria monocytogenes or Leishmania major [27, 28]. Regulatory T [Treg] cells comprise a population of CD4+CD25+ cells that exert an inhibitory effect on the function of other T cells, and attenuate immune responses against self-antigens or non-self-antigens.[29] Moreover, inadequate Treg cell responses have been associated with H. pylori[30] IL-27R is expressed at a high level on regulatory cells and the inhibitory effect of IL-27 on Treg generation has been also demonstrated [31]. Accordingly, IL-27 may also have an important role in the pathogenesis of H. pylori-associated via down regulating Treg cell responses. Collectively, H.pylori-derived components produce IL-27 in the gastric mucosa of H pylori-infected patients. IL-27 has been presented as a main inducer of Th1 cells and accordingly the IL-27/Th1 axis may play an important role in the development of chronic inflammation. Furthermore, the inhibitory effects of IL-27 on Th2 and Treg cells may also play a role in H. pylori-associated diseases. Thus, the higher serum levels of IL-27 that were observed in H. pylori-infected patients is consistent with the up regulation of Th1 cells responses and the down regulation of Th2 or Treg functions, or both [32]. The results shows increasing significantly the levels of Matrix metalloproteinases-9 in H pylori patients in comparison with healthy control in a general, Matrix metalloproteinase-9 [MMP-9] is an enzyme whose primary function is the degradation of extracellular matrix proteins. Under nor mal conditions, MMP-9 is involved in tissue remodeling and mucosal healing. However, during chronic inflammation induced by H. pylori, the enzyme's activity increases sharply. Excess MMP9 damages the gastric mucosa by degrading its structural proteins [such as type IV and V collagens], which exacerbates inflammation and contributes to the progression of CG [33, 34]. Also, [35] has identified a mechanism whereby H. pylori activate the transcription factor STAT1, which directly binds to the MMP-9 gene promoter, enhancing its expression. This STAT1/MMP-9 pathway is considered key in the development of mucosal damage. H. pylori infection leads to a significant increase in MMP-9 and MMP-2 activity in the gastric mucosa, which is likely largely mediated by

tissue macrophages. This increased activity is thought to contribute to tissue damage during H. pylori-associated gastritis [36].

CONCLUSION

The result indicated presence the of H pylori antigen in 115 cases 69 cases of males and 46 of females, Also, the result indicated increasing levels of IL-27 and Matrix metalloproteinase 9 [MMP-9] in patients' sera in comparison with healthy control.

REFERENCES

1. Marshall, B., & Warren, J. R. [1984]. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. The lancet, 323[8390], 1311-1315.
2. Schreiber, S., Konradt, M., Groll, C., Scheid, P., Hanauer, G., Werling, H. O., & Suerbaum, S. [2004]. The spatial orientation of Helicobacter pylori in the gastric mucus. Proceedings of the National Academy of Sciences, 101[14], 5024-5029.
3. Hazell, S. L., Lee, A., Brady, L., & Hennessy, W. [1986]. Campylobacter pyloridis and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. Journal of Infectious Diseases, 153[4], 658-663
4. Bauerfeind, P., Garner, R., Dunn, B. E., & Mobley, H. L. [1997]. Synthesis and activity of Helicobacter pylori urease and catalase at low pH. Gut, 40[1], 25 30.
5. Wen, Y., Feng, J., Scott, D. R., Marcus, E. A., & Sachs, G. [2007]. The HP0165-HP0166 two component system [ArsRS] regulates acid-induced expression of HP1186 α-carbonic anhydrase in Helicobacter pylori by activating the pH-dependent promoter. Journal of bacteriology, 189[6], 2426 2434.
6. Wolle, K., & Malfertheiner, P. [2007]. Treatment of Helicobacter pylori. Best Practice & Research Clinical Gastroenterology, 21[2], 315-324.
7. Graham, D. Y., Opekun, A. R., Osato, M. S., El Zimaity, H. M. T., Lee, C. K., Yamaoka, Y., ... & Monath, T. P. [2004]. Challenge model for Helicobacter pylori infection in human volunteers. Gut, 53[9], 1235-1243.
8. Ford, A., Delaney, B., Forman, D., & Moayyedi, P. [2004] Eradication therapy for peptic ulcer disease in Helicobacter pylori positive patients. Cochrane Database Syst Rev, 4, CD003840 [PMID: 15495066 DOI: 10.1002/14651858.CD003840.pub2].
9. Nurgalieva, Z. Z., Conner, M. E., Opekun, A. R., Zheng, C. Q., Elliott, S. N., Ernst, P. B., ... & Graham, D. Y. [2005]. B-cell and T-cell immune responses to experimental Helicobacter pylori infection in humans. Infection and immunity, 73[5], 2999-3006
10. Lindholm, C., Quiding-Jarbrink, M., Lonroth, H., Hamlet, A., & Svennerholm, A. M. [1998]. Local cytokine response in Helicobacter pylori-infected subjects. Infection and immunity, 66[12], 5964 5971.
11. Cua, D. J., & Tato, C. M. [2010]. Innate IL-17 producing cells: the sentinels of the immune system. Nature Reviews Immunology, 10[7], 479-489.
12. Harris, P. R., Wright, S. W., Serrano, C., Riera, F., Duarte, I., Torres, J., ... & Smith, P. D. [2008]. Helicobacter pylori gastritis in children is associated with a regulatory T-cell response. Gastroenterology, 134[2], 491-499.
13. Wan YY. [2010] multi-tasking of helper T cells. Immunology. 130: 166–71.
14. Yoshida H, Miyazaki Y. [2008] Regulation of immune responses by interleukin-27. Immunol Rev.226: 234–47.
15. Zhu CL, Cao YH, Zhang R *et al.,*[2010]. Stimulatory effect of LPS and feedback effect of PGE2 on IL-27 production. Scand J Immunol. 72: 469–75
16. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. [1991] Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investi gation. Br Med J; 302:1302–1305.
17. Phadnis SH, Ilver D, Janzon L, Normark S, Westblom TU. [1994] Pathological significance and molecular characterization of the vacuolating toxin gene of Helicobacter pylori. Infect Immun.62: 1557–1565.
18. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N, Rappuoli R. [1993] Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc Natl Acad SciUSA90:5791–5795
19. Lempinen M, Inkinen K, Wolff H, Ahonen J. [2000] Matrix metalloprotein ases 2 and 9 in indomethacin-induced rat gastric ulcer. Eur Surg Res 32:169–176.
20. Kabashima A, Maehara Y, Kakeji Y, Baba H, Koga T, Sugimachi K. [2000] Clinico pathological features and overexpression of matrix metalloproteinases in intramucosal gastric carcinoma with lymph node metastasis. Clin Cancer Res 6:3581–3584.
21. Yokoyama T, Otani Y, Kurihara N, Sakurai Y, Kameyama K, Suzuki H, Igarashi N, Kimata M, Wada N, Kubota T, Kumai K, Kitajima M. [2000] Matrix metalloproteinase expression in cultured human gastric wall fibroblasts-interactions with Helicobacter pylori isolated from patients with ulcers. Aliment Pharmacol Ther 14:193–198.
22. Menges M, Chan CC, Zeitz M, Stallmach A. [2000]. Higher concentration of matrix-metalloproteinase 1 [interstitial collagenase] in H. py lori- compared to NSAID-induced gastric ulcers. Z Gastroenterol 38:887–891

23. Al-Ezzy, A. I. A. [2015]. Evaluation of Clinicopathological and Risk Factors for Nonmalignant H. Pylori Associated Gastroduodenal Disorders in Iraqi Patients. Open access Macedonian journal of medical sciences, 3[4], 645
24. Mohammed,K . I A, Ameen Abdul Hasan AL-Alwany, Sarmad M. Zeiny, Saad Hasan Mohammed Ali, Wifaq M. Ali, Suha A. AL-Fakhar, Jinan M. Mousa [2025]. Levels of Interleukin-39, C–Reactive Protein in Patient's Serum with H. Pylori Infections. Saudi J Med, 10[3]: 119-122.
25. Jung HK, Kang SJ, Lee YC, et al [2021]. Evidence-based guidelines for thetreatment of helicobacter pylori infection in Korea. Gut andLiver. 15[2]:168-19513
26. Yoshida H, Miyazaki Y. [2008] Regulation of immune responses byinterleukin-27. Immunol Rev 226: 234–47
27. Yoshida H, Hamano S, Senaldi G *et al.,* WSX-1 is requiredfor the initiation of Th1 responses and resistance to L. majorinfection. Immunity 2001; 15: 569–78.
28. Chen Q, Ghilardi N, Wang H *et al.,* [2000] Development ofTh1-type immune responses require the type I cytokinereceptor TCCR. Nature. 407: 916–20.
29. Costantino CM, Baecher-Allan CM, Hafler DA. [2008] Human regulatory T cells and autoimmunity. Eur J Immunol. 38: 921–4.
30. Robinson K, Kenefeck R, Pidgeon EL *et al.,* [2008]. Helicobacter pylori-induced peptic ulcer disease is associated with inadequate regulatory T cell responses. Gut 57: 1375–85.
31. Huber M, Steinwald V, Guralnik A *et al.,* [2008] IL-27 inhibits the development of regulatory T cells via STAT3. Int Immunol. 20: 223–34.
32. Molnar B, Galamb O, Sipos F *et al.,*[2010]. Molecular pathogenesis of Helicobacter pylori infection: the role of bacterial virulence factors. Dig Dis. 28: 604–8.
33. KRISHNAVENI D., BHAYAL A.C., SRI MANJARI K., VIDYASAGAR A., UMA DEVI M., RAMANNA M., JYOTHY A., NALLARI P. & VENKATESHWARI A. [2012] MMP- 9 Gene Promoter Polymorphism in Gastric Cancer. Indian Journal of Clinical Biochemistry. 27[3], 259–264.
34. SOKOLOVA O. & NAUMANN M. [2022]: Matrix Metalloproteinases in Helicobacter Pylori-Associated Gastritis and Gastric Cancer. International Journal of Molecular Sciences. 23[3], 1883.
35. LI Q., ZHU W. & KANG M. [2025]: Helicobacter pylori enhance MMP-9 expression through STAT1 to promote the development of gastritis. Journal of Radiation Research and Applied Sciences 18[3], 101670
36. BERGIN P.J., ANDERS E., SICHENG W., ERIK J., JENNIE A., HANS L., PIERRE M., QIANG P.H. & MARIANNE Q.J. [2004]: Increased production of matrix metalloproteinases in Helicobacter pylori associated human gastritis. Helicobacter 9[3], 201–210.