

Immune and Molecular Alterations Associated with *Helicobacter pylori* Infection in Patients with Gastric Ulcer

Qahtan adnan qader¹ , Semaa A. Shaban² , Firas faris rija³

Email : qahtan.adnan@st.tu.edu.iq , sema.alsham@tu.edu.iq

^{1,2,3} Biology Department, College of Science, Tikrti University, Iraq

Abstract

A gastric ulcer is a localised lesion in the stomach mucosa resulting from an imbalance between harmful factors and protective mechanisms. Infection with *Helicobacter pylori* (*H. pylori*) is a primary causative factor, as it triggers a chronic inflammatory response that leads to tissue damage. This study investigates changes in the expression of certain immune molecules and microRNAs (miRNAs) in patients with gastric ulcers and *H. pylori* infection, and evaluates their diagnostic potential. Materials and Methods: This study was conducted in Kirkuk city from November 2024 to September 2025 and included 50 blood samples from patients with gastric ulcer (32 males and 18 females), in addition to 40 samples from healthy individuals as a control group. *H. pylori* infection was diagnosed using the rapid urease test (RUT), serological tests, and faecal antigen testing. The levels of NLRP4 and CXCL12 in the serum were measured using the ELISA technique. The relative expression levels of miR-9, miR-362-5p, miR-502-5p and miR-650 were also determined using real-time quantitative polymerase chain reaction (qRT-PCR). Results: Diagnostic methods for *H. pylori*, including the rapid urease test (RUT), serological tests and faecal antigen testing, showed high positive rates among patients (82%, 78% and 76%, respectively). NLRP4 expression was significantly elevated in patients compared with healthy controls, with excellent diagnostic performance (AUC = 0.9965). CXCL12 levels were significantly lower in patients than in healthy controls. miRNA analysis revealed higher expression levels of miR-362-5p, miR-650, and miR-9 in patients compared with healthy controls, although these differences did not reach statistical significance. ROC analyses demonstrated diagnostic performance ranging from moderate to good. Meanwhile, miR-502-5p showed a trend toward reduced expression in patients with moderate diagnostic efficiency. Conclusion: *H. pylori* infection in patients with gastric ulcer is associated with a dysregulation of NOD-like receptors, particularly NLRP4, as well as certain microRNAs such as miR-9, miR-362-5p, and miR-650, reflecting a state of chronic inflammation and tissue damage. Some of these molecules, particularly NLRP4 and miR-650, may serve as promising biomarkers for diagnostic purposes. These findings underscore the complexity of host-pathogen interactions in the development of gastric ulcer and open avenues for exploring novel therapeutic and diagnostic strategies.

Keywords: Gastric ulcer, *H. pylori*, microRNAs Chemokines, NOD-like receptors

الخلاصة:

تعد قرحة المعدة آفة موضعية في الغشاء المخاطي للمعدة تنتج عن اختلال التوازن بين العوامل الضارة وآليات الحماية. تعد عدوى المولوية البوابية (*H. pylori*) عاملاً رئيسياً مسبباً، حيث تحفز استجابة التهابية مزمنة تؤدي إلى تلف الأنسجة. تستقصي هذه الدراسة التغيرات في تعبير بعض الجزيئات المناعية (miRNAs) لدى مرضى قرحة المعدة المصابين بعدوى *H. pylori*، كما تقيم إمكاناتها التشخيصية. المواد وطرق العمل: أجريت هذه الدراسة في مدينة كركوك خلال الفترة من تشرين الثاني 2024 إلى أيلول 2025، وشملت 50 عينة دم من مرضى قرحة المعدة (32 ذكراً و18 أنثى)، إضافة إلى 40 عينة من أفراد أصحاء كمجموعة ضابطة. تم تشخيص عدوى المولوية البوابية (*H. pylori*) باستخدام اختبار اليوريز السريع (RUT)، والاختبارات المصلية، وفحص مستضد البراز. تم قياس مستويات NLRP4 و CXCL12 في المصل باستخدام تقنية الإليزا (ELISA). كما تم تحديد مستويات التعبير النسبي لكل من miR-9 و miR-362-5p و miR-502-5p و miR-650 باستخدام تفاعل البوليميريز المتسلسل الكمي في الوقت الحقيقي (qRT-PCR). النتائج: أظهرت طرق تشخيص *H. pylori* بما في ذلك اختبار اليوريز السريع (RUT)، والاختبارات المصلية، وفحص مستضد البراز معدلات إيجابية مرتفعة بين المرضى (82%، 78%، و76% على التوالي). ، يظهر تعبير NLRP4 مرتفعاً بشكل ملحوظ لدى المرضى مقارنة بالأصحاء، مع أداء تشخيصي عالٍ (AUC = 0.9965)، مما يبرز دوره المحتمل في الالتهاب المرتبط بقرحة المعدة. كانت مستويات CXCL12 أقل بكثير في المرضى منها في الأصحاء. كشف تحليل miRNA عن مستويات تعبير أعلى لـ miR-362-5p و miR-650 و miR-9 في المرضى مقارنة بالأصحاء، على الرغم من أن هذه الاختلافات لم تصل إلى دلالة إحصائية. أظهرت تحليلات ROC أداءً تشخيصياً يتراوح من المعتدل إلى الجيد. وفي الوقت نفسه، أظهر miR-502-5p اتجاهًا نحو انخفاض التعبير لدى المرضى ذوي الكفاءة التشخيصية المعتدلة. الاستنتاج: ترتبط عدوى *H. pylori* لدى مرضى قرحة المعدة باضطراب في تعبير مستقبلات NOD (NOD-like receptors) وخاصة NLRP4، بالإضافة إلى بعض الجزيئات الدقيقة من نوع microRNA مثل miR-9 و miR-362-5p و miR-650، مما يعكس حالة الالتهاب المزمن وتلف الأنسجة. وقد تُعد بعض هذه الجزيئات، ولا سيما NLRP4 و miR-650، مؤشرات حيوية واعدة لأغراض التشخيص. تؤكد هذه النتائج تفيد العلاقات بين العائل والممرض في تطور قرحة المعدة، وتفتح آفاقاً لاستكشاف استراتيجيات علاجية وتشخيصية جديدة. الكلمات المفتاحية: قرحة المعدة المولوية البوابية . الرنا الميكروي. الكيموكينات . مستقبلات NOD.

Introduction

Gastric ulcer refers to a localised lesion and tear in the gastric mucosa resulting from an imbalance between harmful factors and protective mechanisms, where the erosion extends through the muscularis mucosae of the stomach wall into the deeper layers (1). Gastric ulcer is a common global disease, with an estimated prevalence of approximately 10% of the population. If not diagnosed and treated early, it can lead to serious complications with high mortality rates, such as acute bleeding, gastric perforation, and gastrointestinal obstruction, which necessitate emergency medical intervention (2). A gastric ulcer is an inflammatory disease affecting the stomach mucosa, resulting from several factors, the most important of which is *H. pylori* infection, alongside the use of certain medications, and unhealthy lifestyle habits such as smoking and alcohol consumption, as well as psychological stress and autoimmune diseases. Infection with *H. pylori* is considered one of the most common causes of gastric ulcers. The development of the disease is linked to a disruption in the balance between harmful factors, such as hydrochloric acid and pepsin, and defensive factors, which include mucus, bicarbonate, tight junctions, and prostaglandins, leading to damage of the stomach lining and progression of the condition. (3) MicroRNA is a small, non-coding ribonucleic acid molecule that plays an important role in regulating gene expression after transcription. This is achieved by binding to the untranslated region at the 3' end of messenger RNA (mRNA) molecules, which helps to regulate or suppress gene translation. (4,5). MicroRNAs act as important immune regulators that control the immune and inflammatory responses associated with gastric ulceration, by regulating the gene expression of numerous immune and inflammatory factors, such as IL-8 and TNF- α , as well as transcription factors like NF- κ B, and pathogen recognition receptors such as TLRs. Through these mechanisms, miRNAs contribute to maintaining a balance between the activation and suppression of the immune response, which affects the severity of inflammation and the development of gastric ulcers, and may also be

linked to fibrosis in some pathological conditions (6). Studies indicate that miR-9 and miR-650 are associated with the inflammatory response in infected gastric tissue, with miR-9 thought to play an anti-inflammatory role by regulating IL-8 expression and influencing the TLR/NF- κ B signalling pathway involved in the inflammatory immune response. It may also contribute to modulating the microenvironment of gastric epithelial cells, making it one of the key molecular factors associated with the development of inflammation and mucosal damage that can lead to the formation of gastric ulcers (7). Chemokines are inflammatory cytokines with a molecular weight of approximately 8–12 kDa, secreted by immune cells and epithelial cells in response to microbial exposure or inflammatory stimuli. Their primary role is to stimulate and regulate the migration of immune cells, particularly white blood cells, towards sites of infection or inflammation through a process known as chemotaxis (8). NLRP4 is a member of the NOD-like receptor family and functions as a negative regulator of the immune response. Evidence suggests that it has an inhibitory role in Type I interferon (IFN) signaling pathways and in the induction of autophagy, reflecting its regulatory effect in limiting inflammation. This role may contribute to regulating the inflammatory response within the gastric mucosa, which could be particularly important in chronic or complex gastric disorders. NLRP4 has also begun to attract increasing interest as a promising target for future immunotherapeutic interventions (9,10).

2. Materials and Methods

This study was conducted in the city of Kirkuk during the period from November 2024 to September 2025. The study targeted patients attending the Gastroenterology Unit at Azadi Teaching Hospital, who presented with varied clinical symptoms including: loss of appetite, weight loss, diarrhea, vomiting, as well as dyspepsia and epigastric pain.

Fifty blood samples were collected from patients of both sexes (32 males and 18 females), aged between 16 and 74 years. The diagnosis was confirmed by specialist doctors

based on clinical and endoscopic findings, as well as other diagnostic investigations (invasive and non-invasive). In contrast, 40 samples were collected from healthy individuals as a control group, aged between 19 and 63 years, with confirmation of the absence of any acute or chronic diseases. *H. pylori* diagnostic tests, including the rapid urease test (RUT), serological assay, and stool antigen test, were performed only for the patient group to confirm infection status. Healthy controls were selected based on the absence of gastrointestinal symptoms, medical history, and clinical evaluation and were therefore not subjected to invasive diagnostic procedures.

Sample Collection

Biological samples collected from participants included venous blood, stool specimens, and gastric swab samples. Blood samples were used for serological analysis, ELISA assays, and gene expression studies. Stool specimens were collected for *H. pylori* antigen detection, while gastric swabs were obtained for the rapid urease test (RUT).

Measurement of biomarkers in serum

The concentration levels of both NLRP4 and CXCL12 in the serum were measured using an enzyme-linked immunosorbent assay (ELISA), employing kits supplied by Sunlong Biotech (China), with full adherence to the manufacturer's protocols and instructions.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Quantitative real-time PCR (qRT-PCR) was performed using PerfectStar Green qPCR

SuperMix (TRANS, China) on an Applied Biosystems Real-Time PCR System (USA). Total RNA was extracted from blood samples using the TransZol extraction method according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using EasyScript First-Strand cDNA Synthesis Kit (TRANS, China). Specific primers were used for the amplification of miR-9, miR-362-5p, miR-502-5p, and miR-650, and their sequences are presented in Table (3-7). U6 was used as the endogenous housekeeping gene for normalization. Relative gene expression levels were calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method..

Statistical analysis

was performed using GraphPad Prism software (version 10.6.1). Quantitative data were expressed as mean $\pm$ standard deviation (SD). Differences between patients and healthy controls were assessed using the unpaired Student's t-test. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the studied biomarkers and microRNAs by determining the area under the curve (AUC), sensitivity, specificity, and optimal cut-off values. Pearson correlation analysis was used to assess the strength and direction of associations between continuous variables. A P-value of less than 0.05 was considered statistically significant in all analyses.

Table (1). Primer sequences used for qRT-PCR analysis

miRNA	Primer sequences
miR -9	F :5- CTTTGGTTATCTAGCTGTATGAGTCGT-3 R:5- ATCCAGTGCAGGGTCCGA-3
MiR 362-5p	F:5- AATCCTTGGAACCTAGGTGTGAGTAA -3 R:5- ATCCTTGGAACCTAGGTGTGAGT-3
miR-502	F: 5'- CGGGCATCCTTGCTATCTG-3 R: 5'-CAGCCACAAAAGAGCACAAT-3'
miR-650	F:5 TGTTCAGGAGGCAGCGCT-3 R:5-TATGGTTGTTACGACTCCTTCAC -3
U6	F: 5- GTGCTCGCTTCGGCAGCA-3 R:5- CAAAATATGGAACGCTTC-3

Ethical Approval

The study protocol was approved by the Scientific and Ethical Committee of the College of Science, University of Tikrit . Written informed consent was obtained from all participants prior to sample collection, and all procedures were conducted in accordance with the ethical principles outlined

3. Results and Discussion

3.1 Results of tests for the detection of *H. pylori* bacteria

The results of the serological test for *H. pylori* antibodies in blood serum showed that 39 out of 50 samples (78%) were positive. recorded positive results, and this finding is largely consistent with those of (11,12) who reported rates of 80% and 83% respectively. In contrast, the

se results differ from those of (13). which recorded a lower positive rate of 56%. This increase can be attributed to the persistence of antibodies (IgG) in the bloodstream for extended periods even after the bacteria have been eliminated (which limits the test's ability to distinguish between active infection and previous infection(14). Regarding the stool antigen test (SAT), it showed that 38 out of 50 samples were positive (76%). The infection was diagnosed using the faecal antigen test, which is an effective diagnostic method, and the present study was consistent with the findings of (15), where their results reached 74.19%, whereas it was inconsistent with the study by (12) which recorded a much lower rate (40%). These findings confirm the efficacy of the

(SAT) test as a rapid, non-invasive diagnostic tool, although its accuracy may be affected by factors such as the use of proton pump inhibitors (PPIs) or low bacterial colonisation density (16).

In contrast, the urea breath test (UBT) recorded the lowest positivity rate of the three tests, with 26 out of 50 samples (52%) being positive, a result consistent with (12). which recorded a rate of 65%. Despite this low rate, the UBT is considered the gold standard among non-invasive tests due to its high sensitivity, approaching 100%, and its specificity in detecting exclusively active infections (1). Negative results in some samples may be attributed to drug interactions or gastric emptying, which reduces the contact time between the administered urea and the bacteria (18).

2.3 Analysis of immune biomarkers

The study evaluated the diagnostic ability of both immune indicators NLRP4 and CXCL12 in people with stomach ulcers. The results revealed a noticeable variation in their expression patterns, in addition to their different levels of efficiency in diagnostic accuracy

2.3.1 Analysis of NLRP4 Levels

The study results revealed a significant increase ($P=0.0001$) in the levels of the immune protein NLRP4 in the patient group (345.8 ± 45.92) compared with the healthy group (135.8 ± 40.64), as shown in Figure 1-A and Table -2. The receiver operating characteristic (ROC) curve analysis demonstrated superior diagnostic performance, with an area under the curve (AUC) a value of 0.9965, with a sensitivity of 100% and a specificity of 94% at a 95% confidence interval. The cut-off value was 260.3, as shown in Figure (1-B).

The results of this study were consistent with those of (19) suggesting that the mucosal damage caused by *H. pylori* stimulates NLR receptor networks and inflammatory programmed cell death (pyroptosis) pathways.

Although (20) previously classified NLRP4 as a negative regulator of inflammation, The high levels observed in this study may represent a compensatory regulatory response aimed at alleviating chronic mucosal inflammation (21). The exceptional AUC value confirms that NLRP4 is a highly promising biomarker for the diagnosis of gastric ulcer.

2.3.2 Analysis of CXCL12 Levels

The results of the present study revealed a significant difference ($P=0.0003$) in CXCL12 levels in the patient group (535.5 ± 147.8) compared to the healthy group (625.6 ± 70.81). as shown in Figure (1-A) and Table -2. The ROC curve analysis indicated a moderate diagnostic efficiency, with an area under the curve (AUC) value of 0.6793, yielding a sensitivity of 90% and a specificity of 52%, and the value reached (Cut-off value) 551.5, as shown in Figure (1-B).

This study was consistent with the findings of (22). suggesting that chemokine levels may decrease during active inflammation due to local consumption at the site of injury or proteolytic degradation. Since the CXCL12/CXCR4 axis is vital for angiogenesis and tissue repair, its systemic depletion may indicate a defect in mucosal healing (23). In contrast to the strong diagnostic power of NLRP4, CXCL12 functions more effectively as a complementary marker due to its moderate specificity and its influence by various clinical stages (24).

3.3Molecular Study

3.3.1 Gene Expression Level of miR-362-5p

The relative gene expression level of miR-362-5p was measured using the quantitative polymerase chain reaction (qPCR) technique. The results indicated an increased gene expression in the patient group (1.676 ± 1.288) compared to the healthy group (0.85 ± 0.3324) as shown in Figure 2-A and Table -3.

To evaluate the diagnostic performance of miR-362-5p, receiver operating characteristic (ROC) curve analysis was performed. The

analysis yielded an area under the curve (AUC) of 0.7014, indicating a moderate ability to discriminate between patients with gastric ulcer and healthy controls. The sensitivity and specificity were 75% and 66.67%, respectively, at a cut-off value of 0.885, as shown in Figure (2-B). Although miR-362-5p expression was higher in patients than in healthy controls, the observed difference did not reach statistical significance ($P = 0.1072$). Therefore, the diagnostic potential of miR-362-5p should be interpreted with caution and requires further validation in larger cohorts.

The increased expression of miR-362-5p reflects a regulatory response at the tissue level associated with chronic inflammation and gastric mucosal damage. These findings are consistent with recent studies indicating that dysregulation of microRNA patterns is a hallmark of inflammatory gastrointestinal diseases (25). Moreover, this increase is consistent with the findings of (26), who reported that infection with the gastric bacterium *H. pylori* can stimulate miR-362-5p expression as part of the molecular changes within the inflammatory environment of the stomach. Regarding diagnostic value, the results support the potential use (27), who noted that microRNAs serve as biomarkers in the diagnosis of gastric ulcer; however, their diagnostic efficacy is superior when used as part of a panel of multiple molecular markers rather than as a single independent marker.

3.3.2 miR-650 Gene Expression Level

The study results revealed an increase in the relative gene expression of miR-650 in the patient group (3.016 ± 1.307) compared to the healthy group (1.778 ± 0.3354), as shown in Figure (2-A) and Table -3.

To evaluate the diagnostic performance of miR-650, receiver operating characteristic (ROC) curve analysis was performed. The analysis yielded an area under the curve (AUC) of 0.8088, indicating good discriminatory

ability between patients with gastric ulcer and healthy controls. The sensitivity and specificity were 100% and 76.47%, respectively, at a cut-off value of 2.155, as shown in Figure (2-B). Although miR-650 expression was higher in patients than in healthy controls, the observed difference did not reach statistical significance ($P = 0.06$). Therefore, while the ROC analysis suggests potential diagnostic value for miR-650, these findings should be interpreted with caution and confirmed in studies involving larger sample sizes.

This increase reflects the molecular changes associated with the active inflammatory environment resulting from *H. pylori* infection and the subsequent damage to the mucosal membrane. These findings are consistent with those of (28,29) who linked increased miR-650 expression to the degree of inflammatory activity and the activation of pathogenic pathways in gastric ulcer. The observed discrepancy compared to other studies may be attributed to the heterogeneous nature of ulcer disease, where miR-650 expression is influenced by the delicate balance between mucosal damage and tissue regeneration (30).

3.3.3 miR-9 Gene Expression Level

The study showed an increase in the relative gene expression of miR-9 in the patient group (2.618 ± 1.683) compared with the healthy group (1.135 ± 0.7007), as shown in Figure 2-A and Table-3. To evaluate the diagnostic performance of miR-9, receiver operating characteristic (ROC) curve analysis was performed. The analysis demonstrated good discriminatory ability between patients with gastric ulcer and healthy controls, with an area under the curve (AUC) of 0.8056. The sensitivity and specificity were 75% and 83.33%, respectively, at a cut-off value of 1.145, as shown in Figure (2-B). Although miR-9 expression was higher in patients than in healthy controls, the observed difference did not

reach statistical significance ($P = 0.0611$). Therefore, the diagnostic potential of miR-9 should be interpreted cautiously and warrants further validation in larger study populations. Elevated miR-9 levels indicate an impairment in the integrity of the gastric mucosal barrier and increased inflammatory stress. These findings were consistent with those of (31,32) who demonstrated that miR-9 is intrinsically linked to the regulation of inflammation and tissue response to damage. Conversely, discrepancies with other studies, such as that by (33) can be explained by the high sensitivity of miR-9 to individual variations in local inflammatory responses. This suggests that miR-9 functions more as a molecular indicator of active inflammatory status than as a definitive diagnostic marker for the ulcer itself.

3.3.4 Gene Expression Level of miR-502-5p

The results of the study showed a decrease in the relative gene expression of miR-502-5p in the patient group (1.372 ± 0.8878) compared to the healthy group (1.853 ± 0.6108), as shown in Figure (2-A) and Table-3. To evaluate the diagnostic performance of miR-502-5p, receiver operating characteristic (ROC) curve analysis was performed. The analysis demonstrated a moderate ability to discriminate between patients with gastric ulcer and healthy controls, with an area under the curve (AUC) of

[insert AUC value]. The sensitivity and specificity were 100% and 52.94%, respectively, at a cut-off value of 1.085, as shown in Figure (2-B). Although miR-502-5p expression tended to be lower in patients than in healthy controls, the observed difference did not reach statistical significance ($P = 0.2000$). Therefore, the diagnostic utility of miR-502-5p should be interpreted with caution and requires further validation in larger cohorts.

The decrease in miR-502-5p expression suggests a potential loss of its protective regulatory role as a result of the chronic inflammation associated with gastric ulcer. This trend is consistent with the findings of (27), who noted that decreased expression of certain miRNAs is a common feature in mucosal injuries. Functionally, these findings support the hypothesis proposed by (34) regarding the inhibitory role of miR-502-5p in inflammatory responses, where its downregulation may lead to uncontrolled inflammation via pathways such as TRAF2.

The discrepancy with studies reporting increased gene expression can be attributed to differences in clinical characteristics, disease stages, and the influence of pharmacological interventions such as proton pump inhibitors (PPIs) and antibiotics (35). Nevertheless, the mean AUC value confirms that the molecule.

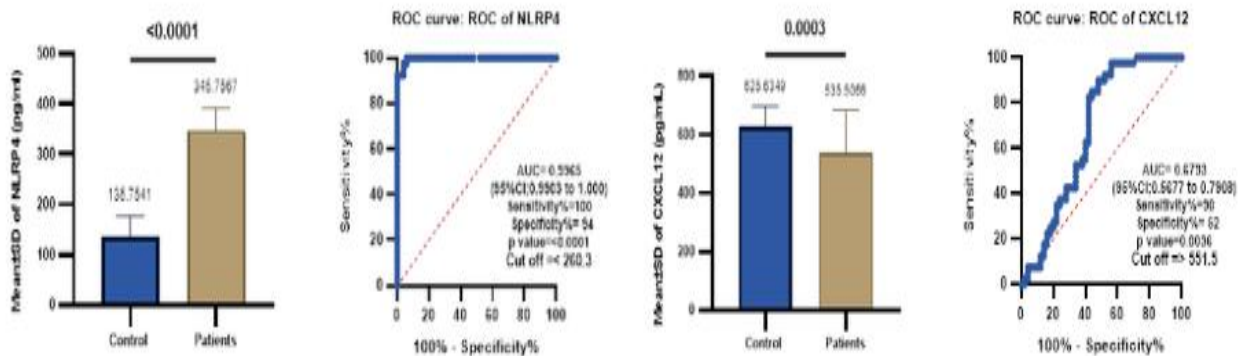


Figure -1

A-Serum NLRP4 , CXCL12 levels in patients and healthy controls.

B. ROC curve showing the diagnostic performance of,NLRP4 , CXCL12 in discriminating patients from healthy individuals.

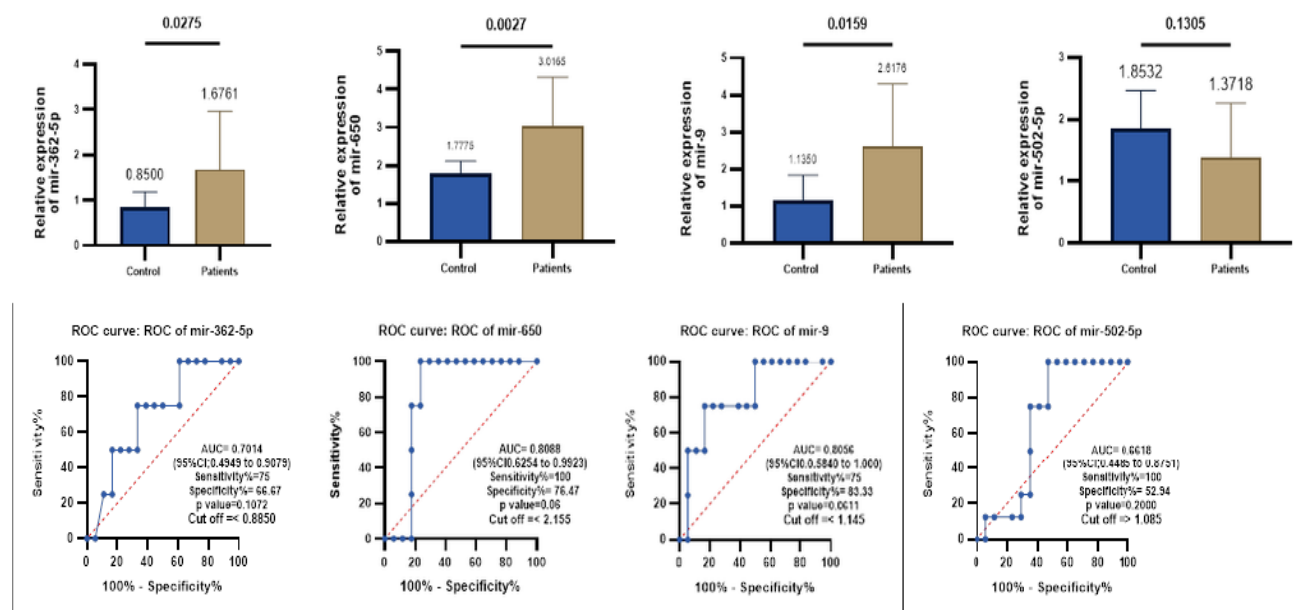


Figure -2

A) Relative expression of microRNA miR362-5P ,miR-650 , miR-9and miR-502-5p in patients with gastric ulcer and healthy controls.

B) ROC curve of microRNA miR362-5P ,miR-650 , miR-9and miR-502-5p for discriminating between patients with gastric ulcer and healthy controls

Table-2 Statistical distribution of NLRP4 and CXCL12 levels in control and patient population

NLRP4	Control	Patients	CXCL12	Control	Patients
Number of values	40	50	Number of values	40	50
Minimum	49.10	242.7	Minimum	455.1	195.4
25% Percentile	117.9	313.8	25% Percentile	579.2	429.8
Median	131.1	355.0	Median	611.0	550.0
75% Percentile	144.4	383.9	75% Percentile	678.8	648.2
Maximum	259.1	423.7	Maximum	791.1	800.0
Range	210.0	181.0	Range	336.0	604.5
Mean	135.8	345.8	Mean	625.6	535.5
Std. Deviation	40.64	45.92	Std. Deviation	70.81	147.8
Std. Error of Mean	6.427	6.495	Std. Error of Mean	11.20	20.91

Table -3 Statistical distribution of relative expression levels ($2^{-\Delta\Delta Ct}$) of the studied miRNAs in patients and healthy controls.

Parameter	miR-650 Control	miR-650 Patients	miR-362- 5p Control	miR-362- 5p Patients	miR-502- 5p Control	miR-502- 5p Patients	miR-9 Control	miR-9 Patients
Minimum	1.320	0.9000	0.5200	0.2000	1.220	0.3700	0.6500	0.6000
25% Percentile	1.433	2.105	0.5675	0.7875	1.340	0.6250	0.6525	1.268
Median	1.840	3.070	0.7900	1.160	1.755	0.9500	0.8750	2.025
75% Percentile	2.060	4.070	1.193	2.830	2.137	2.320	1.878	4.484
Maximum	2.110	4.990	1.300	5.060	3.120	3.200	2.140	5.390
Range	0.7900	4.090	0.7800	4.860	1.900	2.830	1.490	4.790
Mean	1.778	3.016	0.8500	1.676	1.853	1.372	1.135	2.618
Std. Deviation	0.3354	1.307	0.3324	1.288	0.6108	0.8878	0.7007	1.683
Std. Error of Mean	0.1677	0.3171	0.1662	0.3035	0.2159	0.2153	0.3503	0.3967

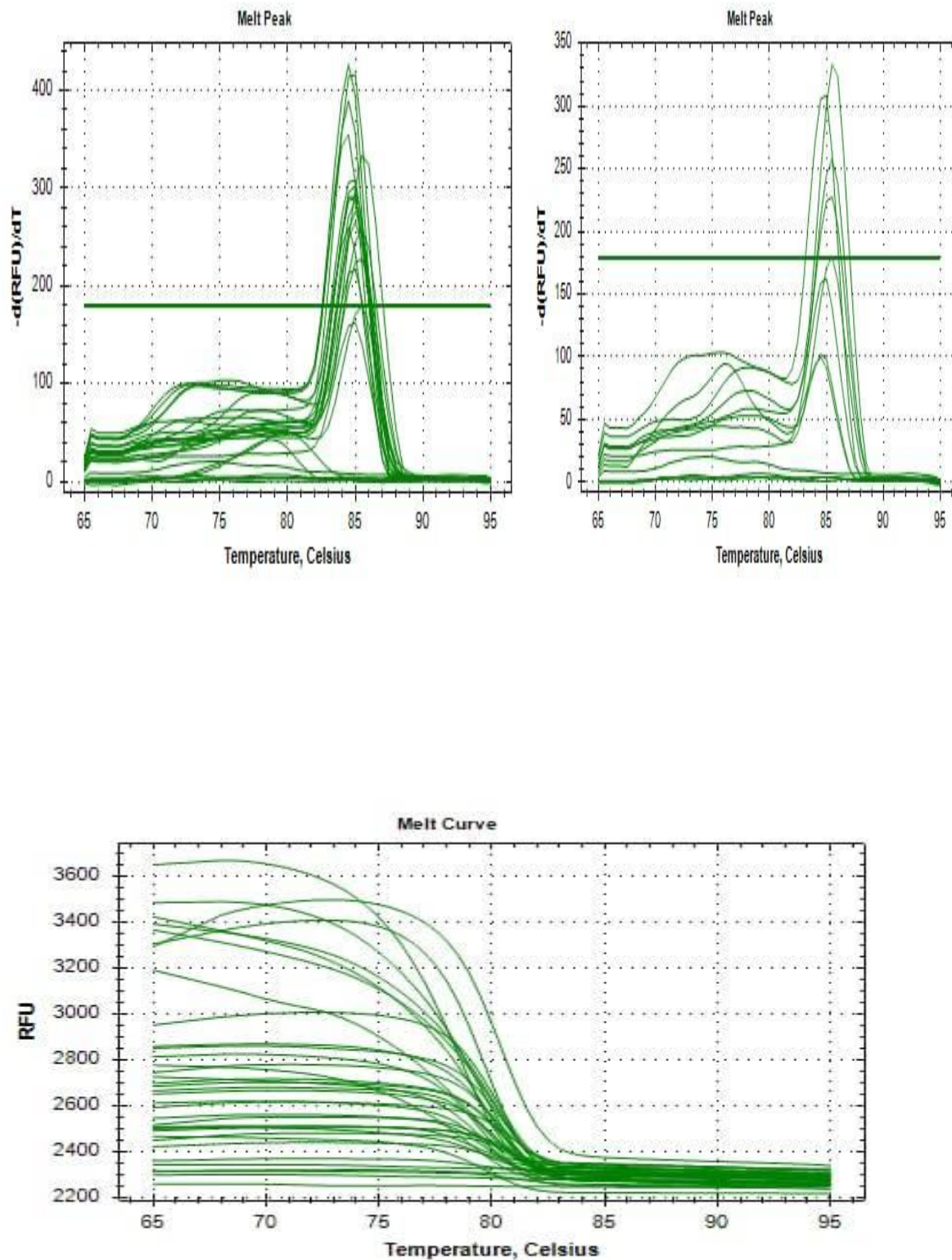


Figure (1-3). Representative melting curve analysis of the qRT-PCR products showing the specificity of amplification for the investigated microRNAs.

4. onclusions

The study summarises that the development of gastric ulcer in patients, accompanied by *H. pylori* infection, leads to significant changes in immune mediators, as well as a disruption in miRNA-dependent molecular regulation.

Serum NLRP4 levels were significantly elevated, demonstrating strong diagnostic performance indicative of its active involvement in ulcer-associated inflammation, suggesting its potential clinical application as an immunological tool for gastrointestinal cancer research and as a potential therapeutic target. On the other hand, a marked reduction in CXCL12 levels may impair the recruitment of immune cells and tissue repair processes, ultimately contributing to prolonged inflammation and hindering healing.

Regarding miRNAs, the notable upregulation of miR-362-5p and downregulation of miR-502-5p are significant for understanding the pathogenesis of chronic inflammation and tissue destruction, representing a complementary diagnostic tool with potential applications, Particularly concerning miR-650 and miR-9 within the mechanisms of epigenetic inheritance, which play a pivotal role in shaping the biological response of tissues to chronic injuries.

5-Reference

1. Gong, H., Zhao, N., Zhu, C., Luo, L., & Liu, S. (2024). Treatment of gastric ulcer: Traditional Chinese medicine may be a better choice. *Journal of Ethnopharmacology*, 324, 117793
2. Hassan, H. M., et al. (2023). Genistein ameliorated experimentally induced gastric ulcer in rats via modulation of Wnt/ β -catenin pathway. *Redox Report*, 28, 2218679
3. Feng, L., A, L., Li, H., Mu, X., Ta, N., Bai, L., Fu, M., & Chen, Y. (2023). Pharmacological mechanism of *Aucklandiae Radix* against gastric ulcer based on network pharmacology and in vivo experiment. *Medicina*, 59(4), 666
4. O'Brien, J., Hayder, H., Zayed, Y., & Peng, C. (2018). Overview of microRNA biogenesis, mechanisms of action, and circulation. *Frontiers in Endocrinology*, 9, 402.
5. Dexheimer, P. J., & Cochella, L. (2020). MicroRNAs: From mechanism to organism. *Frontiers in Cell and Developmental Biology*, 8, 409.
6. Săsăran, M. O., Meliț, L. E., & Dobru, E. D. (2021). MicroRNA modulation of host immune response triggered by *Helicobacter pylori*. *International Journal of Molecular Sciences*, 22, 1406
7. Ma, Y., Zhang, J., Xu, Y., & Liu, Y. (2021). Role of miR-9 in inflammation and gastric pathology. *World Journal of Gastroenterology*, 27(20), 2751–2760.
8. Miller, M. C., & Mayo, K. H. (2017). Chemokines from a structural perspective. *International Journal of Molecular Sciences*, 18, 2088.
9. Wang, H., Xia, L., Yao, C., Dong, H., Yang, Y., Li, C., ... Lu, S. (2022). NLRP4 negatively regulates type I interferon response and influences the outcome in anti-programmed cell death protein (PD)-1/PD-ligand 1 therapy. *Cancer Science*, 113(3), 838–851.
10. Sun, C.-C., Li, L., Tao, H.-Q., Jiang, Z.-C., Wang, L., & Wang, H.-J. (2022). The role of NLRP3 inflammasome in digestive system malignancy. *Frontiers in Cell and Developmental Biology*, 10, 1051612.
11. Ali, H. J. (2021). Immunogenetic study of *H. pylori* among patients with

- coronary heart disease (Master's thesis, University of Al-Qasim Green).
12. Zeina, A. A. W. (2025). *Evaluation of diagnostic, immunologic and molecular approaches of H. pylori* (Doctoral dissertation, University of Tikrit).
 13. Ahood, A. H. (2023). *Comparison between molecular and serological methods for identification of H. pylori isolated from gastric patients and relation with some immunological parameters* (Master's thesis, University of Tikrit, College of Science).
 14. Mouliou, D. S. (2022). Managing viral emerging infectious diseases via current molecular diagnostics. *Expert Review of Anti-Infective Therapy*, 20(9), 1163–1169.
 15. Radhi, M. H., & Mohammed, G. J. (2022). Genotyping and virulence genes of *H. pylori* isolated from patients. *International Journal of Health Sciences*.
 16. Qiu, E., Li, Z., & Han, S. (2021). Methods for detection of *H. pylori* from stool samples. *Brazilian Journal of Microbiology*, 52(4), 2057–2062.
 17. Said, Z. N. A., & El-Nasser, A. M. (2024). Evaluation of urea breath test as a diagnostic tool for *H. pylori* infection. *World Journal of Gastroenterology*, 30(17), 2302.
 18. Buyukcolak-Cebeci, Y., Timucin, E., Akcelik-Deveci, S., Mansur-Ozen, N., Aydinlar, T., Tiftikci, A., & Oktem-Okullu, S. (2025). Determination of the roles of *H. pylori* outer membrane virulence factors and pyroptosis-associated markers in ulcer and gastritis pathogenesis. *Biology*, 14(6), 634.
 19. Cui, J., Li, Y., Zhu, L., Liu, D., Songyang, Z., Wang, H., & Wang, R.-F. (2012). NLRP4 negatively regulates type I interferon signaling by targeting TBK1. *Nature Immunology*, 13(4), 387–395.
 20. Cui, J., Zhu, L., Xia, X., Wang, H. Y., Legras, X., Hong, J., & Chen, Z. J. (2020). NLRC5 negatively regulates the NF- κ B and type I interferon signaling pathways. *Cell*, 141(3), 483–496.
 21. Bagheri, V., Hassanshahi, G., Mirzaee, V., & Khorramdelazad, H. (2016). CXC chemokine CXCL12 tissue expression and circulating levels in peptic ulcer patients with *H. pylori* infection. *Cytokine*, 85, 14–18.
 22. Lu, Q., & Tang, H. (2024). Overexpression of HSP27 accelerates stress-induced gastric ulcer healing via the CXCL12/CXCR4 axis. *Clinical and Experimental Pharmacology and Physiology*, 51(5).
 23. Cambier, S., Gouwy, M., & Proost, P. (2023). The chemokines CXCL8 and CXCL12: Molecular and functional properties, role in disease and efforts toward pharmacological intervention. *Cellular & Molecular Immunology*.
 24. Popa, M. L., Ichim, C., Anderco, P., Todor, S. B., & Pop-Lodromanean, D. (2025). MicroRNAs in the diagnosis of digestive diseases: A comprehensive review. *Journal of Clinical Medicine*, 14(6), 2054.
 25. Zhang, J., Liu, S., Chen, R., Wang, J., & Wei, M. (2025a). Exosomal miR-362-5p induced by *H. pylori* promotes gastric carcinogenesis. *Frontiers in Cellular and Infection Microbiology*, 15(3), 120–135.
 26. Sagadatova, Y. R., et al. (2023). The role of microRNAs in the development of gastric and duodenal ulcers. *Kazan Medical Journal*, 104(4), 534–540.
 27. Lario, S., Ramírez-Lázaro, M. J., Aransay, A. M., Lozano, J. J., Montserrat, A., Casals, Á., & Calvet,

- X. (2012). MicroRNA profiling in duodenal ulcer disease caused by *Helicobacter pylori* infection. *Clinical Microbiology and Infection*, 18(8), E273–E282.
28. Vasapolli, R., Venerito, M., Schirrmester, W., Thon, C., Weigt, J., Wex, T., & Malfertheiner, P. (2021). Inflammatory microRNAs in gastric mucosa. *PLoS ONE*, 16(4), e0249282.
29. Link, A., Kupcinkas, J., Wex, T., & Malfertheiner, P. (2015). Macro-role of microRNA in gastric diseases. *Digestive Diseases*, 33(2), 227–235.
30. Joshi, D. C., Joshi, N., Kumar, A., & Maheshwari, S. (2024). Recent advances in molecular pathways and therapeutic implications for peptic ulcer management: A comprehensive review. *Hormone and Metabolic Research*, 56(9), 615–624. <https://doi.org/10.1055/a-2256-659>.
31. Zhou, J., Wang, G., Han, R., Wang, R., Kong, Y., Zhang, R., Hou, L., & Meng, M. (2021). Glycopeptides from *Paecilomyces sinensis* ameliorate ethanol-induced gastric ulcers via anti-inflammation and the miR-9-5p–MEK/ERK signaling pathway. *Food & Function*, 12(17), 7664–7675.
32. Li, X., Zhang, Y., Wang, H., Liu, J., Chen, S., Li, Y., & Zhao, H. (2022). Roles of microRNAs and exosomes in *Helicobacter pylori*–associated gastric diseases. *Molecular Biology Reports*, 49, 1–15. <https://doi.org/10.1007/s11033-022-07123-x>.
33. Wang, Y., Li, Y., Feng, Y., & Wang, F. (2016). miR-502-5p inhibits IL-1 β -induced inflammatory responses by targeting TRAF2. *Biochemical and Biophysical Research Communications*, 478(4), 1321–1327. <https://doi.org/10.1016/j.bbrc.2016.08.053>.
34. Li, H., Chen, L., & Zhao, X. (2024). Mechanisms of NLRP3 inflammasome activation in gastric mucosal injury. *Frontiers in Immunology*, 15, 112034.