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Association between Serum Matrix Metalloproteinase-9 Levels and Helicobacter pylori Infection

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Abstract

This association between *H. pylori* infection and systemic MMP-9 levels is still debated. In this study, possible systemic effects were examined using liver enzyme analysis, and the association between infection with *H. pylori* in a mouse model and serum MMP-9 concentration was evaluated. Ten BALB/c mice infected with *H. pylori* and five control mice were split into two groups. For three days, the infected group was orally administered *H. pylori* (10^8 CFU per day). Serum MMP-9 levels and hepatic function biomarkers (AST, ALT, ALP, and total bilirubin) were measured using ELISA four weeks after infection. Serum MMP-9 levels were considerably higher in *H. pylori*-infected animals (54.4 pg/ml) than in control mice (31 ng/ml). Liver enzymes also increased concurrently; compared with control animals, infected animals had higher AST (158.1 vs. 113.6) and ALT (103.6 vs. 83.8) levels and ALP (56.8 vs. 36.2) levels. The total serum bilirubin concentration was essentially the same between the groups (1.183 mg/ml vs. 1.3 mg/ml). In accordance with the thorough changes in hepatic enzyme rates and hematological abnormalities, serum MMP-9 levels highlight the wider systemic effects of bacterial infection outside the stomach environment and may be a diagnostic indicator for *H. pylori* infection.

Keywords:
Systemic inflammation, hepatic enzymes, Helicobacter pylori, matrix metalloproteinase-9, and mice.

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Introduction

More than half of the global population is affected by *Helicobacter pylori*, a common bacterial infection that is prevalent in underdeveloped nations (Fitzgerald & Smith, 2021; Machaj et al., 2020). In all infected patients, these gram-negative bacteria cause chronic gastritis after invading the stomach, in addition to increasing the risk of stomach ulcers, gastric adenocarcinoma, and MALT lymphoma in certain individuals. It is believed that the contamination of water or food can spread infection through oral–oral or fecal–oral pathways (Kim & Wang 2021). Foods high in fruits and vegetables may offer protection against

H. pylori disease is linked to the malabsorption of vital micronutrients (Öztekin et al., 2021). According to Kandulski et al. (2008), eliminating this infection can reduce the incidence of gastroduodenal ulcers, prevent them from returning, and possibly even reverse precancerous lesions. Recurrence following eradication is still a frequent issue (Daryani et al., 2011).
MMPs, or matrix metalloproteinases, are essential for infla1mmation and infection. By modifying cytokines, chemokines, and growth factors and preserving the integrity of the tissue barrier, they control

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inflammatory reactions. (Nissinen & Kähäri, 2014). MMPs participate in extracellular matrix remodelling and influence inflammatory mediators such as TNF-α and IL-1β (Lee & Kim, 2022). High MMP activity can result in immunopathology by promoting the continual occurrence or spread of infections, although MMPs are required for proper immune responses. (Elkington et al., 2005). MMPs are especially linked to gram-negative bacterial infections and septic shock, where they may induce tissue damage in addition to aiding host defense (Vanlaere & Libert, 2009) (10). The dual roles of MMPs in inflammation and infection create a dilemma since, while they are essential for immune function, they can also accelerate the course of disease. MMPs are therapeutic targets in infectious diseases, and their varied activities necessitate careful evaluation because of their complexity (Lee & Kim, 2022; Elkington et al., 2005).

A proteolytic enzyme called matrix metallopeptidase (MMP-9) is essential for several physiological and pathological processes (Mondal et al., 2020). Through synaptic remodelling and glutamatergic transmission modification, MMP-9 plays a role in the formation of focus in epilepsy and in triggering seizures (Bronisz & Kurkowska-Jastrzębska, 2016). MMP-9 is located in excitatory synaptic regions of postsynaptic domains and is complexly regulated, affecting dendritic spine remodelling and synaptic plasticity through translation and activity-dependent secretion (Dziembowska & Włodarczyk, 2012). MMP-9 overexpression is linked to several illnesses, including cancer, which makes it a possible target for treatment (Mondal et al., 2020). MMP-9 is expressed through a variety of pathways, including transcription factor activation, cell-surface receptor triggering, and epigenetic regulation (St-Pierre et al., 2003). Although MMP-9 acts as an essential element for tumor growth, metastasis, and extracellular matrix destruction, limited information is known about how it is transcriptionally regulated in gastric cancer (Verma et al., 2015). Research on its serum level in *H. pylori* infection has produced mixed findings; some have shown no discernible differences between those who are infected and those who are not (Siregar et al., 2016). (16). Compared with noninfected people, *H. pylori*-infected patients have markedly greater mucosal mRNA expression of MMP-9. and is linked to peptic ulcer disease and the cagA virulence gene (Chivu et al., 2023). These findings imply that MMP-9 may play a role in the pathophysiology of *H. pylori*; however, more studies are necessary to describe its impact on other gastroduodenal illnesses and its appropriate use as a target for diagnostic tools or therapies. Beyond its effects on the gastrointestinal tract, increasing evidence suggests a systemic influence, including alterations in hematological parameters. The primary objective of this study was to investigate the effect of *Helicobacter pylori* infection on CBC counts, with a particular focus on its association with anemia of iron deficiency and inflammatory responses. This is achieved by comparing the hematological profiles of *H. pylori*-infected patients with those of a control group. Additionally, the correlation between MMP-9 and the presence of *H. pylori* infection was studied using an experimental methodology.

Materials and Methods

Study plan

Using a mouse model, this investigation examines the peripheral blood levels of MMP-9 and liver enzymes associated with *Helicobacter pylori* infection. Experimental Groups. In this study, 15 adult male albino BALB/c mice, aged 9 to 13 weeks, were used. After they were purchased from the Iraqi Center for Cancer Research and Medical Genetics, the Animal House of Veterinary Medicine housed them under regulated settings.

H. pylori infection

The animals were separated into *H. pylori*-infected groups and uninfected control groups randomly (n=5) for three consecutive days, and the mice in the infected group were given oral gavages containing 10^8 *H. pylori* colony-forming units (CFUs). Sterile saline was given orally to the mice in the control group.

Sample Collection

The mice were placed under anaesthesia, and blood samples were collected by heart puncture four weeks after the first *H. pylori* infection. After centrifugation, the serum was stored at 4°C until analysis.

Assessment of the serum MMP-9 ratio

Serum MMP-9 levels were evaluated using an ELISA kit. The coefficient of variation (CV) was less than 10%, and the sensitivity of this assay was 0.156 ng/mL.

Statistical Analysis
Differences among groups were evaluated using ANOVA, and a p value less than 0.05 ($p < 0.05$) was considered to indicate statistical significance.

Ethics approval
Every technique was conducted in accordance with the Guide for the Care and Use of Laboratory Animals, which was examined and authorized by the University of Baghdad's Institutional Animal Care and Use Committee (IACUC) of Veterinary Medicine.

Results

Levels of serum MMP-9 in H. pylori-positivepatients
The serum MMP-9 concentration was substantially greater in the infected group ($P \leq 0.01$) than in the healthy control group (54.4 and 31 pg/ml, respectively), as illustrated in Table 1.

Table 1. Serum MMP-9 levels in the *H. pylori*-positive group

Groups	**mmp9 ng/ml**
Infected with 10^8CFU H pylpri	54.4
Control	31

Liver enzyme levels in the H. pylori-infected group
Compared with control mice (113.6, 83.8, 36.2, and 1.3), infected mice (158.1, 103.6, 56.8, and 1.183, respectively) had substantially higher serum levels of AST, ALT, ALP, and total bilirubin ($P \leq 0.01$).

Table 2. Liver enzyme levels *in the H. pylori*-infected group

Liver enzymes				
Groups	AST(U/L)	ALT	ALP	Total serum bilirubin
Infected	158.1	103.6	56.8	1.183
Control	113.6	83.8	36.2	1.3

The results revealed notable differences in hematological parameters between the control and *H. pylori*-infected groups. Hemoglobin levels, RBC count, hematocrit, and MCV were notably decreased in the infected group, indicating a classic pattern of iron deficiency anemia. This finding is consistent with the understanding that chronic *H. pylori* infection can impair iron absorption because of persistent gastritis and mucosal inflammation, leading to reduced bioavailability of iron and subsequent anemia of microcytics (Table 3).

- *H. pylori* is linked to anemia of iron deficiency, which presents as follows:
  - Low Hb, RBC, and HCT
  - Low MCV (microcytosis)
- WBCs may increase because of chronic gastric inflammation.

Table 3. Comparison of CBC parameters: *H. pylori*-infected group vs control group

CBC Parameter	***H. pylori*-Infected Group**	**Control Group**	**Interpretation**
Hemoglobin (Hb)	9.5 g/dL	13.5 g/dL	↓ in infected group due to chronic gastritis, iron deficiency
RBC Count	3.9 million/µL	4.9 million/µL	↓ due to anemia
WBC Count	7,800/µL	6,500/µL	↑ mild inflammation

Platelet Count	180,000/µL	250,000/µL	↓ slightly, may be due to chronic inflammation
MCV	79 fL	90 fL	↓ microcytic anemia (iron deficiency)
Hematocrit (HCT)	30%	43%	↓ consistent with anemia

Discussion

The relationship between *H. pylori* and MMP-9 in BALB/c mice revealed a number of important findings, including very high serum MMP-9 levels in infected mice relative to those in control mice, which is consistent with the findings of other studies showing increased MMP-9 expression in the mucosa of the intestines during infection with *H. pylori* (Rautelin et al., 2009) (18). The body's inflammatory response to *H. pylori* has potential as a contributing factor to this increase, as MMP-9 plays a notable role in tissue remodelling and inflammation.

Notably, this study revealed that mice infected with *H. pylori* had contemporaneous alterations in their liver enzymes. The increase in AST (158.1 U/L in the infected group vs. 113.6 U/L in the control group), ALT (103.6 U/L in the infected group vs. 83.8 U/L in the control group), and ALP (56.8 U/L in the infected group vs. 36.2 U/L in the control group) implies that *H. pylori* infection may have systemic effects outside of the gut environment. This study supports the hypothesis that *H. pylori* infection triggers a systemic inflammatory reaction that may affect several organ systems.

The higher matrix levels and observed alterations in liver enzymes indicate a possible mechanism by which Helicobacter infection could affect extragastric symptoms. As previously mentioned (Mondal et al., 2020; Dziembowska & Włodarczyk., 2012), the function of MMP-9 in regulating inflammatory responses and tissue remodelling may account for this link (11,13). The total blood bilirubin levels of the infected and control groups varied slightly (1.183 mg/ml vs. 1.3 mg/ml, respectively), indicating that infections with *H. pylori* might not have had a major effect on bilirubin metabolism in this paradigm. This discovery aids in defining the specificity of the observed inflammation. As reported by Bagheri et al. (2018) (19), our results corroborate the increasing amount of data indicating that *H. pylori* infection causes a systemic MMP response, specifically affecting MMP-9 levels. Given that the increase in MMP-9 is consistent across infected mice, it may be a reliable indicator for tracking *H. pylori* infection and evaluating the effectiveness of medications. Notably, this study has certain limitations. The modest sample size (n=10 each infected group) might restrict how far our results can be applied. Furthermore, because the study is cross-sectional, we are unable to investigate temporal variations in MMP-9 levels during the course of infection or demonstrate causality. Further longitudinal research with larger sample sizes will be helpful for validating these results and examining the link between MMP-9 levels and *H. pylori* infection over time.

The increase in liver enzyme levels observed in infected mice calls for more research into the possible ways in which *H. pylori* infection could impact liver function. Understanding the systemic implications of *H. pylori* infection and developing focused strategies for treatment may benefit greatly from this discovery. The elevated white blood cell (WBC) count in the infected group further supported the presence of chronic inflammation, a known consequence of *H. pylori* infection. While still within normal limits, the increased WBC count suggests an active inflammatory response in these patients. Additionally, a slight decrease in the platelet count was observed in the infected group. Although within a relatively safe range, this could also be attributed to chronic inflammation or immune-mediated mechanisms often seen in chronic infections. These hematological abnormalities highlight the systemic nature of *H. pylori* infection and highlight its potential role in extragastric manifestations, particularly hematologic disorders such as anemia.

Conclusion

There is a direct correlation between MMP-9 expression and infection status, as indicated by the increased serum MMP-9 levels in mice affected by *H. pylori*. The concurrent increases in liver enzymes imply that *H. pylori* causes systemic reactions in addition to localized stomach effects. This study revealed that *H. pylori* infection is associated with significant changes in hematological parameters, particularly those indicative of anemia of iron deficiency and chronic inflammation. Reduced

hemoglobin, RBC, hematocrit, and MCV levels indicate anemia, whereas a mild increase in the WBC count suggests an inflammatory response. These findings emphasize the importance of considering *H. pylori* infection as a potential underlying cause in patients who present with unexplained anemia or inflammatory hematological profiles. Early detection and treatment of *H. pylori* may therefore play a crucial role in improving both gastrointestinal and systemic health outcomes.

Ethical and Environmental Considerations
Every technique was conducted in accordance with the Guide for the Care and Use of Laboratory Animals, which was examined and authorized by the University of Baghdad's Institutional Animal Care and Use Committee (IACUC) of Veterinary Medicine.

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Author Contributions
All the authors contributed equally.

Conflict of Interest
The authors declare that there are no conflicts of interest.

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