Original Article

Immunological and Molecular Detection of *Helicobacter pylori* in Patients Clinically Diagnosed with Multiple Sclerosis

Abrar J. Hassan, Rana Saady Aboud
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Background: Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease affecting the central nervous system (CNS). MS damages the myelinated axons; in the CNS; partially damaging the myelin sheath and axons. *Helicobacter pylori* promotes stomach inflammation, and due to the maintenance of chronic inflammation in the gastric mucosa, the virulence factors of bacteria indirectly affect the development of symptoms of multiple sclerosis. **Objective:** The goal of the present study was to evaluate the association of *H. pylori* infection with multiple sclerosis. **Materials and Methods:** One hundred blood samples were collected from people aged 13–65 years from January 2022 to June 2022, and these patients were examined by the consultant medical staff of Dr. Saad Al-Witry Hospital for Neurosciences. These patients were compared with 30 apparently healthy controls. The patients were divided into three groups: the first group consisted of 36 multiple sclerosis patients with *H. pylori*, the second consisted of 34 multiple sclerosis patients without *H. pylori*, and the third group consisted of apparently healthy controls. Anti-*H. pylori* IgA and IgG antibodies were measured using enzyme-linked immunosorbent assay (ELISA), and *TLR-4* and *IL-8* genes were detected in the serum of multiple sclerosis patients and the control group using real-time polymerase chain reaction (RT-PCR) technique. **Results:** The statistical analysis shows a highly significant increase ($P \leq 0.01$) in anti-*H. pylori* IgA and IgG antibodies in sera from patients with MS disorders with *H. pylori* compared to the control group. It also shows a highly significant increase ($P \leq 0.01$) in MS disorders without *H. pylori* compared to the control group. *TLR-4* and *IL-8* gene expression indicate a highly significant difference ($P \leq 0.001$) in sera of patients with MS disorders and *H. pylori* compared to the control group, and highly significant elevation ($P \leq 0.01$) in MS diseases without *H. pylori*. **Conclusion:** The present study shows that *H. pylori* infection has a significant role in MS.

Keywords: IgA, IgG, *IL-8*, multiple sclerosis, *TLR-4*

Introduction

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the central nervous system (CNS). MS damages the myelinated axons of the CNS and causes some damage to both the myelin sheath and the axons.[1,2]

Patients suffering from MS may present a wide range of symptoms that reflect CNS involvement. Each patient may present MS in a different way. Symptoms do not always occur at the same time or in the same space; there may be months or even years that separate the appearance of each episode and may affect different anatomic locations. MS is extremely random and almost impossible to predict for each individual patient.[3,4] Although signs and symptoms of MS are unpredictable, there are certain clinical presentations that are common in MS and are reflective of the primarily white matter location of most lesions.[5,6]

Helicobacter pylori is a Gram-negative, microaerophilic bacterium. It measures 2–4 μm in length and 0.5–1 μm in width and is spiral shaped. The bacterium is mobile due to two to six polar sheathed flagella of approximately 3

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_993_23

Address for correspondence: Dr. Abrar J. Hassan, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
E-mail: abrar.jassem1102a@sc.uobaghdad.edu.iq

Submission: 13-Jul-2023 **Accepted:** 16-Dec-2023 **Published:** 23-Jan-2026

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License (CC BY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Hassan AJ, Aboud RS. Immunological and molecular detection of *Helicobacter pylori* in patients clinically diagnosed with multiple sclerosis. Med J Babylon 2025;22:1278-84.

μm length.[5] The bacterium can survive brief exposure to low pH, but only grows at neutral pH, which is found in the mucous layer of the gastric mucosa.[7,8] The effect of *H. pylori* infection on gastric acid production depends on the severity and anatomic location, in some individuals the infection spreads further to the corpus, followed by the development of atrophy that destroys the parietal cells, leading to hypochlorhydria caused mediated by the elevated levels of inflammatory cytokines, such as IL1-β and TNF-α.[9,10]

Some studies suggest an association between *H. pylori* infection and neurological diseases such as multiple sclerosis,[11,12] MS is associated with the parallel up-regulation of proinflammatory (interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], lymphotoxin-α, and interleukin [IL]-12) and systemic down-regulation of immune response (transforming growth factor-β [TGF-β], IL-10) cytokines.[13,14] Cytokine production is elevated to an even higher degree in the CSF than systemically, underlining the autonomy of the immune responses in the CSF. All cytokine abnormalities are demonstrable already in very early MS, manifested by acute unilateral optic neuritis associated with more than two MS-like lesions on brain magnetic resonance imaging and oligoclonal IgG bands in CSF.[15,16]

The innate immune system is the first line of defense against invading pathogens. The sentinels of the innate immune system are the Toll-like receptors (TLRs). The TLRs survey the cellular environment for molecular patterns commonly associated with pathogens.[17] Once a TLR interacts with its ligand, the receptor complex initiates signaling cascades that lead to transcription and secretion of antimicrobials and immune-modulating cytokines and chemokines. The innate immune responses activate and instruct the adaptive immune system to respond in a pathogen-appropriate manner.[18] Infection by *H. pylori* can cause gastritis and is also highly associated with gastric ulcers, gastric adenocarcinomas, neurological diseases, and mucosa-associated lymphoid tissue lymphomas. Upon *H. pylori* infection, gastric epithelial cells respond to *H. pylori* by activating many signaling cascades.[19]

These lead to the secretion of cytokines and chemokines, which recruit innate and adaptive immune cells to the site of infection. Despite a vigorous host immune response, *H. pylori* infection is persistent and can persist for life without medical intervention.[20]

Interleukin-8 (IL-8) is an important chemokine in mediating the inflammatory response to *H. pylori*. Demonstrated that both the NF-β and activating protein 1 (AP1) DNA binding sites within the IL-8 promoter are required for optimal transcription in response to *H. pylori* infection of gastric epithelial cells.[21] During *H. pylori* infections, both NF-β and members of the mitogen-activated protein kinase (MAPK) family become activated. Activated MAPKs then phosphorylate AP-1 complexes, which results in increased AP-1-dependent transcription. Therefore, signaling pathways that activate NF-β and/or AP-1 could result in increased IL-8 secretion.[22,23]

The present study was to determine the relationship between *H. pylori* infection and multiple sclerosis.

Materials and Methods

Study participants

This group included 70 patients (48 females, 22 males) between the ages of 13 and 65 years, who suffered from multiple sclerosis and were treated at the Dr. Saad Al-Witry Hospital for Neurosciences between January 2022 and June 2022. Those patients were examined by the consultant medical staff at the clinic. The patient groups were divided into three categories: First class: 36 multiple sclerosis patients with *H. pylori* (13 males and 23 females with a mean age of 39 years). Second class: 34 multiple sclerosis patients without *H. pylori* (9 males and 25 females with a mean age of 41.5 years). Control group: This group involved 30 apparently healthy individuals (13 males and 17 females with a mean age of 35.5 years), and the participants had no gastrointestinal diseases and no other complaints.

Blood samples collection

For each patient and healthy individual, 300 μL of serum was added into a 2 mL tube containing 500 μL trizol and the tube was inverted many times for mixing. For the molecular study, trizol has been used to maintain the integrity of RNA and inhibit the action of RNase.

Immunological detection of anti-*H. pyloi* IgA, IgG by ELISA method

All the studied groups include multiple sclerosis patients with *H. pylori*, without *H. pylori* and apparently healthy individuals (control group). They were submitted to estimate the anti-*H. pyloi* IgA and IgG antibody levels using enzyme-linked immunosorbent assay (ELISA) test (MyBioSource, USA).

Molecular detection of *TLR-4* and *IL-8* genes in serum of multiple sclerosis patients and for the control group by real-time polymerase chain reaction (RT-PCR) technique

a) **RNA extraction:** RNA was extracted from serum samples that were collected from the patients clinically diagnosed with multiple sclerosis patients with *H. pylori*, without *H. pylori* and apparently healthy individuals using a commercial Qubit RNA HS Assay Kit and according to the manufacturer's instructions. All the extracted DNA samples were stored at −20°C until use.
b) **RNA quantitation by Qubit 4.0**: The assay is highly selective for RNA and it is accurate for initial sample

concentrations from 10 pg/μL to 100 ng/μL. The assay is performed at room temperature, and the signal is stable for 3 h. Common contaminants such as salts, free nucleotides, solvents, detergents, or proteins are well tolerated in the assay.

c) **RT-qPCR protocol**: This is the main step in our project, which has been divided into two phases. The first is through the synthesis of cDNA from RNA, as mentioned in Table 1. The second section of this protocol is done by choosing the cDNA sample from the patient and control at the same run, for each sample there are three PCR tubes, one tube for each gene, IL-8, TLR-4, and B2M (beta-2-microglobulin), which is considered a house-keeping gene in this study. The detection of quantity is based on the fluorescent power of SyberGreen.[24] The reaction mixture consists of components with their quantity, as mentioned in Table 1.

Spin the PCR quickly tubes to remove the bubbles and collect the liquid (1 min at 2000*g*, then the program was set up for real-time PCR with the indicated thermocycling protocol as shown in Table 2.

Statistical analysis

The statistical analysis system (SAS, 2018) program was used to determine the effect of different factors on study parameters. The least significant difference (LSD) (analysis of variation [ANOVA]) test was used to significantly compare the means. Chi-square test was used to significantly compare percentages (0.05 and 0.01 probability). Estimation of the correlation coefficient between difference parameters in this study.[25]

Ethical approval

The study was conducted in accordance with the ethical principles originating in the Declaration of Helsinki. Verbal and analytical approval from patients were obtained before sample collection. The study protocol and subject information and consent form were reviewed and approved by a local ethics committee according to document number CSEC/0122/0005 (January 10, 2022) to get this approval.

Table 1: Components of RT-qPCR protocol

Component	20 μL Reaction
Luna Universal qPCR Master Mix	10 μL
Forward primer (10 μM)	1 μL
Reverse primer (10 μM)	1 μL
cDNA	5 μL
Nuclease-free water	3 μL

Table 2: Program for real-time PCR with thermocycling protocol

Cycle step	Temperature (°C)	Time	Cycles
Initial denaturation	95	60 s	1
Denaturation	95	15 s	40–45
Annealing	60	30 s (+plate read)	
Melt Curve	60–95	40 min	1

Results

Figures 1 and 2 illustrate the levels of IgA and IgG in the different groups of the study. There was a highly significant difference ($P \leq 0.01$) in the level of IgA Ab in the *H. pylori* + MS groups (0.595 ± 0.08 U/mL) compared to the healthy control group (0.152 ± 0.01 U/mL) and a highly significant difference ($P \leq 0.01$) in the level of IgA Ab in the MS groups (0.175 ± 0.02 U/mL) compared to the healthy control group (0.152 ± 0.01 U/mL). There was a highly significant difference ($P \leq 0.01$) in the level of IgG Ab in the *H. pylori* + MS groups (0.857 ± 0.07 U/mL) compared to the healthy control group (0.166 ± 0.01 U/mL) and a highly significant difference ($P \leq 0.01$) in the level of IgG Ab in the MS groups (0.323 ± 0.06 U/mL) compared to the healthy control group (0.166 ± 0.01 U/mL).

Highly significant differences ($P \leq 0.01$) were recorded in the level of *IL-8* gene expression in different groups studied as shown in Table 3. There was a highly significant difference ($P \leq 0.01$) in the level of *IL-8* gene expression in the *H. pylori* + M.S groups (4.90 ± 0.72 U/mL) compared to the healthy control group (1.00 ± 0.00 U/mL) and a highly significant difference ($P \leq 0.01$) in the level of *IL-8* gene expression in the MS groups (3.03 ± 0.59 U/mL) compared to the healthy control group (1.00 ± 0.00 U/mL).

Highly significant differences ($P \leq 0.01$) were recorded in the level of *TLR-4* gene expression in different groups studied, as shown in Table 4. There was a highly significant difference ($P \leq 0.01$) in the level of *TLR-4* gene expression in the *H. pylori* + MS groups (5.07 ± 0.85 U/mL) compared to the healthy control group (1.00 ± 0.00 U/mL) and a highly significant difference ($P \leq 0.01$) in the level of *TLR-4* gene expression in the MS groups (3.98 ± 0.71 U/mL) compared to the healthy control group (1.00 ± 0.00 U/mL).

Discussion

The results of the current study were consistent with those of several previous research investigations. Aboud *et al.*[26] discovered a significant ($P \leq 0.05$) increase in the concentration of anti-*H. pylori* IgA Abs 7.15 ± 1.04 U/mL and IgG Abs 7.81 ± 0.96 U/mL compared to the control group 4.99 ± 0.80 U/mL. Furthermore, a study was conducted on the mean titer of anti-*H. pylori* IgG in 25 patients. Studies utilizing ELISA showed a substantial protective association (OR, 0.59; 95% CI, 0.46–0.77).

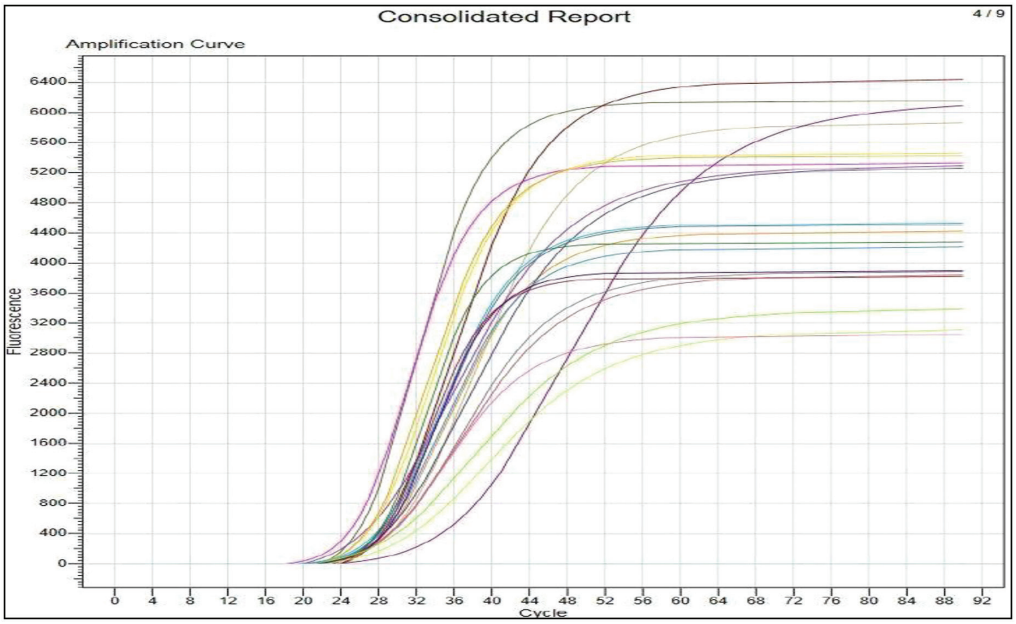

Figure 1: Schematic representation of the data of qualitative RT-PCR for *H. pylori* detection (*TLR-4* gene)

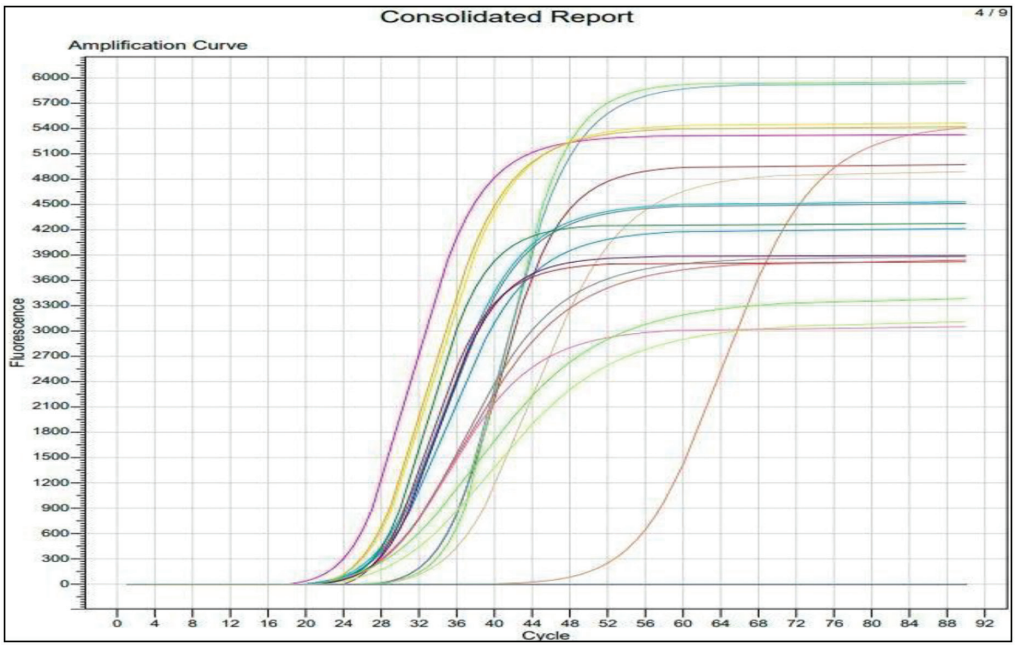

Figure 2: Schematic representation of the data of qualitative RT-PCR for *H. pylori* detection (*IL-8* gene)

Table 3: Results of *IL-8* gene expression in difference groups

Group	HKG	$\Delta ct_{patient}$	ΔΔct	Fold of expression
H. pylori + MS	26.69	11.47	1.60	4.90 ± 0.72 a
MS	27.86	12.40	3.52	3.03 ± 0.59 b
Control	37.10	7.96	0.882	1.00 ± 0.00 c
t test (*P* value)	–	–	–	0.873** (0.0026)

Means having the different letters in the same column differed significantly; $^{**}P \leq 0.01$

M.S = Multiple sclerosis, HP = *Helicobacter pylori*

Table 4: Results of *TLR-4* gene expression in difference groups

Group	HKG	$\Delta ct_{patient}$	ΔΔct	Fold of expression
H. pylori + MS	26.69	1.71	1.30	5.07 ± 0.85 a
MS	27.86	2.03	0.012	3.98 ± 0.71 b
Control	37.10	1.70	0.0038	1.00 ± 0.00 c
t test (*P* value)	–	–	–	0.905** (0.0019)

Means having the different letters in the same column differed significantly; $^{**}P \leq 0.01$

M.S = multiple sclerosis, *H. pylori* = *Helicobacter pylori*

Several studies in which *H. pylori* infection was detected using histological techniques found, a substantial positive correlation (OR, 6.64; 95% CI, 2.40–13.76). Activated *H. pylori* infection may be a risk factor for the development of MS.[27]

According to Cam and Akcal,[28] the MS and control groups had 105 and 79 participants, respectively. *H. pylori* IgG levels were significantly higher in individuals with tiredness than in patients without fatigue in the MS group. The MS group had considerably higher beck depression scale and expanded disability status scale scores.

Another study found a link between *H. pylori*-induced inflammation and neurological illnesses such as multiple sclerosis, Guillain–Barré syndrome, Parkinson's disease, Alzheimer's disease, and other inflammatory diseases such as ischemic stroke.[29] *H. pylori* infection frequently lasts a lifetime, producing a chronic inflammatory response with local release of various inflammatory mediators such as chemokines (IL-8, macrophage chemotactic protein [MCP]-1, growth-regulated oncogene [GRO]-α) and cytokines (IL-1β, TNF-α, IL-6, IL-12, IFN-γ), which can enter the bloodstream, and have a systemic effect. It is believed that the outcome of neurological illnesses will be influenced by the persistence of quantifiable systemic, and local inflammatory mediator concentrations.[30]

Proinflammatory cytokines can promote brain inflammation and neuron death, which have been associated with Parkinson's disease and may play a role in the development of Alzheimer's disease. The systemic inflammatory response is a common element and determinant in disease initiation, development, and prognosis, despite the fact that the majority of neurological disorders are caused by a mix of variables.[31,32]

However, some research has not discovered a connection between MS and *H. pylori* infection, where the seropositivity rate of *H. pylori* IgA was 11.9% in the study group but 30.2% in the control group and the seropositivity rate of *H. pylori* IgG was 33.3% in the study group but 67.4% in the control group. Therefore, *H. pylori* seropositivity to IgA and IgG showed a significant inverse association with affection to MS. In accordance with the findings of this study, *H. pylori* infection is not a cause of classic MS but it may be a protective factor.[33,34]

The results of the present study were compatible with other studies. A study showed that in response to *H. pylori*, IL-8 is secreted by host cells to attract components of the innate and adaptive immune systems to the site of infection. Toll-like receptor 2 (TLR2) and TLR5 have been shown to recognize *H. pylori* and initiate signaling pathways that result in enhanced activation of NF-kB.[35,36]

Another study assessed the results of new evidence on the different effects of *H. pylori* strains and the possible roles of their CagA variants on IL-8 induction. It seems that not only the carriage of CagA and its expression but also the diversity of EPIYA motifs are involved in IL-8 induction in gastric epithelial cells.[37,38]

Additionally, a study showed the level of IL-8 could be different in MS patients than in controls.[39] These differences may be related to damage to the blood–brain barrier (BBB). BBB damage is quantified by the quotient of albumin (Q-alb). Levels of IL-8 in CSF were significantly higher in MS patients than in controls (Mann–Whitney *U* test, $P < 0.0001$). However, this study is not consistent with our research in serum test, showing that serum levels of IL-8 were significantly lower in MS patients than in controls (Mann–Whitney *U* test, $P = 0.018$).[40,41]

Another study by Al-Sammariey[42] proved the relationship between IL-8 and MS where it was found that the serum value (mean ± SD) of IL-8 level was significantly elevated ($P < 0.001$) in patients with MS in comparison with healthy controls (129.11 ± 17.78 ng/mL, 79.83 ± 7.34 ng/mL).

Furthermore, the results of the present study were in agreement with the study by Manjili *et al.*[43] provide the etiology of the MS disease environmental factors, including pathogen-associated molecular patterns (PAMPs) and internal factors such as damage-associated molecular patterns (DAMPs), the pathogen recognition receptors (PRRs) are the main sensors for the PAMPs and DAMPs. Therefore, it seems that the PRRs have been considered to be the plausible molecules participating in the etiology of MS. Toll-like receptors (TLRs) have been the widely studied PRRs and their roles have been documented in human-related diseases. TLR4 is the main PRR expressed on the cell surface of several immune cells including macrophages and dendritic cells. Several investigations reported that TLR4 is the main molecule

involved in the pathogenesis of proinflammatory-based diseases.[43]

H. pylori colonizes the human stomach, and thus the gastric epithelium is the first barrier to encounter the pathogen. Consequently, gastric epithelial cells are primarily infected; however, various innate immune cells comprising macrophages, conventional dendritic cells (cDCs), and neutrophils also reside in the lamina propriety of infected individuals. Of note, CD1c + conventional DCs (cDC2s) are known to penetrate the gastric epithelial lining and directly interact with *H. pylori* via their luminal end.[44,45]

Analysis of cytokine expression and secretion at early time points revealed that type IV secretion system (T4SS is a sophisticated infection apparatus of *H. pylori* that releases virulence factors and other bacterial products into the host cell) contributes significantly to the expression and release the proinflammatory cytokines IL-1β, CXCL8/IL-8, and TNFα in monocyte-derived DCs (moDCs).[46,47]

TLR signaling is known to be activated during *H. pylori* infection, with TLR2, TLR4, and TLR10 signaling on the *H. pylori* infection at epithelial cells. However, studies investigating TLRs, especially TLR10, in immune cells, are rare. To unravel the impacts of TLR2, TLR4, and TLR10 signaling during *H. pylori* infection of human conventional dendritic cells (cDC2s), cDC2s were treated with blocking antibodies targeting TLR2, TLR4, or TLR10 prior to *H. pylori* infection. Inhibition of TLR10 resulted in enhanced *H. pylori*-induced release of proinflammatory cytokines, including IL-1β, IL-12, GM-CSF, and IFNγ.[48] These data indicate that TLR10 acts in an immuno-modulatory manner, because TLR10 inhibition results in increased induction of numerous cytokines in cDC2s. In contrast, diminished levels of primarily inflammatory cytokines were observed with TLR4 blocking; thus, we propose that TLR4 is a driver of the inflammatory response during *H. pylori* infection of cDC2s. Interestingly, TLR2 drives inflammation by promoting the release of inflammatory cytokines on the one hand and seems to dampen chemotaxis by blocking CXCL1 and CXCL8 secretion on the other hand.[49]

The differences in this study could be attributed to the small sample size, the age of the patients, the location from which the samples were collected, and the psychological conditions of the patients. Other factors that predispose to higher rates include low socioeconomic status and less education, in addition to genetic factors.[50-52]

Conclusion

H. pylori may have a role in the pathogenesis of multiple sclerosis, on the other hand, IL-8 and TLR4 genes, appeared to be a useful molecular tool for the detection of *H. pylori* isolates in multiple sclerosis patients and, they could represent a useful molecular method for detecting *H. pylori* isolates in other human diseases.

Acknowledgments

We would like to thank all patients who participated in this study as well as all of the hospital's healthcare professionals for their efforts in providing for these patients. We are also extremely grateful to all those who are patients suffering from multiple sclerosis suspected with *H. pylori*.

Financial support and sponsorship

Nil.

Conflict of interest

The authors have no conflicts of interest to declare.

References

1. Pedrini MJ, Seewann A, Bennett KA, Wood AJ, James I, Burton J, *et al*. *Helicobacter pylori* infection as a protective factor against multiple sclerosis risk in females. J Neurol Neurosurg Psychiatry Res 2015;86:603-7.
2. Abbas SS, Hadi TM. The relationship between *Helicobacter pylori* infection and childhood idiopathic thrombocytopenic purpura. J Fac Med Baghdad 2007;49:257-60.
3. Lisak RP. Human retrovirus pHEV-W envelope protein and the pathogenesis of multiple sclerosis. Proc Natl Acad Sci USA 2019;116:14791-3.
4. Abdulla AR, Ali AH, Al-Rawaq KJ, Ibraheem AN. IL-10 serum level estimation in Iraqi colorectal and gastric cancer patients. J Fac Med Baghdad 2012;54:167-71.
5. Ahmed IE, Al-Jaberi HK, Alkahlissi MM. Comparison of proton density MRI and T2-weighted fast echo for the detection of cervical spinal cord multiple sclerosis lesions. J Fac Med Baghdad 2018;60:195-201.
6. Al-Derzi AR, Alani MM, AL-Hadithi HS. Epstein-barr virus antibodies in rheumatoid arthritis Iraqi patients. J Fac Med Baghdad 2013;55:358-61.
7. Scalfari A, Lederer C, Daumer M, Nicholas R, Ebers GC, Muraro PA. The relationship of age with the clinical phenotype in multiple sclerosis. Mult Scler 2016;22:1750-8.
8. Al-Hamadani HA, Marah HA, Al-Saffar F. Comparison of familial and sporadic multiple sclerosis in Iraqi patients. J Fac Med Baghdad 2012;54:1-6.
9. Al-Omary HL. Expanded disability status scale in multiple sclerosis: Relationship to visual evoked potential. J Fac Med Baghdad 2022;64:96-101.
10. Fahad QA. Paediatric multiple sclerosis: A case report of missed and dismissed diagnosis. J Fac Med Baghdad 2021;63:36-9.
11. Thamer AA, Mohammed NH, Ibrahim AM. The role of trace elements in multiple sclerosis. J Fac Med Baghdad 2014;56:116-20.
12. Aljawadi ZA, Al-Derzi AR, Abdul-Majeed BA, Almahdawi AM. MicroRNAs (20a, 146a, 155, and 145) expressions in a sample of Iraqi patients with multiple sclerosis. J Fac Med Baghdad 2016;58:371-7.
13. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, *et al*. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292-302.
14. Reshetnyak VI, Burmistrov AI, Maev IV. *Helicobacter pylori*: Commensal, symbiont or pathogen? World J Gastroenterol 2021;27:545-60.
15. De Falco M, Lucariello A, Iaquinto S, Esposito V, Guerra G, De Luca A. Molecular mechanisms of *Helicobacter pylori* pathogenesis. J Cell Physiol 2015;230:1702-7.
16. Mohammed HA, Obaid ZH. Pathogenesis of *Helicobacter pylori*: An overview of bacterial virulence agents and the mechanism of occurrence disease. JUBPAS 2022;30:47-53.

17. Madloom BM, Umran HH. *Helicobacter pylori* serology in a sample of Iraqi patients with chronic renal failure. Med J Babylon 2021;18:28.
18. Smolka AJ, Schubert ML. *Helicobacter pylori*-induced changes in gastric acid secretion and upper gastrointestinal disease. Curr Top Microbiol Immunol 2017;400:227-52.
19. Abdulridha RH, Saud AM, Alosami MH. Assessment of miR-146a gene polymorphisms in patients with systemic lupus erythematosus. Iraqi J Sci 2023;64:573-82.
20. Li N, Wang J, Yu W, Dong K, You F, Si B, *et al*. MicroRNA-146a inhibits the inflammatory responses induced by interleukin-17A during the infection of *Helicobacter pylori*. Mol Med Rep 2019;19:1388-95.
21. Vandana UK, Barlaskar NH, Gulzar AB, Laskar IH, Kumar D, Paul P, *et al*. Linking gut microbiota with the human diseases. Bioinformation 2020;16:196.
22. Meliț LE, Mărginean CO, Mărginean CD, Mărginean MO. The relationship between toll-like receptors and *Helicobacter pylori*-related gastropathies: Still a controversial topic. J Immunol Res 2019;2019:1-10.
23. Congedi J, Williams C, Baldock KL. Epidemiology of *Helicobacter pylori* in Australia: A scoping review. PeerJ 2022;10:e13430.
24. Boonyanugomol W, Chomvarin C, Hahnvajanawong C, Sripa B, Kaparakis-Liaskos M, Ferrero RL. *Helicobacter pylori* cag pathogenicity island (cag PAI) involved in bacterial internalization and IL-8 induced responses via NOD1-and MyD88-dependent mechanisms in human biliary epithelial cells. PLoS One 2013;8:e77358.
25. SAS S. Statistical Analysis System, User's Guide. Statistical. Version 9. 6th ed. Cary, NC: SAS. Inst. Inc.; 2018.
26. Aboud RS, Aboud AS, Saleh GM, Fadil HY. Detection of anti-*Helicobacter pylori* in patients with multiple sclerosis. Curr Res Microbiol Biotechnol 2018;6:1536-40.
27. Zhao Q, Yin W, Zhao R, Wang Y, Song C, Wang H, *et al*. Outer inflammatory protein of *Helicobacter pylori* impacts IL-8 expression, adherence, cell apoptosis and cell cycle of gastric cells independent of its copy number. Med Microbiol Immunol 2020;209:621-30.
28. Cam M, Akcalı A. Relationship between fatigue and *Helicobacter pylori* infection in patients with multiple sclerosis. J Mult Sclerosis Res 2022;2:36-40.
29. Lee HW, Lee HM, Yoon SR, Kim SH, Ha JH. Pretreatment with propidium monoazide/sodium lauroyl sarcosinate improves discrimination of infectious waterborne virus by RT-qPCR combined with magnetic separation. Environ Pollut 2018;233:306-14.
30. Arjmandi D, Abdollahi A, Ardekani A, Razavian I, Razavian E, Sartip B, *et al*. *Helicobacter pylori* infection and risk of multiple sclerosis: An updated meta-analysis. Helicobacter 2022;27:e12927.
31. Al Ghuraibawi ZA, Sharquie IK, Gorial FI. A novel link of serum IL-39 levels in patients with rheumatoid arthritis. Iraqi J Sci 2023;30:1651-61.
32. Abd-Alrahman TZ, Aboud RS, Al-Ahmer SD. Immunological and molecular detection of *Helicobacter pylori* in patients clinically diagnosed with chronic urticarial and atopic dermatitis. Curr Res Microbiol Biotechnol 2017;5:1082-7.
33. Álvarez-Arellano L, Maldonado-Bernal C. *Helicobacter pylori* and neurological diseases: Married by the laws of inflammation. World J Gastrointest Pathophysiol 2014;5:400-4.
34. Kira JI, Isobe N. *Helicobacter pylori* infection and demyelinating disease of the central nervous system. J Neuroimmunol 2019;329:14-9.
35. Jasim RZ. Positive and negative aspects of copaxone (Glatiramer acetate) action on TC, TG HbA1c and iron levels in the sera of Iraqi women with multiple sclerosis in Baghdad. Baghdad Sci J 2017;14:0157.
36. Alvestegui A, Olivares-Morales M, Muñoz E, Smith R, Nataro JP, Ruiz-Perez F, *et al*. TLR4 participates in the inflammatory response induced by the AAF/II fimbriae from enteroaggregative Escherichia coli on intestinal epithelial cells. Front Cell Infect Microbiol 2019;9:1143.
37. Choi MS, Ze EY, Park JY, Shin TS, Kim JG. *Helicobacter pylori*-derived outer membrane vesicles stimulate interleukin 8 secretion through nuclear factor kappa B activation. Korean J Intern Med 2021;36:857.
38. Al-Naseri MA, Ad'hiah AH, Salman ED. Predictive significance of interleukins 17A and 33 in risk of relapsing–remitting multiple sclerosis. Baghdad Sci J 2022;19:1191.
39. Matejčíková Z, Mareš J, Sládková V, Svrčinová T, Vysloužilová J, Zapletalová J, *et al*. Cerebrospinal fluid and serum levels of interleukin-8 in patients with multiple sclerosis and its correlation with Q-albumin. Mult Scler Relat Disord 2017;14:12-5.
40. Neuper T, Frauenlob T, Sarajlic M, Posselt G, Wessler S, Horejs-Hoeck J. TLR2, TLR4 and TLR10 shape the cytokine and chemokine release of *H. pylori*-infected human DCs. Int J Mol Sci 2020;21:3897.
41. Shehab MJ, Mahdi BM, Al-Tabra RH, Namaa DS. Evaluation of serum levels of Proinflammatory Cytokines IL-8, IL-17, and IL-22 in *Helicobacter pylori* infection and their association with the degree of gastritis histopathology in a sample of Iraqi patients. Baghdad Sci J 2023;20:0937.
42. AL-Sammariey KA. Serum interleukin-8 and Hs-C reactive protein determination in Iraqi multiple sclerosis patients: Case control study. Iraqi Postgraduate Med J 2014;13:153-5.
43. Manjili FA, Yousefi-Ahmadipour A, Arababadi MK. The roles played by TLR4 in the pathogenesis of multiple sclerosis; a systematic review article. Immunol Lett 2020;220:63-70.
44. Robinson K, Kaneko K, Andersen LP. Helicobacter: Inflammation, immunology and vaccines. Helicobacter 2017;22:e12406.
45. Liu M, Hu Z, Wang C, Zhang Y. The TLR/MyD88 signalling cascade in inflammation and gastric cancer: The immune regulatory network of *Helicobacter pylori*. J Mol Med 2023;101:767-81.
46. Moghadam MA, Honarmand H, Hatamian H, Roudbary A. *Helicobacter pylori* infection might be a potential protective factor against classic multiple sclerosis in Guilan, Iran. Ann Res Rev Biol 2014;4:4502-10.
47. Chapelle N, Martin J, Osmola M, Hémont C, Leroy M, Vibet MA, *et al*. Serum pepsinogens can help to discriminate between *H. pylori*-induced and auto-immune atrophic gastritis: Results from a prospective multicenter study. Dig Liver Dis 2023;55:1345-51.
48. Dore MP, Fanciulli G, Manca A, Pes GM. Association of *Helicobacter pylori* infection with autoimmune thyroid disease in the female sex. J Clin Med 2023;12:5150.
49. Rugge M, Bricca L, Guzzinati S, Sacchi D, Pizzi M, Savarino E, *et al*. Autoimmune gastritis: Long-term natural history in naïve *Helicobacter pylori*-negative patients. Gut 2023;72:30-8.
50. Waldum HL. Conclusion that autoimmune gastritis does not predispose to gastric cancer is unproven. Gut 2024;73:379.
51. Saqi HA, Nooruldeen MY, Al-kadhi NA. Impact of Helicobacter pylori virulence on development of autoimmune thyroid diseases. Med J Babylon 2023;20(Supplement 1):S168-S172.
52. Jasim ZM, Shemran KA, ALhamdani HSM. Prevalence of Cobalamin (Vitamin B12) Deficiency Associated with Autoimmune Gastritis in Patients Suffering from Autoimmune Disorders. Med J Babylon 2025;22(Suppl 1):S49-S54.