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The Relationship between *Helicobacter pylori* Infection and Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is a chronic autoimmune; inflammatory neurological illness affecting the central nervous system (CNS). MS damages the myelinated axons; in the central nervous system; partially damaging the myelin sheath and axons. *Helicobacter pylori* promotes stomach inflammation and because of the maintenance of chronic inflammation in the gastric mucosa therefore, the virulence factors of bacteria directly affect the development of symptoms of multiple sclerosis. The aim of present study was to evaluate the association of *H. pylori* infection with multiple sclerosis disease One hundred blood samples were collected between January 2022 and June 2022 from individuals aged 13 to 65. These samples were evaluated and diagnosed by the consulting medical team at Dr. Saad Al-Witry Hospital for Neurosciences. The patients were subjected to a comparative analysis with a control group consisting of 30 individuals who were deemed to be the healthy control group. The patients were divided into three groups. The first group consisted of 36 patients diagnosed with multiple sclerosis who were also found to have *H. pylori* infection. The second group comprised 34 patients with multiple sclerosis who did not exhibit *H. pylori* infection. Lastly, a control group consisting of apparently healthy individuals was included in the study. The levels of anti-*H.pylori* IgA, IgG, TNF-α, IL-1β, and IFN-γ antibodies were quantified utilizing an enzyme-linked immunosorbent assay (ELISA), and IL-8 genes were detected in the serum of multiple sclerosis patients and the control group using a Real-Time Polymerase Chain Reaction (RT-PCR) technique. The statistical analysis demonstrates a substantial and statistically significant rise ($P \leq 0.01$) in the levels of anti-*H. pylori* IgA, IgG, TNF-α, IL-1β, and IFN-γ antibodies in the blood samples of individuals diagnosed with multiple sclerosis who also have *H. pylori* infection. In comparison to the control group, the experimental group exhibits a statistically significant rise ($P \leq 0.01$) in multiple sclerosis (MS) conditions in the absence of *H. pylori*. *IL-8* gene expression indicate 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 disease without *H.pylori*. It was concluded demonstrates that *H.pylori* infection plays a significant role as a triggering factor for multiple sclerosis disease.

Keywords: IgA, TNF-α, IL-1β, IFN-γ, IL-8, Multiple sclerosis.

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune and inflammatory neurological disease condition that impacts the central nervous system (CNS). Multiple sclerosis is distinguished by the pathological targeting of myelinated axons within the central nervous system, leading to the degradation of both the myelin sheath and the axons to different degrees. The trajectory of multiple sclerosis exhibits a high degree of unpredictability and variability. In the majority of patients, the disease manifests initially through episodes of reversible nervous impairments, subsequently leading to a progressive decline in nervous

function(1). *Helicobacter pylori* is a bacterium that belongs to the Gram-negative group and exhibits microaerophilic characteristics. The dimensions of the object under observation vary between 2-4 μm in length and 0.5-1 μm in width, displaying a spherical morphology. The microorganism demonstrates motility due to the presence of 2-6 polar sheathed flagella, each flagellum is around three μm in length. *H. pylori* has the ability to undergo morphological changes and transition into a non-culturable, coccoid form under conditions of starvation (2). The bacterium has the ability to endure short-term exposure to low pH levels, but it can only thrive under conditions of neutral pH, which are typically present within the mucus layer of the gastric mucosa. The impact of *H. pylori* infection on gastric acid production is contingent upon several factors that include the severity of the infection and its anatomical location. In certain individuals, the infection may progress to the corpus, resulting in the subsequent emergence of atrophy. This atrophy leads to the destruction of parietal cells, ultimately causing hypochlorhydria (3).

The elevation of inflammatory cytokines, such as IL1-β and TNF-α, plays a crucial role in mediating this process. The vast majority of individuals infected with *H.pylori* produce distinct antibodies that can be detected in serum, as well as in gastric aspirates or stomach extracts. As a result, individuals who are infected with *H.pylori* have been observed to display elevated levels of IgG and IgA antibodies that specifically target various proteins present in the bacterial membrane, such as flagellin, urease, lipopolysaccharide (LPS), and *H.pylori* adhesin A (HpaA) (4).

Tumor necrosis factor alpha (TNF-α) was first recognized for its involvement in the induction of tumor necrosis. However, recent research has revealed that TNF-α also plays a significant role as a pathological component in autoimmune diseases. Tumor necrosis factor-alpha (TNF-α) exhibits binding affinity towards two distinct receptors, thereby initiating the activation of signal transduction pathways (5). These signaling pathways give rise to diverse cellular outcomes, encompassing cell viability, differentiation and proliferation. Nevertheless, the inappropriate or excessive activation of tumor necrosis factor-alpha (TNF-α) signaling has been linked to chronic inflammation, which in turn can potentially give rise to the emergence of pathological complications, including autoimmune diseases. The comprehension of the signaling mechanism of TNF-α has been enhanced and utilized in the management of immune disorders, leading to the creation of efficacious therapeutic interventions, such as TNF-α inhibitors (6).

Interleukin-1β (IL-1β) is a highly potent pro-inflammatory cytokine that exerts diverse effects on the immune response in various immune-mediated disorders, including multiple sclerosis (7).

IL-1β is a key participant in the neuro-inflammatory process associated with multiple sclerosis. It is synthesized by various cell types including monocytes, microglial cells, astrocytes, and cerebral endothelial cells. IL-1β plays a crucial role in promoting the movement of activated leukocytes across the endothelial layer and into the central nervous system (CNS) (8).

Interferon-gamma (IFN-γ) is a cytokine that is predominantly synthesized by various immune cell

types. The aforementioned entities encompass different types of lymphocytes, including innate-like lymphocytes such as natural killer (NK) cells and innate lymphoid cells (ILCs), as well as adaptive immune cells like T helper 1 (TH1) cells and CD8+ cytotoxic T lymphocytes (CTLs) (9).

Interleukin-8 (IL-8) is an important chemokine in mediating the inflammatory response to *H.pylori*. Demonstrated that both the TNF-β and activating protein 1 (AP1) DNA binding sites within the IL-8 promoter are required for optimal transcription in response to infection of gastric epithelial cells by *H. pylori* (10). During *H.pylori* infections, both NF-β and members of the mitogen-activated protein kinase (MAPK) family become activated. Activated MAPKs then phosphorylate AP-1complexes, which results in increased AP-1-dependent transcription. As such, signaling pathways that activate TNF-β and/or AP-1 could result in increased IL-8 secretion (11).

The primary aim of the current investigation was to ascertain the potential correlation between *H. pylori* infection and the occurrence of multiple sclerosis.

Materials and methods

Studied subject samples

The study sample consisted of 70 patients, comprising 48 females and 22 males, who were diagnosed with multiple sclerosis. The patients' ages ranged from 13 to 65 years. The data was collected from Dr. Saad Al-Witry Hospital for Neurosciences over a period spanning from January 2022 to June 2022. The patients were diagnosed by the medical staff consultants at the clinic. The patient groups were classified into three distinct categories. In the initial cohort, there were 36 individuals diagnosed with multiple sclerosis who were also found to have *H. pylori* infection. Thirteen of these individuals were male and 23 were female, with a mean age of 39 years. The second group consisted of 34 individuals diagnosed with multiple sclerosis, all of them were negative for *H.pylori* infection. This group comprised of 9 males and 25 females, with a mean age of 41.5 years. The control group refers to a group of subjects or participants in an experiment who do not receive the experimental treatment or intervention. They are the study consisted of a cohort of 30 individuals apparently healthy control groups. The cohort was comprised of 13 males and 17 females, with an average age of 35.5 years. All participants reported no history of gastrointestinal diseases or any other health complaints.

Blood samples collection

Four ml of blood was collected by venipuncture, using plastic disposable 5 ml syringes, from all patients and healthy control. Blood samples was transferred to a gel tube, and then it was let to clot at room temperature (20-25°C) for 15 minutes, centrifuged at 1000 rpm for 10 minutes to separate the serum, the serum sample divided into two parts; the first part was two milliliters transferred into plain tubes stored at -20ºC until carried out to measure the level of IgA, IgG, TNF-α, IL-1B, IFN-γ for patients and healthy control. The second part was three hundred microliter from serum was added into 2 ml tube containing 500 µl Trizol for each patient and healthy individuals and the tube was inverted many times for mixing, for molecular study by using the Trizol to maintain the integrity of the RNA for inhibiting the action of RNase.

Immunological detection of anti-*Helicobacter pyloi* IgA, IgG ,TNF-α, IL-1B, IFN-γ by ELISA method

All the studied groups include multiple sclerosis patients with *H.pylori*, without *H. pylori* and apparently healthy individuals (control group) were submitted to estimate the anti-*Helicobacter pyloi* IgA, IgG antibodies level by using ELISA test (MyBioSource / USA) .

Molecular detection of *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's 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 hours. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay.

c) RT-qPCR protocol: This is the main step in our project have been divided into two phases, the first is done through synthesis of cDNA from RNA as mentioned in Table (1). The second section of this protocol it's done by choosing the cDNA sample from patient and control at the same run, for each sample there are two PCR tubes, one tube for each gene, IL-8 and B2M (Beta-2-Microglobulin) which is consider as a house keeping gene in this study, Table1. The detection of quantity based on fluorescent power of SyberGreen (12). The reaction mix composed from component with their quantity as mentioned in Table2 below:

Table (1). The source of all primers used in this study was macrogen® (Korea). The name and sequence are given.

Name of primer	Sequence	Product size	Reference
IL-8 F **IL-8 R**	(ACTCCAAACCTTTCCACCCC) (CCTCTGCACCCAGTTTTCCT)	143bp	Newly design
B2M F **B2M R**	(CTGGGTTTCATCCATCCGACA) (TCAGTGGGGGTGAATTCAGTG)	122bp	Silver *et al*., 2006 (13)

Table (2): Components of RT-qPCR protocol.

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

Quickly spin for PCR tubes to remove the bubbles and collect the liquid (1 minute at 2000g), then the program for Real-Time PCR was setup with indicated thermocycling protocol as shown in (Table 3).

Table (3): The program for Real-Time PCR with thermocycling protocol.

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	95°C	60 seconds	1
Denaturation **annealing**	95°C 60°C	15 seconds 30 seconds (+plate read)	40-45
Melt Curve	60-95°C	40 minutes	1

Statistical analysis

The Statistical Analysis System (SAS) software was utilized to conduct an analysis on the influence of different variables on study data (14). The analysis of variance (ANOVA) framework was utilize to conduct a least significant difference (LSD) test for the purpose of comparing means. The Chi-square test was utilized to evaluate the statistical significance of variations in percentages, with significance levels established at 0.05 and 0.01. An estimation of the correlation coefficient between various parameters in this study is sought.

Results and discussion

The quantitative measurements of IgA and IgG levels across the different groups examined in the study are presented in Table 4. A statistically highly significant difference ($p \leq 0.01$) was found in the concentration of IgA antibodies between the groups infected with *H.Pylori* and M.S (0.595 ±0.08 U/ml) and the group of individuals who were healthy controls (0.152 ±0.01 U/ml). Furthermore, a statistically highly significant disparity ($p \leq 0.01$) was observed in the levels of IgA antibodies between the groups with Multiple Sclerosis (M.S) (0.175±0.02 U/ml) and the group of individuals without any health conditions (0.152±0.01 U/ml). A statistically highly significant difference ($p \leq 0.01$) was identified in the concentration of IgG antibodies between the groups infected with *H.Pylori* and M.S (0.857±0.07 U/ml) and the group of individuals who were healthy controls (0.166± 0.01 U/ml). Likewise, a statistically highly significant difference ($p \leq 0.01$) was observed in the concentration of IgG antibodies between the groups with M.S (0.323± 0.06 U/ml) and the healthy control group (0.166± 0.01 U/ml).

Table (4): Compare the levels of IgA and IgG antibodies among different groups.

Group	Mean ± SE	
	IgA	**IgG**
H.P+M.S	0.595 ±0.08 a	0.857 ±0.07 a
MS	0.175 ±0.02 b	0.323 ±0.06 b
Control	0.152 ±0.01 b	0.166 ±0.01 b
LSD	0.141 **	0.162 **
P-value	0.0001	0.0001
The presence of distinct letters within the same column exhibited significant variation. ** (P≤0.01)		
M.S= Multiple sclerosis, H.P= *Helicobacter pylori*.		

The results of the current study align with the findings of other previous research investigations. Aboud *et al.* (15) conducted a research in which they detected a statistically significant increase ($p \leq 0.05$) in the concentrations of anti-*H.pylori* IgA antibodies (7.15±1.04 U/ml) and IgG antibodies (7.81±0.96 U/ml) as compared to the control group. The control group displayed antibody levels of 4.99±0.80 U/ml. Furthermore, this study focuses on a research inquiry that was carried out to examine the mean concentration

of anti-*H. pylori* IgG antibodies in a group of 25 participants. The application of the enzyme-linked immunosorbent assay (ELISA) in diverse research investigations has demonstrated a noteworthy protective correlation (odds ratio [OR], 0.59; 95% confidence interval [CI], (0.46-0.77). In a similar vein, other investigations utilizing histological approaches to identify *H.pylori* infection have reported a significant positive connection. (OR, 6.64; 95% CI, 2.40-13.76). The identification of an active *H.pylori* infection has been recognized as a possible risk factor in the pathogenesis of multiple sclerosis (16).

Based on the findings of Cam and Akcal (17), in which the experimental group consisted of 105 participants, while the control group comprised 79 participants, the study observed a significant increase in the levels of *H.pylori* IgG among individuals who reported having tiredness, as compared to patients without fatigue, within the multiple sclerosis group. The cohort who are diagnosed with multiple sclerosis demonstrated notably higher scores on both the Beck Depression Scale and the Expanded Disability Status Scale. The detection of certain antibodies in both blood and stomach aspirates has been found virtually in all persons who are infected with *H. pylori.* In instances when individuals were affected by these bacteria, there was a discernible elevation in the concentrations of IgA and IgG immunoglobulins that selectively recognize membrane proteins, flagellin, urease, lipopolysaccharide (LPS), and *H. pylori* adhesion A. The identification of *H.pylori* infection through innate immune response triggers subsequent signaling cascades that ultimately result in the production of cytokines. Acquiring a thorough comprehension of the immune response to *H.pylori* is of paramount significance in facilitating the progress of more efficacious therapeutic interventions for *H.pylori*-induced pathological states. These conditions encompass a range of diseases, including neurological disorders such as multiple sclerosis. A distinct inquiry has ascertained a connection between inflammation induced by *H.pylori* and diverse neurological disorders, encompassing but not restricted to multiple sclerosis, Guillain-Barré syndrome, Parkinson's disease, Alzheimer's disease, and other inflammatory diseases like ischemic stroke. The infection caused by *H.pylori* commonly endures throughout an individual's lifespan, leading to a continuous inflammatory reaction characterized by the localized release of various inflammatory mediators. These mediators include chemokines such as interleukin (IL)-8, macrophage chemotactic protein (MCP)-1, and growth-regulated oncogene (GRO)-α. Several cytokines, such as IL-1β, tumor necrosis factor (TNF)-α, IL-6, IL-12, and interferon (IFN)-g, have the capacity to enter the circulation and induce systemic reactions. There exists a prevailing idea that the enduring presence of measurable systemic and local concentrations of inflammatory mediators may exert an impact on the prognosis of neurological disorders (18).

Nevertheless, some investigations have been unable to show a significant association between the presence of *H. pylori* infection and the occurrence of multiple sclerosis. In a specific investigation, the prevalence of *H. pylori* IgA seropositivity was seen to be 11.9% among the participants in the study group, whereas it was discovered to be 30.2% among those in the control

group. Similarly, the seropositivity rate of *H. pylori* IgG was 33.3% in the study group, but significantly higher at 67.4% in the control group. Thus, there was a notable negative correlation observed between *H. pylori* seropositivity for IgA and IgG and the incidence of multiple sclerosis. Based on the findings of this inquiry, it has been concluded that the presence of *H. pylori* infection does not function as a causative determinant for classical multiple sclerosis. Nevertheless, there is a possibility that infection with *H. pylori* might serve as a protective factor in regard to this particular illness (19).

The discrepancies seen in this study can be attributed to several variables, such as the sample's restricted size, the dispersion of participant ages, the geographical origins of the gathered samples, and the psychological profiles of the patients. Additional factors that contribute to elevated rates include individuals with lower socioeconomic status and limited educational attainment, in conjunction with genetic predisposition (20).

(Table 5) illustrates the levels of TNF-α, IL-1β, IFN-γ in the study's distinct groups. A statistically highly significant difference was observed ($p \leq 0.01$) in the level of TNF-α, IL-1β, IFN-γ Ab in the *H.Pylori*+M.S groups (501.83 ±52.38, 1426.01 ±150.93 ,392.69 ±40.27) U/ml when compared to the healthy control (148.65 ±6.34, 351.88 ±10.87 ,91.59 ±1.62) U/ml, and a highly significant difference ($p \leq 0.01$) in the level of TNF-α, IL-1β, IFN-γ Ab in the M.S groups (306.15 ±15.07, 647.43 ±20.61, 246.71 ±20.44)U/ml when compared to the healthy control (148.65 ±6.34 , 351.88 ±10.87 ,91.59 ±1.62) U/ml.

Table (5): Comparison between difference groups in TNF-α, IL-1β and IFN-γ.

Group	Mean ± SE		
	TNF-α	IL-1β	IFN-γ
H.P+M.S	501.83 ±52.38 a	1426.01 ±150.93 a	392.69 ±40.27 a
MS	306.15 ±15.07 b	647.43 ±20.61 b	246.71 ±20.44 b
Control	148.65 ±6.34 c	351.88 ±10.87 c	91.59 ±1.62 c
LSD	99.263 **	279.09 **	80.214 **
P-value	0.0001	0.0001	0.0001
The presence of distinct letters within the same column exhibited substantial variation. ** (P≤0.01).			

M.S = Multiple sclerosis, H.P = *Helicobacter pylori*.

The presence of *H.pylori* infection usually persists throughout an individual's lifetime, resulting in a sustained inflammatory response characterized by the localized release of different inflammatory mediators including chemokines (such as interleukin-8, macrophage chemotactic protein-1, and growth-regulated oncogene-alpha) and cytokines (such as interleukin-1 beta, tumor necrosis factor-alpha, interleukin-6, interleukin-12, and interferon-gamma). These mediators have the ability to enter the bloodstream and exert systemic effects. It is probable that the presence of measurable levels of inflammatory mediators in both systemic and local contexts can have an impact on the progression and outcome of neurological disorders. These proinflammatory factors have the potential to initiate brain inflammation and neuronal cell death, potentially leading to the onset of Parkinson's disease. Additionally, they may play a role in the progression of Alzheimer's disease. Nevertheless, it is important to

note that the majority of neurological diseases arise from a confluence of various factors. However, a recurrent factor that significantly influences the onset, development, and final result of these conditions is the systemic inflammatory response (21).

The results of the current study align with the findings of several previous researches. There is an evidence indicating that multiple sclerosis, akin to other autoimmune disorders, that could be potentially instigated by microbial infections. The pathogens that have been linked to the development or worsening of multiple sclerosis encompass various microorganisms. These include bacteria, such as *Chlamydia pneumoniae*, as well as enterotoxins produced by *Staphylococcus aureus* that act as superantigens.

Furthermore, there have been associations between multiple sclerosis and viruses that belong to the Herpesviridae family, including Epstein-Barr virus and human herpes virus. Additionally, viruses from the human endogenous retrovirus families have also been linked to MS. Furthermore, it has been observed that there is a correlation between multiple sclerosis exacerbations and the occurrence of typical upper respiratory, gastrointestinal, and urogenital tract infections. Microbial agents have the potential to influence the neuroimmune system in individuals who are genetically predisposed (22).

A separate investigation revealed a correlation between inflammation caused by *H. pylori* and neurological disorders. *H.pylori* is primarily recognized for its ability to induce various gastrointestinal impairments. However, an increasing body of research indicates that *H. pylori* infection may also have implications in other extragastric illnesses, including neurological, dermatological, hematologic, ophthalmic, cardiovascular, metabolic, hepatobiliary, and allergy diseases. The primary cause of neurological impairments resulting from *H. pylori* infection is attributed to dysfunctions in the gut-brain axis (GBA) and an altered gut microbiota, both of which are facilitated by *H. pylori* colonization(23).

Nevertheless, certain studies have been failed to establish a correlation between multiple sclerosis and *H. pylori* infection. These studies revealed that individuals with MS exhibited significantly lower levels of *H.pylori* seropositivity compared to their healthy counterparts ($P < 0001$). Moreover, this study has uncovered a significant association between the detection of antibodies for *H. pylori* and reduced scores on the Expanded Disability Status Scale (EDSS) in individuals with a diagnosis of multiple sclerosis, when compared to MS patients who do not exhibit these antibodies ($P < .011$). Furthermore, our study demonstrated that the levels of proinflammatory cytokines, including IFN-γ, TNF-α, IL-6, and IL-17, were found to be lower in multiple sclerosis patients infected with *H. pylori* compared to MS patients who tested negative for the infection. Moreover, it was shown that the concentrations of anti-inflammatory cytokines, specifically IL-4 and IL-10, were notably elevated in multiple sclerosis patients who were infected with *H. pylori*, in comparison to MS patients who tested negative for *H. pylori* infection. This study demonstrates a negative correlation between *H. Pylori* infection and multiple sclerosis.

The prevalence of autoimmune diseases has exhibited a consistent upward trend. Concurrently, there has

been a decline in the prevalence of the majority of infectious diseases. The aforementioned observations align with the hygiene hypothesis, a theory positing that a decrease in infection rates is directly linked to the rise in autoimmune and allergic diseases (24). Highly significant difference ($p \leq 0.01$) were recorded in the level of *IL-8* gene expression in different groups in the study as shown in Table 6. 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 (1.00 ±0.00 U/ml) and a highly significant difference ($p \leq 0.01$) in the level of *IL-8* gene expression in M.S groups (3.03 ±0.59 U/ml) compared to healthy control (1.00 ±0.00 U/ml).

Table (6): Results of *IL-8* gene expression in difference groups

Group	HKG	$\Delta ct_{patient}$	ΔΔct	Fold of expression
***H.Pylori*+M.S**	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 with the different letters in same column differed significantly ** (P≤0.01)				

M.S = Multiple sclerosis, H.P = *Helicobacter pylori*.

The results of the present study was compatible with other studies. One study showed in response to *H.pylori*, interleukin-8 (IL-8) is secreted from host cells to attract components of the innate and adaptive immune systems to the site of infection (25). Also other study assessed the results about new evidence at different effects of *H. pylori* strains and possible roles of their CagA variants on IL-8 induction. It seems that not only carriage of cagA and its expression, but also diversity in EPIYA motif be involved in IL-8 induction in the gastric epithelial cells , as well as another study showed that the level of interleukin-8 (IL-8) could be different in MS patients than in controls. These differences may be related to damage of 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, $p<0.0001$), but that is different from this research in serum levels of IL-8 that were significantly lower in MS patients than in controls (Mann-Whitney U test, $p=0.018$) (26). Another study was done by AL-Sammariey (1) proved the relationship between IL-8 and MS, in which it was found that 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±17.78ng/ml, 79.83±7.34ng/ml).

A study was done by Manjili *et al.* (27) provide the etiology of the MS disease which is cause by environmental factors, including pathogen-associated molecular patterns (PAMPs) and the 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. In which *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 propria 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 endings (28).

Analysis of cytokine expression and secretion at early time points reveales that the 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) significantly contributes to the expression and release of the pro-inflammatory cytokines IL-1β, CXCL8, IL-8, and TNFα in monocyte-derived DCs (moDCs) (29).

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 includes low socioeconomic status and less education, in addition to genetic factors(30).

Conclusion

The present study posits that the presence of *H. pylori* infection is a substantial contributing factor in the development of multiple sclerosis.

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