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The protective effect of *Helicobacter Pylori* infection on the susceptibility of multiple sclerosis

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ABSTRACT

In recent years, a possible protective role of *Helicobacter pylori* (Hp) against autoimmune disease has been reported in an experimental murine model of multiple sclerosis (MS), and there are restricted conflicting epidemiologic data concerning Hp serology in MS patients. This study was aimed to determine the seroprevalence of Hp in MS patients and then investigates pro/anti-inflammatory cytokine levels in MS patients infected with HP and seronegative MS patients. Three hundred eighty-seven patients with MS were included in the study and were adjusted by gender and age to 420 healthy subjects. An enzyme-linked immunoassay (ELISA) was used to determine the presence of specific IgG antibodies against *H. pylori* in the serum sample of both groups. Some pro/anti-inflammatory cytokines levels were evaluated in seropositive/seronegative MS patients and healthy individuals. Our result showed that in patients with MS HP seropositivity was significantly lower than the healthy individual ($P < .0001$). Also, we showed that HP seropositive MS patients had lower Expanded Disability Status Scale (EDSS) when compared with seronegative MS patients ($P < .011$). Moreover, we illustrated that proinflammatory cytokine levels include IFN-γ, TNF-α, IL-6, and IL-17 in MS patients infected with HP were lower than seronegative MS patients. Besides, the levels of anti-inflammatory cytokines include IL-4 and IL-10 was significantly higher in MS patients infected with HP when compared with MS patients seronegative for HP infection. In this study, we indicated that HP infection negatively correlated with MS, and conceivably may act as a protective agent against MS. The precise mechanism behind this protective effect remains elusive. However, it seems HP can modulate cytokine signaling, which involved in MS pathogenesis.

1. Introduction

The occurrence of autoimmune diseases has been continuously increasing. Simultaneously, the incidence of most infectious diseases has fallen. These observations are consistent with the hygiene hypothesis, which suggested that a decline in the incidence of infections directly contributes to the development in the incidence of autoimmune and allergic diseases. This hypothesis based on robust epidemiological data, but the underlying mechanisms are uncertain. Pathogens are recognized to be necessary, as the autoimmune disease is hampered in multiple experimental models by infection with diverse bacteria, viruses, and parasites (McAlpine and Compston, 2005). MS is a multifactorial autoimmune disorder of the central nervous system (CNS), which believed results from the incorporation of genetic and environmental factors and their interplays (Ramagopalan et al., 2010). Expansion of autoreactive T cell responses against CNS-derived antigens leads to the entrance of T helper type 1 (TH 1) cells and T helper type 17 (TH17)cells toward the spinal cord and CNS (Baker et al., 2011; Goverman, 2009). These cells create damage to the myelin sheath of neural axons, accompanying inflammation, and degeneration of nerves (Frohman et al., 2006). Previous studies have indicated that the balance between proinflammatory Th1 and Th17 responses and anti-inflammatory Treg responses, either in terms of numbers or functional activity, are essential in MS development and progression (Edström et al., 2011; Sellebjerg et al., 2012). HP is a gram-negative bacterium, which drives infecting nearly half the world's population whose main reservoir is the human stomach. The prevalence of infection varies through the geographic area, age, ethnicity, and socio-economic status; in fact, the prevalence is higher in developing countries and those with poor socio-economic conditions (Ahmed et al., 2006; Go, 2002; Leclerc, 2006; Mandeville et al., 2009). While the majority of HP hosts stay asymptomatic, infection with it has mainly correlated with chronic

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gastritis, peptic ulcer disease, gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma (Banatvala et al., 1993; Kusters et al., 2006).

Furthermore, some reports have figure out a correlation between HP infection and extra-gastrointestinal disorders (such as cirrhosis, pancreatic cancer, coronary heart disease, stroke, migraine, mild cognitive impairment, Alzheimer's and disease and autoimmune diseases) (Yao et al., 2016). Recently, several studies revealed that a steady rise in autoimmune disease incidence in developed countries, which could propose that the concomitant decline in infectious disease prevalence might explain the developed autoimmune disease incidence (Farrokhyar et al., 2001; Poser et al., 1989). HP infection is more common in developing countries, while the incidence is declining in western countries. The lower frequency of infections in the western countries are mainly associated with higher hygiene standards and extensive use of antibiotics (Malaty, 2007). Paradoxically, many studies are reporting the advantages of infection, including a reduction in the likelihood of developing atopic disorders later in life if infected with HP in childhood (McCune et al., 2003). Infected people without gastroduodenal disease lead to possess a more robust Treg response, which may additionally protect against extra-gastric situations such as asthma, allergy, and inflammatory bowel disease (Amberbir et al., 2014; Arnold et al., 2011; Arnold et al., 2012; Kao et al., 2010; Wang et al., 2013). There have been several cross-sectional epidemiological studies reporting a lower prevalence of HP among patients with MS (Li et al., 2007; Mohebi et al., 2013; Wender, 2003; Yoshimura et al., 2014). Recently, several studies demonstrated that patients (Cook et al., 2014) and mice (Kao et al., 2010) infected with HP had promoted Treg populations in their peripheral blood. To date, there is very slight evidence to evaluate the links between MS and HP conclusively. The current study aimed to determine the seroprevalence of HP infection in patients with MS and then assess the levels of proinflammatory cytokines (include IL-1β, IFN-γ, TNF-α, IL-6, IL-12(p70), and IL-17) and anti-inflammatory cytokines (include IL-4 and IL-10) in MS patients infected with HP and MS patients seronegative for HP.

2. Material and method

2.1. Patient and sample collection

The study was conducted in the Isfahan Multiple Sclerosis Society (IMSS). In this case-control study, we first investigated the prevalence of HP infection in MS patients and healthy subjects Table 1. After defining the status of HP infection, the MS patients and healthy individual divided into HP seropositive and seronegative groups Table 2. After grouping our samples, then we evaluate the levels of pro/anti-inflammatory cytokines in the serum of patients with MS and healthy people in order to test whether serum levels of selected cytokines in people with HP infection compared with uninfected people is significant or not. The type of MS disease in MS patients was relapsing-remitting multiple sclerosis (RRMS) as were on IFN-beta. In this study, we measured the levels of inflammatory/anti-inflammatory cytokines in the serum of seropositive/seronegative groups, besides age- and sex-matched between different groups. A trained neurologist determined the Expanded Disability Status Scale (EDSS) of patients. Inclusion criteria were: age 20–60 years, EDSS score < 7.0 and written informed consent from all participants. Exclusion criteria were: taking immunosuppressant drugs at least in the six months preceding study entry and taking any anti-HP medication. MS patients had a relapse-remitting disease course and were on IFN-β treatment (for at least two years). In each group of study, 5 ml of venous blood samples were drawn and then centrifuged to obtain a sufficient amount of serum. Sera were immediately frozen and stored at −70 °C until usage.

2.2. Enzyme-linked immunosorbent assay (ELISA)

2.2.1. ELISA for H. pylori infection

The serum levels of IgG anti-HP was measured in duplicate using a commercially available ELISA kit (IBL, Hamburg, Germany, RE56381) according to the manufacturer's instructions. All standards and samples were carried out in duplicate. Optical density (OD) was measured at 450 nm using a BioRad 650 microplate reader (BioRad Laboratories Inc., USA) and concentrations of IgG anti-HP reported as U/mL. IgG anti-H*P* values > 12 U/mL were considered as positive and 8 U/ml as deemed negative.

2.2.2. ELISA for pro/anti-inflammatory cytokine

The concentration of cytokines in serum was measured by ELISA kits according to the manufacturer's instructions: IL-1β, IFN-γ, TNF-α, IL-2, IL-4, IL-10, IL-12, and IL-17. Briefly, the serum added to a 96-well ELISA plate, and next reacted with their primary cognate antibodies and HRP-conjugated secondary antibodies. Tetramethyl benzidine (TMB) applied as the substrate, and the absorbance was measured at 450 nm using a BioRad 650 microplate reader (BioRad Laboratories Inc., USA). (Of note, IFN-γ, IL-4, IL-6, IL-10, IL-17, and IL-12 (p70) purchased from BOSTER BIOLOGICAL TECHNOLOGY, LTD, Pleasanton, CA, United States, and TNF-α and IL-1β purchased from Abcam, Cambridge, UK).

2.3. Statistical analysis

The analysis of the obtained data was done using the SPSS software version 25. The quantitative variables represented as mean ± SD and qualitative variables as percentages. In the case of normality, the difference between groups was calculated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. In the case of non-normality, the difference between the values of experimental groups was assessed utilizing the Kruskal-Wallis test, followed by Dunn's procedures for pairwise comparisons. The graphs were showed by the GraphPad software version 8. The level of statistical significance was set at $p < .05$.

3. Result

3.1. Demographic and clinical characteristics

As shown in Table 1, all participants, including RRMS patients and healthy individuals, were matched in terms of sex and age, as there were no significant differences among them ($P > .05$). Additionally, the Expanded Disability Status Scale (EDSS) and duration of disease in

Table 1
Demographic and clinical features of MS patients along with healthy subjects.

	RRMS patients (n = 386)	Healthy subjects (n = 420)	P value
Gender (male/female)	186/200	202/218	P > .05
HP seropositivity	188/386 (87/101), 48.7%	298/420 (142/156), 70.9%	P < .05
Age (mean ± SD)	31 ± 8.5	32 ± 7.4	$P > .05$
Disease duration (year ± SD)	5.03 ± 1.2	NA	
EDSS (mean ± SD)	2.6 ± 1.4	NA	

RRMS; relapsing-remitting MS, HP; *Helicobacter pylori*, SD; standard deviation, NA; not applicable.

Table 2
Define four groups after determine HP infection history.

	MS HP positive (n = 120)	MS HP negative (n = 120)	HS HP positive (n = 120)	HS HP negative (n = 120)	P value
Gender (male/female)	60/60	60/60	60/60	60/60	
Age (mean ± SD)	29 ± 9.5	31 ± 7.5	33 ± 6.4	31 ± 7	P > .05
Disease duration (year ± SD)	4.7 ± 1.8	5.1 ± 1.3	NA	NA	P > .05
EDSS (mean ± SD)	2.04 ± 0.21	2.73 ± 0.28	NA	NA	P < .011

MS; multiple sclerosis, HP; *Helicobacter pylori*, HS; healthy subject, NA; not applicable.

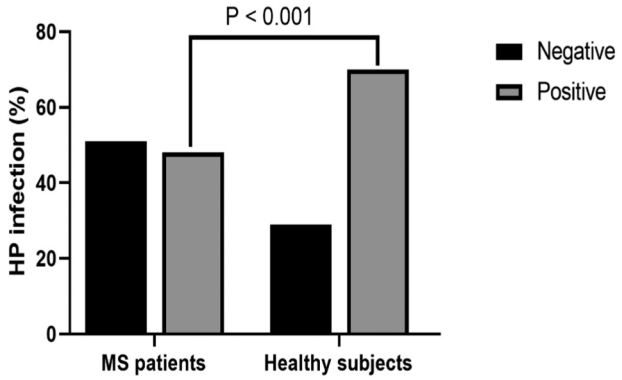

Fig. 1. Comparison of *Helicobacter pylori* seropositivity results between multiple sclerosis patients and healthy subjects. MS; multiple sclerosis, *HP*; *Helicobacter pylori.*

MS patients depicted in Table 1.

3.2. Determine serum IgG anti-HP

We evaluated the seropositivity status of MS patients and controls for the serum IgG anti-HP. HP seropositivity was seen in 48/7% of the MS patients (188/386) and 70/9% of the healthy subject (298/420) (Fig. 1). Our result demonstrated that the HP seropositivity was significantly lower in MS patients when compared to the healthy subjects ($P < .001$) (Fig. 1). Also, in this study, we have shown that the IgG anti-HP levels in MS patients (mean IgG levels = 20.25) was lowered than the healthy individual (mean IgG levels = 30.1) ($P < .001$) (Fig. 2). Moreover, our result displayed that the levels of IgG anti-HP levels in male MS patients (mean IgG levels = 21.5) were significantly higher than female MS patients (mean IgG levels = 19.1) ($P < .01$) (Fig. 3). The EDSS value for our MS patients was 2.6 ± 1.4. Moreover, our result indicated that the EDSS significantly lower in seropositive MS patients (2.04 ± 0.21) as compared to seronegative MS patients (2.73 ± 0.28) ($P < .011$).

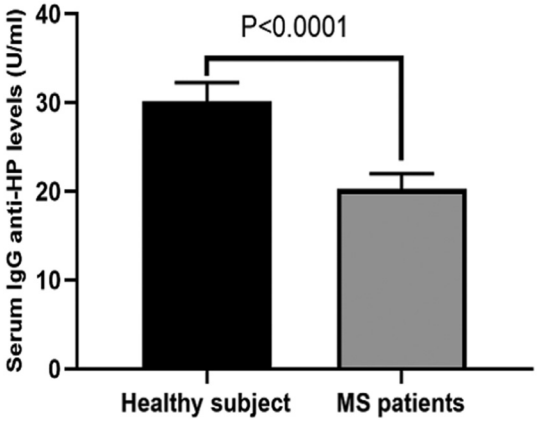

Fig. 2. Comparison of serum IgG anti-*Helicobacter pylori* levels results between multiple sclerosis patients and healthy subjects. MS; multiple sclerosis.

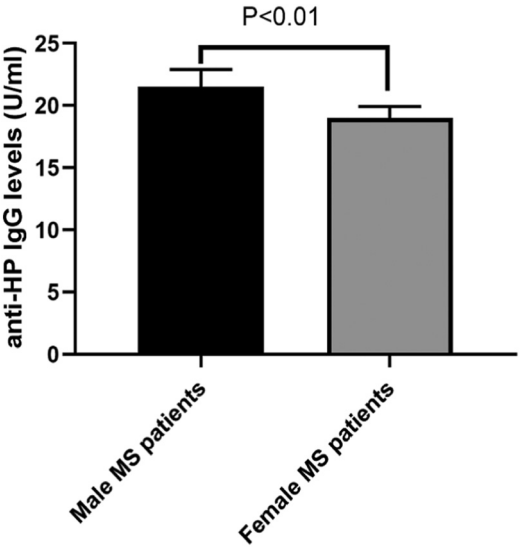

Fig. 3. Comparison of the levels of serum IgG anti-HP between male and female MS patients. MS; multiple sclerosis, *HP*; *Helicobacter pylori.*

3.3. ELISA results for pro/anti-inflammatory cytokines

The concentration of proinflammatory cytokines includes IL-1β, IFN-γ, TNF-α, IL-6, IL-12 (p70), and IL-17 were analyzed in the serum. Our result showed that the levels of IFN-γ, TNF-α, IL-6, IL-17 but not IL-1β and IL-12(p70) significantly lower in seropositive MS patients as compared to seronegative patients. On the other hand, the levels of anti-inflammatory cytokine IL-4 and IL-10 were significantly higher in seropositive MS patients when compared to seronegative patients (Fig. 4A–H).

4. Discussion

MS is a neuroinflammatory disorder of the CNS, which outcome from a combination of environmental and genetic factors and their interplay (Ramagopalan et al., 2010). Environmental factors such as bacterial and viral infections may accelerate, exacerbate, or protect patients from MS (Ascherio and Munger, 2007a,b). The vast majority of seroepidemiological studies suggested that HP infection lower in MS patients as compared to healthy individuals (Jaruvongvanich et al., 2016, Kira and Isobe, 2019, Li et al., 2007, Mohebi et al., 2013, Pedrini et al., 2015, Wender, 2003, Yoshimura et al., 2014). On the other hand, Gavalas et al. showed a positive correlation between HP infection and MS development (Gavalas et al., 2015). Moreover, Long et al. indicated that there was no correlation between HP infection and MS development (Long et al., 2013). In this study, we showed that the seroprevalence of HP infection was significantly lower in MS patients as compared to the healthy subjects. Approximately half of the world's population is infected with HP (Marshall and Warren, 1984). Developing countries have higher infected population than developed ones, and the prevalence of HP infection in some countries may reach 80% (Latif et al., 1991; Mbulaiteye et al., 2009). Moreover, we demonstrated

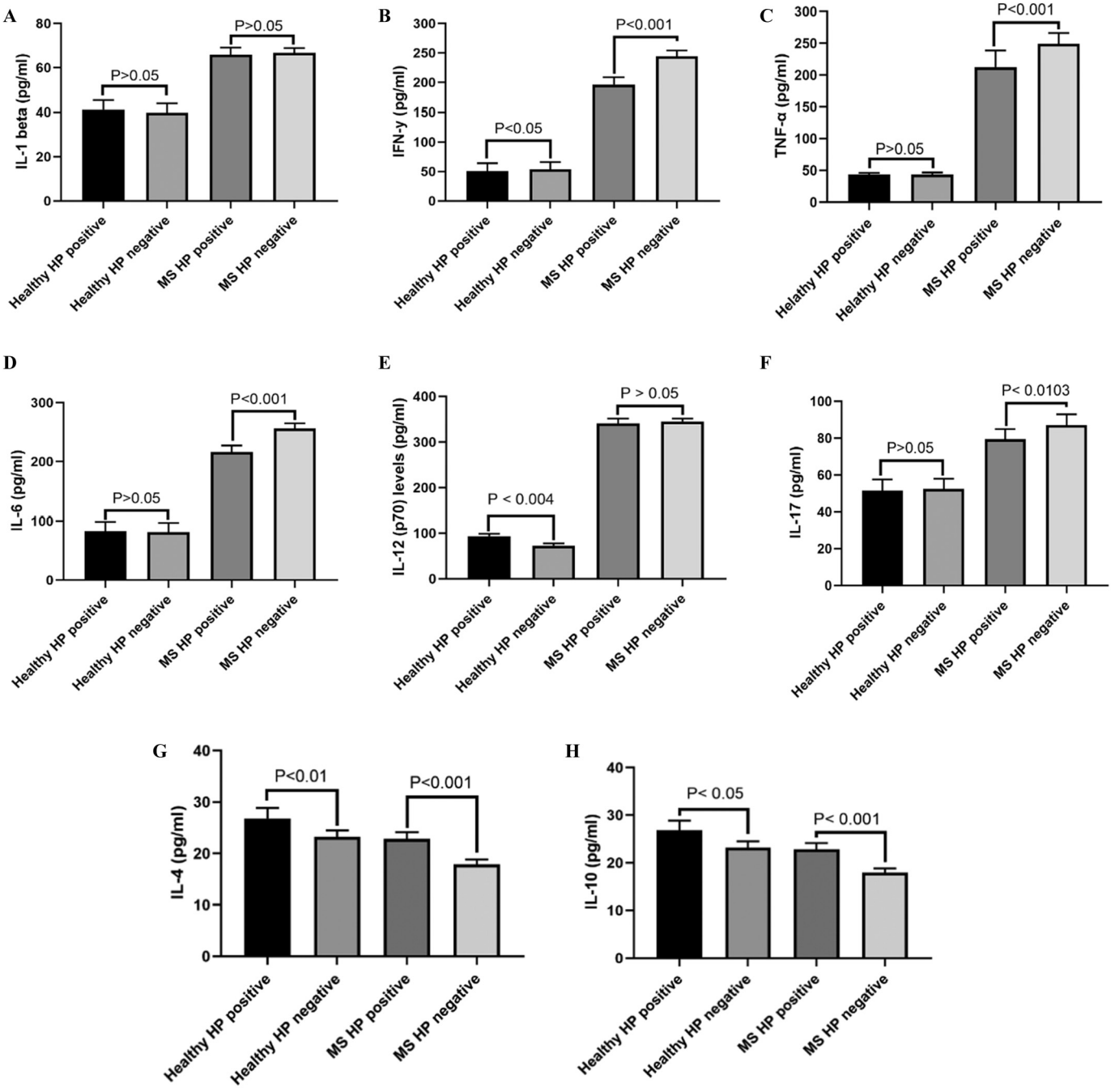

Fig. 4. Comparison of cytokine levels between HP seropositive MS patients and seronegative MS patients (A-H). MS; multiple sclerosis patients, HP; *Helicobacter pylori*.

that the mean of EDSS in HP seropositive MS patients lower than seronegative MS patients. Our findings are in agreement with the studies performed by Mohebi et al. and Pedrini et al. indicated that HP infection negatively correlated with mean of EDSS in MS patients (Mohebi et al., 2013, Pedrini et al., 2015). Furthermore, we showed there was no difference in the degree of EDSS between male and female MS patients infected with HP, while Pedrini et al. demonstrated that in female MS patients seropositive for HP EDSS lower than female MS patients seronegative for HP infection. Current studies have presented insights toward the cytokine signaling pathways that are implicated not only in pathogenesis of neuroinflammatory autoimmune disorders, like as MS but also in neurodegenerative conditions, for instance, Alzheimer's disease (Gogoleva et al., 2018). Recently several studies indicated that the serum and PBMC levels of TNF-α were associated with EDSS scores of MS patients (Kallaur et al., 2017; Rumble et al., 2015). Precise underlying mechanisms of these relationships have not been studied. While TNF-α and IL-1β are linked in microglia-mediated oxidative stress and neuronal loss through apoptosis (Kallaur et al., 2017). TNF-α, CXCL1, and CXCL5 are robust, neutrophil chemotactic factors (Rumble et al., 2015). Hence, these relationships highlight the importance of innate immunity in disability progression in MS. The notable influence of IL-6 on auto-reactive effector T cells has also shown in MS. In relapsing-remitting MS patients during relapse, IL-6 signaling was determined to support T effector cell resistance to handling by regulatory T cells, which may offer to disease worsening (Schneider et al., 2013). The studies were carried out regarding EAE and MS described that the IL-6 might affect the disease pathogenesis through its activity in the peripheral lymphoid organs. Little information is known regarding the

IL-6 role in the CNS. It has recently been determined that mice with an IL-6 deficiency in astrocytes revealed modest amelioration of EAE symptoms and histopathology (Erta et al., 2016). Although TH1 and TH17 cells cytokines play a pivotal role in MS pathogenesis, their impact on disability progression is slightly controversial. The $CD8^+$ cells with high producing IFN-γ along with IL-17 producing $CD4^+$ cells have been associated with poor disease outcome. However, most studies indicate that there were no correlations between EDSS scores and intracellular or serum levels of IL-17 and IL-23. Moreover, in a remarkable study, IL-17 and IL-22 levels have been realized to be correlated with CNS lesion numbers but not EDSS scores of MS patients (Kallaur et al., 2017, Tortorella et al., 2014, Trenova et al., 2014, Wing et al., 2016). On the other hand, lower levels of Th2 and Treg cytokines IL-4 and IL-10 have been correlated with higher EDSS scores in MS. Besides, reduced peripheral blood Treg frequencies are associated with a heightened EDSS score. These findings are corresponding with the concept that T helper type 2 (TH 2), and Treg cells have MS limiting action (Tortorella et al., 2014, Trenova et al., 2014) and thus, individuals with decreased TH 2 and Treg activity are more likely to promote a more severe disease course. In this study, after determination of seroprevalence of HP in MS patients, we investigate some pro/anti-inflammatory cytokines in MS patients infected with HP and seronegative MS patients. Our results demonstrated that some inflammatory cytokine includes IFN-γ, TNF-α, IL-6, and IL-17 levels in serum of seropositive MS patients were reduced as compared to seronegative MS patients. Besides, we found there were no significant differences in the case of IL-1beta and IL-12 (p70) in seropositive MS patients as compared to the seronegative MS patients. Moreover, the anti-inflammatory cytokine includes IL-4 and IL-10 was significantly higher in the serum of seropositive MS patients when compared with seronegative MS patients. Lovett-Racke et al. demonstrated that the myelin-specific TH 1 cells and TH 17 cells are identified in the CNS of mice with EAE and MS patients, and both are believed to play a crucial role in disease pathogenesis (Lovett-Racke et al., 2011). In an interesting study, Cook et al. had infected mice with HP prior to EAE induction. Cook et al. indicate that infection with HP reduced the severity of EAE via decreased the number of $CD4^+$ cells in the CNS. Also, Cook et al. showed that in the CNS and spleen the CD4+ populations which contain $IFN\gamma^+$, $IL\text{-}17^+$, $T\text{-}bet^+$, and $ROR\gamma t^+$ cells notably decreased, but the proportions of Foxp3+ cells were equivalent. Finally, Cook et al. were proposed that HP infection exerted to provides some protection against EAE, such as repressing both TH 1 and TH 17 responses (Cook et al., 2015). In another study, Dlugovitzky and colleague showed that in gastroduodenal diseases, HP enables to alter the serum levels of IL-2, IL-4, IL-10, IFN-γ, and TGF-β for developing pathogenesis. This study demonstrated that the proinflammatory cytokine IL-2 and IFN-γ were reduced in seropositive patients for HP when compared with those seronegative for HP infection. However, the anti-inflammatory cytokine contains IL-4 and IL-10 was increased in seropositive MS patients when compared with seronegative MS patients. Moreover, in this study, the patients were infected with HP when received anti-HP medication, the cytokine levels changes compared to before receiving treatment. (Dlugovitzky et al., 2005). Stent et al., demonstrated that HP superoxide dismutase enables to suppress the production of proinflammatory cytokine during in vivo infection (Stent et al., 2018).

5. Conclusion

In this study, we indicated that HP infection negatively correlated with MS and possibly act as a protective agent against MS; however, the precise mechanism behind this protective effect remains elusive. Moreover, we demonstrated that infection to HP can change the inflammatory and anti-inflammatory cytokines levels in MS patients and healthy individuals. Further studies are required to comprehension and identify a possible therapeutic role for HP as a protective agent against MS development.

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None.

Declaration of Competing Interest

The authors report no conflicts of interest.

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