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RESEARCH ARTICLE

Helicobacter Pylori Infection and Disease Severity in Multiple Sclerosis Patients: Is there a Link with HLA Alleles?

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ABSTRACT

Background: Environmental factors such as bacterial infections, as well as genetic factors—in particular the human leukocyte antigen (HLA) alleles—have been implicated in the etiology of multiple sclerosis (MS).

Aims: This study aims to explore the relationship between *Helicobacter pylori* (*H. pylori*) infection, HLA alleles, and disease severity in Iranian MS patients.

Methods: The study population comprised 125 MS patients and 153 ethnically matched healthy controls. Stool antigen test was used to detect *H. pylori*, and the expanded disability status scale (EDSS) scores were assessed in the patients.*HLA-DRB1* and *DQB1* alleles and haplotypes were determined in both patients and controls groups. The relationships between *H. pylori* infection, HLA alleles, and EDSS were also analyzed.

Results: *HLA-DRB1*15* and *DQB1*06* alleles families and the *DRB1*15~DQB1*06* haplotype were significantly more frequent in MS patients, whereas *HLA-DRB1*14* and *DRB1*14~DQB1*05* haplotype were less frequent. Of the 125 MS patients, 38 were diagnosed with active *H. pylori* infection. We found lower frequencies of *HLA-DRB1*15*(P = .08) and *DRB1*16*(P = .05) alleles and a higher frequency of *DQB1*02*(P = .06) in the *H. pylori*-positive patients. *HLA-DRB1*07*was more prevalent in patients with EDSS≤3.0 (P = .06). More severe MS cases (EDSS>3.0) were linked to *H. pylori* positivity (P = .02), disease chronicity (P = .001), receiving non-steroidal anti-inflammatory drugs (P = .02), and female gender (P = .05).

Conclusion: These preliminary findings suggest a link between *H. pylori* infection and the severity of MS *H. pylori*-positive patients regardless of the type of HLA carriage.

Keywords: multiple sclerosis, *Helicobacter pylori*, HLA-DRB1, HLA-DQB1, EDSS

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system. Although an interplay between genetic and environmental factors is suggested to cause the disease, the exact etiology of MS remains unknown[1, 2, 3]. Among the associations between certain gene loci and MS, the link betweenhuman leukocyte antigen (HLA) alleles and MS has been consistently described[2,4]. While certain HLAclass IIalleles are described as MS risk factors (e.g., HLA-DRB1*15:01, DRB5*0101, DQA1*0102, DQB1*0602), others confer a protective role (e.g., HLA-DRB1*14)[5-7]. Several mechanisms have been suggested for this association; these include antigenbinding and presentation and negative selection of high-avidity autoreactive T cells[4, 8].

Environmental factors such as ultraviolet exposure, smoking, vitamin D intake, and infections can also contribute to MS susceptibility[9]. Among bacterial infections, *Helicobacter pylori* (*H. pylori*) has been associated with MS[10]. *H. pylori* is a gram-negative microaerophilic spiral flagellated bacterium that selectively colonizes the gastric mucosa in more than half of the world's populations[11, 12]. Itcan cause peptic ulcer disease, chronic gastritis, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma[11, 13]. The current evidence, although conflicting, points toward a protective role for *H. pylori* in autoimmune diseases, such as rheumatoid arthritis, systemic sclerosis, systemic lupus erythematosus, and Sjögren syndrome[14, 15]. *H. pylori* seropositivity in MS has been shown to be inversely associated with both the expanded disability status scale (EDSS) and McDonald radiographic criteria for dissemination in space[16]. This may result fromthe central role of helper T cell (Th)1-mediated autoimmune responses in MS pathogenesis and the dominance of regulatory responses in favor of Th2 profile in *H. pylori* infection. On the other hand, several HLA alleles, such as *DRB1*15* and *DQB1*06* contribute to an increased risk of both *H. pylori* infection and MS[17-20]. We designed this cross-sectional study to explore the distributions of HLA-DRB1 and HLA-DQB1 allele families and haplotypes in a group of Iranian MS patients with gastrointestinal manifestations related to *H. pylori* infection.

METHODS

Patients and controls

A total of 125 MS patients who referred to the Department of Neurology of Farshchian (Sina) University Hospital in Hamadan, Iran, between September 2018 and December 2019 were recruited in this cross-sectional and retrospective cohort study. All patients fulfilled the McDonald criteria (a set of clinical, laboratory, and radiographic criteria used to diagnose MS) [21]. Inclusion criteria were *H. pylori*-related gastrointestinal manifestations which examined by laboratory testing (fecal antigen test). Comprehensive demographics and clinical data were collected from the patients' medical records. These included age, gender, ethnicity, the age-at-onset and duration of the disease, history of anti-*H. pylori* treatment(e.g., antibiotics, bismuth compounds, proton pump inhibitors or H2-receptor antagonist) within the past 12 months, EDSS, family history of autoimmune diseases, and smoking history. EDSS values for all patients were assessed based on the Kurtzke expanded disability status scale [22].

The control group comprised 153 ethnically matched healthy subjects with no history for MS or any other autoimmune diseases and absence of *H. pylori* infection. We used the data collected from this group from a previous study on systemic lupus erythematosus patients [23].

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (IR.UMSHA.REC.1397.100) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

HLA-DRB1 and HLA-DQB1 genotyping

The blood sample was collected in ethylene diamine-tetraacetic acid (EDTA)-containing test tubes and DNA was extracted from peripheral blood cells using the salting-out method. In the next step, genotypes of *HLA-DRB1*and *HLA-DQB1* allele families for all subjects were determined by polymerase chain reaction with sequence-specific primer method using low-resolution HLA DR-DQ SSP kits (Olerup SSP®DQ-DR SSP Combi Trays, Stockholm, Sweden) according to the manufacturer's protocol. Determining the specific *HLA-DRB1* and *DQB1* allele families were executed by the SCORE software provided by the company (IMGT/HLA database, release 3.24.0). In addition, *HLA-DRB1* and *DQB1* haplotypes were statistically assignedusing an Expectation-Maximization (EM) algorithm as implemented in the R statistical computing environment (http://www.R-project.org).

Screening test for *H. pylori* infection

Stool samples were collected from all MS patients on the same day as the blood sample collection, and stool *H. pylori* antigen testing was

performed using a commercial enzyme immunoassay kit (Fecal *H. pylori* Antigen Rapid Test Kit, EpiTuub®, ABIN1305189, Germany) as per manufacturer's instructions. Absence of active *H. pylori* infection in the control group was determined through physical examination by an internist.

Statistical analysis

Chi-square test with Yates correction or Fisher's exact test was used to compare *HLA-DRB1* and *DQB1* allele families and haplotype frequencies between patients and controls, as applicable. The risk attributed to haplotypes and genotypes was expressed as odds ratio (OR) with 95% confidence interval. Paired t-test was performed to analyze the quantitative data. We also used the Benjamini–Hochberg method for multiple comparisons to control the false discovery rate (Benjamini and Hochberg, revised version 2010). All these tests were analyzed by SPSS (version 22.0, Chicago, USA) and Epi Info (Version 7.1.3.10) software, and $P < .05$ was considered statistically significant.

RESULTS

Demographics and clinical characteristics of the MS patients

Of the 125 patients (90 females and 35 males; mean [SD] age, 34.92 [1.72]), 38 were diagnosed with active *H. pylori* infection (i.e., positive stool antigen test), all of whom were either receiving anti-*H. pylori* treatment at the time of sample collection or had received treatment within the past 12 months (*H. pylori*-positive).The remaining 87 cases were negative for fecal antigen testing and only developed at least one symptom (Bloating, heartburn or stomachache) in relation to *H. pylori* infection (*H. pylori*-negative).Patient demographics and main clinical features are summarized in Table 1.We observed a significant difference between *H. pylori*-positive and -negative MS patients, in terms of gastrointestinal manifestations (P=0.04) and the history of NSAID use (P=0.004, Table 1).

Table 1. Demographics and the main clinical characteristics of multiple sclerosis patients with and without *Helicobacter pylori* infection; Data presented as mean±SD.

Variables	***H pylori* Positive (n=38)**	***H pylori* Negative (n=87)**	**P values**
Gender (F/M)	31 / 7	59 / 28	0.17
Age (years)	36.2 ± 6.6	34.4 ± 8.0	0.21
Age-at-onset of disease (years)	26.6 ± 7.8	26.3 ± 8.7	0.83
Mean of disease duration in year	9.64 ± 6.09	8.10 ± 4.73	0.16
EDSS (%)			
>3	16 (42.1%)	22 (22.2%)	
≤3	22 (57.9%)	65 (77.8%)	0.09
Gastrointestinal symptoms			
Bloating	20	65	
Bloating +Heart burn	9	10	
Bloating + Heartburn + Stomachache	9	12	0.04
Medication history			
Methyl prednisone	4	10	
Fingolimode	3	6	
Rituximab	28	61	
Natalizumab	0	4	
Interferon-1β	2	2	
Gelatimer acetate	2	1	0.83
Smoking history (Pos/Neg)	5 / 33	12 / 75	0.85
History of NSAID use (Pos/Neg)	27 / 11	36 / 51	0.004

EDSS, expanded disability status scale; NSAID, non-steroidal anti-inflammatory drug.

Distributions of HLA–DRB1 and HLA-DQB1 alleles and haplotypes in MS patients vs. controls

The likelihood of MS diagnosis increased with *HLA-DRB1*15* (OR, 1.66; P = .02) and *DQB1*06* (OR, 1.58; P = .02) alleles, and*DRB1*15~DQB1*06* haplotype (OR, 1.88; P = .009) carriage; conversely, the likelihood decreased in association with the *DRB1*14* (OR, 0.13; P= .002, corrected P = .02) allele and *DRB1*14~DQB1*05* haplotype (OR, 0.13; P=.002; corrected P = 0.03) (Tables 2 & 3).

Table 2. Comparison of *HLA-DRB1* allelic frequencies between multiple sclerosis patients and healthy controls.

HLA-DRB1 Alleles	MS patients 2n=250(%)	Controls 2n=306(%)	OR (95% CI)	P-value	Pc
DRB1*01	13 (5.2%)	20 (6.5%)	0.784 [0.382-1.610]	*0.59*	*0.95*
DRB1*03	19 (7.6%)	29 (9.5%)	0.785 [0.429-1.437]	*0.45*	*0.95*
DRB1*04	29 (11.6%)	33 (10.8%)	1.085 [0.639-1.843]	*0.78*	*0.95*
DRB1*07	23 (9.2%)	30 (9.8%)	0.932 [0.526-1.649]	*0.88*	*0.95*
DRB1*08	9 (3.6%)	9 (2.9%)	1.232 [0.481-3.153]	*0.81*	*0.95*
DRB1*09	3 (1.2%)	4 (1.3%)	0.917 [0.203-4.136]	*1.00*	*1.00*
DRB1*10	9 (3.6%)	9 (2.9%)	1.232 [0.481-3.153]	*0.81*	*0.95*
DRB1*11	50 (20.0%)	65 (21.3%)	0.926 [0.612-1.401]	*0.75*	*0.95*
DRB1*12	0 (0.0)	3 (0.9%)	0.30 [0.03 – 2.72]	*0.38*	*0.95*
DRB1*13	34 (13.6%)	38 (12.5%)	1.110 [0.675-1.823]	*0.70*	*0.95*
DRB1*14	2 (0.8%)	17 (5.5%)	0.137 [0.031-0.599]	***0.002***	***0.02***
DRB1*15	50 (20.0%)	40 (13.1%)	1.662 [1.055-2.618]	***0.02***	*0.13*
DRB1*16	9 (3.6%)	7 (2.3%)	1.595 [0.585-4.345]	*0.44*	*0.95*
HLA-DQB1 alleles					
DQB1*02	42 (16.8%)	58 (19.0%)	0.863 [0.557-1.337]	*0.58*	*0.65*
DQB1*03	86 (34.4%)	111(36.5%)	0.921 [0.649-1.307]	*0.65*	*0.65*
DQB1*04	9 (3.6%)	8 (2.6%)	1.391 [0.528-3.660]	*0.62*	*0.65*
DQB1*05	34 (13.6%)	58 (19.0%)	0.673 [0.424-1.067]	*0.10*	*0.25*
DQB1*06	79 (31.6%)	69 (22.6%)	1.586 [1.087-2.316]	***0.02***	*0.10*

HLA, human leukocyte antigen; MS, multiple sclerosis; OR, odds ratio.

Table 3. Distributions of the frequent *DRB1-DQB1* haplotypes among MS patients and healthy controls.

HLA Haplotypes	MS patients n=250	Controls n=306	P value	Pc
DRB1*01-DQB1*05	13 (5.2%)	19 (6.2%)	*0.71*	*0.96*
DRB1*03-DQB1*02	19 (7.6%)	28 (9.1%)	*0.54*	*0.96*
DRB1*03-DQB1*03	0 (0%)	1 (0.8%)	*1.00*	*1.00*
DRB1*04-DQB1*02	0 (0%)	3 (1%)	*0.38*	*0.96*
DRB1*04-DQB1*04	1 (0.4%)	0 (0%)	*0.59*	*0.96*
DRB1*04-DQB1*03	28 (11.2%)	27 (8.8%)	*0.39*	*0.96*
DRB1*07-DQB1*02	23 (9.2%)	28 (9.1%)	*1.00*	*1.00*
DRB1*07-DQB1*03	0 (0%)	3 (1%)	*0.38*	*0.96*
DRB1*08-DQB1*03	1 (0.4%)	0 (0%)	*0.59*	*0.96*
DRB1*08-DQB1*04	8 (3.2%)	5 (1.6%)	*0.26*	*0.96*
DRB1*09-DQB1*03	3 (1.2%)	4 (1.3%)	*1.00*	*1.00*
DRB1*10-DQB1*05	9 (3.6%)	8 (26%)	*0.62*	*0.96*
DRB1*11-DQB1*03	50 (20%)	66 (21.5%)	*0.67*	*0.96*
DRB1*13-DQB1*03	4 (1.6%)	4 (1.3%)	*1.00*	*1.00*
DRB1*13-DQB1*06	30 (12.0%)	35 (16.4%)	*0.89*	*0.96*
DRB1*14-DQB1*05	2 (0.8%)	17 (5.5%)	***0.002§***	***0.03***
DRB1*15-DQB1*05	1 (0.4%)	6 (2.0%)	*0.13*	*0.82*
DRB1*15-DQB1*06	49 (19.6%)	35 (16.4%)	***0.009§§***	***0.08***
DRB1*16-DQB1*05	9 (3.6%)	7 (2.3%)	*0.44*	*0.96*

§: OR 0.13 [0.03-0.59], §§: OR 1.88 [1.17-3.02]. HLA, human leukocyte antigen; MS, multiple sclerosis, OR, odds ratio; Pc, corrected P value

EDSS≤3.0 was also associated with higher frequencies of *HLA-DRB1*07*and *DQB1*02* alleles (OR, 3.16; P = .06) compared with EDSS>3.0 (OR, 2.06; P = .09; Table 4).

Table 4. Comparisons of the frequencies of *HLA-DRB1* and *DQB1* alleles between multiple sclerosis with EDSS≤3 and EDSS>3;

HLA-DRB1 Alleles	EDSS		P Value	OR (95% CI)
	≤3 (2n=170)	>3 (2n=76)		
DRB1*01	11 (6.4%)	3 (3.9%)	0.56	1.64 (0.44-6.06)
DRB1*03	14 (8.2%)	5 (6.5%)	0.80	1.24 (0.43-3.58)
DRB1*04	19 (10.9%)	10 (13.1%)	0.66	0.80 (0.35-1.83)
DRB1*07	20 (11.5%)	3 (3.9 %)	**0.06**	3.16 (0.91-10.97)
DRB1*08	4 (2.3%)	5 (6.5%)	0.13	0.33 (0.08-1.28)
DRB1*09	1 (0.6%)	2 (2.6%)	0.22	0.21 (0.01-2.39)
DRB1*10	6 (3.4%)	3 (3.9%)	1.00	0.86 (0.21-3.57)
DRB1*11	30 (17.2%)	20 (26.3%)	0.12	0.58 (0.30-1.11)
DRB1*13	28 (16.1%)	6 (7.9%)	0.10	2.23 (0.88-5.65)
DRB1*14	2 (1.1%)	0 (0.0%)	1.00	1.33 (0.13-13.04)
DRB1*15	34 (19.5%)	16 (21.0%)	0.86	0.91 (0.46-1.77)
DRB1*16	5 (2.8%)	4 (5.2%)	0.46	0.53 (0.13-2.04)
DQB1 Alleles				
DQB1*02	34 (19.5%)	8 (10.5%)	0.09	2.06 (0.90-4.70)
DQB1*03	54 (31.0%)	32 (42.1%)	0.11	0.61 (0.35-1.08)
DQB1*04	4 (2.3%)	5 (6.5%)	0.13	0.33 (0.08-1.28)
DQB1*05	24 (13.8%)	10 (13.1%)	1.00	1.05 (0.47- 2.33)
DQB1*06	58 (33.3%)	21 (27.6%)	0.46	1.31 (0.72-2.37)

CI, confidence interval; EDSS, expanded disability status scale; HLA, human leukocyte antigen; OR, odds ratio.

Associations of HLA-DRB1 and HLA-DQB1 alleles with H. pylori infection in MS patients

*HLA-DRB1*15* and *DRB1*16* alleles families were more frequent among *H. pylori*-negative MS patients where only the*DRB1*16*allele showed a marginally significant difference (P=.059, Table 5).In addition, the *DQB1*02* allele was more frequent among *H. pylori*-positive patients (OR, 1.94; P=0.06; Table 5).

Table 5. Distributions of *HLA-DRB1* and *DQB1* allele groups among *Helicobacter pylori*-positive and negative multiple sclerosis patients.

***HLA-DRB1* Alleles**	***Helicobacter pylori* Infection**		**P value**	**RR (95% CI)**
	Positive (2n=76)	Negative (2n=174)		
DRB1*01	4 (5.3%)	9 (5.2%)	1.00	1.02 (0.29-3.54)
DRB1*03	9 (11.8%)	10 (5.7%)	0.11	2.80 (0.85 – 5.66)
DRB1*04	11 (14.5%)	18 (10.3%)	0.46	1.40 (0.69 – 2.82)
DRB1*07	9 (11.8%)	14 (8.0%)	0.32	1.61 (0.63-4.14)
DRB1*08	3 (3.9%)	6 (3.4%)	1.00	1.15 (0.27-4.89)
DRB1*09	2 (2.6%)	1 (0.5%)	0.22	4.58 (0.42-49.74)
DRB1*10	2 (2.6%)	7 (3.9%)	0.72	0.63 (0.12-3.20)
DRB1*11	14 (18.4%)	36 (20.7%)	0.80	0.89 (0.51-1.55)
DRB1*13	11 (14.5%)	23 (13.2%)	0.55	1.33 (0.66-2.65)
DRB1*14	1 (1.3%)	1 (0.5%)	0.51	2.29 (0.15-36.13)
DRB1*15	10 (13.1%)	40 (23.0%)	0.08	0.50 (0.23-1.07)
DRB1*16	0 (0.0%)	9 (5.2%)	**0.05**	-
***HLA-DQB1* Alleles**				
DQB1*02	18 (23.6%)	24 (13.8%)	**0.06**	1.94 (0.98-3.83)
DQB1*03	27 (35.5%)	59 (33.9%)	0.91	1.05 (0.73-1.51)

DQB1*04	3 (3.9%)	6 (3.4%)	1.00	1.14 (0.29-4.46)
DQB1*05	7 (9.2%)	27 (15.5%)	0.23	0.55 (0.22-1.33)
DQB1*06	21 (27.6%)	58 (33.3%)	0.45	0.83 (0.54-1.26)

Comparisons of the alleles with low frequency (2n< 5) were calculated based on two-tailed P values by Fisher's exact test. ; CI, confidence interval; HLA, human leukocyte antigen; RR, relative risk.

Similar associations were found in the corresponding haplotypes–*DRB1*15~DQB1*06* (P=.08) and *DRB1*16~DQB1*05* (P=.059)–with lower frequencies in *H. pylori*-positive patients (Table S1). We found no statistically significant difference in DRB1 and DQB1 allele groups among *H. pylori*-positive cases with EDSS>3 and *H. pylori*-negative cases with EDSS≤3 (Table S2). Of note, multiple logistic regression analysis revealed a significant association of disease severity (EDSS > 3.0) with *H. pylori* infection (P = .02), disease duration (P = .001), receiving NSAIDs (P = .02) and female gender (P = .05, Table 6).

Table 6: Multiple logistic regression analysis of the associations between disease severity (EDSS>3), HLA alleles, *Helicobacter pylori* infection, and other covariates;

Variables	OR	95% CI	P value
HLA-DRB1*15	0.94	0.34 – 2.60	0.91
HLA-DRB1*16	0.32	0.06 - 1.66	0.17
HLA-DQB1*02	2.29	0.52 – 10.11	0.27
HLA-DRB1*07	2.06	0.32 – 13.18	0.44
***H pylori* Infection**	3.17	1.12 – 8.92	**0.02**
Disease Duration	8.84	2.33 – 33.58	**0.001**
NSAID use	2.94	1.13 - 7.63	**0.02**
Gender	0.35	0.12 - 1.00	**0.05**

CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio; NSAID, nonsteroidal anti-inflammatory drug.

DISCUSSION

Genetic and environmental factors play a central role in the pathogenesis and severity of MS. Among the genetic factors, studies have shown a strong association with HLA alleles [24-26]. Among the environmental factors, a link has been described with bacterial infections such as *H. pylori* [10, 16]. In this study, we analyzed the association between a history of *H. pylori* infection, the HLA class II alleles whose links with MS are well described, and the severity of MS.

We observed a positive association of *HLA-DRB1*15* and *DQB1*06* alleles groups as well as *DRB1*15~DQB1*06* haplotype, and a negative association of *DRB1*14* allele group and *DRB1*14~DQB1*05* haplotype with MS disease. Moreover, homozygosity for *DQB1*06/*06* were more frequent in the patients versus controls.

These results are in line with similar studies particularly in Caucasians. Masterman *et al.*[27], Patruccoet *al.*[28], Barcelloset *al.*[29] and Romero-Pinelet *al.*[30]demonstrated the strong positive associations of HLA-DRB1*15:01 and a negative association of *HLA-DRB1*14* with MS in different ethnicities. Also, a meta-analysis by Zhang *et al.*[31] described *HLA-DRB1*14* and *DRB1*07*as protective alleles, and *DRB1*03* and *DRB1*15* as risk alleles in MS patients. Likewise, studies on Middle Eastern and North African populations documented the protective roles for DRB1*10:01 [32], *DRB1*07*, *DRB1*11*[33], DQA1*01:02[34] alleles. Other studies in the Asian and European populations have shown that *DQB1*06* can be associated with MS, independent of *DRB1*15* allele group [27, 31, 35, 36]. Noteworthy, elucidation of the possible protective role of DRB1*14 allele group in our patients had not been reported previously in our population, that could be indicative for genetic hetergenties among different ethnic groups in our country.

We also examined the association of these HLA class II alleles with MS disease severity, and found a lower frequency of *DRB1*07* and *DQB1*02* – previously described as protective alleles [33,37] – in patients with EDSS>3.0. The other alleles that have been linked with disease severity were HLA-DRB1*15:01[38], *DRB1*01, DRB1*04* and *DRB1*03* allele groups[39]. However, two studies in Iranian population, demonstrated the higher frequencies of *DRB1*15*[40] and DQB1*03:03 allele[32] among the severe cases of MS patients which are not consistent with our results in the current study. Taken together, the role of allelic heterogeneities for *HLA-DRB1* and *DQB1* loci in

disease severity is not well understood and may be different from the allelic susceptibility pattern for MS disease.

Considering the probable effect of *H. pylori* infection in the progression of MS, we examined the MS patients suspected for this infection and found a 30.4% *H. pylori* positivity rate based on antigen testing. Interestingly, *H. pylori*-positive MS patients showed lower frequencies of *HLA-DRB1*15* and **16* alleles and a higher frequency of HLA-DQB1*02 compared to *H. pylori*-negative patients. However, these associations were marginally significant probably due to the small number of patients. Although not statistically significant, the disease was more severe in the *H. pylori*-positive cases and *HLA-DRB1*07* negative patients. Of note, multiple logistic regression analysis revealed a significant correlation of disease severity (EDSS >3) with *H. pylori* infection, longer disease duration, NSAIDs usage and female gender of the patients. However, this analysis did not show significant associations with HLA risk/non-risk alleles families in the presence of above-mentioned covariates. A possible explanation for the lack of clear association between HLA alleles, *H. pylori* infection and disease severity in our MS patients could be the sample size in our study which deserves further studies on larger cohort of MS patients.

A lipoprotein known as Lpp20 present in the *H. pylori* can bind to *HLA-DR2* molecule and function as an immunodominant antigen. The dominant Lpp20 epitopes consist of L57-69 and L83-95 which interact with the peptide binding clefts of DRB1*15:01 and DRB1*16:02 respectively. This, in turn, induces a CD4T cell response which could exacerbate the helper T cell (Th)1 responses in *H. pylori*-positive MS patients carrying *HLA-DR2*[42]. Less severe *H. pylori* related gastric diseases have also been linked to specific CD4T cell responses restricted to the DRB1*1501–HpaA88-100 complex [41]. In support of these findings, we observed a more severe form of MS in those *H. pylori*-positive patients, but it was not correlated with the presence of HLA risk alleles. This is in accordance with low frequency of *DRB1*15* (P = .08) and *DRB1*16* (P = .05) alleles among *H pylori*-positive MS patients. These results point out further complexities in the nature of relationship between *H pylori* and MS disease in the genetically susceptible individuals.

In contrast to our results, several clinical and pre-clinical studies have shown a possible protective role for *H. pylori* in MS susceptibility. For instance, the downregulations of Th1 and Th17 cells-mediated inflammatory responses have been reported in the *H. pylori* infected mice with experimental autoimmune encephalomyelitis[43], which can be indicative for a protective role of chronic *H. pylori* infection against MS disease.

The literature review provided above shows inconsistencies between our findings and previous reports which deserves further studies in this area of research. However, the possible explanations for these discrepancies might be recruiting small number of patients (a major limitation in our study), different *H. pylori* detection methods (serological versus antigen screening) in each study, different clinical status of MS patients, variations in drug regimens and other unknown factors.

In conclusion, the patterns for the risk and protective HLA alleles in relation to MS susceptibility were comparable in this sample of Iranian MS patients and other ethnic groups, particularly Caucasians. Moreover, we observed different but non-significant results regarding the *H. pylori* infection, HLA risk alleles status and MS disability scores; namely lower frequencies of *HLA-DRB1*15* and **16* allele groups in the *H. pylori*-positive patients as well as in the patients with higher EDSS scores. Also, the lower frequencies of *HLA-DRB1*07* and *DQB1*02* allele groups in those patients with higher EDSS scores could be indicative for milder disease progression in these patients. Finally, our results can put forward a possible interplay between the inflammatory responses caused by *H. pylori* and the inflammatory process in MS patients regardless of the HLA carriage status. Although, different patterns for the frequencies of HLA alleles in relation to *H. pylori* infection and EDSS score in MS patients separately rises an important point of view to design more exact studies using larger cohort of MS patients among our population and other ethnic groups.

Conflict of interests: None to declare.

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ABBREVEATIONS

EAE: Experimental autoimmune encephalomyelitis
EBV: Epstein-Barr virus
EDSS: expanded disability status scale
EDTA: Ethylene diamide tetra acetic acid
H pylori: Helicobacter pylori
HLA: Human leukocyte antigens
MHC: major histocompatibility complex
MRI: Magnetic resonance imaging
MS: multiple sclerosis
NSAID: non-steroidal anti-inflammatory drug
SSP:sequence-specific primer
Th: T helper

SUPPLEMENTARY MATERIAL

Supplementary Table 1: Distributions of the more frequent DRB1~DQB1 haplotypes among multiple sclerosis patients with and without *H.pylori* infection.

HLA Haplotypes	*H.pylori*^{Pos} MS 2n=78	*H.pylori*^{Neg} MS 2n=174	P-value	RR (95% CI)
DRB1*01-DQB1*05	4 (5.3%)	9 (5.2%)	1.00	1.02 (0.29-3.54)
DRB1*03-DQB1*02	9 (11.8%)	10 (5.7%)	0.15	2.06 (0.87 – 4.87)
DRB1*04-DQB1*04	11 (14.5%)	18 (10.3%)[a]	0.46	1.40 (0.69 – 2.82)
DRB1*07-DQB1*02	9 (11.8%)	14 (8.0%)	0.32	1.61 (0.63-4.14)
DRB1*08-DQB1*04	3 (3.9%)	6 (3.4%) [b]	1.00	1.15 (0.27-4.89)
DRB1*09-DQB1*03	2 (2.6%)	1 (0.5%)	0.22	4.58 (0.42-49.74)
DRB1*10-DQB1*05	2 (2.6%)	7 (3.9%)	0.72	0.63 (0.12-3.20)
DRB1*11-DQB1*03	14 (18.4%)	36 (20.7%)	0.80	0.89 (0.51-1.55)
DRB1*13-DQB1*06	11 (14.5%)	23 (13.2%)[c]	0.55	1.33 (0.66-2.65)
DRB1*14-DQB1*05	1 (1.3%)	1 (0.5%)	0.51	2.29 (0.15-36.13)
DRB1*15-DQB1*06	10 (13.1%)	40 (23.0%)[d]	0.10	0.57 (0.30-1.08)
DRB1*16-DQB1*05	0 (0.0%)	9 (5.2%)	**0.05**	

a: one case had DRB1*04-DQB1*04, b: one case had DRB1*08-DQB1*03, c: four cases had DRB1*13-DQB1*03, d: one case had DRB1*15-DQB1*05. Comparisons for the alleles with low frequency (2n< 5) were calculated based on Two-tailed P values by Fisher's exact test.

Supplementary Table 2: Comparisons of the HLA allele groups frequencies between *H. pylori* ^{Pos} MS patients with EDSS>3 and *H. pylori*^{Neg} patients with EDSS≤3.

HLA-DRB1 **Alleles**	***H. pylori*** ^{Pos} **EDSS>3 (2n=32)**	***H. pylori*** ^{Neg} **EDSS≤3 (2n=130)**	**P-Values**
DRB1*01	2 (6.2%)	9 (6.9%)	1.00
DRB1*03	2 (6.2%)	7 (5.4%)	1.00
DRB1*04	7 (21.8%)	15 (11.5%)	0.15
DRB1*07	2 (6.2%)	13 (10.0%)	0.72
DRB1*08	2 (6.2%)	3 (2.3%)	0.25
DRB1*09	1 (3.1%)	1 (0.7%)	0.35
DRB1*10	1 (3.1%)	5 (3.8%)	1.00
DRB1*11	6 (18.7%)	20 (15.4%)	0.82
DRB1*13	3 (9.3%)	19 (14.6%)	0.53
DRB1*14	0 (0.0%)	1 (0.7%)	1.00
DRB1*15	4 (12.5%)	26 (20.0%)	0.40
DRB1*16	0 (0.0%)	5 (3.8%)	0.57
HLA-DQB1 **Alleles**			
DQB1*02	4 (12.5%)	19 (14.6%)	0.08
DQB1*03	15 (46.8%)	42 (32.3%)	0.10
DQB1*04	2 (6.2%)	3 (2.3%)	1.00
DQB1*05	3 (9.3%)	20 (15.4%)	0.25
DQB1*06	7 (21.8%)	36 (27.6%)	0.45