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Evaluation of Seropositivity Developed Against Specific Antigens of *Helicobacter pylori* in Neurodegenerative Diseases

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ABSTRACT

Introduction: It is suggested that Helicobacter pylori (Hp) can reach the brain via the oral-nasal-olfactory route, through Hp-infected monocytes in the disrupted blood-brain barrier (BBB), or through a rapid retrograde neural network leading to neurodegeneration from the gastrointestinal tract (GIS) and may lead to neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and Multiple sclerosis (MS). In this study, we aimed to evaluate the possible immunopathogenesis relationship between Hp-specific antigens and neurodegenerative diseases by determining the frequency of seropositivity against different specific antigens of Hp in diseases such as AD, PD and MS.

Methods: In our cross-sectional, retrospective case-control study, the immunoreactivity frequencies of Hp-specific and non-specific CagA (p120), VacA (p95), p75, FSH (p67), UreB (p66), HSP homolog (p57), flagellin (p54), p50, p41, p33, OMP (p30), UreA (p29), p26, OMP (p19), p17 antigens were determined by Western Blot method in 36 AD, 35 PD, 91 MS cases with Hp-IgG reactivity, and 55 controls without a neurodegenerative/demyelinating by ELISA method.

Results: No significant difference was found between the immunoreactivity frequencies of Hp antigens between AD and control groups (p>0.05). In the multivariate logistic analysis performed for PD cases, age ≥ 50 and immunoreactivity frequency of p19 were found to be independent risk factors (OR: 36.752, p<0.05) (OR: 5.570, p<0.05). In MS cases, immunoreactivity frequency of p17 antigen was found to be a risk factor (OR: 2.646, p<0.05). In addition, the mean level of Hp-IgG reactivity was found to be negatively associated with MS development (indicating an inverse correlation) in the control group compared to the MS group (OR: 0.585, p < 0.05). Furthermore, logistic regression analysis in the total study group revealed that the immunoreactivity frequency of the p17 antigen was identified as a risk factor for MS (OR: 2.438, p<0.05).

Conclusion: Our data on AD cases are insufficient. In PD cases, the significantly higher frequency of immunoreactivity to the Hp-p19 antigen in individuals aged ≥50 years (OR=5.570) is noteworthy. In the MS group, the significantly high detection of Hp p17 antigen and its presence as a risk factor (OR=2.646), and the significantly high detection of p26 antigen suggest the relationship between these antigens and the MS development process. However, it is a fact that new and many prospective cohort-based case-control studies are needed to reveal this more clearly.

Keywords: Alzheimer's disease, Helicobacter pylori, multiple sclerosis, Parkinson's disease

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INTRODUCTION

Helicobacter pylori (Hp) is a Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes certain regions of the stomach, duodenum, and esophagus. It is known to cause primary gastroduodenal pathologies such as gastritis, peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma, as well as various malignant complications (1,2). Recent studies have suggested that beyond gastroduodenal diseases, Hp may also be associated with extra-gastric conditions such as iron deficiency anemia and thrombocytopenic purpura, as well as with neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (2). Several hypotheses have been proposed regarding the potential relationship between Hp and these neurodegenerative diseases. It has been suggested that Hp may access the brain via several routes: through the olfactory-oral-nasal pathways; via circulating monocytes crossing a disrupted blood–brain barrier (BBB); or through a rapid retrograde neural route originating from the gastrointestinal system. These mechanisms may contribute to the development or progression of neurodegenerative disorders such as AD, PD, and MS (3–7). Hp may induce various inflammatory mediators, including TNF-α, and promote the release of matrix metalloproteinases

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Highlights

- **Helicobacter pylori may be a risk factor for the development of Parkinson's disease and Multiple Sclerosis.**
- **We found no association between Helicobacter pylori and Alzheimer's disease.**
- **Helicobacter pylori Hp-p19 antigen may be associated with Parkinson's disease.**
- **Helicobacter pylori Hp-p33 antigen may be associated with Parkinson's disease.**
- **Helicobacter pylori Hp-p26 antigen may be associated with Multiple sclerosis.**

(MMPs), leading to the breakdown of the blood–brain barrier and blood-ocular barrier. Through these mechanisms, the bacterium may reach the brain inside activated monocytes and trigger the onset or progression of neuropathological processes. Furthermore, the oral–nasal–olfactory system is considered a direct portal of entry for bacterial pathogens into the central nervous system (4, 6–10). In addition, Hp may reach the brain through its reservoirs in the oral cavity—such as dental plaque, saliva, tongue, tonsillar tissue, root canals, and oral mucosa—via the disrupted olfactory bulb (11, 12). Intranasal inoculation of pathogens, transport through infected blood cells, and retrograde axonal transmission may also play critical roles in the pathophysiology of Hp-associated neurodegenerative disorders (13).

It is known that *Helicobacter pylori* (Hp)-specific pathogenic and virulence antigenic factors (such as CagA/EPIYA, VacA) show different effects in both Hp pathogenesis and antimicrobial resistance, particularly depending on geographic regions and countries (1). In light of these data, it is clear that conducting different studies based on various geographic regions and countries regarding the possible relationship between Hp and neurodegenerative diseases will contribute to clarifying this association. For these reasons, in this study—conducted for the first time in Türkiye and based on cases primarily diagnosed with neurodegenerative diseases (AD, PD, MS) from the Marmara region, especially from the city of Istanbul—it was aimed to determine the frequency of seropositivity developed against different specific antigens of Hp in neurodegenerative diseases such as AD, PD, and MS, and, in addition, to evaluate the possible immunopathogenetic relationship between Hp-specific virulence antigens and neurodegenerative diseases.

METHODS

Study Design and Groups

Our study was planned as a cross-sectional, retrospective, case–control-based study between 17.11.2020 and 29.12.2021. A total of 36 AD, 35 PD, and 91 MS (68 RRMS, 10 SPMS, 9 PPMS, 3 SAPMS, 1 RIS) patient cases, who were monitored by İstanbul University-Cerrahpasa, Cerrahpasa Medical Faculty Neurology Department and found to be Hp-IgG reactive, were included in the study. The AD group was defined based on the standardized Mini-Mental State Examination (MMSE) score, the PD group was evaluated within the framework of the Unified Parkinson's Disease Rating Scale (UPDRS), and the MS group was formed based on the Expanded Disability Status Scale (EDSS) and neurologists' general evaluations. MS patients included in the study were those with a definitive diagnosis according to the Lublin criteria (8, 9, 10, 11). No patients who were receiving or had received any immunosuppressive medications (such as corticosteroids, tacrolimus, cyclosporine, etc.) within the last two months or as part of their routine physician-determined treatment plans were included in the study. Additionally, patients with more than one neurodegenerative disease and those under the age of 20 were excluded. Demographic data (age and sex), disease durations, and information on medications used by AD, PD, and MS patients included in the study were obtained from patient records. In our study, to investigate the role of age in the potential association between Hp and these neurodegenerative diseases, threshold values from the literature were used: 65 years as the proposed earliest common age of onset for AD, 50 years as the peak incidence age for PD, and 50 years as the threshold for early vs. late onset in MS. Based on these, 50 years was used as the cut-off age (12, 13, 14). During the study period, the control group was formed from 55 individuals who had previously experienced Hp infection (Hp-IgG reactive), who were referred from various departments of the Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty Hospital for Hp-IgG testing, had given informed consent, and from whom medical history was obtained. The control group was matched with the total patient group (AD, PD, and MS) based on demographic data (age and sex). Individuals under the age of 20 and those with a personal or family history of neurodegenerative/demyelinating, autoimmune disorders or cancer were excluded.

The study was ethically approved by the İstanbul University-Cerrahpaşa, Clinical Research Ethics Committee under decision number 162174 dated 11.12.2020 and by the İstanbul University-Cerrahpaşa Specialty in Medicine Education Board under decision number 505 dated 06.04.2021.

Helicobacter pylori IgG Detection

Hp IgG antibody reactivity was determined using a *Helicobacter pylori* ELISA-based IgG kit (Vircell G1022, Granada, Spain) in patient samples that had been stored at −20 °C until the day of analysis. The optical densities (OD) of the sera were calculated, and in samples with an index value below 0.9, the absence of IgG antibodies against Hp was accepted. In samples with index values above 1.1, the presence of Hp-specific IgG antibodies was accepted, and the results were evaluated semi-quantitatively.

Antibody index = (Sample O.D. / Mean Cut-off Serum O.D.)

Anti-*Helicobacter pylori* Western Blot (IgG) Test

To detect both specific and non-specific antigens of Helicobacter pylori (Hp), the Anti-*Helicobacter pylori* EUROLINE-WB IgG (Euroimmun, Lübeck, Germany) test kit was used. The Western blot test kit includes test strips containing electrophoretically separated antigen extracts from the *Helicobacter pylori* ATCC 43504 strain. These test strips contain the specific Hp antigens CagA (p120), VacA (p95), p33, OMP (p30), UreA (p29), p26, OMP (p19), p17, as well as non-specific antigens p75, FSH (p67), UreB (p66), HSP homolog (p57), flagellin (p54), p50, and p41. The Western blot test strips were incubated with diluted patient samples according to the kit insert. In positive samples, a second incubation was performed using an enzyme-labeled anti-human IgG (enzyme conjugate) to detect antibodies bound to the antigens, catalyzing a color reaction. In accordance with the manufacturer's instructions, the incubated test strips were evaluated using a desktop scanner integrated with a computer (EUROIMMUN AG, Euroimmun, Lübeck, Germany) and the EUROLineScan (Euroimmun, Lübeck, Germany) software. The results were evaluated as positive/negative and semi-quantitative using EUROLineScan. According to the kit insert, the Western blot test result was considered positive when at least two specific Hp antigen bands were reactive. Additionally, the semi-quantitative results of the tested sera were expressed in Reaction Intensity (RI) units, and evaluated as follows based on the commercial kit: 0–12 RI as non-reactive, 13–22 RI as borderline, and 23–256 RI as reactive.

Statistical Analysis

Statistical analyses were performed using the SPSS 21.0 for Windows (IBM Corporation, Armonk, New York, USA) software. The ANOVA test was used to compare the study and control group cases. For categorical variables identified in the study and control groups—specifically the comparison of Hp-specific antigens (CagA, VacA, p33, p30, p29, p26, p19, p17) with other group variables [age group variables (≥ 50 and < 50 for MS, ≥ 50 for PD, ≥ 65 for AD) and gender (female/male)]—Fisher's exact test and Chi-square tests were applied for the analysis of qualitative data. To determine the causal relationship between explanatory variables and outcomes, binary logistic regression (multivariate logistic regression) analysis was performed using the Enter method. For PD, age ≥ 50, gender, and the p33 and p19 values were included as independent variables. For MS cases, the variables found to be statistically significant or near-significant in univariate analysis—Hp-IgG, age (< 50 and ≥ 50), p26, p19, p17, and gender—were analyzed as independent variables. For the total study group, gender, p26, p19, p17, and Hp-IgG reactivity, which were found to be significant or borderline in univariate analysis, were used as independent variables in the model. All variables were evaluated at a 95% confidence level, and a p-value of < 0.05 was considered statistically significant.

RESULTS

The demographic data, presence of comorbidities, Hp-IgG reactivity, and Western Blot (WB)/Hp-IgG positivity/negativity results of the total 217 subjects in the study and control groups who were found to be Hp IgG reactive are shown in Table 1. The distribution of immunoreactivity frequencies for specific and non-specific antigens of Hp in the study and control group subjects who tested positive for WB/Hp-IgG is shown in Table 2. When comparing the distribution of WB/Hp-IgG positivity, the mean levels of Hp-IgG reactivity, the mean levels of WB/Hp-IgG reaction intensity, and the results of immunoreactivity to specific antigens of Hp between AD and control group cases, age ≥ 65 was found to be significantly higher in the AD group (p < 0.05), whereas no statistically significant difference was observed in the frequencies of immunoreactivity to specific antigens of Hp (p>0.05). The results of the distribution of WB/Hp-IgG positivity by sex and age, the mean levels of Hp-IgG reactivity, the mean levels of WB/Hp-IgG reaction intensity, and the frequencies of immunoreactivity to specific antigens of Hp (CagA, VacA, p33, p30, p29, p26, p19, p17) in PD and control group subjects are shown in Table 3. Accordingly, age ≥ 50 and male sex were found to be significantly higher in the PD group (p < 0.05), and the immunoreactivity frequency and reaction intensity level of Hp-p33 were found to be significantly higher in PD cases compared to the control group (p<0.05). The results of the distribution of WB/Hp-IgG positivity by age and sex, the mean levels of Hp-IgG reactivity, the mean levels of WB/Hp-IgG reaction intensity, and the frequencies of immunoreactivity to specific antigens of Hp in MS and control group cases are shown in Table 4. Accordingly, the immunoreactivity frequency and reaction intensity level of Hp-p26, and the immunoreactivity frequency of p17 alone, were found to be significantly higher in the MS group compared to the control group (p<0.05), whereas the mean levels of Hp-IgG reactivity were found to be significantly higher in the control group compared to the MS group (p<0.05).

When the MS subgroups of RRMS, SPMS, and PPMS were compared in terms of the frequencies of immunoreactivity to specific Hp antigens, the immunoreactivity frequency and reaction intensity level of Hp-p33 were found to be significantly higher in RRMS cases (p < 0.05). The comparison of the distribution of WB/Hp-IgG positivity by sex, the mean levels of Hp-IgG reactivity, the mean levels of WB/Hp-IgG reaction intensity, and the frequencies of immunoreactivity to specific antigens of Hp between the total study and control group cases is shown in Table 5. Accordingly, WB/Hp-IgG positivity was significantly higher in males in the total study group (p<0.05), whereas no statistically significant difference was found in the frequencies of immunoreactivity to specific antigens of Hp (p>0.05). The reaction intensity level of Hp-p26 was found to be significantly higher (p<0.05). In PD cases who tested positive for WB/Hp-IgG, according to the multivariate logistic regression analysis of the variables that were found to be statistically significant or borderline significant in the univariate analysis compared to the control group—namely age ≥ 50, sex, p33, and p19—age ≥ 50 and immunoreactivity to Hp-p19 were identified as risk factors (OR: 36.752, p<0.05; OR: 5.570, p<0.05) (Table 6a). In MS cases, based on the multivariate logistic regression analysis results of the variables that were found to be statistically significant or borderline significant in the univariate analysis compared to the control group—namely age < 50, sex, p26, p19, and p17, as well as the mean levels of Hp-

Table 1. Demographic data, Hp-IgG reactivity, and WB/Hp-IgG positivity/negativity results of the study and control group cases found to be Hp-IgG reactive.

Clinical Characteristics	Study Groups			Total Study Group (n=162)	CG (n=55)	p
	AD (n=36)	PD (n=35)	MS (n=91)			
Age (Mean±SD)	71.67±8.906	67.00±9.753	41.37±9.513	53.64±16.86	50.64±16.754	0.254
Gender						
Male; n (%)	17 (47.2%)	24 (68.6%)	26 (28.6%)	67 (41.4%)	27 (49.1%)	0.317
Female; n (%)	19 (52.8%)	11 (31.4%)	65 (71.4%)	95 (58.6%)	28 (50.9%)	
Disease Duration (Years) (Mean ± SD)	3.06±2.735	5.06±3.514	10.07±6.241	7.43±5.96		
Presence of Comorbidity						
Present; n (%)	11 (30.6%)	7 (20%)	14 (15.4%)	32 (19.8%)	7 (12.7%)	0.241
Absent; n (%)	25 (69.4%)	28 (80%)	77 (84.6%)	130 (80.2%)	48 (87.3%)	
Hp-IgG (Mean±SD)	2.97±2.249	2.40±1.143	2.03±0.823	2.32±1.37	2.47±1.834	0.519
WB IgG						
Positive; n (%)	29 (80.6%)	31 (88.6%)	72 (%79.1)	132 (81.4%)	39 (70.9%)	0.097
Negative; n (%)	7 (19.4%)	4 (11.4%)	19 (%20.9)	30 (18.6%)	16 (29.1%)	

AD: Alzheimer's Disease, PD: Parkinson's Disease, MS: Multiple Sclerosis, CG: Control Group, WB: Western Blot, Hp: *Helicobacter pylori*, SD: Standard Deviation

Table 2. Distribution of Immunoreactivity Frequencies for Specific and Non-specific *H. pylori* Antigens in WB/Hp-IgG Positive Study and Control Groups

Antigens of Hp	Study Groups			Total Study Group (n = 132)	Control Group (n=39)
	AD (n = 29)	PD (n = 31)	MS (n = 72)		
Specific Antigens					
CagA	21 (72.41%)	27 (87.09%)	55 (76.39%)	103 (78.03%)	28 (71.79%)
VacA	5 (17.24%)	2 (6.45%)	10 (13.88%)	17 (12.87%)	6 (15.38%)
p33	5 (17.24%)	12 (38.70%)	23 (31.94%)	40 (30.30%)	7 (18%)
p30	11 (38%)	9 (29.03%)	24 (33.33%)	44 (33.33%)	8 (20.50%)
p29	18 (62.06%)	20 (64.51%)	53 (73.61%)	91 (68.93%)	28 (71.79%)
p26	19 (65.52%)	19 (61.29%)	53 (73.61%)	91 (68.93%)	21 (72.41%)
p19	23 (79.31%)	28 (90.32%)	63 (87.5 %)	114 (86.36%)	29 (74.35%)
p17	13 (44.82%)	13 (41.93%)	40 (55.55%)	66 (50%)	13 (33.33%)
Non-specific Antigens					
p75	11 (37.93%)	18 (58.06%)	34 (47.22%)	63 (47.72%)	12 (30.76%)
p67	17 (58.62%)	23 (74.19%)	49 (68.05%)	89 (67.42%)	24 (61.53%)
p66	26 (89.65%)	30 (96.77%)	62 (86.11%)	118 (89.39%)	37 (84.87%)
p57	27 (93.10%)	30 (96.77%)	68 (94.44%)	125 (94.69%)	28 (71.79%)
p54	22 (75.86%)	22 (70.96%)	40 (55.55%)	84 (63.63%)	18 (46.15%)
p50	15 (51.72%)	14 (45.16%)	48 (66.66%)	77 (58.33%)	15 (38.46%)
p41	17 (58.62%)	24 (77.41%)	54 (75%)	102 (77.27%)	24 (61.53%)

AD: Alzheimer's Disease, PD: Parkinson's Disease, MS: Multiple Sclerosis, CG: Control Group, WB: Western Blot, Hp: Helicobacter pylori, SD: Standard Deviation

Table 3. Comparison of the distribution of WB/Hp-IgG positivity according to demographic data and the frequencies of immunoreactivity to specific Helicobacter pylori antigens between PD and control group cases.

Variables	PD (n=35)		CG (n=55)		p
	WB (+) (n=31)	WB (-) (n=4)	WB (+) (n=39)	WB (-) (n=16)	
Age < 50 n (%)	1 (100%)	0	22 (88%)	3 (12%)	-
Age ≥ 50 n (%)	30 (88.23%)	4 (11.77%)	17 (56.66%)	13 (43.34%)	**0.004**
Gender					
Male; n (%)	23 (95.83%)	1 (4.17%)	17 (62.96%)	10 (37.04%)	**0.004**
Female; n (%)	8 (72.72%)	3 (27.28%)	22 (78.58%)	6 (21.42%)	0.693
Hp-IgG (Mean±SD)	2.53±0.984		2.83±1.993		0.654
WB IgG (Mean±SD)	70.44±26.647		63.33±24.393		0.350
Specific Antigens of Hp	**WB (+) (n=31)**		**WB (+) (n=39)**		
CagA	27 (87.09%)		28 (71.79%)		0.121
VacA	2 (6.45%)		6 (15.38%)		0.287
p33	14 (45.16%)		7 (18%)		**0.014**
p30	9 (29.03%)		8 (20.50%)		0.409
p29	20 (64.51%)		28 (71.79%)		0.515
p26	19 (61.29%)		21 (53.84%)		0.532
p19	28 (90.32%)		29 (74.35%)		0.088
p17	13 (41.93%)		13 (33.33%)		0.459

PD: Parkinson's Disease, CG: Control Group, WB: Western Blot, Hp: *Helicobacter pylori,* SD: Standard Deviation

IgG reactivity, which were significantly higher in the control group—the immunoreactivity frequency of the p17 antigen was identified as a risk factor for MS. Additionally, the mean Hp-IgG reactivity level was found to be inversely and significantly higher in the control group compared to the MS group (OR: 2.646, $p < 0.05$; OR: 0.585, $p < 0.05$) (Table 6b). As a result of the multivariate logistic regression analysis conducted between the total study and control group subjects who tested positive for WB/Hp-IgG, based on the variables that were found to be statistically significant or borderline significant in the univariate analysis—namely sex, p26, p19, p17 antigens, and the mean levels of Hp-IgG reactivity—the immunoreactivity frequency of the Hp-p17 antigen was identified as a risk factor for the total study group (OR: 2.438, $p < 0.05$).

Table 4. Comparison of the distribution of WB/Hp-IgG positivity by age and gender and the frequencies of immunoreactivity to specific Helicobacter pylori antigens between MS and control group cases.

Variables	MS (n=91)		CG (n=55)		p
	WB (+) (n=72)	WB (-) (n=19)	WB (+) (n=39)	WB (-) (n=16)	
Age < 50 n (%)	57 (79.16%)	15 (20.84%)	22 (88%)	3 (12%)	0.389
Age ≥ 50 n (%)	15 (78.94%)	4 (21.06%)	17 (56.67%)	13 (43.33%)	0.110
Gender					
Male; n (%)	19 (73.07%)	7 (26.93%)	17 (62.96%)	10 (37.04%)	0.430
Female; n (%)	53 (81.53%)	12 (18.47%)	22 (78.57%)	6 (21.43%)	0.740
Hp-IgG (Mean±SD)	2.18±0.732		2.83±1.993		**0.030**
WB IgG (Mean±SD)	62.60±20.874		63.33±24.393		0.941
Specific Antigens of Hp	**WB (+) (n=72)**		**WB (+) (n=39)**		
CagA	55 (76.39%)		28 (71.79%)		0.595
VacA	10 (13.88%)		6 (15.38%)		0.830
p33	23 (31.94%)		7 (18%)		0.113
p30	24 (33.33%)		8 (20.50%)		0.155
p29	53 (73.61%)		28 (71.79%)		0.837
p26	53 (73.61%)		21 (53.84%)		**0.035**
p19	63 (87.5%)		29 (74.35%)		0.079
p17	40 (55.55%)		13 (33.33%)		**0.025**

MS: Multiple Sclerosis, CG: Control Group, WB: Western Blot, Hp: *Helicobacter pylori*, SD: Standard Deviation

Table 5. Comparison of the distribution of WB/Hp-IgG positivity by gender and the frequencies of immunoreactivity to specific Helicobacter pylori antigens between the total study group and control group cases.

Variables	Total Study Group (n=162)		CG (n=55)		p
	WB (+) (n=132)	WB (-) (n=30)	WB (+) (n=39)	WB (-) (n=16)	
Gender					
Male; n (%)	55 (82.08%)	12 (17.92%)	17 (62.96%)	10 (37.04%)	**0.048**
Female; n (%)	77 (81.05%)	18 (18.95%)	22 (78.57%)	6 (21.43%)	0.771
Hp-IgG (Mean±SD)	2.512±1.3623		2.829±1.993		0.234
WB IgG (Mean±SD)	66.73±22.627		63.33±24.393		0.382
Specific Antigens of Hp	**WB (+) (n=132)**		**WB (+) (n=39)**		
CagA	103 (78.03%)		28 (71.79%)		0.419
VacA	17 (12.87%)		6 (15.38%)		0.687
p33	42 (31.18%)		7 (18%)		0.129
p30	44 (33.33%)		8 (20.50%)		0.126
p29	91 (68.93%)		28 (71.79%)		0.733
p26	91 (68.93%)		21 (53.84%)		0.081
p19	114 (86.36%)		29 (74.35%)		0.075
p17	66 (50%)		13 (33.33%)		0.067

CG: Control Group, WB: Western Blot, Hp: *Helicobacter pylori*, SD: Standart Devition.

DISCUSSION

In recent years, many researchers have suggested that Helicobacter pylori (Hp) may be associated with neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (6). In our study, WB/Hp-IgG positivity was detected in 29 (80%) cases in the AD group and in 39 (71%) cases in the control group. When compared according to age and gender, the distribution of WB/Hp-IgG positivity was found to be significantly higher in AD cases aged ≥ 65 years. In addition, when comparing 29 WB/Hp-IgG-positive AD cases with 39 controls in terms of Hp-IgG reactivity levels, WB/Hp-IgG reaction intensity levels, and the frequencies of immunoreactivity to specific Hp antigens, no statistically significant difference was found. A study conducted in Japan reported no difference in the prevalence of Hp infection between the AD group (385/917) and the control group, but suggested that older age, independent of Hp infection, might be associated with the development of AD (15). In a prospective cohort study in Sweden conducted to determine the association between chronic gastritis and AD, AD developed in 25 out of 488 participants, and similar to our results, no difference was found in Hp prevalence between participants with and without AD (16). In contrast to these results, Malaguarnera et al. (17) reported that Hp-IgG reactivity was higher in

Table 6a. Multivariate logistic regression analysis results of the variables found to be significant in univariate analysis in the PD group.

Variables	B	SE	*Sig.*	Exp(B)	95% CI for EXP(B)	
					Lower	Upper
Age ≥ 50	**3.604**	**1.094**	**0.001**	**36.752**	**4.302**	**313.970**
Gender (1)	0.929	0.676	0.170	2.532	0.673	9.532
p33	0.968	0.718	0.178	2.632	0.644	10.758
p19	**1.717**	**0.846**	**0.042**	**5.570**	**1.062**	**29.213**
Constant	-5.309	1.420	0.000	0.005		

B: Beta Regression Coefficient SE: Standard Error, df: Degree of Freedom, CI: Confidence Interval, Sig.: Sigma, Exp (B): Exponantiate

Table 6b. Multivariate logistic regression analysis results of the variables found to be significant in univariate analysis in the MS group.

Variables	B	SE	*Sig.*	Exp(B)	95% CI for EXP(B)	
					Lower	Upper
Age<50	-0.640	0.499	0.200	0.528	0.198	1.403
Gender (1)	-0.473	0.491	0.336	0.623	0.238	1.632
Hp-IgG	**-0.536**	**0.269**	**0.047**	**0.585**	**0.345**	**0.992**
p26	0.356	0.495	0.472	1.428	0.541	3.767
p19	0.368	0.586	0.530	1.445	0.458	4.560
p17	**0.973**	**0.466**	**0.037**	**2.646**	**1.061**	**6.598**
Constant	1.294	0.862	0.133	3.646		

B: Beta Regression Coefficient SE: Standard Error df: Degree of Freedom CI: Confidence Interval, Sig.: Sigma, Exp (B): Exponantiate

cases of vascular dementia and AD compared to controls among 30 AD, 30 vascular dementia, and 30 control cases. Unlike our study, in 2017, Efthymiou et al. (18) detected Hp-IgG reactivity in 10 of 21 AD cases and in 33 of 68 controls and found that the frequency of immunoreactivity to the specific Hp-p19 antigen was higher in the AD group. Although some epidemiological and pathogenetic mechanisms have been proposed regarding the AD–Hp relationship, our data and the limited number of studies suggest that it is still too early to make a definitive causal inference regarding this association (14, 15, 16, 17).

In the PD group, WB/Hp-IgG positivity was detected in 31 cases (88.57%) and in 39 cases (71%) in the control group. Based on age and gender comparisons between the PD and control groups, WB/Hp-IgG positivity was significantly higher in males and in those aged ≥ 50 years. According to multivariate logistic regression analysis, WB/Hp-IgG positivity was significantly associated with PD cases aged ≥ 50 years. In line with our study, Lolekha et al. (19) found increased Hp prevalence among PD cases aged 40–60 years, and Huang et al. (20) reported an association between increased PD risk and age ≥ 60 years. In our study, among WB/Hp-IgG-positive PD and control group cases, the frequency of immunoreactivity to the Hp-p33 antigen, which is a domain of VacA, was found to be significantly higher in PD cases. In contrast, the immunoreactivity frequency of p19, one of Hp's outer membrane proteins and a major immunostimulatory antigen involved in Hp colonization, was found to be borderline significant. Moreover, multivariate logistic regression analysis revealed that the immunoreactivity frequency of Hp-p19 was significantly higher and that Hp-p19 was identified as an independent risk factor, increasing the risk of PD by approximately sixfold (OR = 5.570, $p < 0.05$). Similar to our findings, Efthymiou et al. (18) detected Hp-IgG reactivity in 14 of 39 PD cases (36%) and in 33 of 68 controls (48%) and reported a higher frequency of immunoreactivity to Hp-p19 in the PD group (57.1%) than in the control group (30.3%). The 33 kDa domain of the 140 kDa VacA protein, known to cause vacuolization, apoptosis, and activation of proinflammatory signaling pathways in human epithelial cells, allows VacA to enter cells and induce vacuolization by forming anion-selective channels (21). Hp may enter dopaminergic neurons via the p33 domain of VacA and induce the mitochondrial apoptotic pathway through proapoptotic proteins such as Bax and Bak and certain caspases (22, 23). Hp-p33 may also play a role in the synthesis of MPTP-like substances, which are toxic to dopaminergic neurons and may contribute to PD development by destroying dopaminergic neurons located in the substantia nigra (24). Hp-p19, one of the outer membrane proteins (OMPs) typical of Gram-negative bacteria, is thought to be an important immunostimulatory antigen that interacts with host defense mechanisms and facilitates Hp colonization, protecting it from the gastric immune response and allowing its persistence (25). p19 also functions as an immunostimulant. Along with other OMPs, p19 facilitates Hp localization and colonization in the host, leading to excessive secretion of pro-inflammatory cytokines (21). This excessive cytokine secretion may induce the release of matrix metalloproteinases, disrupt the physiological structure of the blood–brain barrier (BBB), and increase its permeability. As a result, activated monocytes infected with Hp may cross the BBB, reach the brain, and contribute to the development of neurodegeneration (22). Hp may also exert a proactive role in PD pathophysiology by utilizing L-dopa synthesis in neurons reached through the BBB and transported by monocytes, as well as in its natural habitat—the gastrointestinal system.

In our study, WB/Hp-IgG positivity was found in 72 cases (79%) in the MS group and in 39 cases (71%) in the control group, and no significant difference was detected between the groups in the distribution of WB/Hp-IgG positivity by age and sex in univariate and multivariate analyses. Contrary to our findings, Pedrini et al. (21) found that Hp seropositivity was lower in female MS patients compared to controls. Although we did not find significant differences in the frequency of Hp infection or demographic parameters between groups, we observed that the mean Hp-IgG reactivity level was inversely and significantly higher in the control group. The results of a meta-analysis including nine studies support our findings (22). These nine studies reported a lower prevalence of Hp in MS patients compared to controls. Moreover, while Hp prevalence was lower in MS patients than controls in Western countries, the opposite was observed in Eastern countries. Although our country represents both Eastern and Western geographic characteristics (Eurasia), the data we obtained from MS cases seem to align with the conclusion of the meta-analysis that suggests a negative association between Hp

infection and MS in Western countries. In addition, in our study, the immunoreactivity frequencies of Hp-specific antigens p26 and p17 were found to be significantly higher in the MS group compared to controls among WB/Hp-IgG-positive cases. According to multivariate logistic regression analysis, the p17 antigen—one of Hp's neutrophil-activating protein subunits—was identified as a risk factor for MS, increasing the risk approximately threefold. Similarly, Efthymiou et al. (18) found no statistically significant difference in Hp-IgG reactivity between 60 of 139 MS cases (43%) and 33 of 68 controls (48%), but reported significantly higher immunoreactivity to Hp-p29 in the MS group.

When we examined the WB/Hp-IgG-positive MS subgroups, WB/Hp-IgG positivity was detected in 57 RRMS cases (83.87%), 7 SPMS cases (70%), 7 PPMS cases (70%), and in 1 of 3 SAPMS cases (33.33%). Among SPMS cases with disease duration ≥ 10 years, WB/Hp-IgG positivity was found to be significantly higher, while among PPMS cases with disease duration < 10 years, it was also significantly higher. It is known that some RRMS cases may progress to SPMS within approximately 10 years (23), whereas PPMS is observed in only 10% of MS patients and typically appears in the early stages of the disease (23). In our comparison of WB/Hp-IgG-positive RRMS, SPMS, and PPMS groups, the immunoreactivity frequency of Hp-p33 was found to be significantly higher in the RRMS group. While Efthymiou et al. (13) reported that VacA was significantly higher in the SPMS group, they detected the p33 antigen, a VacA subdomain, in 9 RRMS cases (21.9%) and in 6 SPMS cases (31.6%), but found no statistically significant difference. They also found that the p29 antigen, a subdomain of the UreA protein, was significantly higher in the SPMS group. In contrast, in our study, the immunoreactivity frequencies of the p29 antigen were 43 cases (75.43%) in RRMS, 4 cases (57.14%) in SPMS, and 6 cases (85.71%) in PPMS, with no statistically significant difference between the groups.

Finally, in our study, comparing the Hp-IgG-reactive total study group (81.48%) and the control group (71%) by age and gender, WB/Hp-IgG positivity was found to be significantly higher in males. However, this significance disappeared in multivariate logistic regression analysis. while the immunoreactivity frequencies of Hp-p26, p19, and p17 were found to be borderline significant only; none of these antigens showed a statistically significant difference between the study and control groups. According to multivariate logistic regression analysis, the immunoreactivity frequencies of Hp-p26 and p17 lost their significance as risk factors, but the p17 antigen remained a potential risk factor. Additionally, the mean Hp-IgG reactivity level, which was found to be borderline significant in univariate statistical analysis, was identified as a risk factor for the control group in multivariate analysis. Among the WB/Hp-IgG-positive total study group, the MS group had the highest number of cases (72), and in multivariate logistic regression analysis, the p17 antigen was identified as a risk factor for MS, while the mean Hp-IgG reactivity level was found to be inversely and significantly higher in the control group compared to the MS group. Therefore, the reporting of Hp-p17 as a risk factor for the total study group and the inverse significance of the mean Hp-IgG reactivity level may be due to the higher number of MS cases.

Finding Hp infection in PD cases aged ≥ 50 years in line with the literature, and the significantly high immunoreactivity frequency and OR = 5.570 value of the Hp-p19 antigen, suggest that this virulence antigen should be emphasized in this clinical picture. Furthermore, the significant effect of the Hp-p33 antigen (according to univariate analysis) is also a noteworthy finding. In the MS group, the significant detection of Hp-p17 according to both univariate and multivariate logistic regression analyses as an independent risk factor, and the significantly higher frequency of Hp-p26 (in univariate analysis), suggest that these antigens may be associated with MS pathogenesis.

Whether the onset of neurodegenerative diseases occurs before or after Hp infection remains a topic of debate, and this issue is also present in our study. Our study is a retrospective cohort study in terms of Hp, and, as specified in the inclusion and exclusion criteria in the materials and methods section, cases with past gastroduodenal complaints who had recently been diagnosed with a neurodegenerative disease were included. In general, considering the multifactorial etiology of neurodegenerative diseases, we believe that research on the role of microorganisms—especially Helicobacter pylori—along with genetic predispositions and environmental factors, should continue without interruption.

Limitations: As a limitation of the study, the lack of serological tests such as Hp-IgM, which may indicate recent or active Hp infection and allow for the detection of humoral and cellular immune-mediated autoimmune responses that may damage neurons via Hp's homologous epitopes (molecular mimicry), and the inability of conventional invasive or non-invasive tests to detect the presence of Hp in GIT tissue or stool, may limit the demonstration of immunopathogenic processes due to Hp during active infection. To more clearly define this relationship, large-scale, novel experimental (in vitro or animal-based) and prospective cohort-based case–control and molecular studies are needed.

Ethics Committee Approval: The study was ethically approved by the İstanbul University-Cerrahpaşa, Clinical Research Ethics Committee under decision number 162174 dated 11.12.2020 and by the İstanbul University-Cerrahpaşa Specialty in Medicine Education Board under decision number 505 dated 06.04.2021.

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