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Research Paper

Association Between Helicobacter Pylori Infection and Seronegative Neuromyelitis Optica Spectrum Disorder

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Running Title *H. pylori* Infection and Seronegative NMOSD

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ABSTRACT

Background: Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune demyelinating disease in the central nervous system. Association between NMOSD and *Helicobacter pylori* (*H. pylori*) infection has been investigated, but few studies have assessed the relationship between *H. pylori* and seronegative AQP4-Ab NMOSD.

Objectives: This study aimed to survey the association between *H. pylori* infection and NMOSD patients with seronegative AQP4-Ab status, as well as the possible relationship between the presence of *H. pylor*i and clinical characteristics.

Materials & Methods: This cross-sectional study was carried out in Kashani Hospital affiliated with the Isfahan University of Medical Sciences, Isfahan, Iran, from October 2017 to May 2019. A total of 35 consecutive seronegative AQP4-Ab NMOSD patients and 37 sex and age-matched healthy controls participated in the study. Demographic and clinical characteristics were obtained from all participants. We assessed participants' seroprevalence of IgG and IgM antibodies against *H. pylori*. The Association of *H. pylori* with NMOSD was determined.

Results: The frequency of IgG and IgM Ab *H. pylori* seropositivity in NMOSD patients was 22.9% and 40.0%, respectively. Among HC, 11(29.7%) and 20(54.1%) were positive for IgG and IgM Ab *H. pylori*. Although the rate of *H. pylori* IgG (OR=0.700, 95%, CI=0.243, 2.017, P=0.420) and IgM Ab (OR=0.567, 95%, CI=0.222, 1.444, P=0.233) seropositivity in NMOSD were lower than NMOSD, these differences were not statistically different. No clinical variables associated with *H. pylori* IgG and IgM seropositivity infection seropositivity.

Conclusion: These findings show that possibly there is no relationship between *H. pylori* infection and seronegative AQP4-Ab NMOSD.

Keywords: Neuromyelitis optica, Aquaporin 4, Helicobacter pylori

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Highlights

• Frequency of IgG and IgM antibodies against Helicobacter pylori infection in NMOSD patients with seronegative AQP4 antibody was not different compared to the control population.

• Helicobacter pylori infection seropositivity had no association with the disability of NMOSD with seronegative AQP4 Ab.

Introduction

Neuromyelitis optica spectrum disorder (NMOSD), a severe autoimmune condition in which inflammatory demyelination is directed towards individuals' central nervous system, typically involves optic nerves and the spinal cord [1, 2]. Demyelination, neuronal loss, and astrocytic damage in addition to necrosis are indicated as histopathological characteristics of NMOSD [3]. The majority of the patients show seropositivity for anti-aquaporin 4 water channel immunoglobulin G (AQP4-IgG), which is suggested to own important roles in the disease pathogenicity in these patients [4, 5]. A wide spectrum of studies has investigated different infectious agents including bacteria, viruses, and parasites, and the common flora correlation with autoimmunity and have proposed several pathways through which exposure to each could trigger or exacerbate the disorder [6].

Helicobacter pylori (*H. pylori*) is a gram-negative spiral bacterium colonizing the luminal surface of the gastric epithelium in childhood, with an average infection rate as high as 50% among the human population [7]. This infectious agent is assumed to own probable associations with several extra-gastric autoimmune disorders [8].

H. pylori infection has been extensively studied concerning its probable involvement in susceptibility or resistance to autoimmune diseases, including MS [9, 10]. Generally, infections such as *H. pylori* can trigger or induce NMOSD exacerbation via two pathways. *H. pylori* pathogen may disturb the Th1/Th17 balance and regulatory T cell through AQP4 mimicry and stimulate AQP4-IgG production or induce non-specific inflammatory mediators such as interleukin (IL) 17, IL 6 [11]. Previous studies showed *H. pylori* was more common in NMOSD patients with positive AQP4-Ab compared to controls. However, there are limited studies assessing the association between NMOSD with AQP4-IgG negative and *H. pylori* infection. Their findings regarding the difference between AQP4-IgG negative NMOSD and controls were different compared to seropositive patients [12, 13].

In this paper, we evaluated the presence of *H. pylori* antibodies in sera samples of AQP4-Ab negative NMOSD patients and healthy controls (HC) and compared the acquired results to determine if *H. pylori* infection is associated with the disease. We further investigated any possible relationship between the presence of *H. pylori* and clinical characteristics.

Material and Methods

Study population

We studied consecutive NMOSD patients who visited the outpatient MS clinic of Kashani Hospital, affiliated with the Isfahan University of Medical Sciences, Isfahan, Iran, from October 2017 to May 2019. The inclusion criteria were as follows: diagnosed with NMOSD according to international consensus diagnostic criteria in 2015 [14], and seronegative antibody against AQP4 and Myelin Oligodendrocyte Glycoprotein (MOG) in the cell-based assay. Thirty-seven age and sex-matched HC group were comprised of the general population. HC was excluded from the study if they had been diagnosed with neurological diseases other than NMOSD. Further exclusion criteria for NMOSD patients and HC were as follows: the use of corticosteroids, proton pump inhibitors, H_2-receptor antagonists, antibiotics, and non-steroidal anti-inflammatory drugs (within 3 months).

To calculate sample size, we used the equation for the comparison of two frequencies. Assumptions in two groups were pre-specified based on Long and colleagues' study. We hypothesized that an equivalent result will be detected in the groups. The sample size was calculated as 35 participants in each group.

We documented the demographical features of each participant including age and sex. The severity of disability was assessed in terms of the expanded disability status scale (EDSS) [15]. Annualized relapse rate (ARR) was calculated. ARR was defined as the total number of relapses divided by the duration of the disease. Disease-modifying therapies were also obtained.

Serology

The IgG and IgM antibodies (Ab) against *H. pylori* status were determined using a commercial kit (Euroimmun, Lubeck, Germany), according to the manufacturer's instructions. A positive result for the persistence of *H. pylori* IgG Ab was accepted for a serum level greater than 20 RU/mL. Serum positive for *H. pylori* IgG was considered as *H. pylori* seropositivity. Furthermore, a measure of 40 RU/mL was set as the cut-off value for IgM Ab.

Statistical analysis

All data are presented as Mean±SD and categorical variables are reported as frequency (%). We used unpaired t-tests or the Mann-Whitney U test to compare the means of normally and non-normally distributed continuous variables, respectively. A chi-square test was used for categorical variables. Binary logistic regression analysis was applied to determine the association of *H. pylori* infection with NMOSD and clinical features. The results of logistic regression analyses were recorded as odds ratio (OR), 95% confidence interval (CI), and P value. All statistical calculations were done using the SPSS software, version 20, for Windows (SPSS, Chicago, IL, USA) and P<0.05 was considered significant.

Results

Demographic and clinical features

A total of 35 NMOSD patients and 37 healthy controls were enrolled in the study. Demographic and clinical features are presented in Table 1. There was no difference in mean age and sex ratio between NMOSD patients and HC (P>0.05). The median disease duration was 6.0 (4.0-8.0). The median of EDSS was 2.0 (0.0-2.12). Rituximab was the most frequent disease-modifying treatment.

Frequency of *H. pylori* seropositivity

As shown in Figure 1, the frequency of IgG *H. pylori* seropositivity in NMOSD patients 8.35(22.9%) was lower than HC 11.37(29.7%), though this difference was not statically significant (OR: 0.700, 95%, CI:0.243, 2.017, P=0.420). No significant difference was observed regarding the *H. pylori* IgM Ab seropositivity frequencies among NMOSD patients 14.35(40.0%) and HC 20.37(54.1%) (OR=0.567, 95%, CI=0.222, 1.444, P=0.233).

We also divided NMOSD patients into *H. pylori* seropositive and seronegative to assess the association of *H. pylori* with demographic and clinical characteristics. No significant association was found between *H. pylori* seropositivity and variables (Table 2).

Discussion

The major findings of this study are as follows: (I) frequency of *H. pylori* infection among NMOSD patients negative for anti-AQP4 antibody did not yield any significant difference when compared to HCs and (II) *H. pylori* infection showed significant association neither with clinical features (including EDSS upon the last follow-up session, disease duration, and ARR) nor with individuals' demographic information (age, sex.) in anti-AQP4 negative patients. This result suggests that *H. pylori* infection may not be a risk factor for developing of AQP4-Ab seronegative NMOSD.

Table 1. Demographic and clinical features of participants

Dimensions		Mean±SD/No. (%)/Median (IQR)		P
		NMOSD (n=35)	HC (n=37)	
Age (y)		36.63±9.34	37.57±9.75	0.768
Sex	female	27(77.1)	28(75.7)	0.884
	male	8(22.8)	9(24.3)	
Disease duration (y)		6(4.0-8.0)	NA	-
EDSS		2(0.0-2.12)	NA	-
Annualized relapse rate		0.54±0.29	NA	-
Medication	Rituximab	29(82.8)	NA	-
	Azathioprine	6(17.2)	NA	-

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EDSS: expanded disability status scale; HC: healthy control; NMOSD: neuromyelitis optica spectrum disorder.

Table 2. Binary logistic regression of clinical and demographic variables for IgG and IgM H. pylori seropositivity

Variables		OR (95% CI)	P	OR (95% CI)	P
		IgG *H. pylori* Seropositivity		IgM *H. pylori* Seropositivity	
Sex	Female	1.400(0.215-9.121)	0.725	1.222 (0.240-6.233)	0.809
Age (y)		0.991(0.907-1.083)	0.837	0.952 (0.866-1.047)	0.311
Disease duration		1.529(0.862-2.712)	0.146	0.969 (0.683-1.375)	0.861
EDSS		1.200(0.553-2.607)	0.644	0.710 (0.332-1.517)	0.377
ARR		0.003(0.000-2.135)	0.083	0.158 (0.003-8.691)	0.367

ARR: annualized relapse rate; EDSS: expanded disability status scale; OR: odds ratio.

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Evidence regarding the existence of any association between *H. pylori* infection and NMOSD is scarce. Results from a study designed to investigate whether the genetic and infectious backgrounds of NMOSD patients by Yoshimura et al. reported the prevalence of *H. pylori* infection in 41.81% of HCs and 36.11% in seronegative NMOSD patients. This finding was in the line with our results [13]. Findings from Li et al. study showed *H. pylori* seropositivity in corticospinal multiple sclerosis (OSMS), who are positive for anti-AQP4 IgG, significantly higher when compared to those with negative status for serum anti-AQP4 IgG. However, seropositivity for *H. pylori* antibodies was shown to be significantly higher in AQP-4 antibody-negative OSMS patients than in controls [12]. Furthermore, a study conducted by Long et al. analyzed the sera samples from the Chinese population and showed that the frequency of *H. pylori* seropositivity was higher among the NMO spectrum compared to MS patients and controls. Upon analyzing separately, based on AQP4 antibody status and clinical phenotype, seropositivity for *H. pylori* was significantly higher in AQP4-positive compared to AQP4-negative patients. Nevertheless, the frequency of *H. pylori* seropositivity in NMO patients with negative AQP4 antibody was higher than HCs [16]. These studies suggested a potential role for non-specific pathways in the association of NMOSD with *H. pylori* infection.

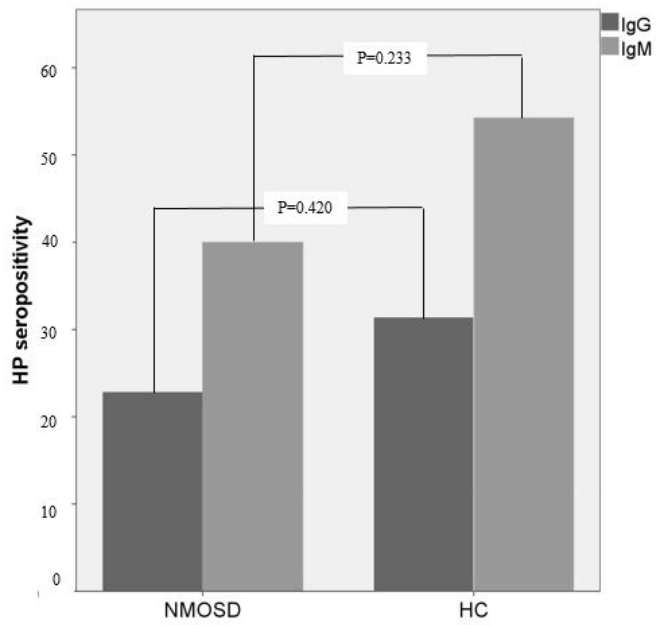

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Figure 1. Frequency of *H. pylori* seropositivity in neuromyelitis optica spectrum disorder and health control subjects

HC: healthy controls; NMOSD: neuromyelitis optica spectrum disorder.

Additionally, a recent study conducted by Wei et al. found significant correlations between IL-6 CSF titers and seropositivity for anti-AQP4-IgG, as this cytokine was reported considerably more elevated among seropositive NMOSD patients, but not among seronegative individuals. Moreover, IL-6 showed a strong correlation with the tissue damage biomarkers such as neurofilament light protein and glial fibrillary acidic protein, leading the authors to conclude that seropositive and seronegative subtypes of NMOSD possess distinct pathogenesis pathways in the initial phases of the disease and IL-6 is of prominent roles in pathways related to seropositive NMOSD [17]. IL-6 is known as a pro-inflammatory cytokine with various functions, such as the synthesis of Ig in activated B-cells and the differentiation of naive T-cells into Th-17 or cytotoxic T-cells [18-20]. Another recent study, involving MS, acute disseminated encephalomyelitis, and seropositive NMOSD patients, reported the aforementioned association of IL-6 elevated levels in CSF with anti-AQP4-IgG seropositive NMOSD, as well [21]. Taken together, these results and prior findings hypothesize non-specific pathways may have a role in the relationship between *H. pylori* infection and NMOSD with positive AQP4-IgG. Further studies are required to elucidate the role of non-specific pathways in the association of *H. pylori* with anti-AQP4-IgG seronegative NMOSD.

Results presented by our study do not contribute much additional information in favor of an existing NMOSD and *H. pylori* association. However, it does strengthen the idea that, in patients negative for anti-AQP4 antibody, infection with *H. pylori* is not associated with NMOSD development, duration of the disease, and other clinical parameters. Other than the different proportions of seropositive/seronegative NMOSD patients, another possible explanation of the different observations in the current study when compared to previous research might be the different methods utilized in studies, including indirect immunofluorescence, fluoro-immunoprecipitation assays cell-based assays, and radio-immunoprecipitation assays. The gold standard test in terms of anti-AQP4 IgG identification has not been demonstrated yet [22]. Different ethnicities can also generate widely varied sensitivities of NMO-IgG tests, explaining the high variability in results from studies with samples from ethnically different populations, to some extent [23].

The limited sample size and power of the current study restrict the possibility of drawing solid conclusions from the obtained results. Additionally, *H. pylori* seropositivity status evaluations in the present study were performed by ELISA, which is considered a rather less appropriate method for such undertakings. Moreover, cross-sectional studies are not designed to assist us to evaluate the causality of the associations observed, which appears to be of less importance in this case, since our study yielded no such association that would necessitate a causality assessment to be performed.

Conclusion

Conclusively, no possible role for *H. pylori* infection in NMOSD susceptibility or pathogenesis was found among patients seronegative for anti-AQP4 Ab. More studies applying larger sample sizes that can generate more powerful findings are needed to complete our knowledge regarding such probable associations and to further elucidate the pathways through which the pathogen affects the human body.

Ethical Considerations

Compliance with ethical guidelines

All study procedures were in compliance with the ethical guidelines of the Declaration of Helsinki 2013. The study protocol was approved by the regional bioethics committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.200) and written informed consent was obtained from all subjects.

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Authors contributions

Conceptualization: Mahdi Barzegar, Hosein Nouri, Vahid Shaygannejad; Methodology: Mahdi Barzegar, Hossein Motedayyen, Vahid Shaygannejad; Investigation: Mahdi Barzegar, Hosein Nouri, Nasim Nehzat; Writing the original draft: Omid Mirmosayyeb, Vahid Shaygannejad; Funding acquisition: Mahdi Barzegar, Hossein Motedayyen, Vahid Shaygannejad; Resources: Mahdi Barzegar, Vahid Shaygannejad; Supervision, Writing, review, and final approval: Vahid Shaygannejad.

Conflict of interest

All authors declare no conflict of interest.

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