

Full Length Research Paper

## Relationship between *Helicobacter pylori* infection and Multiple sclerosis (MS) in South-east Iran

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*Helicobacter pylori* (*H. pylori*) are one of the most common, well-known pathogenic agents in the development of peptic ulcers. Some investigators have shown a relationship between *H. pylori* and Multiple sclerosis (MS). However, this relationship is controversial. The aim of this study was to determine the association between *H. pylori* infection and MS. In a prospective case-control study, we studied 78 patients with MS and 123 healthy blood donors (HBDs) for bacterial DNA detection and antibody assay. DNA extracted from samples (serum and saliva) and real-time polymerase chain reaction (PCR) was employed in the detection of *H. pylori* genome. The presence of anti *H. pylori* CagA and VacA Immunoglobulin G (IgG) was measured in serum by Western blot technique. We found *H. pylori* DNA in both samples of the 32.05% (25/78) and 32.52% (40/123) of patients and HBDs, respectively ( $P=NS$ ). Furthermore, anti *H. pylori* IgG for both antigens were detected in 21.95% (27/123) of HBDs in contrast with 25.64% (20/78) of patients ( $P=NS$ ). Moreover, genome copy number of *H. pylori* was not significantly changed in patients (140 copies/ml) and HBDs (147 copies/ml). There was no significant correlation between *H. pylori* infection in both groups, but *H. pylori* CagA/VacA-IgG was found in patient quite more than HBDs ( $P<0.05$ ) and these patients showed more positively for serum *H. pylori* genome. Although, these results indicate a lack of connection between the *Helicobacter pylori* infection and multiple sclerosis, the role of immune response against *H. pylori* in the modulation of MS requires further study.

**Key words:** *Helicobacter. pylori*, multiple sclerosis (MS), real time- polymerase chain reaction (PCR), Western blot.

### INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). The factors thought to contribute to the debilitating symptoms of MS are poorly understood. While general consensus seems to be that environmental and genetic factors are the

predominant risk-determining elements, a multi-faceted view incorporating many other factors is probably more accurate (Fox et al., 2006; Kantarci and Wingerchuk 2006). *Helicobacter pylori* (*H. pylori*) are spiral, gram-negative, micro-aerophilic bacteria that are important pathogens causing infections in human gastrointestinal system. The bacteria spread through oral-oral and oral-fecal ways, and virulence factors are related to pathogenicity, as well as host factors were defined (Dunn et al., 1997; Gatti et al., 2005). The outcome of *H. pylori* infection is dependent on host, environmental, and

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bacterial factors (Pietrojusti et al., 2002; Franceschi et al., 2002; El-Omar et al., 2000; Blaser and Berg, 2001). *H. pylori* strains possessing the cytotoxin-associated gene-A (CagA) A, encoding a type IV bacterial protein secretion system (Asahi et al., 2000; Odenbreit et al., 2000; Segal et al., 1999; Stein et al., 2000), is more strongly associated with increased levels of inflammation and disease, as are those producing an active form of VacA, a pore forming toxin that induces cytoplasmic vacuolation *in vitro* (Atherton, 1997; Leunk et al., 1988; Papini et al., 1994; Szabo et al., 1999). *H. pylori* causes chronic infections in a large proportion of the world's population (Dunn et al., 1997) and are associated with a number of different clinical conditions. *H. pylori* infections has been linked to MS and demyelinating peripheral neuropathies, as it may trigger cellular and humoral immunity due to the sharing of similar epitopes present in the nervous tissue (Kountouras et al., 2005; Gavalas et al., 2007). These antibodies cross-react with different components of central and peripheral nerves resulting in their damage. Li et al. (2007) studied the prevalence of *H. pylori* infection in different MS subtypes including classic (CMS) and opticospinal MS (OSMS) in the Japanese population and demonstrated a difference in *H. pylori* seropositivity between Japanese patients with OSMS and those with CMS. *H. pylori* infection was significantly lower in patients with CMS than in healthy controls or patients with OSMS (Li et al., 2007). This study suggested that the differences in childhood environment might exert distinct effects on the development of each MS subtype later in life and *H. pylori* might be a protective factor against CMS. Wender (2003) also reported lower frequency of *H. pylori* infection in MS as compared with controls. In this case-control study, we attempted to determine the seroprevalence of anti-*H. pylori* antibodies (CagA-IgG and VacA-IgG) and distribution of *H. pylori* genome in varied specimens to determine the role of systemic active *H. pylori* infection in pathogenesis of MS.

## MATERIALS AND METHODS

### Patients and samples

This study was approved by the Zabol University Multiple Institutional Review Board, and was conducted with all clinical samples from MS patients who were treated at the Department of Neurology, Ali-ebn Abitaleb Hospital, Zahedan, Iran, and also, healthy blood donors (HBDs) who voluntarily submitted for research at the central medical laboratory of Zahedan from December, 2008 to July, 2009. MS patients (in southeast of Iran) were diagnosed with magnetic resonance imaging (MRI) and McDonald criteria were recruited (Polman et al., 2005). 201 different samples were analyzed; 78 patients and 123 people as the HBDs, sample details have been described previously (Sanadgol et al., 2010). Serum and un-stimulated whole saliva samples were collected by standard methods. Serum samples from 15 patients were obtained during periods of disease activity (exacerbation). All Specimens were stored at -70°C until the experiment was performed. Multiple specimens were submitted for one patient, and all of them were tested. If possible, clinical materials were tested more than once.

### DNA extraction and quantitative real-time PCR (qPCR)

*H. pylori* DNA extraction was performed for 100 µl of samples using RIBO-prep nucleic acid extraction kit (Interlabservice, Moscow, Russia) according to the manufacturer's procedure. Positive controls consisted of genomic DNA extracted from *H. pylori* colonies (reference strains ATCC 43504) and negative controls were provided by DNA isolated from blood samples and gastric mucosa of uninfected mice (strain C57BL/6). Real-time PCR was performed with QuantiTect SYBR Green PCR (QIAGEN, Hilden, Germany) in a standard PCR reaction mixture. The amplification primers were: *H. pylori*-specific *ureA* (77-bp PCR fragment) forward primer (5'-CGTGGCAAGCATGATCCAT-3', positions 2877 to 2895, GenBank accession no. M60398) and reverse primer (5'-GGGTATGCACGGTTACGAGTTT-3', positions 2953 to 2932, GenBank accession no. M60398). Amplification and detection were performed in a Rotor-Gene 3000 Instrument (Corbett Research, Sydney, Australia). The conditions were as follows: 10 min, 95°C; 55 cycling steps of 30 s at 95°C, 20 s at 60°C and 40 s at 72°C. And Fluorescence measurement was taken at each extension step. The crossing points (Cp), the cycles when the fluorescence of a given sample significantly exceeded baseline signal, were recorded and expressed as a function of the cycle number. Melting curve analysis was also performed to assess the specificity of the amplicon. Calculations of Ct, preparation of standard curve and quantification of DNA in each sample were performed by Rotor-Gene Operating Software, version 1.8 (Corbett Research).

### *H. pylori* Western blot

CagA and VacA-IgG antibodies from all samples were detected by the Western blot technique, using a commercially available kit, according to the manufacturer's instructions (Immunoblot *Helicobacter* IgG, Mikrogen, Germany). This test detects antibodies for proteins from different *H. pylori* strains (Andersen and Espersen, 1992).

### Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 16 was used for statistical analysis.  $\chi^2$  analysis was applied to analyze categorical variables and *t* tests for continuous variables. All P-values are two-tailed and significant at  $P < 0.05$  or  $P < 0.01$  depending on statistical method.

### Ethical considerations

The study conformed to the Helsinki declaration and was reviewed and approved by the local research committee; written informed consent was obtained from all subjects.

## RESULTS

### *H. pylori* CagA and VacA immunoglobulin G (IgG) response

This study studies have demonstrated that at least 48.71% (38/78) of MS patients are positive for *H. pylori* CagA-specific IgG antibodies in contrast with 29.26% (36/123) of HBDs (Table 1). Moreover, 32.05% (25/78) of MS patients are positive for *H. pylori* VacA-specific IgG antibodies in contrast with 44.71% (55/123) of HBDs

**Table 1.** Prevalence of *H. pylori*, DNA and antibodies amongst controls and multiple sclerosis (MS) patients. *H. pylori* genome was analyzed via Real time-PCR as described previously. Concentrations of serum *H. pylori* IgG were measured according to the manufacturer's instructions. Data are representative of three independent experiments.

	Patients (n=78)		Control (n=123)		Sig. (2-tailed)
	P/N (%) [mean ± SD]				
CagA -IgG (+/-)	25/53 (32.05) [-]		55/68 (44.71) [-]		P<0.01
VacA -IgG (+/-)	38/40 (48.71) [-]		36/87 (29.26) [-]		P<0.05
Saliva-DNA (copies/ml)	43/35 (55.12) [144±27.73]		73/50 (59.34) [148±26.117]		NS
Serum-DNA (copies/ml)	39/39 (50) [135±24.02]		40/83 (32.52) [147±26.57]		P<0.05

P=positive; N=negative; NS=not significant.

(Table 1). Furthermore, 25.64% (20/78) of patients and 21.95% (27/123) of HBDs showed IgG against both CagA and VacA antigens. On the other hand, patients had higher concentration of *H. pylori* CagA but lower concentration of *H. pylori* VacA -specific IgG in contrast with controls. When comparing, *H. pylori* CagA/VacA-IgG immune response in patients and controls, data showed that there is statistically significant relationship between these groups ( $P<0.05$ ). An increase in CagA seropositivity with rising DNA concentration in serum was also observed in patients with disease exacerbation ( $P<0.01$ ).

#### Load of *H. pylori* genome in clinical samples

*H. pylori* DNA was detected in the serum of 50% (39/78) of patients with MS and in 32.52% (40/123) of HBDs (Table 1). In the saliva samples, 55.12% (43/78) of patients had *H. pylori* DNA compared to 59.34% (73/123) of the HBDs (Table 1). Moreover, cell-free (in both serum and saliva) bacterial DNA was detected in 32.05% (25/78) of MS patients compared with 32.52% (40/123) of healthy controls ( $P=NS$ ). Bacterial DNA was found in all serum samples that previously were positive for CagA-specific IgG antibodies in patients but not in controls. Saliva showed much higher prevalence of bacterial sequence than serum samples in both groups ( $P<0.05$ ). Furthermore, among patients samples, 10.25% (8/78) individuals showed positive results in all specimens in contrast with none of the controls ( $P<0.001$ ).

#### DISCUSSION

Prevalence of *H. pylori* infection correlates with socio-economic status rather than race (Mitchell and Megraud, 2002). From a global aspect, 30-40% of people in the United States and Western Europe are infected with *H. pylori*, compared to 70-80% of people in China (Yang et al., 1999) and Indochina. In developing countries, most adults are infected and the acquisition rate occurs in about 2% of children per annum between the ages of 2-8 years. Several mechanisms have been proposed for how pathogens might induce activation and critical expansion

of autoreactive T cells and led to autoimmune diseases such as MS (Rose and Bona, 1993; Oldstone, 1998; Theofilopoulos and Kono, 1998; Lori and Inman, 1999; Benoist and Mathis, 2001; Wucherpfennig, 2004). Activation of resting autoreactive T cells may be achieved by viral and bacterial super antigens that bind a variety of MHC class II molecules and activate large numbers of T cells, irrespective of their specificity (Schrer et al., 1993). Pathogen-induced tissue inflammation may result in local activation of APCs and enhanced processing/presentation of self antigens that causes T cell priming, followed by T cell activation and expansion of additional specificities (epitope spreading) (Lehmann et al., 1992; Miller et al., 1997). Another mechanism would imply that the inflammatory setting and the paracrine secretion of T cell growth factors induce the expansion of activated autoreactive T cells, whose small number was previously insufficient to drive an autoimmune disease. Such a mechanism is referred to as bystander activation (Murali-Krishna et al., 1998). Moreover, a microbial antigen can include an epitope, that is, structurally similar to an autoantigen epitope, providing the basic element of the mechanism referred to as molecular mimicry (Bachmaier et al., 1999; Rose and Mackay, 2000; Hemmer et al., 1999; Martin et al., 2001). TNF- $\alpha$  is a proinflammatory cytokine, that is, induced *in vivo* by *H. pylori* (Crabtree et al., 1991). Translocation and phosphorylation of CagA stimulates MAP kinases and the cag encoded type IV secretion system itself stimulates nuclear factor  $\kappa$ B activation and interleukin 8 transcription (Meyer-ter-Vehn et al., 2000; Naumann et al., 1999). Lim et al. (2003) reported that the increased expression of cell adhesion molecules (galectin 1, aldolase A, integrin  $\alpha$ 5, LMO7) and the decrease in E-cad expression induced by *H. pylori* might contribute to cell adhesion, invasion, and possibly cell proliferation in gastric epithelial cells (Lim et al., 2003). Kitadai et al. (2003) reported that coculture with *H. pylori* increased the expression of interleukin-8, vascular endothelial growth factor (VEGF), angiogenin, uPA, and MMP-9 and increased angiogenic and collagenase activities in gastric carcinoma cells. Sharma et al. (1998) reported that the activation of interleukin-8 gene expression by *H. pylori* is regulated by the transcription factor, nuclear factor-kappa B, in gastric epithelial cells

Akhtar et al. (2001). Some scientists reported that promoter methylation regulates *H. pylori*-stimulated cyclooxygenase-2 expression in gastric epithelial cells. There are some case reports of a relation between *H. pylori* infection and systemic sclerosis and multiple sclerosis (Wender, 2003; Gavalas et al., 2007); however there is a need for a large, double-blind study with proper control groups before we can draw any conclusions. Our study findings suggest that *H. pylori* infection is not a causative factor for MS at least in southeast Iran, but may help to established MS progression. As prevalence of *H. pylori* CagA-specific IgG was higher in patients especially in those suffering exacerbating, we propose that immune response against these antigens antigen could have a critical role in the development of MS. This hypothesis is suggested by the higher ratio of CagA/VacA-IgG in patients compare with controls. Alternatively, because of relatively lower copy number of *H. pylori* genome and titer of *H. pylori* VacA-specific IgG in controls and in compared to patients with MS exacerbation; we propose that VacA immune response could have a protective role against MS. The association of *H. pylori* with MS remains controversial and a more extensive understanding of *H. pylori* neurotropism and its association with the disease process is required.

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