

Current Research Journal of Biological Sciences 4(4): 422-426, 2012

ISSN: 2041-0778

© Maxwell Scientific Organization, 2012

Submitted: February 09, 2012

Accepted: March 01, 2012

Published: July 10, 2012

Relationship Between *Helicobacter pylori* (*H. pylori*) Infection and Multiple Sclerosis (MS) in Southeast of Iran

¹Nima Sanadgol, ²Ebrahim Shahraki and ¹Jasem Estakhr

¹Department of Biology, Faculty of Science,

²Research Center of Domestic Animals, University of Zabol, Zabol, Islamic Republic of Iran.

Abstract: *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 PCR was employed to detection of *H. pylori* genome. The present of anti *H. pylori* CagA and VacA Immunoglobulin G (IgG) were 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 change in patients (140 copies/mL) and HBDs (147copies/mL). We didn't see 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 this 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.

Keywords: *H. pylori*, MS, real time-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 bacterium spreads 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 bacterial factors (Pietrojusti *et al.*, 2002; Franceschi *et al.*, 2002; El-Omar *et al.*, 2000; Blaser and Berg, 2001). *H. pylori* strains that 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 infection 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 maybe 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

Corresponding Author: Nima Sanadgol, Department of Biology, Faculty of Sciences, University of Zabol, Zabol, Islamic Republic of Iran, Tel.: 00989157444696; Fax: 00985422242503

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 (Wender, 2003). 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: The study, approved by the Zabol University Multiple Institutional Review Board, 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 through July 2009. MS patients (in southeast of Iran) were diagnosed with Magnetic Resonance Imaging (MRI) and McDonald criteria were collected (Polman *et al.*, 2005). We analyzed 201 different samples; 78 patients and 123 people as the HBDs, Sample details have been described previously (Sanadgol *et al.*, 2011). 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 protocol. 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'-GGGT ATGCACGGTTACGAGTTT-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 minutes, 95°C; 55 cycling steps of 30 at 95, 20 at 60 and 40 sec at

72°C 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 considerations: The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 16 was used for statistical analysis. χ^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:** Recent 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). 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 were compared, *H. pylori* CagA/VacA-IgG immune response in patients and controls, data showed statically 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

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 measurement according to the manufacturer's instructions. Data are representative of three independent experiments

Patients (n = 78) Controls (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) 73/50(59.34) NS [144±27.73][148±26.117]
Serum-DNA (copies/mL)	39/39(50) 40/83(32.52) p<0.05 [135±24.02] [147±26.57]

P: Positive; N: Negative; NS: Not significant

patients with MS and in 32.52% (40/123) of HBDs (Table 1). In the saliva samples, 55.12% (43/78) of patients were *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) healthy controls (p = NS). Bacterial DNA was found in all serum samples that previously were positive for CagA-specific IgG antibodies in patient 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 with 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 start autoimmune disease 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 superantigens 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- α 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 kB 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 α 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 (Kitadai *et al.*, 2003). 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 (Sharma *et al.*, 1998). Akhtar *et al.* (2001) reported that promoter methylation regulates *H. pylori*-stimulated cyclooxygenase-2 expression in gastric epithelial cells (Akhtar *et al.*, 2001). There are some case reports of a relation between *H. pylori* infection and systemic sclerosis and multiple sclerosis (Wender, 2003; Gavalas *et al.*, 2007; 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. Totally, my findings suggest that *H. pylori* infection is not a causative factor for MS at least in southeast Iran, but maybe help to established of MS progression. As prevalence of *H. pylori* CagA-specific IgG was more in patients especially in whose suffer exacerbate period, we propose that immune response against this antigen could have a critical role in MS development. This hypothesis confirmed by higher among of CagA/VacA-IgG ration in patient compare with patients. Alternatively because of relatively lower copy number of *H. pylori* genome and titer of *H. pylori* VacA-specific IgG in controls contrast with patient with MS exacerbation we propose that VacA immune response possibly 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.

ACKNOWLEDGMENT

This research was financially supported by Zabol University, Zabol, Iran. We appreciate Dr. A. Moghtaderi for his helpful efforts in sample collecting, Mrs. V. Khajeh for their helpful comments on the laboratory and Dr. E. Sanadgol for data assortment.

REFERENCES

- Akhtar, M., Y. Cheng, R.M. Magno, H. Ashktorab, D.T. Smoot, S.J. Meltzer and K.T. Wilson, 2001. Promoter methylation regulates *Helicobacter pylori* stimulated cyclooxygenase-2 expression in gastric epithelial cells. *Cancer Res.*, 61: 2399-2403.
- Andersen, L.P. and F. Espersen, 1992. Immunoglobulin G antibodies to *Helicobacter pylori* in patients with dyspeptic symptoms investigated by the western immunoblot technique. *J. Clin. Microbiol.*, 30: 1743-1751.
- Asahi, M., T. Azuma, S. Ito, Y. Ito, H. Suto, *et al.*, 2000. *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. *J. Exp. Med.*, 191(4): 593-602.
- Atherton, J.C., 1997. The clinical relevance of strain types of *H. pylori*. *Gut*, 40: 701-703.
- Bachmaier, K., N. Neu, L.M. De la Maza, S. Pal, A. Hessel and J.M. Penninger, 1999. Chlamydia infections and heart disease linked through antigenic mimicry. *Science*, 283: 1335-1339.
- Benoist, C. and D. Mathis, 2001. Autoimmunity provoked by infection: How good is the case for T cell epitope mimicry? *Nat. Immunol.*, 2: 797-801.
- Blaser, M.J. and D.E. Berg, 2001. *Helicobacter pylori* genetic diversity and risk of human disease. *J. Clin. Invest*, 107: 767-773.
- Crabtree, J.E., T.M. Shallcross, R.V. Heatley and J.I. Wyatt, 1991. Mucosal tumour necrosis factor alpha and interleukin-6 in patients with *Helicobacter pylori* associated gastritis. *Gut*, 32(12): 1473-1477.
- Dunn, B.E., B. Cohen and M.J. Blaser, 1997. *Helicobacter pylori*. *Clin. Microbiol. Rev.*, 10: 720-741.
- El-Omar, E.M, M. Carrington, W.H. Chow, K.E.L. McColl, J.H. Bream, *et al.*, 2000. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, 404: 398-402, DOI: 10.1038/35006081
- Fox, R.J., F. Bethoux, M.D. Goldman and J.A. Cohen, 2006. Multiple sclerosis: Advances in understanding, diagnosing and treating the underlying disease. *Cleve Clin. J. Med.*, 73(1): 91-102. DOI: 10.3949/ccjm.73.1.91.
- Franceschi, F., A.R. Sepulveda, A. Gasbarrini, P. Pola, N.G. Silveri, *et al.*, 2002. Cross-reactivity of anti-caga antibodies with vascular wall antigens: Possible pathogenic link between *Helicobacter pylori* infection and atherosclerosis. *Circulation*, 106: 430-434.
- Gatti, L.L., E.K.F.E. Souza, K.R.M. Leite, K. Moreira-Leite, E.L.S. Bastos, L.R. Vicentini, *et al.*, 2005. CagA vacA alleles and babA2 genotypes of *Helicobacter pylori* associated with gastric disease in Brazilian adult patients. *Diagn. Microbiol Infect. Dis.*, 51(4): 231-235.
- Gavalas, E., J. Kountouras, G. Deretzi, M. Boziki, N. Grigoriadis, C. Zavos and I. Venizelos, 2007. *Helicobacter pylori* and multiple sclerosis. *J. Neuroimmunol.*, 188(1-2): 187-189.
- Hemmer, B., B. Gran, Y. Zhao, A. Marques, J. Pascal, A. Tzou, T. Kondo, I. Cortese, B. Bielekova, S.E. Straus, *et al.*, 1999. Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease. *Nat. Med.*, 5: 1375-1382.
- Kantarci, O. and D. Wingerchuk, 2006. Epidemiology and natural history of multiple sclerosis: New insights. *Curr. Opin. Neurol.*, 19(3): 248-254.
- Kitadai, Y., A. Sasaki, M. Ito, S. Tanaka, N. Oue, W. Yasui, M. Aihara, K. Imagawa, K. Haruma and K. Chayama, 2003. *Helicobacter pylori* infection influences expression of genes related to angiogenesis and invasion in human gastric carcinoma cells. *Biochem. Biophys. Res. Commun.*, 311: 809-814.
- Kountouras, J., G. Deretzi, C. Zavos, P. Karatzoqlou, L. Touloumis, *et al.*, 2005. Association between *Helicobacter pylori* infection and acute inflammatory demyelinating polyradiculoneuropathy. *Eur. J. Neurol.*, 12(2): 139-143.
- Lehmann, P.V., T. Forsthuber, A. Miller and E.E. Sercarz, 1992. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature*, 358: 155-157.
- Leunk, R.D., P.T. Johnson, B.C. David, W.G. Kraft and D.R. Morqan, *et al.*, 1988. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. *J. Med. Microbiol.*, 26(2): 93-99.
- Li, W., M. Minohara, J.J. Su, T. Matsuoka, M. Osoegawa, *et al.*, 2007. *Helicobacter pylori* infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. *J. Neuroimmunol.*, 184(1-2): 227-231.
- Lim, J.W., H. Kim and K.H. Kim, 2003. Cell adhesion-related gene expression by *Helicobacter pylori* in gastric epithelial AGS cells. *Int. J. Biochem. Cell Biol.*, 35: 1284-1296.
- Lori, J.A. and R.D. Inman, 1999. Molecular mimicry and autoimmunity. *N. Engl. J. Med.*, 341: 2068-2074.

- Martin, R., B. Gran, Y. Zhao, S. Markovic-Plese, B. Bielekova, A. Marques, M.H. Sung, B. Hemmer, R. Simon, H.F. McFarland and C. Pinilla, 2001. Molecular mimicry and antigen-specific T cell responses in multiple sclerosis and chronic CNS Lyme disease. *J. Autoimmun.*, 16: 187-192.
- Meyer-ter-Vehn, T., A. Covacci, M. Kist and H.L. Pahl, 2000. *Helicobacter pylori* activates mitogen-activated protein kinase cascades and induces expression of the proto-oncogenes c-fos and c-jun. *J. Biol. Chem.*, 275(21): 16064-16072.
- Miller, S.D., C.L. Vanderlugt, W.S. Begolka, W. Pao, R.L. Yauch, K.L. Neville, Y. Katz-Levy, A. Carrizosa and B.S. Kim, 1997. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat. Med.*, 3: 1133-1136.
- Mitchell, H. and F. Megraud, 2002. Epidemiology and diagnosis of *Helicobacter pylori* infection. *Helicobacter*, 7(1): 8-16.
- Murali-Krishna, K., J.D. Altman, M. Suresh, D.J. Sourdive, A.J. Zajac, J.D. Miller, J. Slansky and R. Ahmed, 1998. Counting antigen-specific CD8 T cells: A reevaluation of bystander activation during viral infection. *Immunity*, 8: 177-187.
- Naumann, M., S. Wessler, C. Bartsch, B. Wieland, A. Covacci, *et al.*, 1999. Activation of activator protein 1 and stress response kinases in epithelial cells colonized by *Helicobacter pylori* encoding the cag pathogenicity island. *J. Biol. Chem.*, 274(44): 31655-31662.
- Odenbreit, S., J. Puls, B. Sedlmaier, E. Gerland, W. Fischer, *et al.*, 2000. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science*, 287: 1497-1500, DOI: 10.1126/Science.287.5457.1497.
- Oldstone, M.B.A., 1998. Molecular mimicry and immunemediated diseases. *FASEB J.*, 12: 1255-1265.
- Papini, E., M. De Bernard, E. Milia, M. Bugnoli, M. Zerial, *et al.*, 1994. Cellular vacuoles induced by *Helicobacter pylori* originate from late endosomal compartments. *Proc. Natl. Acad. Sci. USA*, 91(21): 9720-9724.
- Pietrojusti, A., M. Diomed, M. Silvestrini, L.M. Cupini, I. Luzzi, *et al.*, 2002. Cytotoxin-associated gene-a-positive *Helicobacter pylori* strains are associated with atherosclerotic stroke. *Circulation*, 106(5): 580-584.
- Polman, C.H., S.C. Reingold, G. Edan, *et al.*, 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald criteria." *Ann. Neurol.*, 58: 840-846.
- Rose, N.R. and C. Bona, 1993. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today*, 14: 426-430.
- Rose, N.R. and I.R. Mackay, 2000. Molecular mimicry: a critical look at exemplary instances in human diseases. *Cell. Mol. Life Sci.*, 57(4): 542-551. DOI: 10.1007/PL00000716.
- Sanadgol, N., N. Ramroodi, G.A. Ahmadi, M. Komijani, A. Moghtaderi, *et al.*, 2011. Prevalence of cytomegalovirus infection and its role in total immunoglobulin pattern in Iranian patients with different subtypes of multiple sclerosis. *New Microbiol.*, 34(3): 263-274.
- Segal, E.D., J. Cha, J. Lo, S. Falkow and L.S. Tompkins, 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. *Proc. Natl. Acad. Sci. USA*, 96(25): 14559-14564.
- Schrer, M.T., L. Ignatowicz, G.M. Winslow, J.W. Kappler and P. Marrack, 1993. Superantigens: Bacterial and viral proteins that manipulate the immune system. *Annu. Rev. Cell Biol.*, 9: 101-128.
- Sharma, S.A., M.K. Tummuru, M.J. Blaser, L.D. Kerr, 1998. Activation of IL-8 gene expression by *Helicobacter pylori* is regulated by transcription factor, nuclear factor-kappa B in gastric epithelial cells. *J. Immunol.*, 160: 2401-2407.
- Stein, M., R. Rappuoli and A. Covacci, 2000. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after cag-driven host cell translocation. *Proc. Natl. Acad. Sci. USA*, 97: 1263-1268.
- Szabo, I., S. Brutsche, F. Tombola, M. Maschioni, B. Satin, *et al.*, 1999. Formation of anion-selective channels in the cell plasma membrane by the toxin VacA of *Helicobacter pylori* is required for its biological activity. *EMBO J.*, 18(20): 5517-5527, DOI: 10.1093/emboj/18.20.5517.
- Theofilopoulos, A.N. and D.H. Kono, 1998. Mechanisms and genetics of autoimmunity. *Ann. N.Y. Acad. Sci.*, 841: 225-235.
- Wender, M., 2003. Prevalence of *Helicobacter pylori* infection among patients with multiple sclerosis. *Neurol Neurochir Pol.* 2003; 37(1):45-48.
- Wucherpfennig, K.W., 2004. Mechanisms for the induction of autoimmunity by infectious agents. *J. Clin. Invest.*, 108: 1097-1104.
- Yang, H., S.V. Wu, S. Pichuanes, M. Song, J. Wang, *et al.*, 1999. High prevalence of cagA-positive strains in *Helicobacter pylori*-infected, healthy, young Chinese adults. *J. Gastroenterol. Hepatol.*, 14: 476-480.