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Association of *Helicobacter pylori* Infection with Systemic Lupus Erythematosus

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Abstract

Objective: Systemic Lupus Erythematosus (SLE) is an autoimmune disease of unknown etiology. However, a complex combination of host and environmental factors are believed to play a pivotal role. In the pathogenesis of SLE, several infectious agents have been held responsible such as *Cytomegalovirus*, Parvovirus B19, Epstein Barr virus and Retrovirus. There is a variable relationship between SLE and *Helicobacter*, which is different from that of lupus and other infections. The aim of this study was to investigate the association between *Helicobacter pylori* (*H.pylori*) infection and SLE development.

Method: In this study, 82 serum samples and 65 stool samples were collected from the SLE patients as well as the control group, respectively. An enzyme-linked immunosorbent assay (ELISA) was used to detect the presence of specific IgG/IgM antibodies against *H.pylori* in all serum samples. The presence of *H.pylori* antigen was examined in all stool samples by using stool antigen test. Suitable statistical analysis was applied.

Results: Thirteen (15.9%) out of 82 SLE patients and 30 (36.6%) out of 82 control group were anti-*H.pylori* IgM seropositive. There was a significant difference between the level of IgM in SLE patients and control group ($p<0.05$). Anti-*H.pylori* IgG antibodies were present in 37 (45.1%) of SLE patients and in 41 (50%) of the control group without any significant difference. Regarding stool antigen examination, there were 24 (36.9%) and 26 (42/6%) positive samples among the SLE patients and control group, respectively. There was no significant difference between the patients and controls samples in the number of stool antigen test positive samples ($p>0.05$).

Conclusions: Based on data obtained in this study, it is concluded that there was a significant difference between the number of IgM seropositive in SLE patients and control group, which shows that SLE disease may have an inhibitory role in *H.pylori* infection occurance.

Keywords: Systemic lupus erythematosus; Autoimmune; Infection; *Helicobacter pylori*; ELISA; Antibodies; Antigen

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that can affect any organ of the body including the skin, mucous membranes, joints, kidneys, blood and brain [1-7]. SLE is the perfect model of autoimmune disorder, begin from innate immunity to adaptive immunity and leading to loss of self-tolerance [8,9]. The exact etiology of SLE is unknown, however, several genetic and environmental factors might have a role [3,10,11]. Epidemiologic studies showed variations in the prevalence and course of SLE between genders, different ages, races and geographic locations [7,12]. The incidence of SLE is estimated to be about 5 per 100,000 people per year with a prevalence of 40 to 80 per 100,000 [8]. Women are more frequently affected than men, with a sex ratio of approximately 9:1 between the ages of 15–45 years [1,3,12,13]. The disease is more common in Native American, African-American, Asian and Hispanic descent [2,14].

Amongst the various infectious agents proposed as autoimmunity triggering agents, *Helicobacter pylori* (*H.pylori*) is one of the most widely studied [15-18].

H.pylori is a gram-negative, spiral-shaped, flagellated and microaerophilic bacterium that colonizes the human gastric mucosa [19-22]. *H.pylori* infection is one of the most usual bacterial infections in humans. *H.pylori* infection is usually acquired in early childhood and continues life-long in the lack of medicine treatment [21-23]. *H.pylori* and humans have a lengthy period of coevolution, as long as human migration out of Africa about 60,000 years ago [24]. The prevalence of *H.pylori* infection differs broadly by geographic area, age, race, and socioeconomic status [19,22,25]. The overall prevalence of this bacterium is as high as 80% in many developing countries to 20-50% in industrialized countries [26]. *H.pylori* infection not only causes a several of gastrointestinal diseases including peptic ulcers, noncardia gastric adenocarcinoma and gastric mucosa associated lymphoid tissue (MALT) lymphoma, but also plays a role in the pathogenesis of various autoimmune disorders such as idiopathic thrombocytopenic purpura, rheumatoid arthritis (RA), autoimmune gastric atrophy, Sjogren´s syndrome and autoimmune thyroiditis [17,27]. This extended coexistence of *H.pylori* in humans may be in part beneficial to humans. Because, in some instances, there is some

evidences explaining a protective effect for *H.pylori* infection against diseases progression such as inflammatory bowel disease (IBD), multiple sclerosis (MS), SLE and allergic diseases [27,28].

Group	Number of Anti-*H.pylori* IgM		Number of Anti-*H.pylori* IgG		Number of Stool antigen test	
	Positive	Negative	Positive	Negative	Positive	Negative
SLE patients	13 (15.9%)	69 (84.1%)	37 (45.1%)	45 (54.9%)	24 (36.9%)	41 (63.1%)
Control	30 (36.6%)	52 (63.4%)	41 (50%)	41 (50%)	26 (42.6%)	35 (57.4%)
p value	0.003		0.53		0.58	

Table 1: The prevalence of *Helicobacter pylori* in SLE patients and control group

There are several methods for the diagnosis of *H.pylori*, each with its own benefits, weaknesses and limitations [29]. Invasive techniques based on gastric specimens for histology, culture and a rapid urease test, and non-invasive techniques based on peripheral samples, such as blood, stools, urine, saliva or breath samples, for detection of bacterial antigens, antibodies or urease activity are used to diagnosis of *H.pylori* infection [22,30,31]. In this study, in order to investigate the possible association between *H.pylori* infection and the development of SLE, the prevalence of *H.pylori* Infection in SLE patients compared with the healthy individuals was evaluated.

Materials and Methods

Patients and controls

Eighty-two SLE patients (76 females and 6 males) who fulfilled American college of rheumatology revised criteria for the classification of SLE were followed up at Rheumatology Clinic affiliated to Isfahan University of Medical Sciences [32]. The demographic data such as age; gender; education; age at the SLE onset; duration of disease; history of gastrointestinal disease and previous administration of anti-*H.pylori* medication were recorded. Disease activity was determined by the SLE disease activity index (SLEDAI-2k) [33]. In addition, eighty-two healthy subjects (77 females and 5 males) of matched gender were used as healthy control group. Sera of all patients and controls were obtained and stored at -20°C. Also, stool specimens at the same time with serum specimens were collected from 65 patients and controls in sterile containers and kept at -70°C.

		Anti-*H.pylori* IgM		p value	Anti-*H.pylori* IgG		p value	Stool antigen test		p value
		Positive	Negative		positive	Negative		positive	Negative	
Mean age of onset of SLE		26.54	26.94	0.89	25	29.19	0.1	27.11	26.69	0.84
Average of disease duration		12.38	11.64	0.66	11.84	11.69	0.9	11.93	11.25	0.65
History of gastrointestinal disease	Yes	2	18	0.5	11	9	0.43	4	12	0.37
	No	11	51		26	36		20	29	
Used prescribed medications for *H.pylori* infection	Yes	0	8	0.34	5	3	0.45	2	5	1
	No	13	61		42	43		22	36	
Used antibiotics	Yes	2	20	0.49	11	11	0.62	5	14	0.39
	No	11	49		26	34		19	27	

Table 2: Associations between various factors and *H.pylori* infection in SLE patients

Serology

The presence of IgM and IgG anti *H.pylori* antibodies in the all serum samples were determined by an enzyme-linked immunosorbent assay (ELISA) based on the manufacturer's instructions (Monobind Inc., USA). Diluted serum and reference samples were pipetted into the streptavidin coated wells and then *H.pylori* biotin reagent solution was added. The microplates were covered and incubated for 60 min at room temperature. The contents of the wells were discharged and washed the wells three times. Then, an enzyme anti-human IgG/IgM conjugate was added into each well. After 30 min of incubation and again washing, substrate solution was added to all wells and was incubated for 15 min at room temperature. The reaction was stopped

by adding stopping solution and the microplate was read at 450 nm with a reference wavelength of 620-630 nm by ELISA reader. A positive result for IgG and IgM anti *H.pylori* antibodies was defined as a concentration greater than 20 U/ml and 40 U/ml respectively.

Stool antigen test

H.pylori antigen was detected in all specimens by stool antigen kit according to the manufacturer's instructions (Farafan Diagnostic kit, Inc; Iran). A little portion of stool specimen was transferred to a dropper vial containing of diluent buffer and later one minute of shaking, about one to two droplets was dispensed into the oval cavity of the test cassette. The test result was read at least after 15 min and based on the appearance of colored lines. The appearance of two purple lines C (control) and T (test) indicated that test was positive and only one purple line in C area indicated the negative result. Also, a pale colored line in T area was considered positive.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 20). The prevalence of *H.pylori* infection among groups was compared by chi-square testing. Quantitative data were compared by student's t-test and described as mean ± SD. A p value of <0.05 was considered to be statistically significant for all data analyses.

	Anti-*H.pylori* IgM		p value	Anti-*H.pylori* IgG		p value	Stool antigen test		p value
	positive	Negative	positive	Negative	positive	Negative			
Arthritis	2	3	0.17	3	2	0.65	1	3	1
Proteinuria	2	2	0.11	1	3	0.62	1	2	1
Rash	2	5	0.3	3	4	1	2	3	1
Low complement	1	6	1	3	4	1	3	3	0.66
Increase DNA binding	2	14	1	7	9	1	4	9	0.75
Pyuria				1	0	0.45			
Hematuria							1	0	0.36
Thrombocytopenia							1	1	1

Table 3: Associations between lupus manifestations and *H.pylori* infection in SLE patients

Results

The mean age of the patients and their mean duration of disease at examination were 38.35 ± 10.2 years (range: 24 to 74) and 11 ± 5 years (range: 2 to 31) respectively. The mean age at disease onset was 26.9 ± 9.8 years (range: 14 to 59 years). SLE disease activity index (SLEDAI-2k) scores ranged from 0 to 10 (mean: 1 ± 2). The frequency of important SLE manifestations (according to SLEDAI-2k) in patients was: increased DNA binding (19.5%), Rash/low complement (8.5%), arthritis (6.1%), proteinuria (4.9%), thrombocytopenia (3.7%), urinary casts/hematuria /pyuria (1.2%). The mean age of healthy control group at sampling was 42.38 ± 12.8 years (range: 18 to 68 years).

Anti-*H.pylori* IgG antibodies were detected in 37 (45.1%) of SLE patients and in 41 (50%) of healthy controls. There was no significant difference between the patients and controls in the number of anti-*H.pylori* IgG seropositive samples (p=0.53). In addition, 13 (15.9%) of SLE patients and 30 (36.6%) of the control group were seropositive for anti-*H.pylori* IgM antibodies. Therefore, anti-*H.pylori* IgM seropositivity was found significantly higher in the control group than SLE patients (p=0.003). Regarding stool antigen test, there were 24 (36.9%) *H.pylori* positive samples among the 65 of SLE patients and 26 (42/6%) among the 61 of healthy controls which showed no statistically significant difference between two groups (p=0.58). In general, 3 (4.6%) of SLE patients and 10 (15.3%) of the control group were positive for anti-*H.pylori* IgM and IgG antibodies and stool antigen test. Results are shown in Table 1.

We investigated the possible associations of mean age of SLE onset, an average of disease duration, the frequency of lupus manifestations, history of gastrointestinal disease, used prescribed medications for *H.pylori* infection and used antibiotics between the SLE patients infected with *H.pylori* and *H.pylori* negative SLE patients. It was shown that there was no significant difference between both groups for all the above-mentioned factors (p>0.05). (Tables 2 and 3)

Discussion

Autoimmune diseases affect 5-10% of the general population and are a significant cause of morbidity and mortality worldwide [26]. The cause of these diseases such as SLE is unknown; although it might be resulted from the interaction between environmental and host factors [15]. The association between infection and autoimmune diseases over the last twenty years has been increasingly supposed [16,27]. Several infectious agents such as *Cytomegalovirus*, Parvovirus B19, Epstein Barr virus and Retrovirus, might play a major role in the pathogenesis of SLE [18,34]. However, there is a different relationship between SLE and *Helicobacter*, from that of other infections. Amongst bacteria, *H.pylori* seems to play a pathogenetic role in autoimmune diseases such as autoimmune gastritis, Sjögren's syndrome, atherosclerosis, immune thrombocytopenia purpura, inflammatory bowel diseases and autoimmune pancreatitis [15,17,27]. Also, *H.pylori* might have a protective role in allergic disorders [7,28], inflammatory bowel disease [35], asthma [36], immune thrombocytopenic purpura [37] and multiple sclerosis [38].

Fewer studies have conducted in order to understand a correlation between *H.pylori* infection and SLE. In an animal study, it was found that exposure to *H.pylori* urease can lead to production of anti-single stranded DNA antibodies [39]. Kalabay and colleagues studied the prevalence of anti-*H.pylori* antibodies in various autoimmune rheumatic diseases and they found the comparable prevalence of *H.pylori* in SLE patients and healthy controls [40]. Showji et al. showed that the titers of anti-*H.pylori* antibodies were lower in SLE than other autoimmune diseases [41]. In another study, Sawalha et al. compared the prevalence of *H.pylori* seropositivity in 466 SLE patients to matched controls and realized SLE patients were less frequently positive (36.5%) for *H.pylori* as compared with healthy controls (42.9%). This negative association for African–American female SLE patients as compared with controls was even severed (38.1 versus 60.2%; p=0.0009). It was noted that *H.pylori* seropositive African-American females compared to *H.pylori* negative SLE patients prone to develop SLE at an older age. The average age of the beginning SLE in the seropositive and seronegative group was 34.4 and 28 years, respectively [42]. The authors suggest that exposure to *H.pylori* may oer some protective benefit against developing SLE in this specific population. In the current study, our data showed no significant difference between the number of positive samples of anti-*H.pylori* IgG and stool antigen among SLE patients and healthy controls.

These studies have some limitations but nevertheless, all of them showed a negative relationship between SLE and *H.pylori* infection. This difference might be resulted from the study population, diversity in the prevalence of *H.pylori* infection in the different part of the world and laboratory methods which applied to detective it.

In conclusion, our study shows that patients with SLE had a lower prevalence of acute *H.pylori* infection than healthy controls and there was a negative association between *H.pylori* infection and SLE. Based on obtained data, it could be concluded that SLE disease might have an inhibitory role in *H.pylori* infection occurrence. More studies are required to elucidate the possible association between the *H.pylori* infection and SLE.

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Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Ben Menachem E (2010) Systemic lupus erythematosus: a review for anesthesiologists. Anesthesia Analgesia 111: 665-676.
2. Bernknopf A, Rowley K, Bailey T (2011) A review of systemic lupus erythematosus and current treatment options. Formulary 46: 178–194.
3. Bertsias G, Cervera R, Boumpas DT (2012) Systemic lupus erythematosus: pathogenesis and clinical features. EULAR textbook on rheumatic diseases, Geneva, Switzerland: European League Against Rheumatism 2012: 476-505.
4. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD (2011) Manifestations of systemic lupus erythematosus. Maedica 6: 330-336.
5. Tsokos GC1 (2011) Systemic lupus erythematosus. N Engl J Med 365: 2110-2121.
6. Manson JJ, Isenberg DA (2003) The pathogenesis of systemic lupus erythematosus. Netherlands Journal of Medicine 61: 343-346.
7. Smith PP, Gordon C (2010) Systemic lupus erythematosus: clinical presentations. Autoimmunity Reviews 10: 43-45.
8. Ahmadpoor P, Dalili N, Rostami M (2014) An update on pathogenesis of systemic lupus erythematosus. Iran J Kidney Dis 8: 171-184.
9. Choi J, Kim ST, Craft J (2012) The pathogenesis of systemic lupus erythematosus—an update. Curr Opin Immunol 24: 651-657.
10. Fernández-Nebro A, Marsal S, Chatham W, Rahman A (2012) Systemic lupus erythematosus: genomics, mechanisms, and therapies. Clin Dev Immunol 2012: 926-931.
11. Mok CC, Lau CS (2003) Pathogenesis of systemic lupus erythematosus. J Clin Pathol 56: 481-490.
12. O'Neill S, Cervera R (2010) Systemic lupus erythematosus. Best Pract Res Clin Rheumatol 24: 841–855.
13. Rahman A, Isenberg DA (2008) Systemic lupus erythematosus. N Engl J Med 358: 929-939.
14. Habibi S, Saleem MA, Ramanan AV (2011) Juvenile systemic lupus erythematosus: review of clinical features and management. Indian Pediatr 48: 879-887.
15. Hasni S, Ippolito A, Illei GG (2011) Helicobacter pylori and autoimmune diseases. Oral Dis 17: 621-627.
16. Radic M (2014) Role of Helicobacter pylori infection in autoimmune systemic rheumatic diseases. World J Gastroenterol 20: 12839.
17. Smyk DS, Koutsoumpas AL, Mytilinaiou MG, Rigopoulou EI, Sakkas LI, et al. (2014) Helicobacter pylori and autoimmune disease: cause or bystander. World J Gastroenterol 20: 613-629.
18. Yildirim T, Yildirim O (2014) The Relationship of Systemic Lupus Erythematosus and Helicobacter Pylori. Med-Science 4: 2344-2347.
19. Brown LM (1999) Helicobacter pylori: epidemiology and routes of transmission. Epidemiologic Reviews 22: 283-297.
20. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R (1999) Helicobacter pylori virulence and genetic geography. Science 284: 1328-1333.
21. Kalali B, Mejías-Luque R, Javaheri A, Gerhard M (2014) H.pylori virulence factors: influence on immune system and pathology. Mediators of Inflammation.
22. Logan RP, Walker MM (2001) ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of Helicobacter pylori infection. BMJ 323: 920-922.
23. Dinic M, Đordevic D, Tasic G, Kocic B, Bogdanovic M (2012) Helicobacter Pylori Infection and Upper Gastrointestinal Disease. Acta Facultatis Medicae Naissensis 29: 5-10.
24. Benghezal M, Gauntlett JC, Debowski AW, Fulurija A, Nilsson HO, et al. (2014) Persistence of Helicobacter pylori infection: Genetic and epigenetic diversity. Helicobacter pylori Infection.
25. Bruce MG, Maaroos HI (2008) Epidemiology of Helicobacter pylori infection. Helicobacter 13 Suppl 1: 1-6.
26. Ram M, Barzilai O, Shapira Y, Anaya JM, Tincani A, et al. (2013) Helicobacter pylori serology in autoimmune diseases–fact or fiction? Clin Chem Lab Med 51: 1075-1082.
27. Amital H, Govoni M, Maya R, Meroni PL, Ori B, et al. (2008) Role of infectious agents in systemic rheumatic diseases. Clin Exp Rheumatol 26: S27.
28. Hasni SA (2012) Role of Helicobacter pylori infection in autoimmune diseases. Curr Opin Rheumatol 24: 429-434.
29. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ (2014) A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol 20: 1438.
30. Aktepe OC, Çiftçi IH, Safak B, Uslan I, Dilek FH (2011) Five methods for detection of Helicobacter pylori in the Turkish population. World J Gastroenterol 17: 5172.
31. Ricci C, Holton J, Vaira D (2007) Diagnosis of Helicobacter pylori: Invasive and non-invasive tests. Best Pract Res Clin Gastroenterol 21: 299-313.

32. Hochberg MC (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 40: 1725.
33. Gladman DD, Ibañez D, Urowitz MB (2002) Systemic lupus erythematosus disease activity index 2000. J Rheumatol 29: 288-291.
34. Rigante D, Mazzoni MB, Esposito S (2014) The cryptic interplay between systemic lupus erythematosus and infections. Autoimmun Rev 13: 96-102.
35. Väre PO, Heikius B, Silvennoinen JA, Karttunen R, Niemelä SE, et al. (2001) Seroprevalence of Helicobacter pylori infection in inflammatory bowel disease: is Helicobacter pylori infection a protective factor?. Scand J Gastroenterol 36: 1295-1300.
36. Zevit N, Balicer RD, Cohen HA, Karsh D, Niv Y, et al. (2012) Inverse Association Between Helicobacter pylori and Pediatric Asthma in a High-Prevalence Population. Helicobacter 17: 30-35.
37. Kuwana M, Ikeda Y (2006) Helicobacter pylori and immune thrombocytopenic purpura: unsolved questions and controversies. Int J Hematol 84: 309-315.
38. Li W, Minohara M, Su JJ, Matsuoka T, Osoegawa M, et al. (2007) Helicobacter pylori infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. J Neuroimmunol 184: 227-231.
39. Yamanishi S, Iizumi T, Watanabe E, Shimizu M, Kamiya S, et al. (2006) Implications for induction of autoimmunity via activation of B-1 cells by Helicobacter pylori urease. Infect Immun 74: 248-256.
40. Kalabay L, Fekete B, Czirják L, Horváth L, Daha MR, et al. (2002) Helicobacter pylori infection in connective tissue disorders is associated with high levels of antibodies to mycobacterial hsp65 but not to human hsp60. Helicobacter 7: 250-256.
41. Showji Y, Nozawa R, Sato K, Suzuki H (1996) Seroprevalence of Helicobacter pylori infection in patients with connective tissue diseases. Microbiol Immunol 40: 499-503.
42. Sawalha AH, Schmid WR, Binder SR, Bacino DK, Harley JB (2004) Association between systemic lupus erythematosus and Helicobacter pylori seronegativity. J Rheumatol 31: 1546-1550.