Association Between Systemic Lupus Erythematosus and *Helicobacter pylori* Seronegativity

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ABSTRACT. ***Objective.*** *Helicobacter pylori* is a gram negative spiral bacterium that is clearly associated with a variety of gastrointestinal pathologies. A number of non-gastrointestinal diseases have also been associated with *H. pylori*. We investigated the prevalence of *H. pylori* seropositivity as part of a larger serologic survey in a group of 466 patients with systemic lupus erythematosus (SLE) and 466 controls.

Methods. We studied subjects for seropositivity against 5 antigens including mumps, measles, rubella, varicella zoster, and *H. pylori*. The 466 SLE patients were taken from a total of 290 pedigrees multiplex for SLE and matched to 466 controls for age (± 3 yrs), sex, and ethnicity to non-SLE affected individuals, taken mostly from the same collection of pedigrees multiplex for SLE. Assays for seropositivity were performed using a heterogeneous immunoassay technique. Pearson's chi-square was used to test for association of categorical variables and Student t-test for continuous variables. Logistic regression was used to compute the odds ratio for *H. pylori* seropositivity in patients and controls.

Results. There was a significant difference only in *H. pylori* seropositivity between SLE cases and their controls. The results were not altered by intrafamilial correlation. Subset analysis by race and sex showed that the differences between the African-American female patients with SLE and their matched controls were responsible for this association. Female African-American patients with SLE had a lower prevalence of *H. pylori* seropositivity compared to controls (38.1% vs 60.2%, OR 0.41, p = 0.0009, 95% CI 0.24–0.69). Of the 113 African-American female SLE patients in the study group, 43 were seropositive for *H. pylori*. The mean age of onset for SLE was older in the seropositive group (34.4 yrs) compared to the seronegative SLE patients (28.0 yrs) (t = 2.11, p = 0.039).

Conclusion. Of 5 serologic tests performed, only the frequency of *H. pylori* seropositivity was different between SLE cases and their controls, and then only in African-Americans. We found an association between being seronegative for *H. pylori* and the development of SLE in African-American women, who also tend to be younger at the time of disease onset. These findings suggest that there is a possible protective role for *H. pylori* infection against the development of SLE or that immunoregulatory events leading to *H. pylori* seropositivity are inversely related to the risk of SLE. (J Rheumatol 2004;31:1546–50)

Key Indexing Terms:
LUPUS INFECTION *HELICOBACTER PYLORI* SEROLOGY

Systemic lupus erythematosus (SLE) is an autoimmune multisystem disease characterized by the production of antinuclear antibodies (ANA). Several organ systems can be involved including the skin, mucous membranes, joints, kidneys, blood, and central nervous system. The disease is more common in women, and is also more common and more severe in peoples derived from Africa[1]. A variety of environmental and genetic factors have been postulated to be involved in the pathogenesis of the disease. However, to date, understanding of its etiology and pathogenesis is very incomplete.

Among the environmental factors studied, various viral pathogens have been investigated. Most significantly, an association between SLE and Epstein-Barr virus has been established in both children and adult patients[2-7].

Helicobacter pylori is a gram negative spiral bacterium that colonizes the mucus layer of the human stomach[8]. The prevalence of *H. pylori* is highly variable among different populations, but infection occurs worldwide, with higher prevalence in developing countries[9]. *H. pylori* is transmitted predominantly via fecal-oral route, and infection is mainly acquired during early childhood[8]. Seropositivity for *H.*

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pylori increases with age, low socioeconomic status, low level of education, and poor housing conditions[10-12].

The human is the only species known to be commonly infected with *H. pylori*; the original source of this microorganism is still unknown[13]. Although most people infected with *H. pylori* are asymptomatic, the infection is clearly associated with gastric and duodenal ulcers, gastric adenocarcinoma, and gastrointestinal lymphomas[13]. A variety of invasive and noninvasive tests have been developed for diagnosis of *H. pylori* infection. Noninvasive tests include urea breath test, fecal antigen test, and serology[8].

Serological tests generally detect the circulating IgG directed to *H. pylori* antigens. Although they have high sensitivity and specificity for infection, their results do not identify recent or active infection. Spontaneous resolution of *H. pylori* infection is rare, however, in individuals who have not been treated[14]. After eradication of *H. pylori*, the level of IgG decreases slowly, and most patients will achieve a reduction of 50% or more of the pretreatment titer in one year[15]. Therefore serological tests have limited value in monitoring the success of eradication therapy[8].

We studied the prevalence of *H. pylori* infection in a group of patients with SLE compared to a group of healthy controls. The association with various American College of Rheumatology (ACR) criteria for SLE was also evaluated for *H. pylori* positive and *H. pylori* negative patients. The data show that *H. pylori* seronegativity is associated with SLE in African-Americans. This is the first description of this association. Interestingly, similar association has been noted in inflammatory bowel disease, and particularly in Crohn's disease[16-19].

MATERIALS AND METHODS

Patients and controls. We studied patients who satisfied the ACR classification criteria for SLE[20,21]. Patients were selected from the collection of SLE pedigrees assembled at the Oklahoma Medical Research Foundation. We had a total of 466 patients from a total of 290 SLE pedigrees. Our control group consisted of non-SLE individuals who came mostly from SLE affected families. We had a total of 466 controls matched to each case for age (± 3 yrs), sex, and ethnicity. Patients and controls were 61% European-American, 28% African-American, and 11% Hispanic (Table 1). Clinical data were collected using a questionnaire, from review of medical records, and from interview.

Serology. H. pylori. Serum samples were collected from both cases and controls at the time of enrollment, and were stored at –20°C. Assays for *H. pylori* infection were performed using a heterogeneous immunoassay technique that permitted simultaneous measurement of multiple antibodies (BioRad, Hercules, CA, USA). In this approach, cell lysates prepared from *H. pylori* and other infectious agents were loaded onto distinct 8 µm magnetic color-coded microspheres. These beads were then mixed with the serum samples, incubated 20 min, washed, incubated with mouse anti-IgG antibody labeled with beta-phycoerythrin, and washed again. The antibody concentration on each bead was then determined, for each cell lysate, using a Luminex 100 flow cytometer. The screen for *H. pylori* utililized 2 distinct cell lysates as antigens (supplied by RSL, Salt Lake City, UT, USA, and Bio-Rad Laboratories, Marnes, France) to increase sensitivity. Patients and controls were considered infected with *H. pylori* if they showed seropositivity to either of the 2 lysates tested. The assay has a sensitivity of 97% and specificity of 98%.

Table 1. Characteristics of SLE patients and matched controls. SLE cases were matched one-to-one with controls for age (± 3 years), sex, and self-identified race. No characteristics reported here are different between cases and controls.

	Patients	Controls
Number	466	466
Mean age ± SD, yrs	45.6 ± 13.6	46.3 ± 12.8
Age range, yrs	10–79	11–79
Sex, %		
Female	86.3	86.3
Male	13.7	13.7
Race, %		
European-American	61	61
African-American	28	28
Hispanic	11	11
Individuals in Household, mean no.	3	3
Level of education, %		
High school	44	41
College	34	35
Beyond college	8	8

Viral serologies. The reactivity against mumps, measles, rubella, and varicella zoster viruses was determined for all patients and controls, using the heterogeneous immunoassay technique described above. The multiplex assay format permitted measurement of all IgG antibodies simultaneously, minimizing potential variations in performance due to sample volume, timing, and temperature.

Antinuclear antibodies. ANA was determined for all patients in the clinical immunology laboratory at the Oklahoma Medical Research Foundation by indirect immunofluorescence using human tumor cell line HEp-2. Precipitins were determined by Oüchterlony immunodiffusion assays.

Statistical analysis. Logistic regression was used to compute the odds ratio for *H. pylori* seropositivity in patients and controls. Pearson chi-square test was used to test for association of categorical variables and Student's t test for continuous variables. To determine difference in clinical manifestations of SLE between the *H. pylori* positive and *H. pylori* negative African-American patients with SLE in a subset analysis, only one patient per family was included in the analysis to exclude the influence of intrafamilial correlation. Logistic regression was used to calculate the odds ratio (OR) for any association with the ACR classification criteria for SLE. Independent variables included malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, pericarditis, pleuritis, seizures, psychosis, proteinuria, urine cellular casts, hemolytic anemia, leukopenia, lymphopenia, thrombocytopenia, and anti-double stranded (ds) DNA, anti-Smith, anticardiolipin, anti-Ro, and anti-La antibodies. The dependent variable was *H. pylori* seropositivity.

RESULTS

Patients and controls were similar ($p > 0.05$) for socioeconomic status as measured by the number of people in the household and the level of education. Indeed, many controls came from the same pedigrees as our SLE patient group (Table 1).

There was a significant difference in *H. pylori* seropositivity among the SLE patients and matched controls. SLE patients were less frequently seropositive for *H. pylori* than controls (36.5% vs 42.9%, respectively; OR 0.8, $p = 0.045$). This negative association was especially strong in African-

Americans (39.8% vs 59.4%; OR 0.45, p = 0.0018). Subset analysis further revealed that the difference observed was indeed in the African-American female patients compared to controls (38.1% vs 60.2%; OR 0.41, p = 0.0009, 95% CI 0.24–0.69). On the other hand, African-American male SLE patients showed no difference in *H. pylori* seropositivity compared to controls (53.3% in both patients and controls). There was no difference between cases and controls for the European-American or Hispanic subjects (Tables 2 and 3).

We also tested sera from patients and controls for other common pathogens including mumps, measles, rubella, and varicella zoster viruses. SLE patients were not more frequently seropositive to any of these pathogens than were the controls (Table 4).

A difference in therapeutic experience did not explain the increased seronegative frequency for *H. pylori* observed in African-American female patients with SLE. The *H. pylori* positive and *H. pylori* negative African-American female patients had had nearly identical experience with immunosuppressive medications (Table 5).

The mean age of onset of SLE for African-American female patients infected with *H. pylori* was older at 34.4 years compared to 28.0 years for the *H. pylori* negative patients (t = 2.11, p = 0.039).

We investigated possible associations with the ACR criteria between the *H. pylori* positive and negative African-American female patients. No association was found with *H. pylori* serology and the ACR criteria. In addition, no difference was noted in the frequency of precipitins against extractable nuclear antigens (anti-Ro, anti-La, anti-nRNP, antiribosomal P, or anti-Sm) present at the time of enrollment in the study.

We were concerned with artifact from intrafamilial correlation of *H. pylori* infection, as some of our patients came from the same pedigrees. Our 128 African-American SLE patients were taken from 80 pedigrees and the matched controls from 69 pedigrees. To be certain that our results were not affected by intrafamilial correlation, we randomly selected one SLE patient per pedigree compared to controls, who were also selected one per pedigree. Our results were virtually identical, with lower *H. pylori* infection rate in the SLE group compared to controls (38.8% vs 62.3%; OR 0.4, p = 0.004).

DISCUSSION

Helicobacter pylori, a highly motile gram negative bacterium, is associated with a variety of gastrointestinal pathologies. Although *H. pylori* infects the stomach, a variety of systemic inflammatory effects are seen. These include a higher number of white blood cells, lymphocytes and basophils[22], higher levels of C-reactive protein[23], and higher concentrations of tumor necrosis factor-α in *H. pylori* infected individuals[24]. A variety of cardiovascular, endocrine, skin, blood, and rheumatic disorders have been reported to be associated with *H. pylori* infection; however, in many of these reports data have been inconsistent[25].

In this study, African-American patients with SLE were clearly less likely to react against *H. pylori* antigens tested compared to controls. This indicated a significantly lower prevalence of *H. pylori* infection in African-Americans with SLE, an association not observed in European-Americans or Hispanics. Further, subgroup analysis revealed that this association was present in African-American women and that no difference was noted between African-American male patients with SLE and their matched controls. In Japanese, no difference was noted in the *H. pylori* seropositivity between SLE patients and healthy controls[26].

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Table 2. H. pylori seropositivity in SLE patients and controls.

Population	Total, n*	Anti-*H. pylori* Positive Patients, n (%)	Anti-*H. pylori* Positive Controls, n (%)	OR	Chi-square	p	95% CI
All	466	170 (36.48)	200 (42.92)	0.76	4.03	0.045	0.59 to 0.99
African-American	128	51 (39.84)	76 (59.38)	0.45	9.77	0.0018	0.28 to 0.75
European-American	286	84 (29.37)	89 (31.12)	0.92	0.21	NS	
Hispanic	52	35 (67.31)	35 (67.31)	1	0.00	NS	

* Number of cases and matched controls. NS: nonsignificant by chi-square.

Table 3. H. pylori seropositivity in African-American SLE patients and controls.

	Total, n*	Anti-*H. pylori* Positive Patients, n (%)	Anti-*H. pylori* Positive Controls, n (%)	OR	Chi-square	p	95% CI
Total	128	51 (39.84)	76 (59.38)	0.45	9.77	0.0018	0.28 to 0.75
Female	113	43 (38.05)	68 (60.18)	0.41	11.07	0.0009	0.24 to 0.69
Male	15	8 (53.33)	8 (53.33)	1	0.00	NS	

* Number of cases and matched controls. NS: nonsignificant by chi-square.

Table 4. Rate of seropositivity against various viral antigens tested in SLE patients and matched control group.

	Patients, n (%)	Controls, n (%)	p
Anti-mumps	406 (87)	416 (89)	NS
Anti-measles	452 (97)	442 (95)	NS
Anti-rubella	413 (89)	417 (90)	NS
Anti-VZV	455 (98)	448 (96)	NS

NS: nonsignificant by chi-square; VZV: varicella zoster virus.

Table 5. Frequency of various immunosuppressant medications used by female African-American SLE patients.

Medication	Anti-*H pylori* Positive Patients, %	Anti-*H pylori* Negative Patients, %	p
Prednisone	90.7	90.0	NS
Cyclophosphamide	11.6	18.6	NS
Hydroxychloroquine	55.8	64.3	NS
Azathioprine	27.9	30.0	NS

NS: nonsignificant by chi-square.

whether the observed association of *H. pylori* seronegativity in African-American SLE patients derives from events before or after the onset of SLE autoimmunity. That *H. pylori* infection occurs mostly during childhood[8] argues that it is more likely that African-Americans who are infected with *H. pylori* are less likely to develop SLE and not the opposite. However, no molecular or immune mechanism is known that would influence SLE susceptibility in this way. Parsimony could be used to argue that *H. pylori* infection is probably not directly involved in SLE pathogenesis. Rather, a third factor that influences both SLE and *H. pylori* infection may be the least complex explanation for the observed association.

Interestingly, our study showed that African-American female patients with SLE who are infected with *H. pylori* tend to get SLE at a significantly older age compared to those who lack evidence of *H. pylori* infection. This might further suggest that *H. pylori* seropositivity, for some unknown reason, does not favor the development of SLE. There was no difference in the seroprevalence of antibodies to mumps, measles, rubella, or varicella zoster viruses between the SLE patients and the controls. Further, the *H. pylori* positive and *H. pylori* negative SLE patients were not significantly different in the immunosuppressive medications used. This suggests that the difference in the seroprevalence of antibodies to *H. pylori* between SLE patients and controls is not related to the immunosuppressive medications. Lupus patients, partly due to using immunosuppressive medications, likely received antibiotics capable of treating *H. pylori* infection. However, no difference in the prevalence of *H. pylori* was seen in a variety of other rheumatic conditions also treated with immunosuppressive medications, including rheumatoid arthritis, scleroderma, and Sjögren's disease[25]. However, these data come mostly from Italian and Scandinavian populations and more data are needed from patients of African descent.

In a recent study, a genome-wide linkage analysis revealed a genetic polymorphism in the human interferon-γ receptor gene region in Senegalese siblings phenotyped for *H. pylori* positive serology[27]. The relevance of this polymorphism to the development of SLE is not known. Certain polymorphisms may play an important role in determining the spectrum of *H. pylori* induced disease. Polymorphisms in the interleukin 1 gene are associated with an increased risk of hypochlorhydria and gastric cancer[28]. A recent finding that *H. pylori*-specific CD4+ CD25 (high) regulatory T cells suppress memory T cell response to *H. pylori* in infected individuals is of interest[29]. Several studies have shown that patients with inflammatory bowel disease, especially Crohn's disease, have a lower prevalence of *H. pylori* infection compared to controls[16-19]. Both SLE and inflammatory bowel disease have poorly understood pathogeneses. Perhaps our observations will help identify shared mechanisms.

SLE is associated with a decrease in the expected frequency of serological reactions directed against *H. pylori*, specifically in African-American women with SLE compared to their matched controls. It is not seen in men with SLE, European-Americans, or Hispanics. The biologic or epidemiologic explanation for this relationship may yield insights into the pathogenesis of SLE.

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