Original Research Article

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The effect of *Helicobacter pylori* seropositivity and activity on disease outcome in patients with rheumatoid arthritis, systemic lupus erythematosus and ankylosing spondylitis

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Abstract

The association between infection with Gram-negative bacteria and autoimmune diseases has been investigated with controversies about the role of the organisms especially, *Helicobacter pylori* (*H. pylori*). To evaluate the impact of the presence and activity of *H. pylori* on the disease activity in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and ankylosing spondylitis (AS). This study was carried out on one hundred adult patients and 50 controls. Patients included 40 RA, 40 SLE, and 20 AS. Participants were subjected to clinical examination and laboratory investigations; ESR (spectrophotometric assay), CRP (turbidimetric method), serum *H.pylori* IgG antibody test (enzyme immunoassay), and *H. pylori* antigen test (lateral flow immunoassay, rapid one-step test) in stool. Positive test in stool indicated active current *H. pylori* infection. Mean age of patients was 36.95±10.34; 51.2±6.91, 35.5±3.71, and 48.82±5.81 in SLE, RA, AS, and control groups respectively. Serum *H. pylori* IgG antibodies was 0.803±0.497 U/mL in SLE group, 1.48±0.637 U/mL in RA group and 0.75±0.68 U/mL in AS group while it was 0.2±0.61U/mL in the control group with a significant difference (*P*=0.000). The *H. pylori* antigen in stool was found positive in 30%, 70% and 20% of patients of the three groups respectively while it was positive in 10% of the control group with *P*>0.001. Patients with active SLE (SLEDAI >3) and RA (DAS-28 >3.2) demonstrated higher frequency of positive test for *H. pylori* antigen in stool than patients at remission (66.7% *P*= 0.02 and 83.3% *P*= 0.01 respectively). In contrast, 22.2% of patients with active AS (BASDAI > 4) were positive for *H. pylori* in stool. In conclusion, *H. pylori* infection is associated with increased disease activity in patients with SLE and RA but not AS.

Keywords: *Helicobacter pylori* infection; RA; SLE; AS; Disease activity.

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Introduction

H. pylori is a widespread, spiral-shaped, flagellated Gram-negative bacterium usually infects the gastric mucosa.[1] Its seropositivity increases with age as it affects about eighty percent of the middle-aged adults in the developing countries and about twenty-five to fifty percent in the western populations.[2] The relation between infection and autoimmune diseases has been previously investigated. Many Gram-negative bacteria as salmonella, shigella, and chlamydia were confirmed to be associated with the development of reactive arthritis.[3] These bacteria have been found to stimulate the host immune response due to the presence of lipopolysaccharides and other antigenic molecules that can cause inflammation.[4] It has been previously hypothesized that *H. pylori* can induce such immune host response through many mechanisms as the molecular mimicry and antigenic similarity, disruption of the tolerogenic immune response, and activation of the polyclonal lymphocytes leading ultimately to the imbalance between T regulatory/Th17 cells in addition to the induction of autoantibody production.[5,6,7]

The association between *H. Pylori* and autoimmune diseases has been reported in idiopathic thrombocytopenic purpura,[8] chronic idiopathic urticaria[9], auto-immune thyroiditis[10], autoimmune atrophic gastritis,[11] and some other rheumatic autoimmune disorders as rheumatoid arthritis (RA),[12,13,14] systemic lupus erythematosus (SLE),[15] Sjogren syndrome,[16] and ankylosing spondylitis (AS).[17]

On the other hand, a protective role of *H. Pylori* in some inflammatory conditions as multiple sclerosis,[18] inflammatory bowel disease[19] and allergic conditions as pediatric asthma[20] has been reported. In the same context, low seropositivity of *H. Pylori* has been reported among Korean patients with HLA-B27 positive acute anterior uveitis.[21]

Rheumatoid arthritis, systemic lupus erythematosus, and ankylosing spondylitis are the most encountered rheumatic autoimmune diseases sharing the presence of chronic inflammation, autoantibody production, and breaking of the self-immune tolerance. However, they are completely different in the clinical and laboratory profiles.[22] Although the precise mechanism of the pathogenesis is still unknown, the genetic and epigenetic events of the host with the environmental and infectious factors resemble the most important triggering elements of autoimmunity in the three rheumatic diseases. However, there is still a controversy about the pathogenetic role of *H. Pylori* in these rheumatic autoimmune conditions, and further studies from different areas are needed to support these hypotheses.[23] In this study, we aimed to evaluate the impact of the presence and activity of *H. pylori* on disease activity in patients with RA, SLE, and AS.

Patients and Methods

This study was approved by the ethics committee for medical research, Al-Azhar University, and all participants gave informed consent before enrollment in the study. This study was carried out on one hundred adult patients, randomly selected from those attending the outpatient rheumatology clinic and internal medicine department of our university hospital during the period from January 2018 to December 2019. -Patients were divided into three groups:

-Group A: (RA group): forty RA patients diagnosed according to the American college of rheumatology (ACR)-EULAR RA classification criteria 2010.[24]

-Group B (SLE group): forty SLE patients diagnosed according to the Systemic Lupus International Collaborating Clinics (SLICC) Classification Criteria.[25]

-Group C (AS group): twenty AS patients diagnosed according to the modified New York criteria.[26]

Fifty healthy age and sex matched adults were selected as a control group. Any patient or control known to have treatment for *H. pylori* or taking antibiotic treatment for the previous three months, patients receiving biologic therapy and those known to have diabetes mellitus, autoimmune thyroid disease or autoimmune hepatitis or any other autoimmune

disorders were excluded from the study as well as those subjected to gastrointestinal surgery or endoscopy through the previous three months and those with pregnancy. Complete history taking and thorough clinical examination were done for each patient.

Assessment of disease activity

The following disease activity parameters were used to assess the current activity of each disease:

-Disease activity score (DAS-28)[27] for patients with RA.

-SLE disease activity scale (SLEDAI)[28] for patients with SLE.

-Bath ankylosing spondylitis activity index (BASDAI)[29] for patients with AS.

Laboratory workup

Each patient and control were subjected to do the following laboratory investigations:

Erythrocyte sedimentation rate (ESR) was performed using the spectrophotometric assay, C-reactive protein (CRP) by the turbidimetric method.

H. pylori serum antibody assessment

Quantitative assessment of *H.Pylori* antibodies (IgG) in serum was done by enzyme immunoassay (EIM). The kit was provided by MyBiosource, Southern California, San Diego (USA) cat number: MBS494432. Helicobacter antigen is bound on the surface of the microtiter strips. A binding between the IgG antibodies of the serum and the immobilized Helicobacter antigen takes place and leads to the development of a blue color. This color is changed to yellow after the addition of a stop solution and is measured spectrophotometrically at 450 nm. Negative result was obtained if the concentration was <8 U/mL, equivocal if 8-12 ULmL, and positive if the concentration was > 12 U/mL.

H. pylori stool antigen test

-Fresh stool samples were collected on the same day the serum antibody test was performed.

-*H. pylori* antigen was qualitatively determined in participants' stool by lateral flow immunoassay (rapid one step *H. pylori* antigen test Device, ABON Biopharm (Hangzhou), China lot number: HP0122007). In this test, the membrane was pre-coated with *H. pylori* antibodies on the test line region of the test. The antigen in the specimen was allowed to react with the particle antibody and then this mixture migrates upward on the membrane by capillary action and reacts with the *H. Pylori* antibodies on the membrane generating a colored line in the test region indicating a positive result. A colored line will appear in the control line region as a procedural control.

-*H. pylori* antigen identification in the stool is an indicator of active infection by the organism which has been reported to be a safe alternative method compared to the invasive, endoscopic biopsy examination with more than 90% sensitivity and specificity.[30]

Statistical analysis

Statistical package for social sciences (IBM-SPSS), version 24 IBM- Chicago, USA (May 2016) was used for statistical data analysis. Data expressed as mean, standard deviation (SD), number and percentage. Mean (±standard deviation) was used for the presentation of quantitative data, while number and percentage were used to describe qualitative data. One-way analysis of variance (ANOVA) test was used to compare means of the three groups of RA, SLE, and AS. Mann Whitney test was used instead of Student-t test in case of non-parametric data. Pearson Chi-square was used to compare percentages of qualitative data, and Fisher's Exact test was used for non-parametric data. Pearson correlation test was used to compare two quantitative variables. The value of (r) is explained in the following figures: r <0.2 negligible correlation, r 0.2-0.4 weak correlation, r 0.4-0.7 moderate correlation, while, r 0.7-1 = strong correlation. For all these tests, the level of significance (*P*-value) can be explained as significance; $P < 0.05$.

Results

The mean age of patients was 36.95 ± 10.34 in the SLE group, 51.2 ± 6.91 in the RA group, and 35.5 ± 3.71 in the AS group while in the control group it was 48.82±5.81. The female to male ratio was 19:1, 18:2, 1:19, and 33:17 in each group respectively. The mean (±SD) of *H. Pylori* IgG in the serum was 0.803±0.497 U/mL in the SLE group, 1.48±0.637 U/mL in the RA group, and 0.75±0.68 U/mL in AS group while it was 0.2±0.61 U/mL in the control group with a significant difference (*P*=0.000). The *H. pylori* antigen was found positive (indicating *H. pylori* activity) in the stool of 30%, 70%, and 20% of patients of the three groups respectively while it was positive only in 10% of the control group with a *P*>0.001. The disease activity of SLE patients (SLEDAI) was 0.6±1.6, DAS Score was 2.97±1.05 in the RA group, and BASDAI Score was 3.7±2.45 in the AS group. The demographic, clinical, and laboratory data are summarized in table 1.

Table 1. Demographic data of participants.

	Group A (SLE)	Group B (RA)	Group C (AS)	Control group	*P*-value
	No.%	No.%	No.%	No. %	
Age (years) Mean±SD	36.95 ± 10.34	51.2 ± 6.91	35.5 ± 3.71	48.82±5.81	<0.001
Sex					
Female	38 (95.0%)	36 (90.0%)	2 (5.0%)	33 (66%)	<0.001
Male	2 (5%)	4 (10.0%)	38 (95.0%)	17(44%)	
Disease duration (years) Mean±SD	3.22 ± 1.23	2.22 ± 1.01	1.00 ± 0.6	NA	<0.001
H. Pylori IgG in serum (U/mL) Mean±SD	0.803±0.497	1.48±0.637	0.75±0.68	0.2±0.61	0.000
H. Pylori Antigen in stool (*H. pylori* Activity)					
Positive	12 (30%)	28(70%)	4(20%)	5(10%)	0.000
Negative	28(70%)	12(30%)	16(80%)	45(90%)	
Disease activity Mean±SD	SLEDAI Score 0.6±1.6	DAS-28 Score 2.97±1.05	BASDAI Score 3.7±2.45	NA	NA
ESR (mm/h) Mean±SD	29±17	60.2±25.1	52.3±18.4	19±12.1	0.000
CRP (mg/L) Mean±SD	6.8±29	12.8±58	9.7±12.2	5.8±19.33	NS

AS: Ankylosing spondylitis, CRP: C- reactive protein, ESR: Erythrocyte sedimentation rate, RA: Rheumatoid arthritis, SLE: Systemic lupus erythematosus, NA: Not applicable, BASDAI: Bath ankylosing spondylitis disease activity index, DAS-28: Disease activity score, SLEDAI: SLE disease activity index. *P*>0.05 is not significant (NS).

Regarding the relation between *H. pylori* activity and disease activity, we found that in the SLE group, 66.7% of patients who had active disease (SLEDAI >3) also had positive *H. pylori* and only 33.3% of them had negative *H. pylori* (P = 0.02). In the RA group, 60% of patients had moderate disease activity (DAS 28 >3.2-5.1) and 40% were in remission (DAS 28≤ 2.6); 83.3% of patients who had active RA also had positive *H. pylori* and only 16.7% of them had negative *H. pylori* antigen in the stool (*P*=0.01). In AS group, 45% of patients had active disease (BASFAI > 4), only 20% of them had active *H. pylori* infection (*P* = 1.000). Data are shown in table 2 and figure 1.

Table 2. Relationship between Helicobacter activity and disease activity.

Groups		Parameters		
SLE Group *H.Pylori* (Activity)		Disease Activity		
		Inactive	Active	Total
Negative	Count	26	2	28
	% within HP (Activity)	92.9%	7.1%	100.0%
	% within Dis Activity	76.5%	33.3%	70.0%
Positive	Count	8	4	12
	% within HP(Activity)	66.7%	33.3%	100.0%
	% within Dis Activity	23.5%	66.7%	30.0%
Total	Count	34	6	40
	% within HP (Activity)	85.0%	15.0%	100.0%
	% within Dis Activity	100.0%	100.0%	100.0%
**P* value		0.011		
RA Group *H.Pylori* (Activity)		Disease Activity		
		Inactive	Active	Total
Negative	Count	8	4	12
	%within HP Activity	66.7%	33.3%	100.0%
	% within Dis. Activity	50.0%	16.7%	30.0%
Positive	Count	8	20	28
	%within HP Activity	28.6%	71.4%	100.0%
	%within Dis. Activity	50.0%	83.3%	70.0%
Total	Count	16	24	40
	%within HP Activity	40%	60%	100%
	%within Dis. Activity	100%	100%	100%
**P* value		0.011		
AS Group *H. Pylori* (Activity)		Disease Activity		
		Inactive	Active	Total
Negative	Count	9	7	16
	%within HP(Activity)	56.3%	43.8%	100.0%
	% within Dis. Activity	81.8%	77.8%	80.0%
Positive	Count	2	2	4
	%within HP(Activity)	50.0%	50.0%	100.0%
	% within Dis. Activity	18.2%	22.2%	20.0%
Total	Count	11	9	20
	% within HP (Activity)	55.0%	45.0%	100%
	% within Dis Activity	100%	100%	100%
**P* value		NS		

AS: ankylosing spondylitis, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, S: significant, NS: non-significant. *P*>0.05 is not significant (NS). *Chi square

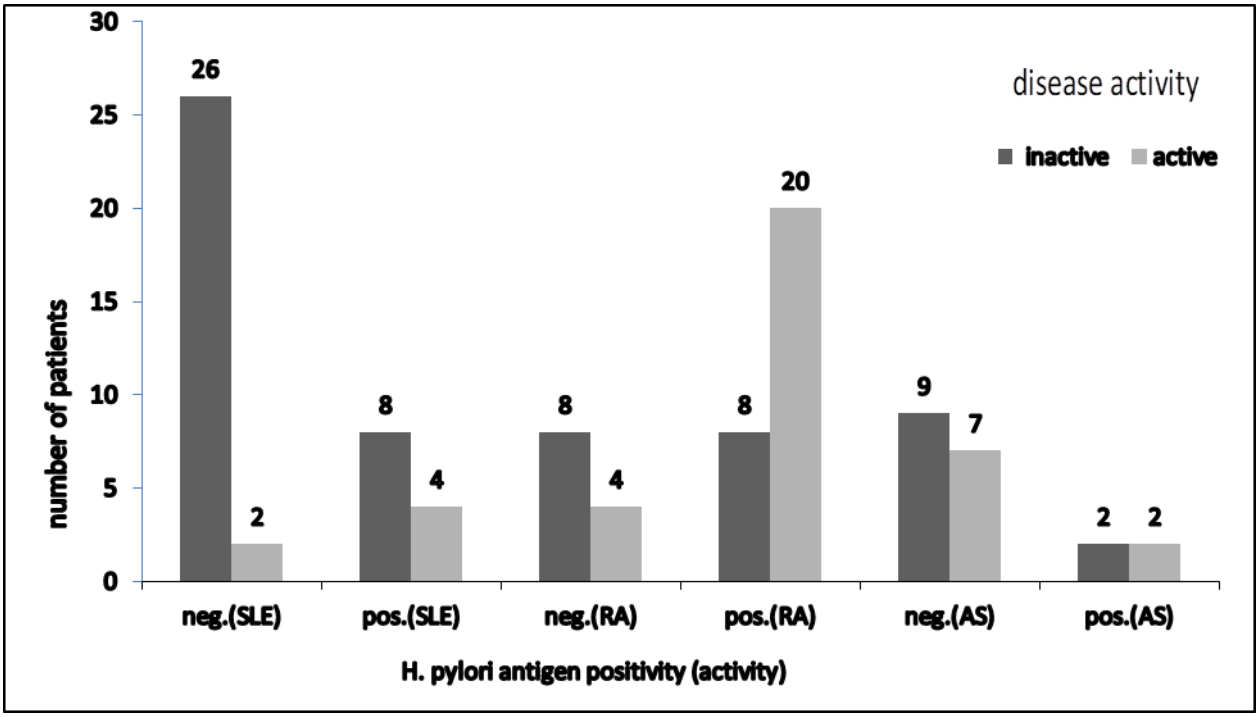

Figure1. *H. pylori* activity and disease activity in the three groups

Discussion

The relationship between infection and autoimmunity has been intensely investigated over the last 20 years. The immune system dysregulation and loss of immune tolerance in most systemic rheumatic diseases are suspected to be caused by many environmental effectors as infectious agents in the genetically predisposed subjects.[31] *H. pylori* is one of the most common pathogens affecting humans, infecting approximately 50% of the world's population.[32] To clarify the impact of *H. pylori* infection on disease activity and severity of RA, SLE and AS, this study was carried out. In the SLE group, the mean *H. pylori* level in serum was 0.803±0.497, and the relevance of active *H. pylori* in stool was 30%. Regarding disease activity, the mean SLEDAI score was 0.6±1.6, and 15% of SLE patients in our study had active disease. Kalabay and colleagues studied the prevalence of anti-*H. pylori* antibodies in various autoimmune rheumatic diseases and found a comparable prevalence of *H. pylori* in SLE patients and healthy controls.[33]

In another study, Sawalha et al.[34] compared the prevalence of *H. pylori* seropositivity in 466 SLE patients to matched controls and reported that SLE patients were less frequently positive (36.5%) for *H. pylori* as compared with healthy controls (42.9%) which is higher than our prevalence. They found a significant negative association for female SLE patients versus controls (38.1 versus 60.2%; *P*=0.0009). It was noted that *H. pylori* seropositive African-American females have been prone to develop SLE at an older age. In the RA group, the mean *H. pylori* level in serum was 1.48±0.637 while the prevalence of active *H. pylori* in stool was 70% which are higher than SLE patients in this study. As regard disease activity, the mean DAS score was 2.97±1.05 and 60% of RA patients in our study had moderately active disease. This prevalence in RA is higher than that reported by Ishikawa et al. as the prevalence of *H. pylori* infection in their RA patients was (61.4%) in a large-scale study on 1290 Japanese subjects.[35] Tanaka et al.[36] reported that the seroprevalence of *H. pylori* in RA patients was 32%. Also, Zentilin et al.[37] reported that the seroprevalence of *H. pylori* in RA patients was 48% which is lower than our prevalence. In the AS group in our study, the mean *H. pylori* in serum was

0.75±0.68 while the prevalence of active *H. pylori* in stool was 20% which is lower than the other 2 groups. Regarding disease activity, the mean BASDAI score was 3.7±2.45, and 45% of AS patients in our study had active disease. In the current study, we demonstrated the relation between Helicobacter activity and disease activity in SLE patients and found that 66.7% of patients who had active disease also had positive *H. pylori* and only 33.3% of them had negative *H. pylori* with a significant difference.

In RA patients, we found that 83.3% of patients who had active disease also had positive *H. pylori* and only 16.7% of them had negative *H. pylori* which suggest a strong effect of *H. pylori* positivity on the severity of arthritis in RA. This was in agreement with a preliminary report of Zentilin et al.[37] who reported on the effect of *H. pylori* eradication on the severity of RA. According to their results, an improvement of the analog score for RA and morning stiffness had been noticed 4 months after eradication of *H. pylori*. However, this improvement could be explained by many factors other than *H. pylori*. In contrast to our results, in the investigation of Ishikawa et al,[35] there were no differences in the clinical severity of RA between *H. pylori*-positive and -negative patients. Additionally, Mizokami et al.[38] reported that anti-rheumatic drugs didn't affect the prevalence of *H. pylori* in patients with RA. In line with our results, El-Hewala et al[14], found that the fecal antigen-positive RA patients had significantly higher activity than the negative group. This is consistent with that reported by Wen et al.[39] who found that patients with *H. pylori* positive have a tendency for severe clinical features characterized by an increased number of painful joints and functional impairment.

Contrarily, Matsukawa et al,[40] reported that RA patients possibly could suffer from an increase in disease activity and the development of joints pain after eradication of H pylori infection. They hypothesized that this eradication may lead to disruption of oral tolerance against mycobacterial heat shock protein-65. In the AS group, we found that 22.2% of patients who had active disease had positive *H. pylori* and 77.8% of them had negative *H. pylori*. This percentage was lower than the other two groups in this study. This is in agreement with a study performed by Bae et al.[41] who stated that AS patients with HLA-B27-positive acute anterior uveitis (AAU) had a significantly lower prevalence of *H. pylori* seropositivity compared to patients with HLA-B27-negative AAU and controls. However, there was no significant difference in the prevalence of *H. pylori* seropositivity between the non-uveitis group and the control group. These results suggest an inverse relationship between *H. pylori* seropositivity and HLA-B27-positive AS which is in line with our results. The HLA-B27 antigen is considered closely related to AS, and exogenous peptides from gram-negative bacteria have been suggested to be environmental triggers for HLA-B27-positive AS[42]. Also, Onal et al,[43] reported that there was no significant association between gram-negative organisms and AS which is consistent with our findings. In contrast to our results, Huhtinen et al. and Otasevic et al. reported a possible role of gram-negative bacteria including *H. pylori* in the development and recurrence of HLA-B27-positive AS.[44,45] This study has many limitations as the small sample size, the lack of confirmative invasive techniques for diagnosing *H. pylori* infection and the difficulty in correlating the individual parameters of each disease with the active *H. pylori* infection.

We concluded that, *H. pylori* infection is associated with increased disease activity in patients with RA and SLE but not in patients with AS.

Author Contributions

AE, MR, AM, MA, MIA, and HA acquired, analyzed, and interpreted the patient data. AE, AM, MR, and MIA were major contributors in writing and editing the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study was approved by the institutional ethical committee of Al-Azhar University Hospitals -Assiut.

Informed consent

A signed consent form was obtained from each study participant.

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