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ORIGINAL ARTICLE

Effect of eradication of *Helicobacter pylori* in rheumatoid arthritis patients

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Abstract: *Helicobactor pylori* (*H. pylori*) is suspected to be one of the factors triggering rheumatoid arthritis. The aim of this study was to evaluate the effect of *H. pylori* eradication on inflammatory disease activity in patients with rheumatoid arthritis. The presence of *H. pylori* was assessed in 59 patients with rheumatoid arthritis from whom *H. pylori* was eradicated. The level of inflammatory disease activity was evaluated before eradication and during a 42-week follow-up period, as well as the inflammatory disease. The activity of *H. pylori* in uninfected patients was evaluated with similar time intervals. A significant improvement was seen in the patients' ESR after *H. pylori* eradication, although this was non-significant after adjustment for multiple comparisons. A significantly reduced number of tender joints was observed in patients unaffected by *H. pylori,* possibly due to altered DMARD-treatment in 17% of the patients. Our results indicate the role of *H. pylori* on the inflammatory state of rheumatoid arthritis, but further studies on larger populations are necessary for final conclusions to be drawn.

Key words: rheumatoid arthritis, *Helicobacter pylori*, inflammatory disease, autoimmune diseases, arthritis

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory, disabling disease with a worldwide prevalence of approximately 1%. The etiology remains unknown and the pathogenesis only partially understood. An infectious etiology has often been proposed [1-13] and in recent decades a plethora of micro-organisms have been investigated as potential inducers of rheumatoid arthritis. Most studies have been disappointing, although some positive results have been reported [14-30].

Warren and Marshall were the first to describe the presence of *Helicobacter pylori* (*H. pylori*), a Gram negative curved and motile bacteria in biopsy specimens from patients with gastritis [31, 32] and during recent decades, *H. pylori* has been found to be the major cause of gastritis and gastro-duodenal ulcers [33]. *H. pylori* can thus be identified in 70-90% of all incidences of gastritis and gastro-duodenal ulcers [34]. Patients with rheumatoid arthritis have an increased risk of developing gastro-intestinal tract lesions [35-37] and for this reason, the prevalence of *H. pylori* in patients with rheumatoid arthritis has previously been the subject of several investigations. The reported prevalence of *H. pylori* in patients with rheumatoid arthritis varies from 22%-68% [38-47].

A possible etiological, pathological, and genetic relationship between *H. pylori* and rheumatoid arthritis, has to our best knowledge, only been considered previously in 2 studies [48, 49]. We believe this is the first prospective study of any length describing the effect of *H. pylori* eradication on inflammatory disease activity in patients with rheumatoid arthritis.

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MATERIALS AND METHODS

Study population. Outpatients from the Department of Rheumatology at the Frederiksberg Hospital in Copenhagen, Denmark, who met the criteria for rheumatoid arthritis – as set by the American College of Rheumatism 1988 [50] – were eligible to participate in the study. A total of 194 patients diagnosed with rheumatoid arthritis were identified using a computer search in the patient database. Of these, 49 patients were not eligible because of age, concurrent disease or not fulfilling the ACR criteria, and 94 patients declined to participate. A total of 51 outpatients consented to participate in the study, and a further 8 voluntarily referred themselves, or were referred by their general practitioner. Consequently, 59 patients – 47 women and 12 men, median age 60 years (range 19-80 years), and median disease duration of 10 years (range 1-42 years), were included in the study. A randomised, controlled and blind study was not possible due to the ethical problems created if the treatment of the *H. pylori* positive group was delayed. Demographic and disease characteristics of the patients are shown in Table 1.

All patients had an upper endoscopy performed using an Olympus GIF Type 100 gastroscope, were pre-medicated with topical xylocaine spray and offered intravenously titrated doses of diazepam. A total of 10 biopsy specimens were taken at random from the antrum (6 biopsy specimens) and the corpus (4 biopsy specimens) for histological and microbiological examination.

Table 1 Demographic and disease characteristics of 59 rheumatoid arthritis patients

Age, years. Median (range)	60 (19-80)
Disease duration, years. Median (range)	10 (1-42)
Women, number (%)	47 (79.7%)
Men, number (%)	12 (20.3%)
Patients with erosive arthritis, number (%)	44 (74.5%)
Patients with seropositive arthritis, number (%)	49 (83.0%)
Patients with epigastric pain, number (%)	28 (47.5%)
Patients with dyspepsia, number (%)	14 (23.7%)
Patients in NSAID-treatment, number (%)	26 (44.5%)
Patients in Acetylsalicylic Acid treatment, number (%)	8 (13.6%)
Patients in Prednisolone treatment, number (%)	18 (30.5%)
Patients in DMARD treatment, number (%)	34 (57.6%)
Gold compounds, number (%)	4 (6.8%)
Sulfasalazine, number (%)	15 (25.4%)
Chloroquine, number (%)	3 (5.1%)
Methotrexate, number (%)	6 (10.2%)
Azathioprine, number (%)	2 (3.4%)
Penicillamine, number (%)	5 (8.4%)
Patients smoking, number (%)	20 (33.9%)
Patients with daily alcohol consumption, number (%)	2 (3.4%)

Four biopsy specimens for histological examination (2 from the antrum and 2 from the corpus) were immediately fixed in formalin and routinely processed. Sections of paraffin-embedded biopsy specimens were stained with hematoxylin-eosin, van Gieson/alcian blue and periodic acid Schiff for morphological examination and detection of *H. pylori*-like organisms (HLO). Inflammation, gland atrophy, intestinal metaplasia and the presence of HLO was evaluated according to the Sydney system [51]. The biopsy specimens were additionally immuno-histochemically stained using polyclonal antibodies to *H. pylori* (DAKO, Copenhagen, Denmark) for further detection of HLO.

Three biopsy specimens for microbiological examination (1 from the antrum and 2 from the corpus), after completion of the endoscopy, were immediately transported in sterile saline and plated within 3 hours on chocolate-agar plates. The plates were incubated under micro-aerobic conditions at 37°C for up to 5 days. *H. pylori* was identified as urease-, oxidase- and catalase-positive Gram-negative, motile curved rods from small translucent colonies.

Treatment. Patients who were found to be *H. pylori*-positive, either by culture or by histology, (hereafter named *H. pylori*/HLO positive) were given eradication therapy for 14 days. The choice of antibiotics was made according to the resistance pattern, and to known allergic reactions towards Penicillin. Ten patients were given amoxicillin 750 mg TID, metronidazole 500 mg TID, and bismuth 120 mg QID. In 2 patients, amoxicillin was substituted by tetracycline 500 mg QID, and in 5 patients clarithromycine 500 mg TID was used as the only antibiotic. To ensure eradication, a second upper endoscopy was performed 6 weeks after completion of eradication therapy. Eradication of *H. pylori* failed in 2 patients who were initially treated with amoxicillin, metronidazol and bismuth. One patient was subsequently successfully treated with clarithromycine and bismuth, while 1 declined further therapy.

The patients were examined for IgG antibodies to *H. pylori* by enzyme-linked immunosorbent assay (ELISA) using a low molecular weight (LMW) antigen prepared by filtration of sonicated bacteria. Microtiter plates were coated overnight with the antigen preparation and washed 5 times. The sera, diluted 1:500, were added to the plates which were incubated for 1 hour, washed 5 times, and then incubated for 1 hour with rabbit antibody to human IgG (DAKO, Copenhagen, Denmark) combined with horseradish peroxidase in a 1:2.000 dilution. The plates were again washed 5 times, and enzyme activity detected by using the ortho-phenyle-diamine dihydrochloric acid-hydrogen peroxidase system. After 30 minutes, the chromogenic reaction was stopped with sulphuric acid, and the absorbance was read on a photometer at 492 nm. The amount of antibody was expressed in ELISA units (EU), which are the absorbance values corrected for plate-to-plate and day-to-day variation. From previous validation of this ELISA, sero-positive was defined as LMW IgG values ≥ 400 EU, and sero-negative as LMW IgG values ≤ 100 EU [52]. Intermediate values were reported as borderline sero-positive.

Absence of *H. pylori* at the second endoscopy and decrease in the LMW IgG-antibody level were used as indicators for successful eradication.

The patients' inflammatory disease activity was assessed at entry to the study, and after 3, 6, 9 and 12 months, which for the initially *H. pylori*/HLO positive patients was 6, 18, 30 and 42 weeks, respectively, after completion of the eradication therapy. The inflammatory-disease activity was referred to as the extent of the current overall inflammation and measured by assessing the number of swollen and tender joints, duration of morning stiffness, level of functional capacity (health-assessment questionnaire score, HAQ) [53], level of pain (visual analogue scale score, VAS), erythrocyte sedimentation rate (ESR), and the level of C-reactive protein (CRP). The joint count system included 28 joints [54]. The joints were evaluated for tenderness and swelling (0 = none, 1 = mild, 2 = moderate and 3 = severe). The joint score was ultimately recorded in a binary manner as recommended by ACR [55].

During the follow-up period, 2 patients died from other medical disorders (*H. pylori*/HLO negative), 1 *H.pylori*/HLO positive patient dropped out due to general weakness, 6 patients (2 *H.pylori*/HLO positive) dropped out due to personal circumstances, and 4 patients (1 *H. pylori*/HLO positive) missed 1-2 follow-up visits. Therefore, a total of 14 *H. pylori*/HLO positive and 32 *H. pylori*/HLO negative patients completed the follow-up period (Figure 1).

Ten patients (of these, 3 *H. pylori*/HLO positive) altered their treatment with a disease modifying anti-rheumatic drug (DMARD) during the follow-up period. The prednisolone dose did not exceed 15 mg/day in any patient, and during the follow-up period, the prednisolone dose was only altered > 5 mg/day in 2 patients (1 *H. pylori*/HLO positive and 1 *H. pylori*/HLO negative).

Statistical analyses. The patients were used as their own control. Data were not normally distributed, and the statistical analyses were carried out by the non-parametic Wilcoxon's signed rank test and Friedman's test, as appropriate. Multiple comparison was adjusted by the Bonferroni method. The level of significance was set at $p < 0.05$.

Ethics. The study was conducted in accordance with the Helsinki II Declaration. All patients received oral and written information before initiation of the study, and all gave written consent for participation. The study was approved by the local

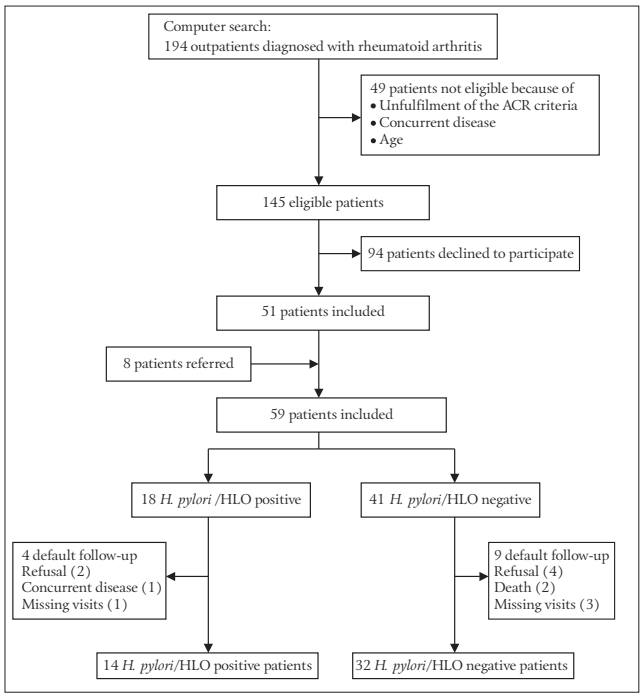

Figure 1 Patient- and follow-up chart.

Ethical Committee for the municipalities of Copenhagen and Frederiksberg.

RESULTS

A total of 18 patients were found to be *H. pylori*/HLO positive. No significant difference was observed between *H. pylori*/HLO positive and *H. pylori*/HLO negative patients with regard to demographic or disease characteristics. The data for the clinical and laboratory parameters of the inflammatory disease activity prior to and 6 weeks after *H. pylori* eradication therapy are shown in Table 2 (on entering the study and 3 months after for *H. pylori*/HLO negative patients). Following the treatment, an improvement was observed in all parameters except for the VAS-score) in *H. pylori*/HLO positive patients, but the improvement was only significant for ESR ($p = 0.02$), and only before adjusting for multiple comparisons ($p = 0.14$). The LMW IgG antibody level to *H. pylori* declined significantly 6 weeks after eradication therapy and during the entire follow-up period ($p < 0.001$), consolidating the absence of *H. pylori* on the second endoscopy, while no significant change was seen in *H. pylori*/HLO negative patients ($p = 0.94$). In *H. pylori*/HLO negative patients, an improvement was also observed in all parameters, except for the ESR, but the improvement was only significant for the number of tender joints ($p = 0.02$), and only before adjusting for multiple comparisons ($p = 0.14$).

Table 3 and Figure 2 show the data of the clinical and laboratory parameters of the inflammatory disease activity at entry and 6, 18, 32 and 42 weeks after *H. pylori* eradication. The data measured 6 weeks after eradication of *H. pylori* show a tendency towards improvement in all parameters, except for the VAS score and CRP level, although this was only significant for the ESR ($p = 0.02$), and only before adjusting for multiple comparisons ($p = 0.14$).

Table 4 and Figure 3 show the recorded clinical and laboratory parameters of the inflammatory disease activity in *H. pylori*/HLO negative patients at entry, and after 3, 6, 9 and 12 months. A significant decline in the number of tender joints was observed ($p < 0.001$), also after adjustment for multiple comparisons ($p < 0.007$).

Table 2 Clinical and laboratory parameters of inflammatory disease activity and LMW IgG-antibodylevel to *H. pylori* in *H. pylori*/HLO positive and in *H.pylori*/HLO negative patients with rheumatoid arthritis prior to and 6 weeks after eradication of *H. pylori* (at entry and after three months) (Wilcoxon's Signed Rank Test).

	H. pylori/HLO positive patients (n = 17)			*H.pylori*/HLO negative patients (n = 37)		
	Before eradication of *H.pylori*	Six weeks after eradication of *H. Pylori*	P	Entry	Three months	p
Morning stiffness (min.) Median (range)	31 (0-249)	24 (0-161)	0.08	0.5 (0-266)	0 (0-386)	0.59
HAQ – score (0-3) Median (range)	1.25 (0-2)	0.875 (0-1.875)	0.34	1.25 (0-2.125)	1.125 (0-2.375)	0.70
VAS – score (0- 100) Median (range)	33 (0-78)	40 (0-74)	0.21	39.5 (9-86)	34.5 (7-75.5)	0.21
Swollen joints (number) Median (range)	4 (0-13)	1 (0-22)	0.12	2 (0-16)	1 (0-8)	0.33
Tender joints (number) Median (range)	9 (0-23)	6 (0-26)	0.29	5 (0-30)	4 (0-27)	0.02
ESR (AE) Median (range)	11.5 (3-47)	8 (1-26)	0.02	14 (1-90)	22 (1-60)	0.68
CRP (nmol/l) Median (range)	57 (48-346)	48 (48-541)	0.16	266 (48-1,875)	218.0 (48-1,496)	0.94
LMW-IgG (EU) Median (range)	578 (146-1339)	178 (0-1150)	< 0.001	56 (0-933)	42 (0-897)	0.46

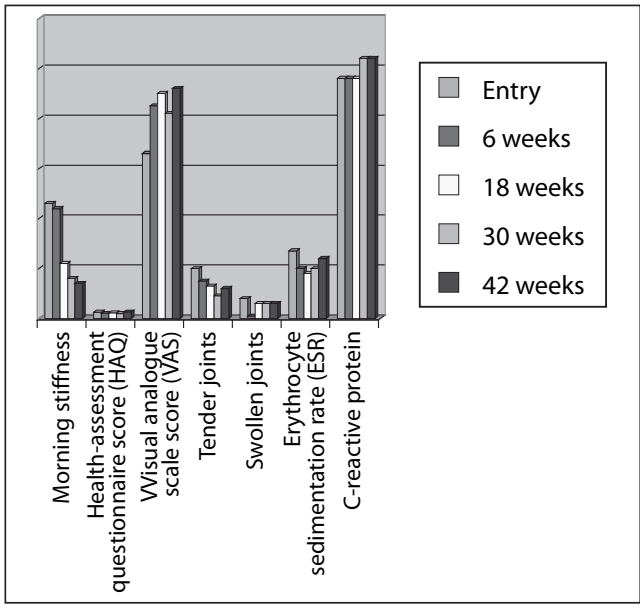

Figure 2 Clinical and laboratory parameters of inflammatory disease activity in *H. pylori*/HLO positive patients with rheumatoid arthritis before and six, eighteen, thirty and forty-two weeks after eradication of *H. pylori*.

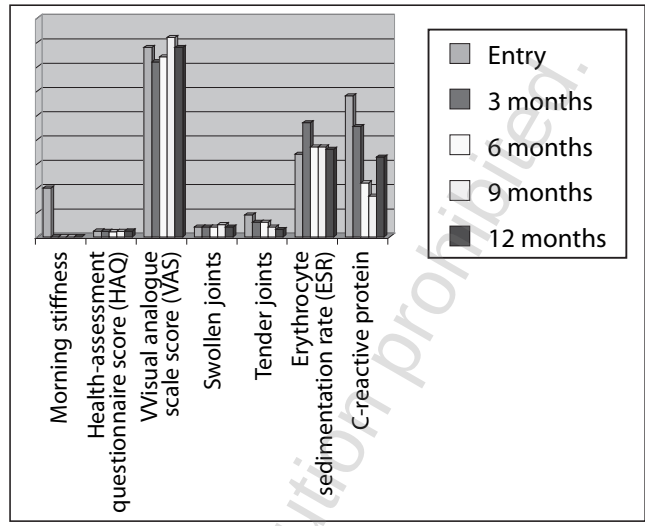

Figure 3 Clinical and laboratory parameters of inflammatory disease activity in *H. pylori*/HLO negative patients with rheumatoid arthritis at entry and after 3, 6, 9 and 12 months.

Table 3 Clinical and laboratory parameters of inflammatory disease activity and LMW-IgG antibody level to *H. pylori* in *H. pylori*/HLO positive patients with rheumatoid arthritis before and 6, 18, 30 and 42 weeks after eradication of *H. pylori* (Friedman's test). N= 14.

	Entry	Six weeks	Eighteen weeks	Thirty weeks	Forty-two weeks	P
Morning stiffness (min.) Median, range	23 (0-179)	22 (0-72)	11 (0–79)	8 (0-112)	7 (0-171)	0.20
HAQ – score (0-3) Median, range	1.25 (0-2)	1.06 (0-1.875)	1.125 (0-1.875)	1 (0-2.125)	1.31 (0-2)	0.37
VAS – score (0-100) Median, range	33 (0-78)	42.5 (0-74)	45 (0-71)	41 (0-61.5)	46 (0-77)	0.46
Swollen joints (number) Median, range	4 (0-13)	0.5 (0-22)	3 (0–16)	3 (0-24)	3 (0-16)	0.27
Tender joints (number) median, range	10 (0-23)	7.5 (0-26)	6.5 (0-28)	4.5 (0-26)	6 (0-27)	0.45
ESR (AE) median, range	13.5 (5-47)	10 (1-26)	9 (3–44)	10 (2-44)	12 (2-42)	0.02
CRP (nmol/l) median, range	48 (48-346)	48 (48-184)	48 (48-676)	52 (48-930)	52 (48-743)	0.18
LMW-IgG (EU) median, range	555 (146-986)	178 (0-856)	183 (25-553)	143 (20-283)	65 (18-292)	< 0.001

Table 4 Clinical and laboratory parameters of inflammatory disease activity and LMW-IgG antibody level to *H. pylori* in *H. pylori*/HLO negative patients with rheumatoid arthritis at entry and after 3, 6, 9 and 12 months (Friedman's test). N = 32.

	Entry	Three months	Six months	Nine months	Twelve months	p
Morning stiffness (min.) Median, range	10 (0-266)	0 (0-264)	0 (0-231)	0 (0-281)	0 (0-289)	0.73
HAQ x– score (0-3) Median, range	1.187 (0-2)	1.125 (0-2.375)	1.062 (0-2.375)	1.125 (0-2.625)	1.187 (0-2.625)	0.45
VAS – score (0-100) Median, range	39 (9-86)	36 (7-75.5)	37 (6.5-76.5)	41 (8-81)	39 (2-79)	0.51
Swollen joints (number) Median, range	2 (0-16)	2 (0-8)	2 (0-14)	2.5 (0-14)	2 (0-9)	0.34
Tender joints (number) Median, range	4.5 (0-24)	3 (0-25)	3 (0-23)	2 (0-30)	1.5 (0-32)	< 0.001
ESR (AE) Median, range	17 (1-82)	23.5 (1-60)	18.5 (1-85)	18.5 (1-87)	18 (1-56)	0.96
CRP (nmol/l) Median, range	290 (48-966)	227 (48-596)	111 (48-1540)	84 (48-1262)	164 (48-957)	0.19
LMW-IgG (EU) Median, range	46.5 (0-933)	37.5 (0-759)	37.5 (0-796)	29.5 (0-772)	22 (0-769)	0.94

DISCUSSION

A total of 59 patients with rheumatoid arthritis entered the study and *H. pylori*/HLO was identified in 18 of them (31%). This prevalence is in accordance with previously described prevalence of *H. pylori* in patients with rheumatoid arthritis [38-47]. The prevalence is also equivalent to the estimated prevalence of *H. pylori* (26%) in the Danish population [56]. The patients were therefore as prone to *H. pylori* infection as other patients with rheumatoid arthritis, and the Danish background population.

When examining the *H. pylori* positive patients, a significant decrease in the ESR of the patients was observed 6 weeks after ended eradication therapy, and also during the whole follow-up period. After adjusting for multiple comparisons, however, the results were not significant. A non-significant improvement was also observed for most of the clinical parameters. It is possible that the observed improvements reached significance if the inclusion criteria in this study had been more restricted. There were no criteria for maximal disease duration, because many of the included patients had diseases of long duration, and consequently many had irreversible damage to joints, which made a clinical improvement difficult to establish. Also, there were no criteria for minimal inflammatory disease activity, because some patients had only mild activity; this too rendering a clinical improvement difficult to establish.

As the improvements observed were most impressive immediately after eradication therapy, the improvement might be due to the temporary suppression of other underlying infections, or may be explained by an anti-inflammatory or immune-modulating effect of the eradication therapy *per se*. Indeed, it has previously been shown that treatment with minocycline (a tetracycline) has a beneficial impact on clinical as well as on laboratory parameters of inflammatory-disease activity [57, 58]; chlarithromycine has been shown to have an important immune-modulating effect on synoviocytes and T-cells [59]. This may also explain the reported improvement of inflammatory-disease activity in patients with rheumatoid arthritis following an eradication of *H. pylori* – as reported by Zentilin et al. [49], though the eradication regimen used in their study is not documented.

In conclusion, the results of this pilot study do not unequivocally reject the hypothesis of an aetiological-pathological-genetic relationship between *H. pylori* and rheumatoid arthritis. Some of the results indicate the role of *H. pylori* in the expression of the inflammatory state of rheumatoid arthritis. Further randomised, controlled and double-blind trials, including a larger number of selected patients, are necessary for confirmation. In a well-planned study, the ethical problems of not treating the control group immediately could be overcome.

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