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## Abstract

**Background** Acne rosacea is a dermatosis with unknown etiology. Some studies have reported a high prevalence of *Helicobacter pylori* infection in acne rosacea. Other studies have reported a decrease in the severity of the lesions of acne rosacea after eradication of *H. pylori*. *H. pylori* is a Gram-negative bacterium which colonizes the gastric mucosa and increases the synthesis of oxygen radicals, such as superoxide and proinflammatory cytokines. These cytokines have been demonstrated to stimulate the synthesis of the inflammatory species nitric oxide (NO). In this study, we examined the role of NO in the possible effect of *H. pylori* in acne rosacea.

**Methods** Thirty-three acne rosacea patients were included in the study and the control group comprised 20 healthy individuals. The levels of immunoglobulin G antibodies against *H. pylori* in the serum samples were measured using the enzyme-linked immunoabsorbent assay method. Measurement of nitrate was performed using chemiluminescence in accordance with the method described by Braman and Hendrix (Braman RS, Hendrix SA. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(iii) reduction with chemiluminescence detection. *Anal Chem* 1989; **61**: 2715–2718). For statistical analysis, the *t*-test was used.

**Results** The seropositivity of *H. pylori* in acne rosacea patients was found to be high; however, the serum nitrate levels were found to be normal.

**Conclusions** The results of the study indicate that the inflammatory species NO, which has been hypothesized to be associated with *H. pylori*, has no role in the inflammatory mechanism of acne rosacea.

## Introduction

Acne rosacea is an inflammatory skin disease that affects the central parts of the face. It is characterized clinically by papules and papulopustules, erythema, telangiectasia, and flushing episodes. The flushing episodes are seen generally in the initial stages of the disease and turn into permanent dark-red erythema in the later stages. The etiopathogenesis has not yet been clarified. Various factors are thought to affect the course of the disease, including the genetic predisposition of the individual, gastric hypochlorhydric dyspepsia, gastrointestinal system infections, hypertension, *Demodex folliculorum*, and psychogenic factors.[1–3] *Helicobacter pylori* is the pathogenic bacterium responsible for the etiology of gastrointestinal system infections from non-ulcer dyspepsia to the activation of peptic ulcer. Recent studies have shown that the prevalence of *H. pylori* infection is higher in acne rosacea patients than in control groups.[4–6] In another study, Utaş *et al*.[7] found that the seroprevalence of *H. pylori* was the same as that of the control group, but a statistically significant decrease in the severity of the disease was reported with the treatment of seropositive acne rosacea patients with a combination of metronidazole, amoxicillin and bismuth compounds.

*H. pylori* increases the synthesis of oxygen metabolites, such as superoxide and proinflammatory cytokines. These cytokines have also been shown to stimulate the synthesis of the inflammatory species nitric oxide (NO).[8–11]

In this study, we investigated the role of NO in the possible effect of *H. pylori* in acne rosacea patients. The seroprevalence of *H. pylori* and the serum nitrate levels in a group of acne rosacea patients were measured and compared with those of a control group consisting of healthy individuals.

## Materials and methods

Thirty-seven acne rosacea patients (between the ages of 26 and 67 years; 27 women and 10 men), who were seen at the Polyclinic



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duration varying between 4 months and 13 years. Twenty healthy individuals of similar gender and age distribution (20–69 years; 15 women and five men) were selected as the control group.

Acne rosacea can be clinically classified into four stages: stage I, episodic erythema and flushing attacks on the central areas of the face, the neck, and the V-shaped area of the chest (rosacea diathesis); stage II, persistent erythema and telangiectases; stage III, persistent erythema, telangiectases, and papules and pustules; stage IV, persistent erythema, telangiectases, papules, pustules, and nodules, and tissue hypertrophy (rhinophyma).

The clinical diagnosis of acne rosacea was made according to the above stages. Patients in stages II, III, and IV were included in the study. All patients were also diagnosed histopathologically according to the clinical stage. The histopathologic diagnoses had been made previously in 13 patients, whereas the diagnoses were made during the study in 20 patients.

Before blood samples were taken from the patients, it was made certain that they had not received topical or systemic drugs for acne rosacea for at least 1 week. This period was determined from the half-lives of the drugs used in the treatment of acne rosacea to avoid the influence of the possible anti-inflammatory effects of these drugs. Four blood samples (three women and one man) could not be analyzed because of hemolysis. From the blood samples of both the patient and control groups, 5 $cm^3$ portions were drawn into test tubes containing gel and centrifuged. These samples were stored at −20 °C until analysis.

The amount of total nitrate in the test samples was determined by a modification of the procedure described by Braman and Hendrix[12] using the purge system of a Sievers Instruments Model 280A Nitric Oxide Analyzer. A standard curve was constructed using various concentrations of $NO_3^-$ (10–100 μM). The nitrate levels of the samples were calculated using the standard curve. Results were expressed as the mean ± standard deviation (SD).

The level of immunoglobulin G (IgG) antibodies against *H. pylori* in the serum samples was measured using the enzyme-linked immunoabsorbent assay (ELISA) method (International Immuno-Diagnostics, USA). The antibody titer of each serum sample was determined using the calibration curve. A sample was considered to be positive if the IgG value was equal to or greater than 100 EU/mL. If the value was between 91 and 99 EU/mL, it was considered to be equivocal. If the value was lower than 90 EU/mL, it was considered to be negative.

## Results

Thirty-three acne rosacea patients were compared with the 20 healthy individuals of the control group in terms of the average age (48.21 and 46.35 years, respectively) and no statistically significant difference was found between the groups. There was also no difference in terms of gender between the patient and control groups: both groups contained 75% women.

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| Patients | 29 | 1 | 3 | 33 |
| Controls | 9 | 3 | 8 | 20 |

IgG antibodies against *H. pylori* were positive in 29 (95.7%) of the acne rosacea patients, whereas in one (3.3%) patient they were found to be probably positive. Among individuals in the control group, nine (45%) were positive, whilst three (15%) were probably positive (Table 1). The seropositivity of *H. pylori* in the patient group was found to be statistically significantly higher than that of the control group ($p < 0.005$). The average nitrate level of the serum samples from the patients was 49.27 ± 9.85 μmol/L, whereas that of the control group was 46.06 ± 18.44 μmol/L. No statistically significant difference was found between the nitrate levels of the two groups ($p > 0.5$).

## Discussion

Acne rosacea is a dermatosis with unknown etiology. The disease is associated with hypochlorhydria, gastritis and jejunal mucosal abnormalities, such as gastrointestinal system disturbances. *H. pylori* infection plays an etiologic role in the development of chronic active gastritis, peptic ulcer disease, and gastric malignancy. *H. pylori* is a Gram-negative bacterium that colonizes the gastric mucosa. In various studies, it has been shown that inducible nitric oxide synthetase protein is expressed in the gastric mucosa of patients with active gastritis and *H. pylori* infection, and that large amounts of NO are excreted from the gastric mucosa of these patients.[8–11]

NO is a colorless paramagnetic gas. It has radical characteristics due to the uncoupled single electron it carries in its outer orbital. In fluid media, it diffuses freely and passes through cell membranes easily. The half-life in tissues varies between 10 and 60 s. In water and plasma, it reacts with oxygen to form the nitrite, whilst in whole blood it reacts with hemoglobin to form the nitrate which is excreted in urine. The nitrites and nitrates have no biological activity. In biological systems, the measurement of NO is generally performed indirectly via its physiological activity. To determine the amount of NO in an organism, the serum or plasma nitrate and nitrite levels, the end-products of NO, are used.[13,14] NO is thought to play a role in vasodilatation, inflammation, and immunomodulation in the skin.[13,14]

Knowledge of the etiopathogenesis of acne rosacea is still very limited, and treatment is performed on a symptomatic basis. Studies on *H. pylori* in acne rosacea suggest that this

effect of *H. pylori* in patients with acne rosacea. The seropositivity of *H. pylori* in acne rosacea patients was found to be significantly high, whereas the serum nitrate levels of the patients were normal. Based on the results of the study, we conclude that the inflammatory species NO, which has been hypothesized to be associated with *H. pylori*, has no role in the inflammatory mechanism of acne rosacea.


## Acknowledgments

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## References


1 Plewig G, Jansen T. Rosacea. In: Freedberg IM, Eisen AZ, Wolff K, *et al.*, eds. *Dermatology in General Medicine*, 5th edn. Philadelphia: McGraw-Hill Company, 1999: 785–794.

2 Wilkin JK. Rosacea pathophysiology and treatment. *Arch Dermatol* 1994; **130**: 359–362.

3 Griffiths WAD. Rosacea. *Retinoids* 1998; **14**: 117.

4 Rebora A, Drago F, Picciotto A. Helicobacter pylori in patients with rosacea. *Am J Gastroenterol* 1994; **89**: 1603–1604.

5 Erel A, Öztaş M, İlter N, *et al.* Helicobacter pylori in rosacea: lack of an association. (letter). *Arch Dermatol* 1998; **134**: 511.

7 Utaş S, Özbakır Ö, Turasan A, Utaş C. Helicobacter pylori eradication treatment reduces the severity of rosacea. *J Am Acad Dermatol* 1999; **40**: 3.

8 Tsuji S, Kawano S, Tsujii M, *et al.* Helicobacter pylori extract stimulates inflammatory nitric oxide production. *Cancer Lett* 1996; **108**: 195–200.

9 Franco L, Talamini G, Carra G, Doria D. Expression of COX-1, COX-2, and inducible nitric oxide synthase protein in human gastric antrum with Helicobacter pylori infection. *Prostaglandins Other Lipid Med* 1999; **58**: 9–17.

10 Mannick EE, Bravo E, Zarama G, *et al.* Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in Helicobacter pylori gastritis: effect of antibiotics and antioxidants. *Cancer Res* 1996; **56**: 3238–3243.

11 Shapiro KB & Hotchkiss JP. Induction of nitric oxide synthesis in murine macrophages by Helicobacter pylori. *Cancer Lett* 1996; **102**: 49–56.

12 Braman RS & Hendrix SA. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection. *Anal Chem* 1989; **61**: 2715–2718.

13 Erbaş D. Nitric oxide in health. *Gazi Med J* 1998; **9**: 1–36.

14 Kiechle F, Malinski T. Nitric oxide. *Clin Chem* 1993; **100**: 567–573.