

Is There A Correlation Between Seroprevalence of Helicobacter Pylori and Plasma Nitric Oxide Levels In Patients With Rosacea?

ROZASEA HASTALARINDA HELICOBACTER PYLORI SEROPREVALANSI İLE PLAZMA NİTRİK OKSİT SEVİYELERİ ARASINDA KORELASYON VAR MI ?

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Summary

Purpose: Recently, there are several investigations suggesting that there might be an etiologic role of *Helicobacter pylori* (*Hp*) in rosacea. However, how *Hp* is related to rosacea remains unclear. It has been shown that *Hp* induces expression of inducible nitric oxide synthase (iNOS) and corresponding inflammatory cell NO synthesis. The aim of this study was to investigate the possible role of NO, related with *Hp* infection, in rosacea.

Methods: Twenty-nine rosacea patients and age and sex-matched 20 healthy controls were examined for specific immunoglobulin G (IgG) and IgM against *Hp* and plasma NOS enzyme activities.

Results: In comparison with controls, the seropositivity of *Hp* for IgM was found to be significantly higher ($p=0.03$) whereas the seropositivity of *Hp* for IgG was lower ($p=0.0001$) in patients. There was no difference between plasma NOS activities of the two groups. Plasma NOS activities were not affected by the seropositivity of *Hp* for IgM and/or IgG in both groups.

Conclusion: Our results suggest that NO, which has been hypothesized to be associated with *Hp*, has no pathogenic role in rosacea.

Key Words: Rosacea, Nitric oxide, *Helicobacter pylori*

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Özet

Amaç: Son zamanlarda rozaseanın etiolojisinde *Helicobacter pylori* (*Hp*)'nin rolü olabileceğini gösteren birtakım çalışmalar mevcuttur. Ancak *Hp* ile rozasea ilişkisinin mekanizması henüz aydınlatılamamıştır. *Hp*'nin nitrik oksit sentaz (iNOS) ve dolayısıyla inflamatuvar hücre nitrik oksit sentezini arttırdığı gösterilmiştir. Bu çalışmada, *Hp* infeksiyonu ile ilişkili NO'nun rozaseadaki olası rolünün belirlenmesi amaçlandı.

Hastalar ve Yöntem: Yirmi dokuz rozasealı hasta ile yaş ve cins dağılımı uyumlu yirmi sağlıklı bireyde, *Hp*'ye karşı oluşmuş spesifik immünglobulin G (IgG) ve IgM antikorları ve plazma NOS enzim aktiviteleri araştırıldı.

Bulgular: Kontrol grubu ile karşılaştırıldığında, hasta grubunda *Hp* IgM seropozitifliği istatistiksel olarak anlamlı olarak daha yüksek ($p=0.03$), *Hp* IgG seropozitifliği ise daha düşük bulundu ($p=0.0001$). Hasta ve kontrol gruplarının plazma NOS enzim aktivite düzeyleri arasında fark saptanmadı. Her iki grupta, plazma NOS enzim aktivite düzeylerinin *Hp* IgM ve/veya IgG seropozitifliğinden etkilenmediği tespit edildi.

Sonuç: Bulgularımız, *Hp* ile ilişkili olduğu varsayılan nitrik oksitin rozaseada patojenik rolü olmadığını göstermektedir.

Anahtar Kelimeler: Rozasea, Nitrik oksit, *Helicobacter pylori*

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Rosacea is a chronic inflammatory skin disease with an unknown etiology usually affecting middle-aged individuals. It is characterized by papules and papulopustules, erythema, and telen-

giectases, preceded by episodes of flushing (1).

Helicobacter pylori (*Hp*) is the primary cause of gastritis and a major contributor to peptic ulcer disease. Recently, there are several investigations

suggesting a possible etiologic role for *Hp* in rosacea (2-8). Some of these investigations showed that the prevalence of *Hp* infection in patients with rosacea was higher than that of control subjects (4-6) or average (7), and the others reported that *Hp* eradication treatment reduced the severity of rosacea (2,3,6,8). How *Hp* is related to rosacea remains unclear. However it has been postulated that NO, known to be produced by *Hp*, may have a pathogenic role in rosacea (5,9). It has already been shown that *Hp* and its cellular products induce expression of inducible nitric oxide synthase (iNOS) and corresponding inflammatory cell NO synthesis by the L-arginine-NO pathway (10,11). Increased serum NO levels have been reported in *Hp* infected subjects (12).

NO is a free radical synthesized from L-arginine by one of the family of nitric oxide synthase (NOS) enzymes. There are three genetically distinct NOS enzymes in humans. These are constitutive mainly endothelial form; the constitutive mainly neuronal form; and the inducible form (iNOS). The latter is involved in several inflammatory and autoimmune diseases and produces larger quantities of NO. Nitric oxide has been found to be important in a number of different physiological processes. Of particular relevance to the skin are the roles of NO in vasodilatation, inflammation and immunomodulation (13,14). Rosacea is a chronic inflammatory skin disease, and vasodilatation and inflammation are among the main characteristics of the disease. Therefore we hypothesized that increased levels of NO, induced by *Hp*, may have a pathogenic role in rosacea, at least as an aggravating factor.

The aim of this study was to investigate the possible role of NO, related with *Hp*, in rosacea patients. For this purpose, the seroprevalence of *Hp* and plasma NOS enzyme activities, as an indicator for plasma NO levels, were measured in rosacea patients and compared with those of healthy control subjects.

Material and Methods

This study was conducted between September 2001 and November 2002 in the Dermatology

Department of Mersin University School of Medicine. Twenty-nine rosacea patients (20 women and 9 men, between the ages of 33 and 70 years) were included in the study. Twenty age and sex-matched healthy individuals (12 women and 8 men, between 30 and 66 years of age) were selected as the controls.

Rosacea was diagnosed by clinical findings and severity of the disease was scored as follows: 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) (5). Patients in stage II (n=9), stage III (n=19), and stage IV (n=1) were included in the study.

All patients and control subjects were examined for specific immunoglobulin G (IgG) and IgM against *Hp* and plasma NOS activity. Both the patients and control group had no history of any topical or systemic drug therapy for at least one month prior to blood collection, and none of them had any systemic disease. There was no difference between the patients and controls with regard to smoking and alcohol intake.

The fasting blood samples of both groups were drawn into citrate (3.5 mg/ml blood) containing glass tubes and centrifuged at 480 x g for 10 minutes, and plasma samples were stored at -20 °C until analysis.

These plasma samples were examined for specific IgG and IgM antibodies against *Hp* by means of enzyme-linked immunosorbent assay (ELISA) method (Immunolab GmbH, Germany). A sample was considered to be positive if the antibody titer (for both IgG and IgM) was equal to or greater than 25 IU/ml.

Plasma NOS enzyme activities were measured based on the diazotization of sulfanilic acid by nitric oxide at acid pH and subsequent coupling to N-(1-naphthyl)-ethylenediamine as described in reference (15), and results were expressed as IU/ml

($\mu\text{mol nitrite/ml min}$). All procedures were performed at $+4^{\circ}\text{C}$ throughout the experiments.

Student-t, Chi-square test and ANOVA test were performed for statistical analysis. Differences between the two groups for IgM and IgG values were detected with Chi-square test. ANOVA test was performed to compare mean values of NOS enzyme activity of the two groups. Descriptive statistics were shown as mean \pm standard deviation.

Results

Twenty-nine rosacea patients with disease duration between 1 and 15 years and 20 healthy control subjects were comparable in terms of the age (48.58 ± 7.89 and 47.85 ± 10.13 years, respectively), and sex (69% and 60% women).

The seropositivity of *Hp* for IgG in the rosacea group was found to be statistically significantly lower ($p=0.0001$), whereas the seropositivity of *Hp* for IgM was found to be higher than that of the control group ($p=0.03$) (Table 1).

There was no statistically significant difference between the plasma NOS enzyme activity of the groups ($p=0.594$) with ANOVA test (Table 2).

The plasma NOS enzyme activity levels were found not to be affected by gender ($p=0.309$) with Chi-square test, and by the seropositivity of *Hp* for IgM ($p=0.119$) and IgG ($p=0.053$) in patients, and by the seropositivity of *Hp* for IgG ($p=0.815$) in controls, with ANOVA test.

Discussion

The etiology of rosacea remains unclear. Many factors including endocrine, psychologic, pharmacologic, immunologic, infectious, thermal, and alimentary have been investigated as pathophysiological sources of the disease, but none of them has been proven to be the actual cause of the disease. Over the past decades, there are several investigations suggesting a possible etiologic role for *Hp* in rosacea. This suggestion was further supported by the response of rosacea patients to metranidazole, a drug that had been used for treatment of the gastric disease. Moreover, it has been noted that peptic ulcer disease and rosacea share their seasonal behavior (16).

Contradictory results of anti-*Hp* serology in rosacea patients have been reported. Some investigators had found a significantly higher prevalence of *Hp* infection in rosacea subjects compared to controls (4-6) or general population (7) while some

Table 1. Helicobacter pylori (*Hp*) immunoglobulin G (IgG) and IgM results in patients and control subjects.

| | | <i>Hp</i> IgM | | <i>Hp</i> IgG | | Total |
|----------|---|---------------|----------|---------------|----------|--------|
| | | Positive | Negative | Positive | Negative | |
| Patients | n | 6 | 23 | 3 | 26 | 29 |
| | % | 20.7% | 79.3% | 10.3% | 89.7% | 100.0% |
| Controls | n | - | 20 | 13 | 7 | 20 |
| | % | - | 100.0% | 65.0% | 35.0% | 100.0% |
| p | | .030 | | .0001 | | - |

Table 2. Plasma NOS enzyme activity (IU/ml) in patients and control subjects.

| | | n | Mean | Std. Error | Minimum | Maximum | p |
|-----|----------|----|-------|------------|---------|---------|------|
| NOS | Patients | 29 | 0,105 | 0,011 | 0,014 | 0,241 | n.s. |
| | Controls | 20 | 0,117 | 0,021 | 0,009 | 0,352 | |

n.s.: non significant

others had not (3,8,17-20). The results of our study revealed a higher seropositivity for IgM, and a lower seropositivity for IgG antibodies against *Hp* in rosacea patients when compared with the control subjects.

Although there are several studies investigating a possible relation between *Hp* and rosacea, a review of the literature revealed only one study aimed to clarify the mechanisms by which *Hp* is involved in the pathogenesis of rosacea. In this study, Güreter et al had investigated the role of NO, which has been hypothesized to be associated with *Hp*, in rosacea patients. They reported that the seropositivity of *Hp* in rosacea patients was found to be high, but the serum nitrate levels were within normal limits (5) and concluded that NO has no role in the inflammatory mechanism of rosacea. In accordance with their results, no statistically significant difference between rosacea patients and controls with regard to plasma NOS enzyme activity was detected in our study.

Our results suggest that NO has no role in the pathogenesis of rosacea. We believe that our data need to be supported by further studies.

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