

# Serum Nitrite Levels in Helicobacter pylori Associated Gastritis in Women

Hüseyin BASKIN(\*), Sevin KIRDAR(\*), İ Hakkı BAHAR(\*), Nuran YULUĞ(\*)

(\* Dokuz Eylül Üniversitesi, Tıp Fakültesi, Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı, İzmir)

## SUMMARY

Helicobacter pylori infection (HPI) is known as a risk factor for gastric cancer. Nitric oxide is produced during non specific defensive events of immune system. In carcinogenesis theories, although the association is known, the role of Helicobacter pylori (HP) in etiology of gastric cancer remains unclear. In this study we examined the serum nitrite contents of female patients (n=46) who were diagnosed to have gastritis endoscopically and the control group of healthy females (n=10). Nitrite levels were significantly higher in HP IgA-IgG positive group, and in conclusion, if women are known to be under significant risk of gastric cancer, periodically monitoring the serum nitrite levels (or nitric oxide synthase activity) of every female patients with HPI during the treatment period may be a valuable marker for a possible gastric cancer risk despite of the possible eradication of the bacteria.

Key Words: Helicobacter pylori, serum nitrite, gastric cancer

## ÖZET

Kadınlarda Helicobacter pylori ile İlişkili Gastritte Serum Nitrit Düzeyleri

Helicobacter pylori enfeksiyonunun (HPI) gastrik kanser için bir risk faktörü olduğu bilinir. Nitrik oksit bağışıklık sisteminin özgül olmayan savunma mekanizmalarınca üretilir. Karsinogenez teorilerinde ilişki bilinmesine karşın, HPI'nin gastrik kanser etiolojisindeki rolü henüz açık değildir. Bu çalışmada endoskopik olarak gastrit tanısı almış kadınların (n=46) ve kontrol grubu olarak sağlıklı kadınların (n=10) serum nitrit değerleri değerlendirilmiştir. HP IgA-IgG pozitif olan gruptaki serum nitrit düzeyleri belirgin olarak yüksek bulunmuştur. Sonuç olarak, eğer kadınların belirgin olarak gastrik kanser riski altında olduğu biliniyorsa, HPI enfeksiyonu olan kadınların sağaltımları sırasında düzenli olarak serumlarındaki nitrit düzeyleri (veya nitrik oksit sentaz aktiviteleri) izlenirse, bu izlem sonuçları bakteri tamamen silinse bile hala olasılığı süren gastrik kanser riski için değerli bir gösterge olabilir.

Anahtar Kelimeler: Helicobacter pylori, serum nitrit, gastrik kanser

## INTRODUCTION

Helicobacter pylori infection (HPI) is known as a risk factor for gastric cancer. Many epidemiological and experimental studies have shown an association between chronic HPI and subsequent development of gastric carcinoma in humans and animals (1,2). Nitric oxide is produced in multiple cells of the human body as a part of non specific immune response in a soluble gas form and shows its effect in seconds, however following the production its defensive effects are not limited and might be harmful to normal cells. One of the main adverse effect of nitric oxide is its precancerous DNA fragmentation (3). In this study we examined the serum nitrite contents of female patients who have gastritis.

## MATERIALS AND METHOD

Methods for determining nitrite and nitrate are applicable to both fresh and archived body fluids such as plasma, serum, urine, bile, synovial fluid, sputum, and cerebrospinal fluid. Fasting for 12 hours is reported to reduce concentrations of plasma nitrate and nitrite by 50 %, and in fasted volunteers approximately 90 % of plasma nitrite and nitrate is derived from NOS-derived NO (4,5). Before endoscopy and serum samples all the subjects were fasted overnight.

The sera of the female patients (n=46) who were diagnosed to have gastritis endoscopically and the control group of healthy females (without gastritis) (n=10) were collected and kept at -20 °C until use. Nitrite levels of these sera were determined by Gri-

ess Reagent. Plasma and urine nitrite and nitrate measurements may have a diagnostic role for individual patients with conditions in which inducible NO synthase (NOS) is massively up-regulated, such as infection, rejection, and inflammation (6). Nitrite is generated by the rapid oxidation of NO. To assay nitrite we used a modification of a previously published method (7). Aliquots of 100  $\mu$ L serum samples were mixed with equal volumes of Griess Reagent in a 96-well microtitre plate (Maxisorb Immunoplate, Nunc). After 10 minutes of incubation at room temperature the absorbance at a wavelength of 540 nm was measured in a microplate reader (Model 230S; Organon Technica). A range of 2-fold dilutions of sodium nitrite (0.05-100 mM) in PBS was run in each assay to generate a standard curve. These sera were screened by Helicobacter pylori (HP) IgA and IgG EIA (Quorum Diagnostics Inc., Canada) according

to manufacturer's instructions. Statistical analyses were made by Kruskal-Wallis one-way ANOVA to determine the differences between groups.

## RESULTS

In HP IgA-IgG negative cases (n=10) nitrite values were  $15.3 \pm 2.6 \mu\text{M}$  (95% confidence interval, C.I: 14.33-16.82). In HP IgA-IgG positive cases (n=36) nitrite values were  $30.8 \pm 6.9 \mu\text{M}$  (C.I: 30.16-35.73). Serum nitrite values of healthy group were  $16.4 \pm 4.3 \mu\text{M}$  (C.I: 14.18-17.82) (Table 1). A significant difference was shown between groups by Kruskal-Wallis one-way ANOVA ( $p < 0.001$ ) (Figure 1).

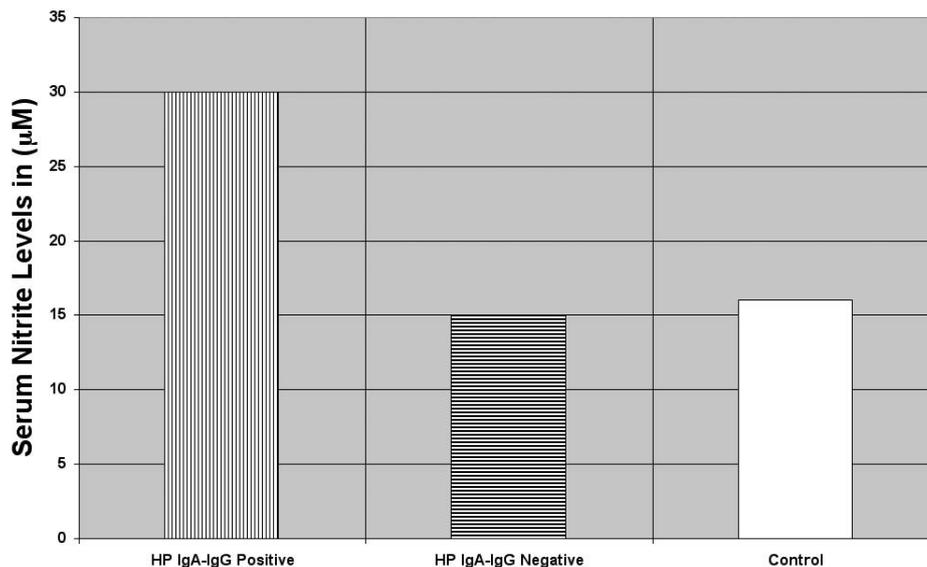
## DISCUSSION

In carcinogenesis theories, there are several explanations about the role of HPI in etiology of gastric can-

Table 1. Serum Nitrite Levels of Helicobacter pylori IgA positive, Helicobacter pylori IgA negative and control subjects.

|                           | Helicobacter pylori<br>IgA-IgG positive (n=36) | Helicobacter pylori<br>IgA-IgG negative (n=10) | Control (n=10) |
|---------------------------|--|--|----------------|
| Serum Nitrite Levels (mM) | $30.8 \pm 6.9$                                 | $15.3 \pm 2.6$                                 | $16.4 \pm 4.3$ |

Figure 1: Serum nitrite levels of Helicobacter pylori (HP) IgA-IgG positive, Helicobacter pylori (HP) IgA-IgG negative, and control groups (in  $\mu\text{M}$ ) ( $p < 0.001$ ).



cer. The most convincing hypothesis shows chronic inflammation as a reason for malignant transformation (8). In recent studies the role of nitroso compounds in the gastric carcinogenesis was shown (9,10).

In this study we found that serum nitrite levels were significantly higher in HP IgA-IgG positive group and it is known that elevated HP IgA levels reflect active chronic gastritis (11) as a non invasive and easy to perform serological test.

Females are known to be under significant risk of cancer (12,13), and if treatment is successful no progression to cancer occur in HPI and it is assumed that 1.2 % of persons would become reinfected with HP annually (14).

In this study, no significant matchings between age, socioeconomic status and serum nitrite levels were observed. These results direct us to think and study more about the problem in developing countries.

In conclusion, periodically monitoring the serum nitrite levels of female patients with HPI after treatment may be a valuable marker for a possible gastric cancer risk despite of the possible eradication of the bacteria. And these results deserve further studies in wide patient groups.

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