Türk Klinik Biyokimya Derg 2023; 21(2): 89-93

https://doi.org/10.56615/tkbd.2023.11

Araştırma Makalesi / *Research Article*

The Relationship Between the Presence of Helicobacter Pylori and Serum Neopterin Levels

Helicobacter Pylori Varlığı ile Serum Neopterin Seviyeleri Arasındaki İlişki

Yakup Dülgeroğlu[1] **Gökhan Bilgehan**[2]

1 Kulu State Hospital, Medical Biochemistry, Konya, Türkiye

2 Bozkır State Hospital, Internal Medicine, Konya, Türkiye

Başvuru Tarihi / *Received:* 21 Şubat 2023 Kabul Tarihi / *Accepted:* 11 Temmuz 2023

ABSTRACT

Aim: In this study, it was aimed to examine the relationship between serum levels of neopterin, which is considered a marker of cellular immunity and oxidative stress, and the presence of H. pylori, which causes both inflammation and increased oxidative stress.

Material and Methods: Fifty-three patients with chronic gastritis and 14 healthy volunteers were included in the study. According to the histopathological examination results, patients with chronic gastritis were divided into two groups as H. pylori positive (n=24) and H. pylori negative (n=29). Neopterin measurements were performed on HPLC device. Whether serum neopterin levels differed between groups and whether neopterin and H. pylori quantity, degree of inflammation and activity were correlated, were analyzed by appropriate statistical methods.

Results: Although the serum neopterin level was higher in the H. pylori positive chronic gastritis group, there was no statistically significant difference between the groups (*P=0.369*). No correlation was found between serum neopterin levels and H. pylori quantity, degree and activity of inflammation (*P>0.05*).

Conclusion: Serum neopterin levels are unchanged in patients with chronic gastritis with H. Pylori positive or negative compared to healthy controls. It was thought that neopterin levels could not be used as a marker of H. pylori positivity or chronic gastritis.

Keywords: Helicobacter pylori; Neopterin; Gastritis

Yazışma adresi: Yakup Dülgeroğlu

Kulu Devlet Hastanesi, Yeni Mahalle Ahmet Baran Caddesi Kozanlı Yolu Kulu / Konya

e-posta: dulgerogluyakup@gmail.com

Etik onay: KTO Karatay Üniversitesi Tıp Fakültesi, İlaç ve Tıbbi Cihaz Dışı Araştırmalar Etik Kurulu, 23.05.2022 tarihli ve 2022/039 sayılı kurul kararı

ÖZET

Amaç: Bu çalışma ile hücresel immünite ve oksidatif stres belirteci olarak kabul edilen neopterin serum seviyelerinin hem inflamasyona hem de oksidatif stres artışına neden olan Helicobacter pylori (H. pylori) varlığı ile ilişkisinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 53 kronik gastritli hasta ve 14 sağlıklı gönüllü dahil edilmiştir. Histopatolojik inceleme sonucuna göre kronik gastritli hastalar H. pylori pozitif (n=24) ve H. pylori negatif (n=29) olarak iki gruba ayrılmıştır. Neopterin ölçümleri HPLC cihazıyla gerçekleştirilmiştir. Serum neopterin seviyelerinin gruplar arasında farklı olup olmadığı ve neopterin ile H. pylori sayısı, inflamasyonun derecesi ve aktivitesinin ilişkili olup olmadığı uygun istatistiksel yöntemler ile analiz edilmiştir.

Bulgular: Serum neopterin düzeyi H. pylori pozitif olan kronik gastritli hasta grubunda daha yüksek olsa da gruplar arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (*P=0.369*). Serum neopterin seviyeleri ile H. pylori sayısı, inflamasyonun derecesi ve aktivitesi arasında bir korelasyon tespit edilmemiştir (*P>0.05*).

Sonuç: Serum neopterin seviyeleri H. Pylori pozitif ya da negatif olan kronik gastritli hastalarda sağlıklı kontrollere kıyasla değişmemektedir. Neopterin düzeylerinin H. pylori pozitifliğinin ya da kronik gastritin bir belirteci olarak kullanılamayacağı düşünülmüştür.

Anahtar kelimeler: Helicobacter pylori; Neopterin; Gastrit

INTRODUCTION

Helicobacter pylori (H. pylori) is a gram-negative, flagellated, microaerophilic bacterium and it is estimated that up to 50% of the world's population is infected with this bacterium. H. pylori can colonize the gastric mucosa and cause gastritis, peptic ulcer, gastric carcinoma and gastric lymphoma (1). The long-term presence of H. pylori in the gastric mucosa results in the activation of the immune system, as well as the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from various cells, primarily neutrophils. ROS production is mainly catalyzed by NADPH oxidase (NOx), while RNS production is primarily catalyzed by the inducible nitric oxide synthase (iNOS) enzyme. Overproduction of ROS and RNS causes oxidative damage in cells, including DNA damage, and ultimately paves the way for neoplastic transformation (2).

Neopterin is a byproduct of tetrahydrobiopterin (BH4) biosynthesis. During BH4 synthesis, guanosine triphosphate (GTP) is first converted to 7,8-dihydroneopterine triphosphate in a reaction catalyzed by the GTP cyclohydrolase 1 enzyme. Then, 6-pruvoyltetrahydrobiopterin is formed by the enzyme 6-pruvoyltetrahydrobiopterin synthase. In the last step, BH4 is formed from 6-pruvoyltetrahydrobiopterin by the enzyme sepiapterin reductase. During this synthesis pathway, the triphosphate part of 7,8-dihydroneopterin triphosphate is cut by phosphatases to form 7,8-dihydroneopterin, and then neopterin is formed by oxidation reactions (3).

The activity of GTP cyclohydrolase 1, the rate-limiting enzyme in neopterin synthesis, is mainly increased by interferon-γ. To a lesser extent, interferon-α, some cytokines and endotoxins increase the activity of this enzyme. Monocyte/macrophages activated by interferon-γ secreted mainly by Th1 lymphocytes are the source of neopterin production in the body. Therefore, neopterin is accepted as an indicator of cellular immune response. Since 7,8-dihydroneopterin must undergo oxidation for its synthesis, neopterin is also considered as an indirect indicator of oxidative stress (4).

In this study, it was aimed to examine the relationship between the presence of H. pylori, which is known to cause oxidation as well as inflammation, and serum neopterin concentrations, a marker of inflammation and oxidative stress, in patients with chronic gastritis.

MATERIALS AND METHODS

Study Groups

Fifty-three patients with chronic gastritis and 14 healthy volunteers were included in the study. According to the histopathological examination results, patients with chronic gastritis were divided into two groups as H. pylori positive gastritis (n=24; 11 men, 13 women) and H. pylori negative gastritis (n=29; 10 men, 19 women). The control group (n=14; 4 men, 10 women) consisted of apparently healthy adults without symptoms of gastritis and with inflammatory markers (CRP, ESR, WBC) within reference limits.

Those who were previously diagnosed with diabetes mellitus, hypertension, peptic ulcer, all kinds of cancer, autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, etc.) were not included in the study. Also, those who had taken a proton pump inhibitor or H2 receptor blocker in the last week were also excluded from the study.

Sample Collection

The patient samples included in the study consisted of serum samples previously collected within the scope of a different study from patients who applied to Kuru State Hospital with complaints of chronic gastritis and underwent upper gastrointestinal endoscopy as a routine diagnostic procedure. During the collection of serums, blood samples were taken after 8-10 hours of fasting and before endoscopy. Blood samples were centrifuged at 1500xg for 10 minutes within 2 hours at the latest and the sera were separated into Eppendorf tubes. These serum samples were stored at -30 °C for approximately 18 months, protected from light and without thawing.

Biopsy samples were evaluated by pathologists in a pathology laboratory for variables such as H. pylori positivity, intensity of inflammation and activation level. These data were used retrospectively in this study.

Informed consent form was signed by the patients during the sample collection phase. This study was approved by KTO Karatay University, Faculty of Medicine, Research Ethics Committee other than Pharmaceutical and Medical Device (No: 2022/039).

Laboratory Analyses of Serum Neopterin Levels

Serum neopterin measurements were carried out by high performance liquid chromatography (HPLC) method. For this purpose, samples were combined with equal amounts of acetonitrile in order to precipitate proteins in the serum. These samples were then mixed with vortex and centrifuged at 10,000 x g for 10 minutes. The supernatant obtained after centrifugation was used for analysis. During the measurement, acetonitrile/water (1/99, (v/v) was used as the mobile phase and the flow rate was adjusted as 1 mL/min. Neopterin measurements were made in a HPLC device with a fluorescence detector (excitation: 353 nm and emission: 438 nm) using a C18 reverse-phase column with a size of 150 x 4.6 mm and a particle size of 5 mm.

Neopterin standard was obtained from Sigma-Aldrich (N3386, Sigma Chemical Company, USA). Intra-assay and inter-assay coefficient of variation were found to be <3.1, <6.3% for neopterin, respectively. Limit of quantitation (LoQ) values were determined to be 0.72 nmol/L, for neopterin. In the recovery studies performed at 2 different levels (7.5 and 20 nmol/L), the recovery value was found to be between 88-102%.

Statistical Analyses

Research data were evaluated through the PASW program (Predictive Analytics Software, Version 18.0. Chicago: SPSS Inc.). Mean, median, standard deviation etc. descriptive statistical data were calculated. Interquartile range (IQR) and box-plot charts were used to detect the outliers. Whether the neopterin values conformed to the normal distribution was evaluated with histogram graphs and

Shapiro Wilks test. It was observed that the data did not fit the normal distribution. Kruskal Wallis analysis of variance was used to determine whether the parameters were different among the groups. Correlation analysis was performed using Spearman Correlation Analysis method, since there were categorical variables among the parameters and the neopterin values were not normally distributed. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

A total of 67 people, 25 men and 42 women, were included in the study. The mean age and SD values of H. pylori negative gastritis, H. pylori positive gastritis and healthy control groups were calculated as 46.14 years (±13.52), 47.54 years (±13.22) and 31.14 years (±6.53), respectively. There was no statistically significant difference between H. pylori negative gastritis and H. pylori positive gastritis groups in terms of age variable. Compared to these two groups, the healthy control group consists of younger individuals.

Median (minimum-maximum) neopterin serum levels in H. pylori negative gastritis, H. pylori positive gastritis and control groups were determined as 4.80 nmol/L (2.80-10.80), 6.75 nmol/L (1.30-12.70) and 5.40 nmol/L (1.70-9.10), respectively. Although the median neopterin level was higher in the H. pylori positive gastritis group, it wasn't statistically significant, and no statistical difference was found between the groups ($P=0.369$).

The generally accepted cut-off value for neopterin serum levels is 10 nmol/L (4). Accordingly, 1 patient in the H. pylori negative gastritis group and 4 patients in the H. pylori positive gastritis group were above this value, while none in the control group was above this value. *Figure 1* shows the median neopterin levels in the study groups.

No statistically significant correlation was found between neopterin levels and age, H. Pylori quantity, degree of inflammation and activity ($P>0.05$).

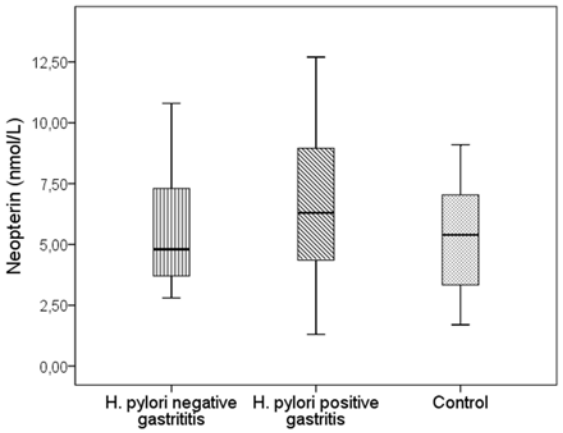

Figure 1. Neopterin serum levels in the study groups

DISCUSSION

Although H. pylori positivity often progresses with chronic inflammation without showing any symptoms, it may cause peptic ulcer in 10%, gastric adenocarcinoma in 1-3% and mucosa-associated lymphoid tissue (MALT) lymphoma in 0.1% as a result of long-term inflammation (5). Therefore, detection and eradication of H. pylori is critical. Today, non-invasive methods such as urea breath test and serological methods are used in the diagnosis of H. pylori in addition to invasive methods such as histological examination, PCR, and culture which performed using biopsy material (6). Although the urea breath test, which is used especially in the control of H. pylori eradication, is a sensitive and specific test, it is not applied in small and medium-sized health centers. Therefore, if there is a biomarker that can be measured minimally invasively in serum will be of great benefit in detecting H. pylori presence and in the follow-up of its treatment.

In our study, it was aimed to reveal the association between serum neopterin levels and the presence of H. pylori in patients with chronic gastritis. The fact that neopterin synthesis in the body is associated with both inflammatory processes and oxidative stress suggests that it may be an indicator of chronic inflammation and oxidative stress induced by H. pylori. However, in our study, no association was found between the

presence of H. pylori and serum neopterin levels. Moreover, no correlation was found between serum neopterin levels and H. pylori quantity, degree of inflammation and activity.

There are few studies in the literature examining the association between H. pylori and serum neopterin levels. In a study by Ledochowski et al., it was reported that neopterin levels (6.38 nM) were found to be higher in patients' sera with H. pylori antibody positive than those with H. pylori antibodies negative (5.74 nM) (*P=0.027*). However, no difference was found in terms of neopterin levels between individuals with ^{13}C-Urea breath test positive for H. pylori and those with negative H. pylori (7). In a study by Fahim et al. reported that 12–18-month-old children, fecal neopterin levels were found to be high in 99% of children with environmental enteric dysfunction that includes intestinal inflammation and immune activation. Fecal neopterin levels were decreased in 99% of the children included in the study with the nutritional supplement. In the same study, no statistical difference was found in fecal neopterin levels in children with H. pylori positive compared to those with negative H. pylori (8). In a study by Kutluana et al., it was reported that serum neopterin levels were found to be higher in patients with both gastric atrophy and gastric intestinal metaplasia compared to the control group consisting of patients with chronic gastritis and 79% H. pylori positivity (9). Along with the results of all these studies, the result of our study also revealed that neopterin levels do not change with the presence of H. pylori.

The most important limitation of our study is the relatively small number of participants. Another limitation is that we could not follow the patients to see if their neopterin levels changed after the treatment.

In conclusion, serum neopterin levels do not change in patients with chronic gastritis with H. pylori positive or negative compared to healthy controls. When the results of this study and other studies in the literature were evaluated together, it was thought that neopterin levels could not be used as a marker of H. pylori positivity or chronic gastritis.

CONFLICT OF INTERESTS None

REFERENCES

1. Hassan TM, Al-Najjar SI, Al-Zahrani IH, Alanazi FI, and Alotibi MG. Helicobacter pylori chronic gastritis updated Sydney grading in relation to endoscopic findings and H. pylori IgG antibody: diagnostic methods. J Microsc Ultrastruct 2016; 4(4): 167-74.
2. Jain U, Saxena K, and Chauhan N. Helicobacter pylori induced reactive oxygen Species: A new and developing platform for detection. Helicobacter 2021; 26(3): e12796.
3. Ghisoni K, de Paula Martins R, Barbeito L and Latini A. Neopterin as a potential cytoprotective brain molecule. J Psychiatr Res 2015; 71: 134-9.
4. Berdowska A and Zwirska- Korczala K. Neopterin measurement in clinical diagnosis. J Clin Pharm Ther 2001; 26(5): 319-29.
5. Wang F, Meng W, Wang B, and Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. Cancer Lett 2014; 345(2): 196-202.
6. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, and Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol 2014; 20(6): 1438-19.
7. Ledochowski M, Murr C, Lass-Flörl C, and Fuchs D. Helicobacter pylori infection and neopterin. Pteridines 2001; 12(3): 126-9.
8. Fahim SM, Das S, Gazi MA, Alam MA, Hasan MM, Hossain MS, et al. Helicobacter pylori infection is associated with fecal biomarkers of environmental enteric dysfunction but not with the nutritional status of children living in Bangladesh. PLoS Negl Trop Dis 2020; 14(4): e0008243.
9. Kutluana U, Kilciler AG, Mizrak S and Dilli U. Can neopterin be a useful immune biomarker for differentiating gastric intestinal metaplasia and gastric atrophy from non-atrophic non-metaplastic chronic gastritis?. Gastroenterol Hepatol 2019; 42(5): 289-95.