ORIGINAL ARTICLE

Neopterin as an indicator of inflammation in chronic viral hepatitis, liver cirrhosis, and hepatocellular carcinoma

Kronik viral hepatitler, karaciğer sirozu ve hepatosellüler karsinomada inflamasyon göstergesi olarak neopterin

Muhammed Fatih AYDIN[1], Şule NAMLI KOÇ[2], Sabiye AKBULUT[3], Dilek OĞUZ[4]

Department of [1]Gastroenterology, Istanbul Altınbaş University, Medical Park Bahçelievler Hospital, Istanbul, Turkey

Department of [2]Gastroenterology, Istanbul Memorial Ataşehir Hospital, Istanbul, Turkey

Department of [3]Gastroenterology, University of Health Sciences, Kartal Koşuyolu High Specialty Training and Research Hospital, Istanbul, Turkey

Department of [4]Gastroenterology, Kırıkkale University School of Medicine, Kırıkkale, Turkey

Background and Aims: *Neopterin is a proinflammatory indicator that plays a role in cell-mediated immunity, and elevated concentrations of neopterin indicate the presence of interferon-γ in body fluids. In this study, neopterin concentrations were determined in patients with a virus-induced chronic liver disease (chronic hepatitis, liver cirrhosis, and hepatocellular cancer), inactive hepatitis B virus carriers, and in a healthy control group to assess whether neopterin can be used as a disease marker in patients with virus-induced chronic liver disease.* ***Materials and Methods:*** *A total of 110 subjects (42 females and 68 males, with an average age of 44±8.90 years) were included in this study. Of these patients, 33 had chronic hepatitis; 22, liver cirrhosis; 22, hepatoma; 18, inactive hepatitis B virus carriers; and 15 were included in the healthy control group. Neopterin levels were measured before and after interferon treatment in patients with chronic hepatitis. Data collected among the groups were analyzed statistically using the Mann-Whitney test, considering $p < 0.05$ as statistically significant.* ***Results:*** *Neopterin concentrations and gender showed no statistically significant correlation. Patients with cirrhosis, hepatocellular cancer, and chronic hepatitis had statistically significantly higher neopterin levels than the healthy control and healthy carrier groups. A positive correlation was observed between neopterin levels and other disease activity indicators such as aspartate transaminase, alanine transaminase, hepatitis B virus deoxyribonucleic acid, hepatitis C virus ribonucleic acid, erythrocyte sedimentation rate, and C-reactive protein levels, and their levels were also high. Although 14 patients, who were also evaluated after interferon treatment, showed good response to the treatment, no statistically significant correlation was observed between their neopterin levels and disease activity indicator levels.* ***Conclusion:*** *Neopterin concentrations were found to be significantly higher in patients with inflammation than in inactive hepatitis B virus carriers who had no ongoing inflammatory activity and in the healthy control group. No correlation was detected between neopterin levels and hepatitis B virus deoxyribonucleic acid and hepatitis C virus ribonucleic acid levels in patients who received interferon treatment and benefitted from it. Although neopterin levels indicate inflammatory activity in cases of chronic hepatitis, liver cirrhosis, and hepatocellular cancer, additional studies are necessary to determine its usefulness in clinical practice.*

Key words: *Neopterin, chronic hepatitis, liver cirrhosis, hepatocellular cancer*

Giriş ve Amaç: *Neopterin hücre aracılı immünitede rol oynayan proinflamatuvar bir belirleyicidir ve konsantrasyonu vücut sıvılarındaki γ interferonun varlığını gösterir. Viral etiyolojili karaciğer hastalığı olan kronik hepatit, karaciğer sirozu, hepatosellüler kanserli olgularda, inaktif hepatit B virüsü taşıyıcısı olanlarda neopterin seviyesini saptamak ve kontrol grubu ile karşılaştırarak; viral etiyolojili kronik karaciğer hastalıklarında yeri olabilecek bir belirteç olabileceğini ispatlamak amacıyla bu çalışma planlandı.* ***Gereç ve Yöntem:*** *Çalışmaya toplam 110 kişi (42 kadın, 68 erkek, yaş ortalaması 44±8.90) alındı. Bunlardan 33 hasta kronik hepatit, 22'si karaciğer sirozu, 22'si hepatoma, 18'i inaktif hepatit B taşıyıcısı ve 15'i sağlıklı kontrol grubu olarak belirlendi. Neopterin düzeyleri kronik hepatitli hastalarda interferon tedavisinden önce ve sonra ölçüldü. Gruplar arasındaki ölçümler istatistiksel olarak Mann-Whitney testiyle araştırıldı. $p<0,05$ istatistiksel olarak anlamlı kabul edildi.* ***Bulgular:*** *Cinsiyet ve neopterin düzeyleri arasında istatistiksel olarak ilişki saptanmadı. Hastalıklar değerlendirildiğinde, neopterin seviyeleri siroz, hepatosellüler kanser, kronik hepatit grubundaki hastalarda sağlıklı taşıyıcı ve sağlıklı kontrol grubundaki hastalara göre istatistiksel olarak anlamlı düzeyde yüksekti. Neopterin düzeyleri, bu hasta gruplarında aktivite göstergeleri olan aspartat aminotransferaz, alanin aminotransferaz, hepatit B virüs deoksiribonükleik asit, hepatit C virüs ribonükleik asit, sedimentasyon ve C-reaktif protein değerleri ile arasında pozitif korelasyon vardı ve yüksek olarak saptandı. İnterferon tedavisi sonrası değerlendirilen 14 hastada ise tedaviye cevap alınmasına rağmen, neopterin düzeyleri ile aktivite belirteçleri arasında anlamlı ilişki saptanmadı.* ***Sonuç:*** *Sonuç olarak neopterin seviyeleri inflamasyonu olan hastalarda sağlıklı kontrol ve inflamasyon aktivitesi beklenmeyen hepatit B taşıyıcılarına göre anlamlı olarak daha yüksekti. İnterferon tedavisi verilen ve tedaviden fayda gören hastalarda hepatit B virüs deoksiribonükleik asit ve hepatit C virüs ribonükleik asit düzeyleri ile neopterin arasında bir korelasyon saptanmadı. Neopterin seviyesi kronik hepatit, karaciğer sirozu ve hepatosellüler kanserli olgularda inflamasyon aktivitesini gösterirken bunun klinik pratikteki yararı konusunda ilave çalışmalara gerek olduğu açıktır.*

Anahtar kelimeler: *Neopterin, kronik hepatitler, karaciğer sirozu, hepatosellüler kanser*

Correspondence: Sabiye AKBULUT
Department of Gastroenterology, University of Health Sciences, Kartal Koşuyolu High Specialty Training and Research Hospital, Istanbul, Turkey
Fax: +90 216 459 63 21 • E-mail: sabiye4@hotmail.com

Manuscript: 28.07.2018 • **Accepted:** 30.10.2018

DOI: 10.17941/agd.502317

INTRODUCTION

Liver homeostasis depends on regulatory factors produced by the hepatocytes and the balance between non-parenchymal liver cells and cells of other organs. The microenvironment within the sinusoid is the where hepatocytes, sinusoidal endothelial cells, Kupffer cells, lymphocytes, and stellate cells interact with each other. The main roles of these cells are to carry out immunologic homeostasis, develop tolerance, select immune cells, activate local immunologic responses, and to maintain immunity. A great number of signal molecules, cytokines and metabolites are released from these cells. Neopterin is one of them (1).

Neopterin is a derived pteridin and is found as united with pyrazino- (2,3-d-) pyrimidine in the living cells (1,2). Pteridin was isolated as biological material in 1889 for the first time. Then, other pteridins were identified. They are categorized as aromatic pteridins, 7,8, dihidropteridins, 5,6,7,8, tetrahidropteridins, and unidentified pteridins. Neopterin takes place in the group of aromatic pteridins in this categorization (3). Neopterin is a reagent which is produced with the activity of monocytes/macrophages and is spread to body fluids (4). There is a relationship between the quantity of neopterin released and the hydrogen peroxide oscillation capacity of these cells because the presence of neopterin is a sign for oxidative stress by the immunologic system (5). Neopterin is metabolized in the liver, excreted by the kidneys, and its clearance is more than inulin. Neopterin is most likely excreted by glomerular filtration and tubular secretion (6).

Neopterin is determined in a variety of body fluids like serum, cerebrospinal fluid, synovial fluid, pancreatic fluid, urine, and saliva. The upper limit of serum neopterin is 10 nmol/l. Neopterin serum level is age dependent. It is high in children and elderly; however, there is no relation between neopterin serum level and gender. Neopterin levels can be found abnormally high in a variety of clinical situations such as infections, allograft rejection, autoimmune diseases, malignancies, cardiac insufficiency, renal insufficiency and myocardial infarctions (7, 8). Increased neopterin concentration is clinically important for the diagnosis and prognosis on cell-mediated immunity cases (9). Neopterin levels increase at different rates according to the inflammatory activity in viral liver infections, acute or chronic liver diseases, and liver cirrhosis (10). These changes at molecular levels translate into high levels of neopterin in blood and urine and therefore can be useful as inflammatory indicators (11).

In this study, we aimed to determine the neopterin levels in cases of chronic hepatitis of viral etiology, liver cirrhosis, hepatocellular cancer, and inactive HBV carriers before and after the interferon treatment. We also obtained neopterin levels in a control group for comparison and see if neopterin can be used as a marker in chronic liver diseases.

MATERIALS and METHODS

The research protocol was reviewed and approved by the ethical committee of the Türkiye High Specialty Hospital. Patients were informed about the study and informed consents obtained. In this study, we included patients that consulted for treatment and follow-up on an outpatient basis and hospitalized patients in the Türkiye High Specialty Hospital Gastroenterology Clinic. We recruited 110 patients (42 female, 68 male). Within these 110 patients, 22 patients had hepatomas, 33 patients had chronic hepatitis, 18 patients were inactive HBV carriers, 22 patients had liver cirrhosis, and 15 patients were included as the healthy control group.

In patients with hepatoma, 21 out of 22 patients were due to HBV. In chronic hepatitis cases, 21 out of 33 patients were due to HCV, while 12 of them had HBV infection. In liver cirrhosis cases, 12 out of 22 patients had cirrhosis due to hepatitis B.

Neopterin levels were measured after interferon treatment in patients with chronic hepatitis. Control serum samples were taken from 11 out of 21 patients having chronic hepatitis C and 3 patients having chronic hepatitis B at 6 months of treatment.

Neopterin Measurement Procedure: Test Principles

We used the competitive ELISA method which is based on the principle of competition between a known amount of enzyme-linked antigen and an unknown amount of antigen. Antigen-antibody complexes are bound to microwells covered with the goat-anti-rabbit antibody. The unbound antigen is removed from the environment during the irrigation. The color intensity after substrate incubation is inversely correlated with the quantity of the antigen in serum. The amount of neopterin in the samples is determined by using a standard curve. There are 6 standard curve in the test: 0, 1.35, 4.0, 12.0, 37.0, and 111 nmol/l. We also included 2 control serums. Direct light was removed from the environment during the test because neopterin can react with light. The values above 10 nmol/l were considered positive (Neoptrein Biochemistry- Methods–clinical Applications; H. Wacher et all 1992, Berlin-Newyork).

Statistical Analysis

The analysis of the data was done using the SPSS 11.5 package program. Continuous measurement variables were shown as mean, ± standard deviation, or median while categorical variables were shown as the number of observation and percentages. The Mann Whitney test was used to assess the values obtained in the measurement between groups. For categorical comparisons, Ki-Kare, Fisher's Exact, and McNemar tests were used. Results were accepted as statistically significant when the p-value is <0.05.

RESULTS

The study was completed with 110 patients, 14 of these patients were called back to repeat neopterin measurements after 6 months of treatment. The average ages and genders of patients and the number of patients with high neopterin levels in the different groups are shown in the Table1.

When the relation between gender and neopterin level was evaluated, 42 out of 68 male patients (61.8%) had high neopterin level while 23 out of 42 female patients (54.8%) had high neopterin level. There was no statistically significant difference between the two groups. Gender had no influence on neopterin level (p >0.05). When the relation between diseases and neopterin levels were evaluated, there was a statistically significant difference in terms of neopterin level among the carrier, healthy control, and other diseases groups (p <0.001). When the carriers and healthy control group were compared, a statistically significant difference was not detected between these two groups (p >0.05).

When the relation between α-Feto Protein (αFP) and neopterin level was analyzed, 20 out of 21 patients with high αFP (95.2%) also had high neopterin level. Then 43 out of 86 patients had a normal αFP level (50%) and high neopterin level, the difference between them was statistically significant (p <0.001).

When the relation between hepatitis B virus (HBV) deoxyribonucleic acid (DNA) and neopterin level was analyzed, 40 out of 49 patients with positive DNA level (81.6%) had high neopterin level. Then 2 out of 13 patients with negative DNA level (15.4%) had high neopterin level. The difference between them was statistically significant (p <0.001).

When the relation between hepatitis C virus (HCV) ribonucleic acid (RNA) and neopterin level was analyzed, 19 out of 30 patients with positive RNA (63.3%) had high neopterin level, 1 patient with negative RNA did not have high neopterin level. Statistical analysis could not be completed because the number of patients having negative RNA was low. In contrast, the patient group with positive RNA had high neopterin level.

When the relation between sedimentation and neopterin level was analyzed, 12 out of 13 patients with high sedimentation level (92.3%) had high neopterin level. Then 53 out of 96 patients with normal sedimentation level (55.2%) had high neopterin level, and the difference between them was statistically significant (p <0.005). Nine out of 9 patients with high CRP level had high neopterin level. Fifty-six out of 100 patients with normal CRP level (56%) had high neopterin level. The difference between them was statistically significant (p <0.005).

When the relation between aspartate aminotransferase (AST) level and neopterin level was analyzed, 43 out of 56 patients with high AST level (76.8%) had high neopterin level, 21 out of 42 patients with normal AST level (50%) had high neopterin level. The difference between them was statistically significant (p <0.001). When the

Table 1. The distribution of patients according to age, gender and high neopterin level

Disease	Total (n)	Average Age (year)	Female (n)	Male (n)	The number of patients with high neopterin level n (%)
Hepatocellular Cancer	22	54±8.40	4	18	20 (90.9)
Chronic Hepatitis C	21	45±7.96	10	11	9 (42.9)
Chronic Hepatitis B	12	38±6.89	4	8	8 (66.7)
Liver Cirrhosis	22	51±8.72	7	15	21 (95.5)
HBV Carrier	18	35±7.80	7	11	3 (16.7)
Healthy Control	15	32±6.71	10	5	4 (26.7)
Total	110	44±8.90	42	68	65 (59.9)

relation between alanine aminotransferase (ALT) level and neopterin level was analyzed, 36 out of 52 patients with high ALT level (69.2%) had high neopterin level, 28 out of 56 patients with normal ALT level (50%) had high neopterin level. The difference between them was statistically significant ($p < 0.003$).

When hepatitis B e antigen (HBeAg) was considered, in 49 out of 56 (91.4%) cases HBeAg was antiHBe (+), 3 out of 7 patients with HBeAg (+) (42.9%) had high neopterin level, and 32 out of 49 patients with HBeAg (-) (65.3%) had high neopterin level. There was no statistically significant difference between them ($p > 0.05$). When the relation between lesion size and neopterin level in cases with hepatocellular cancer was analyzed, in 20 out of 22 cases with cancer, neopterin level was found to be high. The relation between lesion size and neopterin level could not be evaluated statistically. In these patients, the lesion size was found to be 5 cm (2 to 11 cm) on average.

When 14 chronic hepatitis patients that received interferon treatment were called for follow-up, only one of the 14 patients had high sedimentation rates previously, the other patient's sedimentation rate was found normal. When the patients' C-reactive protein (CRP) values were analyzed, CRP values were normal before and after treatment, there was no statistical difference. In 11 out of 12 patients having high AST level (12/14), AST level became normal after the treatment. There was no change in the other 2 patients' AST values, these values were statistically significant. When ALT levels were analyzed, there was no change in 1 patient; in 11 out of 13 patients having high ALT level, ALT level became normal and in 2 patients with normal ALT levels at the beginning, ALT levels were found to be higher afterward. This was statistically significant.

In 14 patients controlled after the treatment, 3 cases with chronic hepatitis B were determined to have HBV DNA (+) in the beginning and to have HBV DNA (-) after the treatment. In 2 of these patients, the initial neopterin level was (+) while in the other one it was (-) before the treatment; after the treatment, there was no change in neopterin levels. In 2 patients, HBeAg was (+), in the other one it was (-). In 3 patients, AST and ALT levels were high before the treatment. After the treatment, they returned to normal. While in 11 patients with chronic hepatitis C, HCV RNA was (+) before the treatment, in 9 patients HCV RNA was (-), and in 2 patients, HCV RNA was (+) after the treatment. In the cases whose HCV RNA stayed (+), in 1 patient neopterin level was (-) before the treatment and after the treatment it was (+). In the other patient, neopterin level was (+), then it became (-) after the treatment. Before the treatment, in each patient, AST and ALT levels were high; after the treatment, they remained high.

The following patients did not benefit from the interferon treatment. In 2 out of 9 patients, neopterin was (+), and after the treatment became (-). In 3 cases it was (-) and stayed (-). In 3 cases, neopterin was (-), and after interferon treatment became (+). In one case, neopterin stayed (+). In 9 patients whose HCV RNA became (-) after the treatment, AST and ALT levels were high but they returned to normal after the treatment. In 4 out of 8 patients whose neopterin level was (-) before the treatment, it stayed (-) after the treatment. In the other 4 patients, neopterin level became (+). One of these cases was the patient with chronic hepatitis C who did not benefit from the treatment and whose HCV RNA continued (+). Although other patients benefited from the treatment, and their HCV RNA became negative, their neopterin level became (+). In 6 cases whose neopterin level was (+) before the treatment, neopterin level of 3 cases became (-) and the other 3 cases continued (+) after interferon treatment.

One of the 3 patients whose neopterin level became (-) was the one that did not benefit from the interferon treatment and still had high ALT and HCV RNA levels after the treatment. Other 2 cases that benefited from the treatment were the cases whose HCV RNA and ALT levels became normal following the treatment. When patients' HCV RNA and HBV DNA were analyzed, it was detected that HCV RNA and HBV DNA became (-) after the treatment in 12 out of 14 cases. In the other two cases, HCV RNA levels stayed (+), and these cases were the ones that did not benefit from the treatment.

DISCUSSION

In previous studies, it was shown that neopterin levels in children and elderly people were higher than the general population. In our study, these high levels of neopterin did not have a significant effect on the results within different patient groups. We also found that gender had not an effect on neopterin level similar to other studies that also did not found that gender had an effect on neopterin level (8).

When the relation between neopterin and diseases were analyzed, the patient groups (hepatocellular cancer, chronic hepatitis B and C, and liver cirrhosis) were expected to have high inflammatory activity and higher neopterin levels than the healthy control group. In hepatitis B carriers and the healthy control group the inflammatory

activities were expected to be normal, there was no significant difference in the neopterin levels. With these findings, we can affirm that measuring neopterin levels is significant for showing inflammation in the body.

In patients whose sedimentation rates and CRP values were high, their neopterin levels were also high. This enables us to use neopterin as an inflammation marker. In the control group, there was no significant change in sedimentation rates and CRP values after the treatment, so their relationship with neopterin could not be analyzed.

When the relation between the patients levels of αFP and their neopterin levels were assessed, we found that patients with significantly high αFP levels had high neopterin levels. In these patients, neopterin measurement can be valuable because it can be used as a marker of hepatocellular carcinoma. In most of the hepatocellular cancer cases, neopterin levels were high, but there was no relation between the lesion size and the neopterin level. However, the correlation was significant when displaying the inflammation in the body (12,13).

When the patients' HBeAg statuses were determined, 91.4% of the cases had anti-HBe positivity. Neopterin positivity was discovered in 65.3% of these patients, neopterin was high in 3 out of 7 cases with HBeAg(+) (42.9%). The difference between them was not statistically significant. We believe this could be due to the fact that the majority of the patients had anti-HBe positivity.

A significant relation was detected between neopterin levels and HBV DNA or HCV RNA levels which indicate viral activity. Especially in patients with high HBV DNA, high neopterin levels were detected. Similarly, a significant relation was detected between neopterin levels and AST or ALT values which indicate liver inflammation. With these findings, we can conclude that neopterin could be used as a marker of inflammation in viral hepatitis. These findings were similar with previous studies that showed a positive correlation among viral replication, cellular immunity, and neopterin levels (14-16).

When the patients' parameters were assessed after interferon treatment, in most HBV DNA and HCV RNA levels decreased (12/14 cases). However, in only 3 patients a decreased neopterin level was observed (3/14 cases). One of these three cases was the patient whose HCV RNA did not improve, ALT levels continued to be as high and the treatment was not effective. In these cases, AST and ALT levels had a positive correlation with HBV DNA and HCV RNA levels. In patients that benefited from the interferon treatment, there was a decrease in AST and ALT levels while there was no decrease in AST and ALT levels in patients that did not benefit from the treatment. However, neopterin levels were not in correlation with HBV DNA and HCV RNA levels following treatment. There was an increase or there was no change in neopterin levels in patients who benefited from the treatment. The number of patients whose neopterin levels were decreased after treatment was 2 out of 14. As a result, while the patients with some kind of inflammatory activity in their liver had significantly higher neopterin levels compared to healthy controls and hepatitis B carriers who were not expected to have any inflammatory activity, there was no correlation with HBV DNA and HCV RNA levels in patients that had interferon treatment and benefited from this treatment.

More significant results can be obtained if the number of control patients is higher and if neopterin levels could also be measured in urine samples. Neopterin levels show the presence of inflammatory activity in cases with chronic hepatitis, liver cirrhosis and hepatocellular cancer, however, it is also clear that additional studies are needed to show the benefits of this information in clinical practice.

REFERENCES

1. Reibnegger G, Auhuber I, Fuchs D, et al. Urinary neopterin levels in acute viral hepatitis. Hepatology 1988;8:771-4.
2. Eisenhut M. Neopterin in diagnosis and monitoring of infectious diseases. J Biomark 2013;2013:196432.
3. Berdowska A, Zwirska-Korczala K. Neopterin measurement in clinical diagnosis. J Clin Pharm Ther 2001;26:319-29.
4. Huber C, Batchelor JR, Fuchs D, et al. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. J Exp Med 1984;160:310-6.
5. Haavik J. [From butterflies to neurobiology and the diagnosis of AIDS. The 100th anniversary of the discovery of pteridines]. Tidsskr Nor Laegeforen 1989;109:1986-9. Norwegian.
6. Oettl K, Reibnegger G. Pteridines as inhibitors of xanthine oxidase: structural requirements. Biochim Biophys Acta 1999;1430:387-95.
7. Weiss G, Widner B, Zoller H, et al. Immune response and iron metabolism. Br J Anaesth 1998;81(Suppl 1):6-9.
8. Oda K, Arai T, Nagase M. Increased serum and urinary neopterin in nephrotic syndrome indicate cell-mediated immune dysfunction. Am J Kidney Dis 1999;34:611-7.
9. Müller MM, Curtius HC, Herold M, Huber CH. Neopterin in clinical practice. Clin Chim Acta 1991;201:1-16.
10. Schennach H, Schoenitzer D, Fuchs D. Association between chronic hepatitis C virus infection and increased neopterin concentrations in blood donations. Clin Chem 1998;44:2225-6.

11. Daito K, Suou T, Kawasaki H. Clinical significance of serum and urinary neopterin levels in patients with various liver diseases. Am J Gastroenterol 1992;87:471-6.
12. Kawasaki H, Watanabe H, Yamada S, et al. Prognostic significance of urinary neopterin levels in patients with hepatocellular carcinoma. Tohoku J Exp Med 1988;155:311-8.
13. Melichar B, Spisarová M, Bartousková M, et al. Neopterin as a biomarker of immune response in cancer patients. Ann Transl Med 2017;5:280.
14. Kaleli I, Demir M, Cevahir N, et al. Serum neopterin levels in patients with replicative and nonreplicative HBV carriers. BMC Infect Dis 2006;6:157.
15. Demirturk N, Demirdal T, Aktepe OC, et al. Serum neopterin levels in patients with HBV infection at various stages. Hepatogastroenterology 2007;54:903-5.
16. Gulcan EM, Tirit I, Anil A, et al. Serum neopterin levels in children with hepatitis-B-related chronic liver disease and its relationship to disease severity. World J Gastroenterol 2008;14:6840-3.