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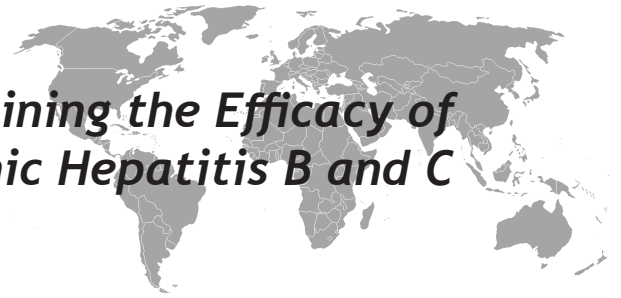

Role of Neopterin in Determining the Efficacy of Interferon Therapy in Chronic Hepatitis B and C

Serap Ozcimen[1], Mehmet Bitirgen[2], Bahar Kandemir[2]

ABSTRACT

Neopterin (NP) is a pteridine derivative that is secreted as a response to gamma interferon stimulation. The purpose in this study was to investigate the relationship between the NP levels and the disease and determining the efficacy of an interferon (IFN) therapy in patients with chronic hepatitis B (CHB) and C (CHC). The study was conducted on 49 cases with CHB, 30 cases with CHC and 72 healthy individuals. Serum samples were taken from the patients receiving treatment at the beginning and at the end of the treatment and only once from the healthy individuals in the control group. The NP levels were found significantly higher in the patients with CHB and CHC than those in the control group. When the pre and post-treatment serum NP levels of the patients who received an interferon therapy were compared, the post-treatment NP levels of the patients who responded to the treatment were significantly higher. When a comparison was made before and after treatment, a decrease was seen in the NP levels in most of the infections due to decreased activation of the immune system. However, when the disease was treated with an IFN therapy, which is a treatment stimulating the immune system, the post-treatment NP level remained high.

Key words: *Neopterin, chronic hepatitis B, chronic hepatitis C*

Kronik Hepatit B ve C'de interferon Tedavisinin Etkinliğini Belirlemede Neopterinin Rolü

ÖZET

Neopterin (NP), hücresel immun sistemin aktivasyonu sonucu, gama interferon stimülasyonuna cevap olarak insan makrofaj ve monositlerinden sekrete edilen bir pteridin derivesidir. Hücresel immünutenin göstergesi olan NP pek çok kanser, infeksiyon hastalığı ve otoimmün hastalıkta vücut sıvılarında çalışılmış ve hücresel immün sistemin aktif durumda olduğu hastalıkların neredeyse tümünde yüksek olarak tespit edilmiştir. Bu çalışmada, kronik hepatit B (KHB) ve kronik hepatit C'li (KHC) hastalarda; NP düzeyleri ile hastalık arasındaki ilişki ve interferon (IFN) tedavisinin etkinliğini belirlemede neopterin rolünün araştırılması amaçlandı. Çalışma, 49 KHB 'li olgu, 30 KHC'li olgu ve 72 sağlıklı birey üzerinde yapıldı. Serum örnekleri tedavi alan hastalardan tedavi başlangıcı ve bitiminde, kontrol grubundaki sağlıklı bireylerden ise bir kez alındı. Tedaviye yanıtsızlık virolojik ve/veya biyokimyasal cevap alınıp alınmamasına göre değerlendirildi. Kronik hepatit B ve KHC'li hastalarda NP düzeyleri kontrol grubundan anlamlı olarak yüksek saptandı. Pegile interferon tedavisi alan KHB'li ve KHC'li hastaların tedavi öncesi ve sonrası serum NP düzeyleri karşılaştırıldığında tedavi sonrası NP düzeyleri anlamlı olarak yüksek saptandı. Tedaviye yanıt veren??? KHB'li 16 hasta ve KHC'li 14 hastada tedavi sonrası NP düzeylerinde anlamlı yükseklik saptanırken, her iki hasta grubunda da tedaviye yanıtsız hastalarda tedavi öncesi ve sonrası serum NP düzeylerinde anlamlı değişiklik saptanmadı. İnfeksiyonların çoğunda infeksiyon tedavisi öncesi ve sonrasında bir kıyaslama yapıldığında tedavi sonunda immün sistemin aktivasyonu azaldığı için NP düzeylerinde azalma görülmektedir. Fakat immün sistemi uyarıcı bir tedavi olan IFN tedavisi ile hastalık tedavi edildiğinde, tedavi sonunda IFN tedavisine bağlı olarak NP düzeyi yüksek kalmaktadır.

Anahtar kelimeler: *Neopterin, kronik hepatit B, kronik hepatit C*

[1]Numune Hospital Infectious Diseases Clinic, Konya, [2]Necmettin Erbakan University, Meram Faculty of Medicine Infectious Diseases Clinic, Konya

Correspondence: *Serap Özçimen*
Konya Numune Hospital
Tel::+905054221980
E-mail:serapdr@yahoo.com

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INTRODUCTION

Neopterin (NP) is a pteridine derivative that is secreted by macrophages and monocytes through a primary IFN-gamma stimulation occurring as a result of the activation of the cellular immune system (1-4). The NP concentrations in body fluids also show interferon-γ presence. Therefore, NP is regarded as a sensitive indicator of cell-mediated immunity (1,3,5). The relationship of NP production with cellular immune activation has been evidenced in a large number of clinical and experimental studies carried out to date and a considerable increase has been found in NP levels in body fluids during bacterial and parasitic infections and particularly in viral infections, all of which trigger cellular immune response (3,6,7). A necrosis is seen in chronic hepatitis, which is composed of lymphocyte and plasma cells that are spread over parenchyma and perilobular areas. The IFN-gamma secreted from T lymphocytes activates macrophages and the NP released from these activated macrophages may be an indicator of inflammation in a chronic liver disease (8,9).

Interferons have various functions such as regulating cellular immunity and antibody synthesis, improving antigen expression and identification, and increasing NK cell activity (10,11). By increasing the Major Histocompatibility Complex (MHC) class I molecules on the surface of the hepatocyte cell, interferon enables identification of virus antigens on the surface of the infected hepatocyte by cytotoxic T cells and destruction of the infected hepatocyte (11,12). The objective in this study was to explore the relationship between the NP levels and the disease and the role of in determining the efficacy of interferon (IFN) therapy in patients with CHB and CHC.

MATERIALS AND METHODS

This study was conducted between October 2008 and December 2009 on 49 cases (28 males and 21 females) who were diagnosed with CHB and 30 cases (17 females and 13 males) who were diagnosed with CHC when they consulted the Microbiology and Infectious Diseases Clinic of Meram Faculty of Medicine and 72 healthy individuals (34 males and 38 females) who had HBsAg negative and Anti-HCV negative with normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values. Presence of physical examination and laboratory results that indicate an alcoholic liver disease, autoimmune hepatitis, HIV-hepatitis D co-infection, hepatocellular carcinoma, malignity, immunosuppressive condition and cirrhosis was considered as an exclusion criterion.

Written consents of the patients and approval of the ethics committee were obtained for the study. Patient blood samples were taken on the day of biopsy from the patients who were included in the study, at the beginning and end of the treatment from the patients who were administered an IFN therapy and at the time of enrollment from the healthy individuals in the control group. The blood samples were centrifuged at 3000 cycles for 5 minutes to obtain their serums. The serum samples were stored at -80oC until the study process. NP level measurements from the patients' serum samples were carried out on a Biotek brand ELISA device using a Brahms brand ELISA kit at the Research Laboratory of Biochemistry Department in Meram Faculty of Medicine. The normal value interval of the kit was 5.4±2.3 nmol/L. The ALT and AST measurements from the blood samples taken from these patients were carried out at the Central Laboratory of Meram Faculty of Medicine, viral markers were studied using the macroELISA method and the HBV DNA test using a Real Time PCR Detection System device at the Microbiology Laboratory.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 13.0 software. Chi-square test was used in analyzing pair groups, T Test in independent groups and Kruskal-Wallis variance analysis in stage-based comparisons, and then a Mann-Whitney U test with Bonferroni correction was conducted. Pearson's and Spearman's correlation analyses were used to assess correlations. $P<0.05$ was considered statistically significant.

RESULTS

A total of 151 cases, 79 being patients and 72 control group members, were assessed in this study. The demographic characteristics of the cases are presented in Table 1. The NP levels in patients with CHB and CHC

***Table 1**. Demographic Characteristics of Cases*

	CHB	CHC	Control
n	49	30	72
Gender (F/M)	21/28	17/13	38/34
Age, mean±SD	36.7±13.1	58.5±9.4	41.1±13.6

***Table 2**. A comparison of the neopterin levels of patients with chronic hepatitis B and chronic hepatitis C versus those of the control group*

	CHB	CHC	Control
Neopterin (nmol/L)	10.02±3.4	11.77±5.0	8.10±2.8

were found significantly higher than those in the control group (p=0.001) (Table 2). An IFN therapy was administered to 22 patients from 49 patients with chronic hepatitis B. When the mean pretreatment and post-treatment serum NP levels of the patients who received an interferon therapy were compared, their post-treatment NP levels were found higher. This was statistically significant (p=0.001) (Table 3). The patients were divided into two groups according to their response to the treatment. Six patients who received interferon therapy were considered non-responder to the treatment due to virologic and/or biochemical lack of response, whereas response was obtained from 16 patients after the treatment. The pretreatment and post-treatment serum NP levels of these patients were compared. The post-treatment serum NP levels were found higher than the pretreatment serum NP levels in the group from which response was obtained and this difference was statistically significant (p=0.001). No statistically significant difference was found between the pre- and post-treatment serum NP levels in the group that did not respond to the treatment (p>0.05) (Table 4). Interferon+ribavirin therapy was administered to 23 patients with chronic hepatitis C. The pretreatment and post-treatment mean serum NP levels of the patients who were administered treatment were compared. Their post-treatment NP levels were found to be higher. This difference was statistically significant (p=0.001) (Table 5). The patients were divided into two groups according to their response to the treatment. While post-treatment response was obtained in 14 patients who were administered treatment, 9 patients were assessed as non-responders due to virologic and/or biochemical lack of response. The pre- and post-treatment serum NP levels of the patients who responded and did not respond to treatment were compared. The post-treatment serum NP levels of the patient group who responded to the treatment were found to be significantly higher than their pretreatment serum NP levels (p=0.003). No significant difference was found between the pre- and post-treatment NP levels in the patient group who did not respond to the treatment (p>0.05) (Table 6).

DISCUSSION

In chronic hepatitis, the IFN-gamma secreted from T lymphocytes activates macrophages and NP is released (13, 14). In the study made by Kalkan and associates (15) on 89 patients and 40 healthy adults, the mean serum NP levels of the patients with CHB were found to be significantly higher than those of the healthy adults (p<0.001). Grüngreiff and associates (16) found in their study on 16 patients with CHC that the serum NP levels of the patients were higher than the serum NP levels of the control group. Demirturk and associates (17) divided the patients into three groups in their study on 90 patients and 30 healthy adults. They included non-replicative HBV carriers in the first group, patients with HBsAg (-), Anti-HBcIgG (+) in the second group and patients with CHB in the third group. The serum NP levels in all these three groups were found significantly higher than those in the healthy control group. In our study, the serum NP levels of the patients with CHB and CHC were also higher than the serum NP levels of the control group. This difference was statistically significant (p=0.001, p=0.001). The high NP level in patients with chronic hepatitis B and chronic hepatitis C was linked to the stimulation of cellular immune system.

The NP levels seem to increase in patients undergoing an immune-stimulator treatment. This may be caused by induction of the immune-regulatory cascade that stimulates IFN-γ secretion (3). With interferon alpha therapy, both monocyte-macrophage activation and NP levels increase. Interferon alpha is a pleiotropic cytokine. It produces a direct antiviral, immune-modulator and pro-anti-inflammatory action. The increase in neopterin during an IFN-alpha based treatment is due to the pro-inflamma-

***Table 3**. Comparison of pretreatment and post-treatment mean neopterin levels of the patients with CHB who received Interferon therapy*

Treatment Given	Pretreatment mean NP levels (nmol/L)	Post-treatment mean NP levels (nmol/L)	p value
Interferon (CHB)	10.47±3.46	15.86±5.83	0.001

***Table 4**. Comparison of pre- and post-treatment serum NP levels of the patients groups with CHB who responded and did not respond to the treatment*

Treatment Given (IFN) (n=22)	Pretreatment mean NP levels (nmol/L)	Post-treatment mean NP levels (nmol/L)	P value
Responders (n=16)	10.67±3.87	16.87±5.33	0.001
Nonresponders (n=6)	9.92±2.21	13.15±6.73	>0.05

tory action of IFN-alpha (18). In the study conducted by Gelderblom et al. (18) with the inclusion of 54 patients with CHC, the patients were administered placebo or telaprevir either together with peg-IFN or as a single agent. While the NP levels increased in the group who received telaprevir+IFN or IFN+placebo, the ALT levels decreased (both were statistically significant). This shows that IFN reduces hepatocyte damage, but increases monocyte/macrophage activity. The decreased levels of NP and ALT in the group who received only telaprevir shows that telaprevir reduced both monocyte/macrophage activity and hepatocyte damage.

In the study made by Grüngreiff and associates (16) on 16 patients with CHC, the patients were administered an IFN-alpha therapy for an average of 7 months. The patients were divided into 3 groups according to their response to the treatment as responders, partial responders and non-responders. The patients' NP values were measured before, during and after the IFN alpha therapy and after a 6-month follow-up. The pretreatment NP levels of all the patient groups were found higher than those of the healthy controls. It was shown that the NP levels of the entire patient groups had increased in the course of the IFN alpha therapy and at the end of the therapy the NP levels of the partial responders were the lowest, whereas the NP levels of non-responders were the highest. In the study of Daito and associates that included 14 patients with chronic HBV infection, the patients were administered an IFN therapy. The serum NP levels of the patients were measured at the beginning, during and after completion of the treatment. A week after the onset of the treatment, the serum NP levels were found significantly higher in both groups as compared to those at the beginning of the treatment. However, after this, the serum NP levels decreased gradually during the IFN therapy and they quickly returned to their baseline levels at the end of the treatment. In the present study, the post-treatment serum NP levels of the patients with CHB who received interferon monotherapy for one year were significantly higher than their pretreatment serum NP levels (p=0.001). The patients were divided into two groups according to their response to the treatment. The pre- and post-treatment serum NP levels of the responders and non-responders were compared. The post-treatment serum NP levels were significantly higher than the pretreatment serum NP levels in the group from whom response was obtained at the end of the treatment (p=0.003). There was no significant difference between the pre- and post-treatment NP levels in the patient group who did not respond to the treatment. In our study, the NP values at the end of this 1-year pegylated interferon alpha and/or ribavirin therapy were found higher than the initial NP values without differentiating between the responders and non-responders (the number of responders were more). This highness was attributed to the immune-modulator effect of interferon. Our results were consistent with those of Gelderblom et al. (18), but did not comply with the results of Daito et al. (19) and Grüngreiff et al. (16). The inconsistency was thought to be due to the fewer number of cases in the latter two studies (14 cases in Daito et al. and 16 cases in Grüngreiff et al.).

In the study made by Oxenkrug et al. (20) on 260 patients with CHC who received peginterferon alpha therapy, they observed that the rate at which the patients responded

***Table 5**. Comparison of pre- and post-treatment mean neopterin levels of the patients with CHC who received an interferon+ribavirin therapy*

Treatment Given	Pretreatment mean NP levels (nmol/L)	Post-treatment mean NP levels (nmol/L)	P value
Interferon+Ribavirin	12.10±5.34	17.61±7.00	0.001

***Table 5**. Comparison of pre- and post-treatment serum NP levels of the patient groups with CHC who responded and did not respond to the treatment*

Treatment Given (IFN+Ribavirin) (n=23)	Pretreatment mean NP levels (nmol/L)	Post-treatment mean NP levels (nmol/L)	P value
Responders (n=14)	12.77±6.45	20.25±6.91	0.003
Nonresponders (n=9)	8.31±4.62	12.70±6.45	>0.05

to the treatment was correlated with neopterin concentrations. They found that before the antiviral treatment the response to treatment rates of the patients whose serum neopterin levels were ≤16 nmol/L were higher than those of the patients whose serum neopterin levels were ≥16 nmol/L. Thus, they concluded that the neopterin levels measured before an antiviral treatment could be used for response to treatment estimates. In most of infections, when a comparison is made before and after an infection treatment, a decrease is seen in NP levels because of the decline in immune system activation at the end of the treatment. However, when the disease is treated with an IFN therapy, which is a treatment that stimulates the immune system, the NP level remains high at the end of the treatment in connection with the IFN therapy. Going through the literature, there was not any study where the measurement of post-treatment neopterin levels of patients with CHB and CHC who received an antiviral treatment was assessed against the patients' response to the antiviral treatment. Our study suggests that neopterin can be used in determining the efficacy of interferon in treating chronic hepatitis. The conflicting results between the NP level and specific parameters of CHB and CHC in the literature show that there is need for further studies.

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