The relationship between neopterin and hepatitis B surface antigen positivity

Introduction

Each year, approximately 1.45 million people die worldwide as a result of viral hepatitis infections [1]. Hepatitis B virus (HBV) is the main cause of various liver diseases such as acute and chronic hepatitis B infections, liver cirrhosis and hepatocellular carcinoma. According to the data from World Health Organization (WHO), hepatitis C virus (HCV) and HBV are responsible for 90% of the deaths due to hepatitis infections. The infection may be chronic in about 5% of adults and 95% of newborns [2].

Hepatitis B virus, settling inside the liver, damages the liver cells. It has various viral surface antigens on the lipid envelope gained from the host cell surface. One of these antigens is the HBsAg [3], which can be detected in the serum approximately 6-8 weeks prior to the occurrence of the disease symptoms and 12 to 20 weeks before levels of transaminases increase. HBsAg is the first indicator to be evaluated in patients with acute hepatitis B. In recovering cases, it decreases in the serum and disappears within 6 months; its presence in the serum after this period indicates that either the disease has become chronic or the patient has become a carrier. In the serum, following the disappearance of HBsAg, hepatitis B surface antibodies (anti-HBs) appear and it can remain detectable for lifetime. The incubation period of the HBV may vary depending on the amount of the viruses exposed and the immunity of the patient. It is usually 60-90 days following the exposure to the virus, whereas it sometimes can be delayed as long as 45-180 days [4,5].

The vast majority of viruses that infect humans are effectively removed by the immune system; however, some viruses develop defense mechanisms against their elimination [6]. Natural killer cells, cytotoxic effectors, and cytokine production are important components of the natural immune system, which serves to eliminate viral infections. Natural killer cells have two primary functions in controlling viral infections. The first function is to attack the infected cell through direct cell-to-cell contact. The other is the secretion of antiviral cytokines such as interferon-gamma (IFN-γ). Studies have suggested that the immune response generated by the natural killer cells...
cells may provide the possibility to control the disease in the early stages of acute HBV infections. Additionally, in HBV infections, the evaluation of the parameters, which are involved in the host defense system also provides information on whether the infection is in its active phase or not [7]. Although HBV does not lead to a significant natural immune response in the liver, it may lead to HBV-specific adaptive T-cell responses. Despite the advancements gained in the studies on HBV, the important aspects of HBV immunology and pathogenesis, particularly the immune mechanisms that the persistent virus-related effects are responsible for, could not yet be adequately identified [8].

The clinical features of the hepatitis-related disorders varies widely. Approximately half of the patients are asymptomatic [9]. At the end of the incubation period, before clinical symptoms occur, neopterin levels increase in body fluids. The highest neopterin levels are observed just before the detection of virus-specific antibodies [10,11]. It is known that immune system activation can be observed following the development of a viral infection. Change in concentration of neopterin in viral infections, which is one of the indicators of immune system activation, provides insight into the relationship between infectious diseases and the immune system [12]. Neopterin, which is secreted by macrophages that are activated by inflammation, can be used as an indicator of liver diseases such as acute hepatitis, chronic hepatitis, and cirrhosis. A positive correlation has been found between the elevated serum neopterin levels and the disease severity in many disorders [12-16].

The aim of our study was to evaluate the serum neopterin levels, which is an indicator of cellular immune system activation in persistent carriers of HBsAg (+) patients.

**Materials and methods**

**Subjects and samples**

A total of 115 serum samples were collected. The study group comprised of 72 HBsAg carriers (male: 44, female: 28) and 43 healthy individuals (male: 26, female: 17). The average age of the patient group was 40.49 ± 0.20 years, whereas control group’s average age was 33.12 ± 0.22 years.

Blood samples were collected from the patients and healthy individuals who applied to Family Medicine Polyclinic. Diagnostic routine hepatitis tests for acute or chronic HBV infections were requested from patients with suspected hepatitis B infection. The patients who had positive HBsAg test results for more than four months were formed patient group. The health status was thoroughly questioned in all of the individuals in the control group. Individuals who applied to Family Medicine Polyclinic without an infectious disease, chronic disease, malignancy and immune system-related disorders were selected for control group. Additional blood was collected from both control group and patient group when the physician requested blood for routine biochemical tests and for the evaluation of hematological parameters.

**Informed consent:** Informed consent has been obtained from all individuals included in this study.

**Ethical approval:** The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the İnönü University’s local ethics committee.

**Measurement of neopterin**

During collection of the blood samples required for routine biochemical and hematological tests, additional blood samples were also taken in biochemistry tubes. Then, the samples were centrifuged at 3,500 rpm for 10 minutes. The serum samples were collected and stored at -20°C until the time of measurement.

Serum neopterin levels were measured with enzyme linked immunoassay (ELISA) method by using commercially available DRG Diagnostics GmbH (Marburg, Germany). Results were expressed as nmol/L.

**Statistics**

All results are presented as means ± standard error of the mean (SEM) using the Statistical Package for the Social Sciences (SPSS) 11.5 program. Mann-Whitney U test was used for the comparison between the two independent groups and Pearson correlation test was used in the evaluation of the correlations. p values < 0.05 were considered statistically significant.

**Results**

The serum level of neopterin in HBsAg (+) group was 17.6 ± 0.13 nmol/L (range 8.25-68.40 nmol/L), whereas it was 9.12 ± 0.09 nmol/L (range 3.66-20.30 nmol/L) in the control
The mean neopterin level of the HBsAg (+) patients increased when compared to the control group, and this increase was found statistically significant (p < 0.001) (Figure 1).

Both in the control group and the patient group, there was no significant relationship between gender and serum neopterin level (both, p > 0.05) (Figure 2).

There were positive correlations between the serum neopterin level and age in both the control group (r = 0.1, p > 0.05) and the patient group (r = 0.09, p > 0.05). However, these correlations were not found to be significant (Figure 3).

**Discussion**

The pathogenesis of the liver diseases caused by hepatitis B virus is mostly based on the immune system-mediated mechanisms. However, it can rarely be caused by direct hepatotoxic damage. The principal mechanism of immune system-mediated hepatotoxicity is the destruction of the infected hepatocytes through cytotoxic T cells [4]. In hepatitis B infection, IFN-γ, which is released from cytotoxic T cells, activates the macrophages in the liver, stimulating the release of neopterin from these macrophages [17]. IFN-γ production increases due to the stimulation of T lymphocytes by various specific antigens, mainly viral antigens [15,18,19].

Neopterin is regarded as an early valuable marker for monitoring of infectious disease activity [20]. Serum neopterin levels were not only correlated with activity of disease but also, they are useful and informative for an early differentiation between infectious and non-infectious patients [21]. It has been suggested that serum neopterin level may be an important indicator for differential diagnosis of viral hepatitis and non-infectious hepatitis [15,22].

Serum neopterin levels in patients with acute and chronic hepatitis B were found to be higher than those of healthy individuals. The relationship between neopterin level and the severity of the disease has been proved, and it has been suggested that neopterin level can be used as a prognostic indicator of the disease progression in combination with clinical data [12,15,23]. High neopterin levels have been found in hepatitis B e antigen positive (HbeAg +) chronic hepatitis B patients. It can be concluded that there is an association between elevated neopterin levels and HbeAg-positivity [23]. The results of a pediatric study suggested that serum neopterin levels can be used as an inflammatory marker in children with hepatitis-

B-related chronic liver disease [15]. In acute hepatitis, chronic inactive hepatitis, chronic active hepatitis, liver cirrhosis, hepatocellular carcinoma, alcoholic liver diseases and in asymptomatic HBsAg carriers, serum and urine neopterin levels were found to be higher than those of the control group. The highest levels of neopterin
were detected in patients with acute hepatitis [15,24]. Our results are in agreement with previous studies [12,15,22-24]. We found a significant increase in serum neopterin levels in the HBsAg carriers due to the activation of the cellular immune system compared to the healthy individuals.

Neopterin levels are reduced when the immune system is suppressed, due to infectious diseases such as chronic hepatitis B and chronic hepatitis C infections. Conversely, when the chronic hepatitis is treated with an IFN therapy, neopterin concentrations increase due to stimulation of the immune system [25]. The development of necroinflammation and fibrosis in chronic hepatitis B infection is caused by the immune response in which T lymphocytes and macrophages play roles. It has been considered that neopterin, which is secreted by macrophages during this immune response, reflects the presence and the severity of fibrosis and necroinflammation in the liver. Neopterin measurement in the body fluids not only informs about the level of the cellular immune response but also helps to predict the progression of the disease [15,26].

Elevated neopterin levels in body fluids can be detected immediately before the occurrence of the clinical symptoms at the end of the incubation period, and it show a significant increase with the emergence of clinical symptoms. High neopterin concentrations are more pronounced in diseases in which monocyte and macrophage activities are intense. Neopterin release begins three days before T lymphocyte proliferation reaches its peak and neopterin can be used as an early indicator of inflammation since an increase in neopterin production is observed approximately one week before the specific antibodies become positive. After the emergence of neutralizing antibodies in the convalescence period, however, neopterin secretion is reduced to its normal level [17,27].

As it is known that cellular immune system activation is observed following cardiovascular diseases, infectious diseases, autoimmune disorders, malignancies, and organ transplantations. HBsAg can be detected in the serum from several weeks before occurrence of the clinical symptoms. We aimed to evaluate the serum neopterin level, which is one of the early indicators of the cellular immune system activation.

In conclusion, we suggest that measurement of serum neopterin level can be used for assessment of the immune system activity in combination with other parameters in hepatitis B-related diseases.

Conflict of interest: Authors state no conflict of interest.

References