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Short Communication

Serum Neopterin Levels in Patients with Chronic Hepatitis B

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SUMMARY: The aim of this study was to determine the neopterin levels and peripheral blood lymphocyte subgroups in HBeAg-positive and -negative chronic hepatitis B patients. A total of 89 patients were included in the study. The mean serum neopterin level of patients with chronic hepatitis B was significantly higher than that of the control group. In HBeAg-positive chronic hepatitis B patients, the mean serum neopterin level was significantly higher than that of anti-HBeAb-positive patients. There was no significant correlation between the serum neopterin levels and alanine aminotransferase and HBV-DNA levels in HBeAg-positive chronic hepatitis B patients. There were no significant differences between the control subjects and patients with HBeAg-positive or anti-HBeAb-positive hepatitis in terms of the percentage of peripheral blood $CD4^+$ or $CD8^+$ or the ratio of $CD4^+/CD8^+$ lymphocytes. Our results suggest an association between elevated neopterin concentrations and HBeAg-positivity in patients with chronic hepatitis B. However, there appears to be no association between the neopterin levels and either hepatocyte damage or viral replication status.

Neopterin is produced by macrophages after stimulation with interferon (IFN)-γ or lipopolysaccharide (1). Increased concentrations of neopterin have been reported in conditions causing a stimulation of cellular immunity, such as viral and other infectious diseases, malignancies, autoimmune diseases, heart and kidney failure, coronary artery disease and allograft rejection (2,3).

The aim of this study was to assess the relation between immune activation status and serum neopterin levels in HBeAg-positive and -negative chronic hepatitis B patients. Furthermore, possible relationships between the neopterin levels and viral load, histologic activity index, liver enzyme levels and peripheral blood lymphocyte subgroups in HBeAg-positive chronic hepatitis B patients were also investigated.

A total of 89 consecutive patient volunteers (58 males, 31 females; age range, 17 - 56 years [32.9 ± 10.1]) were included in the study. Among them, 30 had been diagnosed with HBeAg-positive chronic hepatitis B, and 59 with anti-HBeAb-positive chronic hepatitis B. The 30 patients with HBeAg-positive chronic hepatitis B had been histopathologically diagnosed, and their HBV-DNA and alanine aminotransferase (ALT) values were >2.5 pg/ml and ≥40 IU/L, respectively. The 59 patients with anti-HBeAb-positive chronic hepatitis B were under follow-up, and had HBV-DNA < 2.5 pg/ml and ALT levels ≤40 IU/L. The control group was composed of 40 healthy volunteers (20 males, 20 females; age range, 21 - 59 years [33.1 ± 7.8]). Venous blood (5 ml) was taken from each subject for measurement of viral serological markers (Dia-Sorin, Vercelly, Italy), HBV-DNA (Quantification of HBV DNA in serum using bDNA), ALT (Olympus AU 600 autoanalyzer (Olympus Corp., Tokyo, Japan) and neopterin (Brahms Diagnostica GmbH, Berlin, Germany) levels. For the histopathological evaluation, necro-inflammatory activity (grade) and fibrosis (stage) were determined using modified Knodell scoring (4). Peripheral blood T lymphocyte subpopulations were studied by flow cytometric analyses (Beckman-Coulter, Miami, Fla., USA).

The mean serum neopterin level of patients with chronic hepatitis B (15.6 ± 5.1, n = 89) was significantly higher than that of the control group (7.5 ± 1.4) ($P < 0.001$) (Fig. 1). In HBeAg-positive chronic hepatitis B patients, serum neopterin levels were significantly higher than those in anti-HBe-positive patients ($P < 0.001$, Table 1). There was no significant correlation between neopterin and either ALT or HBV-DNA levels in HBeAg-positive chronic hepatitis B patients. In addition, no significant correlation was found between the serum neopterin levels and Knodell score in HBeAg-positive chronic hepatitis B patients (data not shown).

There were no significant differences between the control subjects and either patients with HBeAg-positive or anti-HBeAb-positive hepatitis in terms of the percentage of peripheral blood $CD8^+$ or $CD4^+$ or the ratio of $CD4^+/CD8^+$

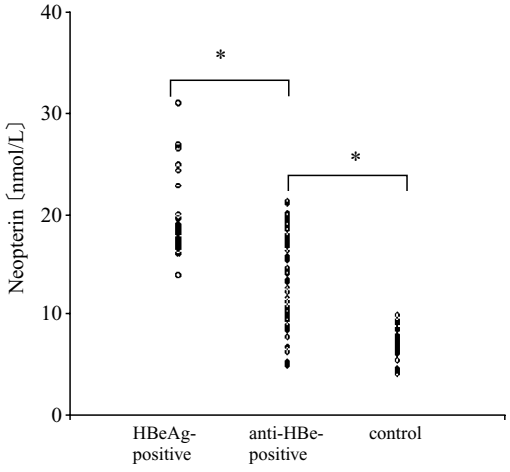

Fig. 1. Comparison of serum neopterin levels between HBeAg-positive and -negative chronic hepatitis B patients and healthy controls. A significant difference was found among the three groups (*$P < 0.05$).

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Table 1. Mean values of ALT, HBV-DNA and neopterin levels; peripheral blood lymphocyte subpopulations of chronic hepatitis B patients

Group	Age (years)	Gender M/F	Neopterin (nmol/L)	Viral load (pg/ml)	ALT (IU/L)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Chronic hepatitis B patients	32.9 ± 10.1	58/31	15.6 ± 5.1[1)]	1455.3 ± 421.1	57.2 ± 5.2	41.1 ± 6.6	29.2 ± 7.1	1.5 ± 0.5
HBeAg+ (n = 30)	30.3 ± 9.3	19/11	19.2 ± 3.7[2)]	4309.9 ± 1082.4	85.9 ± 11.9	40.1 ± 6.5	28.1 ± 5.8	1.5 ± 0.6
anti-HBe+ (n = 59)	34.2 ± 10.2	39/20	13.7 ± 4.5	4.5 ± 2.9	42.6 ± 4.1	41.6 ± 6.6	29.8 ± 7.5	1.5 ± 0.4
Control (n = 40)	33.1 ± 7.8	20/20	7.5 ± 1.4	negative	25.4 ± 8.1	39.6 ± 5.3	28.5 ± 4.6	1.4 ± 0.3

ALT, alanine aminotransferase.
[1)]: $p < 0.001$, vs. control
[2)]: $p < 0.001$, vs. anti-HBeAb positive

lymphocytes (data not shown). There was no significant correlation between serum neopterin levels and the $CD4^+$ or $CD8^+$ levels or the $CD4^+/CD8^+$ ratio for the HBeAg-positive patients (data not shown).

In agreement with previously published studies, the serum neopterin levels of chronic hepatitis B patients were found to be higher than those of the controls (5-7). HBV infection develops in four consecutive phases that can be identified using specific immunological markers. Our study included HBeAg-positive patients whose serum HBV-DNA titers and ALT levels were high and who were at the second stage of the replication phase. The second stage of infection is the active stage of the immune system (8). Neopterin levels of patients at the stage of replication phase being HBeAg-positive, HBV-DNA >5 pg were significantly higher than those of patients with chronic hepatitis B at the non-replication phase (anti-HBeAb-positive chronic hepatitis B patients) ($P < 0.05$). However, there seemed to be no relationship between serum neopterin values and the hepatocyte damage or viral replication status in those patients.

We found an association between elevated neopterin concentrations and HBeAg-positivity in patients with chronic hepatitis B. It has been shown that the serum neopterin levels of patients with chronic hepatitis C are lower in those for whom total viral clearance has been achieved by IFN-α therapy than in those in whom viral RNA is still detectable (7). To our knowledge, there has been no previous study comparing the neopterin concentrations between patients with an HBV-DNA level less than 5 pg/ml and those with an HBV-DNA level greater than 5 pg/ml (based on a PubMed search). Our study is thus the first to investigate the correlation between HBV-DNA and neopterin levels in patients with chronic hepatitis B. Serum neopterin and ALT (which is the best indicator of hepatocellular damage) levels have been significantly correlated in patients with acute viral hepatitis, but not in those with chronic viral hepatitis (6). These results suggest that measurement of serum neopterin can not be used as a sensitive marker for hepatocellular damage in chronic hepatitis B.

Various studies have reported that the $CD4^+$ and $CD8^+$ response and $CD4^+/CD8^+$ ratio identified in peripheral blood in cases with chronic HBV infection decreased, increased or did not change when compared to, respectively, cases of acute HBV infection, cases of self-limited HBV infection and healthy controls (9,10). In this study we could not find any difference between the HBeAg-positive or anti-HBeAb-positive chronic hepatitis B patients and control subjects with regard to the percentage of peripheral blood $CD4^+$ or $CD8^+$ or the $CD4^+/CD8^+$ ratio. Morever, in our study no correlation was found between peripheral blood lymphocyte subgroups and serum neopterin levels. On the other hand, peripheral blood subgroups have been reported to be insufficient in reflecting hepatic immune changes in patients infected with HBV (11). Our data suggest that serum neopterin levels and peripheral blood lymphocyte subgroups poorly reflect hepatocellular damage in HBeAg- positive patients. Finally, this study had several major limitations that bear mentioning, i.e., the heterogeneity of the study population, small sample size and varying times of infection.

In conclusion, we suggest that measurement of the serum neopterin level will provide additional immunologic information about the state of chronic hepatitis B infection. However, this level may not reflect the hepatocyte damage or viral replication status.

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