Plasma Neopterin in Hepatitis C Virus Antibody -positive and -negative Donors with Elevated Alanine Aminotransferase Activity

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Summary

Plasma neopterin was assayed in units from HBs antigen-negative male donors with elevated levels of alanine aminotransferase (ALT, above 35 Karmen units) who were divided into two groups according to whether they were negative (N=162) or positive (N=80) for antibody to hepatitis C virus (HCVAb). Male donors with no abnormalities reflected in screening tests were selected as controls (N98). The average values of plasma neopterin were 5.6 nmol/l in the HCVAb-negative group, 7.9 nmol/l in the HCVAb-positive group and 5.9 nmol/l in the control group. Significant differences were only found between the HCVAb-positive group and each of the other two groups. From the results, the elevated ALT levels in the majority of HCVAb-negative donors with elevated ALT were considered to be due to liver injuries caused by metabolic disturbances such as steatosis due to excess body weight and/or alcohol consumption.

Key words : Plasma neopterin, Hepatitis C virus, Alanine aminotransferase

Introduction

The urinary or serum neopterin level rises in diseases related to cell-mediated immunity (1-3). The mechanism is considered to involve enhanced neopterin excretion from monocytes in the hyperimmune state (4-6). In 1987, Prior *et al.* (7) reported that increased urinary neopterin excretion was a useful marker for diagnosing chronic non-A, non-B hepatitis and for differentiating this disease from fatty liver. Interest in using this fact has declined since the assay of antibodies to hepatitis C virus (HCVAb) was developed as a serologic marker for diagnosing non-A, non-B hepatitis.

Since November 1989, when the assay of c100-3 antibody was adopted in the screening of donated

blood in Japan, the incidence of posttransfusion hepatitis non-A, non-B (PTH-NANB) has greatly decreased (8, 9). An even greater decline in cases is expected following the introduction of the second-generation assay of passive hemagglutination (PHA) in February 1992. We therefore retrospectively assayed neopterin in stored plasma of two groups of HCV Ab -positive and -negative donors with elevated ALT, and attempted to correlate the neopterin values with HCVAb positivity and compared these results to those of normal control donors.

Subjects and Methods

Subjects

Only male donors only were studied so as to avoid the gender differences in ALT, which have been documented (10).

The following 3 groups of HBs antigen-negative male donors were investigated :

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Group A : HCVAb-negative donors with abnormal ALT (above 35 Karmen units), N=162.

Group B : Donors with a high HCVAb titer based on PHA results ($\geq 2^{10}$) and with abnormal ALT, N=80.

Group C (controls) : Donors with no abnormal results in routine screening tests, N=98.

Male donors included in groups A and B were selected at random. Each sample was assayed to ensure that no abnormal results were present other than those for ALT and ALT and HCVAb, respectively.

Mean, standard deviation and ranges of ALT in the 3 groups were as follows : Group A, 55 19 Karmen units (KU), 36-160KU; Group B, 63 32KU, 36-166KU; Group C, 11 6KU, 4-33KU.

Methods

After routine screening at the Hachioji Blood Center, including HCVAb and ALT tests, plasma samples were immediately stored at -30°C in the dark until use for the measurement of neopterin.

Neopterin was directly assayed without oxidation (11) using a RIA kit, IMMUTEST Neopterin (Henning Berlin GMBH, Berlin, Germany). Fifty microliter of plasma, 100 μl of ^{125}I -neopterin and 100 μl of antiserum were mixed and incubated at room temperature for 60 minutes in the dark. After addition of washing solution, the mixture was centrifuged at 2,500 g for 10 minutes, and the radioactivity of the precipitate was measured using a γ -counter. HCVAb was assayed using a commercial second-generation passive hemagglutination assay (PHA) kit (Dinabot Co., Ltd., Tokyo, Japan).

ALT was assayed by the Wroblewsky and La Due method (12) using an autoanalyzer, Olympus ACA 8000 (Tokyo, Japan), and activity was expressed in Karmen units (normal range 3-35).

Results

Distributions of plasma neopterin levels in the 3 groups are shown in Fig. 1 and Table 1. The means are 5.6 nmol/l in group A, 7.9 nmol/l in group B and 5.9 nmol/l in group C. Significant differences by *t*-test were found only between group B and each of the other two groups ($p < 0.01$). When the mean $\pm 2\text{SD}$ (8.2 nmol/l) of the control group C was determined as the cutoff value of plasma neopterin, the frequency of abnormally elevated levels was significantly high at 35% in group B, as compared with those in group A (5.5%) and group C (5.1%). In group A, however, 3 cases were found with plasma neopterin levels higher than 10.3 nmol/l (mean $\pm 4\text{SD}$). No such cases were

Figure 1. Frequency distributions of plasma neopterin levels in three groups.

..... : HCVAb-negative donors with abnormal ALT (group A)
 — : HCVAb-positive donors with abnormal ALT (group B)
 - - - : Controls (group C)

Table 1. Plasma neopterin levels in three groups.

Group	N	Neopterin (nmol/l) Mean $\pm$ SD (Range)	Abnormal rates (%)	P
A : HCVAb-negative donors with abnormal ALT	162	5.6 $\pm$ 2.2 (3.2~25.0)	5.5	* NS
B : HCVAb-positive donors with abnormal ALT	80	7.9 $\pm$ 1.8 (5.2~13.8)	35.0	
C : Controls	98	5.9 $\pm$ 1.1 (4.2~9.8)	5.1	

Abnormal rate (%) shows frequency of donors having measured value over mean $\pm 2\text{SD}$ of normal controls.

* : $p < 0.01$, NS : not significant

found in group C. The neopterin levels of the 3 cases in group A were 11.4, 19.0 and 25.0 nmol/l, and their ALT values were 80, 66, and 160 KU, respectively.

Discussion

Several studies have already shown that urinary neopterin was increased in acute or chronic viral hepatitis, whereas the levels were almost normal in etiologically non-viral liver injuries, such as steatosis (7, 13, 14).

In HCVAb-negative donors with elevated ALT, plasma neopterin levels were not significantly different from those of the control group. The ALT elevation in these donors is considered to be mostly caused by steatosis due to excess body weight and/or alcohol consumption (10, 15).

Based on our observations in 1984, the mean $\pm 2\text{SD}$ of ALT grouped by body weight in male donors were 22 KU for 50~59 kg body weight, 27 KU for 60~69 kg, 40 KU for 70~79 kg and 61 KU for body weight of 80 kg and above (Nojiri *et al.* unpublished). More-

over, we have recently observed that, out of 5671 male donors with elevated ALT found over two years (November 1989~October 1991), 75% (4249) were donors weighing above 70 kg. These findings indicate that most of the units containing elevated ALT in Japan occur in subjects with excess body weight.

In contrast, 3 donors (1.9%) had an increase of plasma neopterin of more than 10 nmol/l in the group of HCVAb-negative donors with elevated ALT. Such donors are suspected as being infectious, or as having autoimmune liver injury. Currently, over 3% of donations are discarded at our blood centers due to elevated ALT levels. From the above results, it is concluded that neopterin assay is occasionally useful for the assessment of a hyperimmune state of these ALT elevated bloods. The majority of HCVAb-positive donors with elevated ALT were considered to be HCV carriers with chronic hepatitis. This supposition was strongly supported by a recent report by Watanabe *et al.* (16) indicating that all donated units with results of both HCVAb positivity based on PHA, and ALT level higher than 35 KU, were HCV-RNA positive.

In this donor group, the average plasma neopterin level was significantly higher than that in the control group. However, definitely increased plasma neopterin levels, that is, above the mean + 2SD of the control group (8.2 nmol/l), were recognized in 35% of the donors. This abnormal rate is considerably low, compared with those of 70~90% for urinary neopterin excretion levels which were found in clinical cases of acute or chronic hepatitis (7, 13, 14). This is attributed to differences in severity of hepatitis between clinical and donor cases.

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