Assessment of HBc antibody in individuals with HBs antigen negative test

HBs antijen testi negatif kişilerde HBc antikor değerlendirmesi

To the Editor,

Hepatitis B virus (HBV) infection is a global public health problem. About 2 billion individuals are infected with HBV globally (1,2). In Iran, about 3% of the population are chronic carriers.

Hepatitis B core antibody (HBc Ab) is the first antibody after HBV infection, and it is the only detectable marker in the window period. It is in the serum of recovered individuals and also those with chronic hepatitis B (CHB). Resolved infection usually reveals hepatitis B surface antibody (HBs Ab).

Isolated HBc Ab is the presence of HBc Ab in the sera without hepatitis B surface antigen (HBs Ag) or HBs Ab. It may be an indicator of resolved HBV infection in which HBs Ab had declined to an undetectable level or of chronic infection such that HBs Ag cannot be detected due to protein mutation, which makes it undetectable by certain diagnostic assays (2). Recent studies clarified that we cannot judge the HBV infection based only on the presence or absence of HBs Ag and HBs Ab (1).

Whole blood samples were collected from 1000 unpaid blood donors of an Iranian blood transfusion service between December 2006 and January 2007 in Hamedan, a western province of Iran.

Samples were centrifuged at 3000 g for 10 minutes (min), and EDTA plasma was separated within 3 hours (h). The samples were tested by enzyme linked immunosorbent assay (ELISA) method with Radim® anti-HBc kit. The samples were retested by the same Radim® anti-HBC kit. All of the results were similar to those of the first test. The reactive samples were tested by Diapro® anti-HBc kit. A total of 70 blood samples positive for anti-HBc by both Radim® and Diapro® kits were tested for HBs Ab by Radim® kit. A titer >15 IU/ml was considered as reactive. Fifty-two samples were reactive and 18 samples were not reactive.

All of the 70 blood samples were tested for HBV DNA. These confirmed samples (70 cases), diagnosed as anti-HBc reactive by Radim® and Diapro®, were tested with a commercial polymerase chain reaction (PCR) assay with primer targeted to the surface (S) nucleotides 338-430 dalton. They were subjected to nucleic acid extraction using the Bioneer® DNA blood mini-kit and amplification by HBV PCR detection kit of Cinnagen® Inc. lot no. PR7831 c in a duplicate manner. We used Tecne® 312 thermocycler with a 36-cycle thermal program. The product was analyzed with gel electrophoresis.

This study was approved by the ethics committee of Hamadan University of Medical Sciences.

A total of 1000 HBs Ag-negative donors of our blood donation service were screened with Radim® and Diapro® anti-HBc. Seventy out of 1000 (7%) samples were reactive by Radim® and Diapro® anti-HBc kit. The majority of these samples were additionally positive for HBs Ab (52.70%). All of these HBs Ab-positive sera were negative for HBV DNA by standard PCR test system. The PCR test was repeated, and the results were similar to the first testing. Summary of test results are shown in Table 1.

The isolated presence of anti-HBc in the absence of Hbs Ag and anti-HBs has been reported in 0.4 to 1.7% of blood donors in low prevalence areas (3,4), in 10 to 20% of the population in endemic countries (5), and in 7% in our region. Isolated detec-

Table 1. Summary of test results in the study group

	Number (n=1000)	Percentage
HBs Ag-Negative	1000	100
HBc Ab Radim-Positive	105	10.5
HBc Ab Diapro-Positive	70	7.0
HBs Ab Radim-Positive	52	5.2
HBs Ab Radim-Negative	18	1.8
HBV-DNA PCR-Positive	0	0.0
HBV-DNA PCR-Negative	70	100

Address for correspondence: Alireza Monsef ESFAHANI
Rajaei Heart Center, Tehran, Iran
Phone: +9821 664 39 463 • Fax: +9821 664 23 304
E-mail: swt_f@yahoo.com

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tion of anti-HBc can occur during the window period of acute hepatitis B; many years after recovery from acute hepatitis B when anti-HBs has fallen to undetectable levels; and after many years of chronic HBV infection when the HBs Ag titer has decreased below the cut-off level for detection. Loss of detectable HBs Ag occurs in 0.5% of patients with CHB per year (6).

Transmission of HBV infection has been reported from blood and organ donors with isolated anti-HBC, but the incidence ranged widely, from 0.4 to 78% (2,7-9).

In this study, we found isolated anti-HBc as part of occult HBV infection in those without any clinical or laboratory finding. They must have no history of blood transfusion or icteric disease and must be in a completely healthy condition.

Individuals with evidence of chronic liver disease should be tested for HBV DNA to exclude low level chronic HBV infection.

Blood donor screening with HBs Ag assays may fail to detect chronic HBV-infected persons, because the low virus burden may remain undetected by these assays. A few patients were infected with CHB after blood transfusion, and studies confirmed they had a latent and low virus level (10). Based on the study results, although undetectable DNA virus particle in HBs Ab-positive donors is present, it seems that the present policy of blood donation in our region is safe and suitable. In a Canadian study (1), they found many samples with isolated HBc Ab positivity, and they did not consider them as safe and harmless. They proposed HBc Ab-negative persons as safe donors.

As we did not find HBV DNA in the isolated HBc Ab persons, we proposed that these bloods be accepted for blood donation. However, they are not suitable for tissue donation because of some reports of hepatitis B after liver transplantation from HBc Ab-positive donors to HBc Ab-negative recipients, at a rate of about 70% (1).

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Alireza Monsef ESFAHANI, Amir MAJLESI, Fatemeh EGHBALIAN

Department of Pathology, Hamedan University of Medical Sciences, Hamedan, Islamic Republic Of Iran