Frequency and significance of antibodies against hepatitis B core (anti-HBc) antigen as the only serological marker for hepatitis B infection in Lebanese blood donors

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SUMMARY
During a 2-year period, blood samples from 2505 Lebanese blood donors were chosen at random, at various periods of time at one blood donation centre (Hotel Dieu de France, Beirut, Lebanon) and were screened for markers of HBV infection (HBsAg, anti-HBc and anti-HBs). The study showed HBsAg positivity of 0.6% and an overall exposure rate to HBV of 10.0%. Out of the 2505 blood donors screened, 56 (2.2%) were found to be ‘anti-HBc alone’ positive which is almost four times the HBsAg positivity. The 56 ‘anti-HBc alone’ samples were retested by another ELISA kit commercially available and 54 samples were ‘anti-HBc alone’ positive by both assays. The 54 samples had no serological markers as evidence of infection with human immunodeficiency virus (HIV) or hepatitis C virus (HCV). Only seven (13%) out of the 54 samples were HBV DNA positive by PCR and all were HBV genotype D. All seven HBV DNA-positive samples had HBV DNA levels below 400 copies/ml. Although any circulating HBV DNA among our ‘anti-HBc alone’ blood donors was below the detection limit of our Amplicor Monitor assay, some of these samples had circulating virus. A national study, where a larger number of blood donors from different blood donation centres across the country will perhaps determine whether screening for anti-HBc in addition to HBsAg detection is needed in Lebanese blood donors.

INTRODUCTION
Antibody to hepatitis B core antigen (anti-HBc) is found in individuals who have experienced natural infection with hepatitis B virus (HBV), and its presence in the absence of hepatitis B surface antigen (HBsAg) is usually interpretable as evidence of past HBV infection. Recently, attention has been drawn to a subgroup of patients with anti-HBc antibodies as the only serological marker of hepatitis B infection (‘anti-HBc alone’) [1, 2]. In areas of low HBV endemcity (e.g. western Europe, United States), ‘anti-HBc alone’ is found to be present in 10–20% of all individuals with HBV markers [3, 4]. The majority of these ‘anti-HBc alone’ individuals seem to be healthy but some of them have HBV DNA that could be detected by PCR [5]. Individuals with ‘anti-HBc alone’ are, therefore, potentially infectious. This is supported by the reports of HBV transmission through sexual contact, perinatal transmission [6] and in blood recipients [7]. The latest studies have shown that the majority of healthy individuals positive for...
‘anti-HBc alone’ were latently infected with the episomal form of HBV accompanied by ongoing viral replication [8]. The reason for the lack of HBsAg in ‘anti-HBc alone’-positive individuals is not clear but several explanations have been offered. These include variation in the pre-S region or mutations in the surface antigen itself especially in the a determinant which makes HBsAg undetectable by conventional ELISA assays [9, 10]. HBsAg may be hidden in circulating immune complexes [11] or present in low concentrations below the detection limit of current serological tests [12]. False-positive anti-HBc results due to the variable sensitivity and specificity of commercial assays [13] can be a problem and HBsAg synthesis may be downgraded by co-infection with HCV [14] or HIV [15].

Recently, an international group met in Zurich, Switzerland, for a workshop on ‘anti-HBc alone’ to discuss the problem and have summarized the present knowledge [1]. In the workshop, there was consensus that testing for anti-HBc should be obligatory for transplant donors. However, regarding blood banking, all participants of the workshop shared the view that it is necessary to develop national criteria that may be applied to the blood donor population to determine if anti-HBc screening is truly needed.

Currently, screening of blood donors for anti-HBc is practised in some countries, including the United States, France and Japan, but in other countries it is only practised in risk groups [1]. In Lebanon, the phenomenon of ‘anti-HBc alone’ has not been evaluated and hence no national criteria have been established. The aim of this study is to evaluate the frequency of the ‘anti-HBc alone’ phenomenon and assess its significance in Lebanese blood donors at one of the major hospitals in the country.

**METHODS**

**Patients**

Blood donors at Hotel Dieu de France Hospital were involved in the study. They were chosen at random during various periods throughout the 2-year period of the study. The number of donors and the period covered are shown in Table 1. All samples were tested for anti-HBc and anti-HBs in addition to the routine testing for HBsAg, anti-HCV and anti-HIV.

<table>
<thead>
<tr>
<th>Period of sampling</th>
<th>No. blood donors</th>
<th>No. HBsAg positive</th>
<th>No. anti-HBc positive and anti-HBs positive</th>
<th>No. ‘anti-HBc alone’ positive</th>
<th>No. anti-HBs alone positive</th>
<th>No. with markers of HBV</th>
<th>No. with no evidence of HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 June 2002 to 16 July 2002</td>
<td>427</td>
<td>2</td>
<td>26</td>
<td>10</td>
<td>4</td>
<td>42</td>
<td>385</td>
</tr>
<tr>
<td>5 Aug. 2003 to 11 Sep. 2003</td>
<td>740</td>
<td>5</td>
<td>51</td>
<td>14</td>
<td>6</td>
<td>76</td>
<td>664</td>
</tr>
<tr>
<td>Total (%)</td>
<td>2505</td>
<td>15</td>
<td>163</td>
<td>56</td>
<td>18</td>
<td>252</td>
<td>2253</td>
</tr>
</tbody>
</table>

SEROLOGY

A 10-ml blood sample was collected from each donor during the donation visit, centrifuged, aliquoted and stored frozen at −70 °C before shipment to the Molecular Virology Laboratory at the Faculty of Health Sciences, American University of Beirut to be tested for anti-HBc IgM and HBV DNA extraction and HBV genotyping. Testing for HBsAg, anti-HCV, anti-HIV, anti-HBc and anti-HBs was performed at Hotel Dieu de France by enzyme linked
immunosorbent assay (ELISA) using commercially available kits (bioMérieux bv Boseind, Boxtel, The Netherlands). The HBsAg ELISA kit used has a sensitivity of 0.18 ng/ml and specificity of 99.9% and that of anti-HBs and anti-HBc have a sensitivity and specificity of 100%. All samples that were found to be ‘anti-HBc alone’ positive were retested by another commercially available ELISA (DiaSorin srl Saluggia, Italy) with a sensitivity of 100% and specificity of 99.5% and only samples positive by both assays were included in the study. Samples with an anti-HBs level ≥ 10 IU/ml were considered positive.

The concentration of alanine aminotransferase (ALT) was determined in all ‘anti-HBc alone’ samples by an autoanalyzer (Hitachi 912), and considered abnormally high if ≥ 60 IU/l.

**HBV DNA detection and genotyping**

DNA extraction was performed using the QIAamp DNA Blood Mini kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer’s instructions. HBV DNA detection was performed using polymerase chain reaction (PCR) with primers P7 and P8 as described by Lindh et al. [16]. HBV genotyping was investigated by restriction fragment length polymorphism (RFLP) analysis of the fragment of the HBV genome which was amplified by PCR as described by Lindh et al. [16], with minor modifications. In brief, 10 μl from the extracted DNA was used for the PCR reaction. Amplified DNA was digested using Tsp 5091 (New England Biolabs, Beverly, MA, USA) and HinfI (Amersham, Biosciences, Uppsala, Sweden). Resulting DNA fragments were separated by electrophoresis in 3% agarose gel, stained by ethidium bromide and the restriction pattern was read according to the description of Lindh et al. [16].

**Hepatitis B viral load**

Samples with detectable HBV DNA by PCR were further tested for HBV viral load using the Amplicor HBV Monitor test (Roche, Switzerland). All manufacturer’s instructions were followed. The detection limit of the assay was 400 genome copies/ml.

**Statistical analysis**

Data were analysed using Stata software version 7.0 (Stata Corporation, TX, USA). χ² test was administered to test whether the proportion of markers was homogenous across the period of investigation. Furthermore, logistic regression was carried out in order to investigate the role of the year on proportion of carriers.

**RESULTS**

Exposure rates to HBV infection in 2505 Lebanese blood donors (2005 males, 500 females; mean age 35±12 years) screened at various periods between 22 June 2002 and 20 November 2003 are shown in Table 1. Approximately 10% of blood donors show evidence of HBV infection with HBsAg positivity at 0.6%. Exposure rate to HBV was similar across the five periods of sampling (P>0.1). HBsAg positivity during the period of investigation was close to that found during the past 6 years (Table 2). Logistic regression analysis revealed that for each additional year the risk for HBsAg positivity decreased by 8% [95% confidence interval (CI) 0.86–0.98]. Among the 2505 blood donors tested, 56 (2.2%) blood donors were ‘anti-HBc alone’, a rate which is almost four times the HBsAg positivity. Out of these 56 samples only two were anti-HBc negative by another commercially available ELISA and hence 54 samples (anti-HBc positive by both ELISA assays) were tested for HBV DNA. HBV DNA was detected in seven (13%) of the 54 ‘anti-HBc alone’ samples and the virus load in all seven was below 400 copies/ml. All seven HBV DNA-positive samples were HBV genotype D. None of the 54 ‘anti-HBc alone’-positive samples was anti-HBc IgM positive and hence recent HBV infections were excluded.

**DISCUSSION**

The results of this study show that out of the 2505 Lebanese blood donors screened, 56 (2.2%) were found to be ‘anti-HBc alone’ positive which is almost
four times the HBsAg positivity rate (0.6%). HBsAg positivity in this study is considerably lower than that reported by Nabulsi et al. [17] in the Lebanese population (2.2%). This could be due to the fact that the subjects tested in the latter study, in contrast to ours, were from various other parts of the country reflecting geographic variation in exposure to HBV in the same country.

Previous reports have shown that a high proportion of ‘anti-HBc alone’ patients had co-existing infection with HIV (33.1%) [4] and HCV (9.3%) [12]. Co-infection of HBV with HIV or HCV could lead to down-regulation or interference of HBsAg production. In contrast to the findings of Jilg et al. [3] where 14.3% and 40.5% of the ‘anti-HBc alone’ samples were also positive for HIV and HCV respectively, none of our ‘anti-HBc alone’ samples had any serological markers of infection with HIV or HCV. This could be due to low prevalence of HIV (<1%) and HCV (<1%) in the Lebanese blood donor population [18, 19] or possibly due to the small number of ‘anti-HBc alone’ patients we investigated. ‘Anti-HBc alone’ could also be due to false-negative HBsAg tests. Very low concentrations of HBsAg (below detection limits) or mutations in the major antigenic determinant (a determinant) of HBsAg [20] may lead to false-negative HBsAg results. Mutation in codon 144 was also reported to give false-negative HBsAg results but these mutants were not investigated in the Lebanese population.

Only seven (13%) of our 54 ‘anti-HBc alone’ samples had circulating HBV DNA detectable by PCR. False-positive HBV DNA results due to contamination were unlikely as our results were reproducible and confirmed independently in another laboratory. The seven HBV DNA-positive samples were genotype D confirming our recent findings of the predominance of HBV genotype D in the Lebanese population [21]. ALT levels were raised in only two of the seven HBV DNA-positive samples indicating no correlation between biochemical evidence of liver damage and HBV DNA detection. Furthermore, all of the seven samples had no detectable viral load, indicating that the amount of virus present was below 400 copies/ml.

The results of this limited study show that a relatively high prevalence of ‘anti-HBc alone’ (2.2%) was found in the 2505 Lebanese blood donors investigated. The fact that 13% of those ‘anti-HBc alone’ positive samples were reactive by PCR means that they had circulating amounts of virus although this amount could not be quantitated by the assay used in this study. Perhaps a national study will evaluate the problem more accurately and will determine whether routine screening for anti-HBc will be justified in Lebanese blood donation centres in addition to HBsAg detection. HBV DNA detection should be attempted on samples from patients with clinical or biochemical evidence of liver disease, or if there is co-infection with another bloodborne virus.

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REFERENCES


