

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

S. RAMIA^{1*}, F. RAMLAWI¹, M. KANAAN², S. KLAYME³ AND R. NAMAN³

¹ *Department of Medical Laboratory Technology, Faculty of Health Sciences, American University of Beirut, Beirut, Lebanon*

² *Department of Epidemiology and Population Health, Faculty of Health Sciences, American University of Beirut, Beirut, Lebanon*

³ *Blood Bank, Hotel Dieu de France Hospital, Beirut, Lebanon*

(Accepted 9 January 2005)

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 endemicity (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

* Author for correspondence: Dr S. Ramia, Faculty of Health Sciences, American University of Beirut, PO Box 11-0236, Riad El Soloh, 1107-2020, Beirut, Lebanon.
(Email: sramia@aub.edu.lb)

Table 1. *The number of Lebanese blood donors with evidence of HBV infection during various periods of 2002 and 2003*

Period of sampling	No. blood donors	No. HBsAg positive	No. anti-HBc positive and anti-HBs positive	No. 'anti-HBc alone' positive	No. anti-HBs positive	No. with markers of HBV	No. with no evidence of HBV
22 June 2002 to 16 July 2002	427	2	26	10	4	42	385
7 Oct. 2002 to 28 Oct. 2002	399	2	27	10	3	42	357
17 Jan. 2003 to 1 Feb. 2003	356	2	22	13	3	40	316
5 Aug. 2003 to 11 Sep. 2003	740	5	51	14	6	76	664
25 Oct. 2003 to 20 Nov. 2003	583	4	37	9	2	52	531
Total (%)	2505	15 (0.6)	163 (6.0)	56 (2.0)	18 (0.7)	252 (10.0)	2253 (89.9)

'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. Samples that were found to be 'anti-HBc alone' positive were tested for anti-HBc IgM and for HBV DNA. Samples that were HBV DNA positive were investigated for their hepatitis B viral load and were genotyped for HBV.

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 Beseind, 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 μ l from the extracted DNA was used for the PCR reaction. Amplified DNA was digested using *Tsp 509I* (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). χ^2 test was administered to test whether the proportion of markers was

Table 2. Carrier rates of HBsAg in Lebanese blood donors over the past 6 years

Year	No. blood donors	No. HBsAg positive (%)
1998	6100	60 (0.98)
1999	5733	43 (0.75)
2000	6229	49 (0.78)
2001	6877	53 (0.77)
2002	6733	45 (0.66)
2003	7576	45 (0.60)

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.

ACKNOWLEDGEMENTS

The authors thank Khayat-Kanaan for supplying medical equipment, and bioMérieux for partial funding of the research project. Special thanks go to the administration and staff of the Blood Bank at Hotel Dieu de France Hospital for their excellent cooperation. In addition, the authors gratefully acknowledge the secretarial help of M. Abul Naja.

REFERENCES

1. **Grob P, Jilg W, Bornhak H, et al.** Serological pattern 'anti-HBc alone'. Report on a workshop. *J Med Virol* 2000; **62**: 450–455.
2. **Weber B, Melchior W, Gehrke R, Doerr HW, Berger A, Rabenau H.** Hepatitis B virus markers in anti-HBc only positive individuals. *J Med Virol* 2001; **64**: 312–319.
3. **Jilg W, Sieger E, Zchoval R, Schatzl H.** Individuals with antibodies against hepatitis B core antigen as the only serological marker for hepatitis B infection: high percentage of carriers of hepatitis B and C virus. *J Hepatol* 1995; **23**: 14–20.
4. **Berger A, Presier W, Doerr HW.** The role of viral load determination for the management of human immunodeficiency virus, hepatitis B virus and hepatitis C virus infection. *J Clin Virol* 2001; **20**: 23–30.
5. **Brechot C.** Applications of molecular biology to the diagnosis of viral hepatitis. In: Zucherman A, Thomas H, eds. *Viral hepatitis 3rd edn.* London: Churchill Livingstone, 1995: 585–604.
6. **Descos B, Scotto J, Fayol V, et al.** Anti-HBc screening for the prevention of perinatal transmission of hepatitis B virus in France. *Infection* 1987; **15**: 434–439.
7. **Moore C, Barbara J, Hewitt PE.** Anti-HBc in blood donors. *Vox Sang* 1998; **74**: 209.
8. **Rokuhara A, Tanaka E, Yagi S, et al.** De novo infection of hepatitis B virus in patients with orthotopic liver transplantation. *J Med Virol* 2000; **62**: 471–478.
9. **Seddigh-Tonekaboni S, Waters JA, Jeffers S, et al.** Effect of variation in the common, 'a' determinant on the antigenicity of hepatitis B surface antigen. *J Med Virol* 2000; **60**: 113–121.

10. **Zuckerman AJ.** Effect of hepatitis B virus mutants on efficacy of vaccination *Lancet* 2000; **355**: 1382–1383.
11. **Joller-Jemelka HI, Wicki AN, Grob PJ.** Detection of HBs antigen in ‘anti-HBc alone’ positive sera. *J Hepatol* 1994; **21**: 269–272.
12. **Carman WF, Van Deursen FJ, Mimms LT, et al.** The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa, and Sardinia. *Hepatology* 1997; **26**: 1658–1666.
13. **Alhababi F, Sallam TA, Tong CYW.** The significance of ‘anti-HBc only’ in the clinical virology laboratory. *J Clin Virol* 2003; **27**: 162–169.
14. **Berger A, Doerr HW, Rabenau HF, Weber B.** High frequency of HCV infection in individuals with isolated antibody to hepatitis B core antigen. *Intervirology* 2000; **43**: 71–76.
15. **Hofer M, Joller-Jemelka HI, Grob PJ, Luthy R, Opravil M, and the Swiss HIV Cohort Study.** Frequent chronic hepatitis B virus infection in HIV infected patients positive for antibody to hepatitis B core antigen only. *Eur J Clin Microbiol Infect Dis* 1998; **17**: 6–13.
16. **Lindh M, Andersson AS, Gusdal A.** Genotypes, nt 1858 variant, and geographic origin of hepatitis B virus large-scale analysis using a new genotyping method. *J Infect Dis* 1997; **175**: 1285–1293.
17. **Nabulsi M, El-Saleeby C, Araj G and the Lebanese Hepatitis B Collaborative Study Group.** The current status of hepatitis B in Lebanon. *Leb Med J* 2003; **51**: 64–70.
18. **Ramia S, Klayme S, Naman R.** Infection with hepatitis B and C viruses and human retroviruses (HTLV-I, HIV) among high-risk Lebanese patients. *Ann Trop Med Parasitol* 2003; **97**: 187–192.
19. **Naman R, Mansour I, Klayme S, Khalil G.** Hepatitis C virus in haemodialysis patients and blood donors in Lebanon [in French]. *Leb Med J* 1996; **44**: 4–9.
20. **Weinberger KM, Bauer T, Bohm S, Jilg W.** High genetic variability of the group-specific a determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. *J Gen Virol* 2000; **81**: 1165–1174.
21. **Sharara AI, Ramia S, Ramlawi F, et al.** Prevalence of restriction fragment length polymorphism (RFLP) patterns of Hepatitis B Virus (HBV) compatible with genotype D in Lebanon. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 861–863.