Prevalence of occult hepatitis B infection in a highly endemic area for chronic hepatitis B: a study of a large blood donor population


ABSTRACT

Background and aims The aim of the present study was to determine the population prevalence of occult hepatitis B (OHB) infection and its clinical profile in a highly endemic area of chronic hepatitis B virus disease.

Methods OHB was first identified by individual sample testing for hepatitis B surface antigen (HBsAg) followed by nucleic acid testing (NAT) and vice versa for 3044 (cohort 1, stored sera from donation within 1 year) and 9990 (cohort 2, prospective study) blood donors, respectively. OHB was confirmed meticulously by ≥2 out of 3 tests with detectable hepatitis B virus (HBV) DNA using a sensitive standardised assay. Detailed serology and viral load in the serum and liver were studied.

Results The prevalence of OHB was 0.13% (4/3044) and 0.11% (11/9967) for cohort 1 and 2, respectively. In cohort 2, 10 out of 11 OHB samples were positive for anti-HBc (hepatitis B core antigen) antibody (all were immunoglobulin G). Seven had detectable anti-HBs. The serum HBV DNA levels were extremely low (highest 14.1 IU/ml). Of the six donors who underwent liver biopsies, all had normal liver biochemistry, extremely low liver HBV DNA (highest 6.21 copies/cell) and nearly normal liver histology. For those with viral sequence generation, none had the common HBsAg mutant G145R.

Conclusions The prevalence of OHB in a highly endemic area of chronic HBV was very low, thus implying a low impact on transfusion services. To implement universal screening, the high cost of NAT should be taken into account. OHB blood donors had very low HBV replication, and normal liver biochemistry and histology, conferring a favourable prognosis.

INTRODUCTION

The prevalence of chronic hepatitis B (CHB) which affects 400 million people worldwide has been fully documented. The highly endemic regions with carrier rates of >8% include Asia and Africa. However, there is a lack of systematic and population studies in Asia on the prevalence of occult hepatitis B (OHB) virus infection, defined as the presence of hepatitis B virus (HBV) DNA in the sera or livers in subjects who are negative for serum hepatitis B surface antigen (HBsAg). The paucity of population-based data on OHB may be due to two main reasons. First, the entity OHB has gained global attention only recently. Secondly, there is no generally accepted assay of HBV DNA detection for OHB in which serum HBV DNA levels are usually extremely low (<200 IU/ml). However, studies on the prevalence of OHB have many implications for the blood transfusion services as the infectivity of blood products from donors with OHB remains...
Hepatologists largely unknown. The prevalence documented in cohort studies is usually <1%.4–7 Large population studies are required in order to define the prevalence with higher confidence of accuracy.

In addition, the serology, virology (in serum and in the liver) and histology of subjects with incidental identification of OHB have not been studied in detail.

We carried out the present large population study in Hong Kong (where 8% of the population has CHB) with the primary aim of determining the prevalence of OHB in our general population by using two different screening strategies. The secondary aims were to examine the viral and disease status of subjects with OHB.

PATIENTS AND METHODS

The present study was carried out in two stages with two cohorts. The first stage (cohort 1) involved testing of 3044 stored sera randomly selected by the Hong Kong Red Cross Transfusion Service. These sera were from blood donors who donated blood within 1 year of the start of the present study. The time of donation of these samples was between 1 June 2005 and 31 May 2006. The samples were retrieved from all the 18 governorates of Hong Kong. All donor sera were first tested negative for HBsAg (Abbott PRISM, Abbott Laboratories, Abbott Park, Illinois, USA), antibody to hepatitis C virus (HCV) and antibody to HIV. These samples were then tested individually (not by pooling) using the COBAS TaqScreen MPX (Roche Molecular Systems, Branchburg, New Jersey, USA) test on the s201 system (Roche Instrument Center, Rotkreuz, Switzerland), a screening nucleic acid testing (NAT) for HBV, HCV and HIV. The lower limit of detection of this assay for HBV is 3.7 IU/ml with a 95% CI of 3.5 to 4.4 IU/ml. Initial positive samples were then quantified for the HBV DNA levels by a standardised commercial HBV DNA assay, Artus HBV RG test (QIAGEN, Hilden, Germany). When used with the QIAamp DSP Virus Kit (QIAGEN) for HBV DNA extraction, the Artus HBV RG test has a 95% lower limit of detection of 3.8 IU/ml, with a linear range of detection of between 1.1 and >4x10^7 IU/ml. Since the serum HBV DNA levels of occult HBV subjects were expected to be very low, in order to minimise the chance of false-positive or false-negative results, the Artus HBV test was performed three times on three separate occasions in all the samples which tested positive by NAT. Definite OHB was defined as two or more of the three runs of assays showing detectable HBV DNA levels. Further serological tests including antibody to HBsAg (anti-HBs), and total and immunoglobulin M (IgM) antibodies to hepatitis B core antigen (anti-HBc) using the Elecsys 2010 system (Roche Diagnostics, Mannheim, Germany) were performed. In cohort 1 with 3044 HBsAg-negative donors (1525 males, 1519 females), the mean age was 33.1 years (SD 10.6, range 16.0–62.3). NAT by the s201 system identified 12 positive samples (0.4%). Four samples had detectable HBV DNA by the Artus HBV RG test (two had 2 out of 3 tests, two had 1 out of 2 tests (sample volume not adequate for the third run)). If the latter two cases were also regarded as having OHB, the prevalence of OHB was 0.13% with a 95% CI of 0.036% to 0.336%.

RESULTS

In cohort 1 with 3044 HBsAg-negative donors (1525 males, 1519 females), the mean age was 53.1 years (SD 10.6, range 16.0–62.3). NAT by the s201 system identified 12 positive samples (0.4%). Four samples had detectable HBV DNA by the Artus HBV RG test (two had 2 out of 3 tests, two had 1 out of 2 tests (sample volume not adequate for the third run)). If the latter two cases were also regarded as having OHB, the prevalence of OHB was 0.13% with a 95% CI of 0.056% to 0.336%. The HBV DNA levels of these six runs were 1.10, 1.21, 1.70, 2.16, 2.47 and 19.40 IU/ml. Of the 12 NAT-positive samples, 10 with adequate volume for further anti-HBc testing, six were positive for anti-HBc (all were negative for IgM anti-HBc) of which two had OHB.
For the prospective cohort 2, the demographics and the report of the questionnaires of the 9990 donors recruited are listed in table 1.

Figure 1 shows the multistep approach by which the donors with OHB were identified. OHB was found in 11 donors with detectable HBV DNA (three in all 3 runs, eight in 2 out of 3 runs). The prevalence of occult HBV was 0.11% (95% CI 0.055% to 0.197%). There were another four donor samples with detectable HBV DNA only in 1 out of 3 runs. They were regarded as suspected OHB.

The demographics, serum viral markers and serum HBV DNA levels of the 11 confirmed occult HBV donors are listed in table 2. Of these, 10 donors had no family history of CHB; one donor was not sure. All had no family history of hepatocellular carcinoma or cirrhosis. Nine had given previous blood donations. Only two were known to have had HBV vaccinations (six had not received HBV vaccinations; three were not sure). When compared with blood donors without hepatitis B (n=9956) and donors with CHB (n=23), OHB donors were significantly older than donors without hepatitis B (mean age 39.9 years (SD 12.1; range 20.4–57) vs 31.5 years (SD 10; range 16–65.8), respectively, p=0.005) and donors with CHB (mean age 25.6 years (SD 7.7; range 18.5–49.0), p=0.005). There were no significant differences in the gender ratio between the three groups (all p>0.05).

Ten of the 11 samples of these OHB donors were positive for total anti-HBc but all were negative for anti-HBc IgM, indicating that these donors were not in the window phase of acute hepatitis B infection, when subjects are positive for HBV DNA and have yet to develop circulatory antibodies to HBsAg.

Nine donors agreed to undergo further liver biochemical tests and viral sequencing, and six consented to liver biopsies. All these OHB donors had normal liver biochemistry, extremely low serum and liver HBV DNA and nearly normal liver histology (table 2). The HBV surface regions of all nine donor samples were successfully sequenced. Three donors carried genotype B HBV and six carried genotype C HBV. All nine showed wild-type glycine at HBsAg amino acid 145, indicating the absence of G145R mutations (the vaccine escape mutants).

**DISCUSSION**

The present study documented a population prevalence of OHB of 0.13% and 0.11% from two large cohorts of blood donors in

**Table 1  Demographics and report of questionnaires of the 9990 donors**

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>9990</th>
</tr>
</thead>
<tbody>
<tr>
<td>M:F (%)</td>
<td>5550:4440 (55.6:44.4)</td>
</tr>
<tr>
<td>Mean age, years (SD, range)</td>
<td>31.5 (10.1, 16–65.8)</td>
</tr>
<tr>
<td>Report of questionnaires</td>
<td></td>
</tr>
<tr>
<td>Previous blood donation (yes: no: not sure)</td>
<td>7714: 2245: 31 (77.2: 22.5: 0.3%)</td>
</tr>
<tr>
<td>HBV vaccination (yes: no: not sure)</td>
<td>2657: 3502: 3831 (26.6: 35.1: 38.4%)</td>
</tr>
<tr>
<td>HBV infection (yes: no: not sure)</td>
<td>11: 9906: 73 (0.1: 99.8: 0.2%)</td>
</tr>
<tr>
<td>HCV infection (yes: no: not sure)</td>
<td>0: 9924: 66 (0: 99.3: 0.7%)</td>
</tr>
<tr>
<td>Jaundice (yes: no: not sure)</td>
<td>44: 9745: 201 (0.4: 97.5: 2%)</td>
</tr>
<tr>
<td>Significant alcohol intake (yes: no)</td>
<td>205: 9785 (2.1: 97.9%)</td>
</tr>
<tr>
<td>Diabetes mellitus (yes: no: not sure)</td>
<td>15: 9839: 36 (0.2: 99.5: 0.4%)</td>
</tr>
<tr>
<td>Hypertension (yes: no: not sure)</td>
<td>29: 9940: 21 (0.3: 99.5: 0.2%)</td>
</tr>
<tr>
<td>Long-term medication (yes: no)</td>
<td>117: 9873 (1.2: 98.8%)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>HBV infection (yes: no: not sure)</td>
<td>727: 9039: 224 (7.3: 90.5: 2.2%)</td>
</tr>
<tr>
<td>Liver cancer (yes: no: not sure)</td>
<td>178: 9638: 172 (1.8: 96.5: 1.7%)</td>
</tr>
<tr>
<td>Chronic liver disease (yes: no: not sure)</td>
<td>372: 9377: 241 (3.7: 93.9: 2.4%)</td>
</tr>
</tbody>
</table>

F, female; HBV, hepatitis B virus; HCV, hepatitis C virus; M, male.

* Incidence of occult HBV: 0.11%
We carried out the present study in two separate cohorts by using two different strategies of screening for OHB. In the first cohort with 3044 donors, all the samples were first tested negative for HBsAg before being subjected to NAT and finally by sensitive HBV DNA assay. In the second prospective cohort with 9990 donors, the samples were first tested by NAT. NAT-positive samples were then tested for HBsAg. For NAT-positive and HBsAg-negative samples, HBV DNA assay was performed to identify OHB. Both strategies yield very similar prevalence rates. This suggests that initial screening by HBsAg followed by NAT has the same yield of detection as with initial screening by NAT. HBsAg testing followed by NAT is obviously cheaper. However, a practical advantage of adopting the second strategy of initial screening by NAT is that there will be a faster release of blood products for use once the NAT is negative since the sensitivity of NAT is superior to detection of HBsAg by enzyme immunoassay (EIA). Another potential advantage of using initial NAT screening is its ability to detect HBV in the presence of antigenically modified HBsAg such as G145R mutants, which may give rise to negative results in HBsAg detection assays. However, our study revealed that none of the occult HBV carried this G145R mutation, a finding consistent with other studies.

Since the cost for universal NAT screening is high, anti-HBc screening has been suggested as an alternative. The positivity rate of isolated anti-HBc in the general population directly correlates with the endemicity of CHB. It is estimated to be up to 17% in areas of intermediate endemicity. In areas which are highly endemic for HBV infection, such as Hong Kong, the prevalence rate of anti-HBs positivity was as high as 40% before the implementation of universal HBV vaccination. A majority of these anti-HBs-positive subjects would also be positive for anti-HBc. In light of the low prevalence rate of OHB observed in the present study, a high proportion of blood donations may be discarded from usage unnecessarily if isolated anti-HBc positivity is used for screening HBV.

In the 11 OHB donors identified in cohort 2, all had normal liver biochemistry and nearly normal liver histology with no or insignificant necroinflammation and fibrosis. Performing liver biopsies on these subjects was to be more confirmatory of the diagnosis of OHB as we could not completely rule out false-positive HBV DNA results obtained by very sensitive assay. In addition, there are no data on the possible liver injury and intrahepatic vireological status in these subjects. The serum HBV DNA levels were very low, with the highest value of 14.1 IU/ml (19.4 IU/ml in cohort 1). This is consistent with the very low or undetectable total intrahepatic HBV DNA, indicating that HBV inside the liver is replicating at an extremely low rate. It has been shown that most patients with CHB with HBsAg seroclearance still have detectable total intrahepatic and cccDNA.

In the present study, the undetectable cccDNA in the liver tissues is likely to be related to the extremely low viral load. This suggests a favourable long-term prognosis in donors with OHB. However, there are still two main concerns for these OHB donors despite the low level of viral replication. There is a possibility of reactivation of the hepatitis B disease if these OHB donors should require immunosuppressive therapy in the future, especially in regimens containing rituximab. Also the low viral load is still of a theoretical concern in transmitting HBV. It has been shown that as few as 1–10 HBV particles can infect chimpanzees.

One limitation of the present study was that the prevalence of OHB elucidated by the present study may not be totally representative for the general population in Hong Kong since it recruited blood donors who were relatively young (mean age of 31.5 years, table 1) and healthy. In conclusion, with the prevalence of only 0.1% of the population having OHB in an endemic area of high HBV prevalence, the impact on the transfusion services is expected to be low. Implementation of universal screening of OHB in blood transfusion services should be determined with the consideration of both the disease prevalence and the cost incurred by the programme.

Acknowledgements The authors are indebted to Elizabeth Kin-Ming Chua, Vincent Wing-Shun Ngai, Connie Wai-Fan Ng, Maggie Shuk-Ying Wong, Mario Man-Lit Tang, Jeannie Yi-Lam Cheung, Cookie Wing-Wa Tang and all the involved staff in the Red Cross Blood Transfusion Centers for their assistance in conducting the study.

Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES
An unusual inflammation of the colon
A 27-year Caucasian man received an unrelated donor bone marrow transplant for severe aplastic anaemia. Six weeks later he was re-admitted to hospital with a febrile illness. He developed profuse non-bloody diarrhoea and cervical lymphadenopathy was detected. Blood tests showed pancytopenia and peak concentrations of C-reactive protein 100 mg/l, alanine aminotransferase 357 U/l and alkaline phosphatase 197 U/l. Standard stool microscopy and culture did not reveal any abnormalities and tests for Clostridium difficile toxins a and b were negative. Flexible sigmoidoscopy revealed the following appearances from the rectum to the extent of the examination (figure 1).

**QUESTION**
What is the cause for this atypical colitis and how would you treat it?

See page 1427 for the answer

**Editor’s quiz: GI snapshot**

**Figure 1** Endoscopic view of the rectal mucosa.
Prevalence of occult hepatitis B infection in a highly endemic area for chronic hepatitis B: a study of a large blood donor population

Man-Fung Yuen, Cheuk-Kwong Lee, Danny Ka-Ho Wong, et al.

Gut 2010 59: 1389-1393 originally published online July 30, 2010
doi: 10.1136/gut.2010.209148

Updated information and services can be found at:
http://gut.bmj.com/content/59/10/1389.full.html

These include:

References
This article cites 34 articles, 2 of which can be accessed free at:
http://gut.bmj.com/content/59/10/1389.full.html#ref-list-1

Article cited in:
http://gut.bmj.com/content/59/10/1389.full.html#related-urls

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Hepatitis B (61 articles)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/