Can Potentially Infectious Specimens Containing Hepatitis B Virus Be Identified on the Basis of Their Biochemical Profile?

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The potential infectivity of 1129 randomly selected plasma specimens was directly assayed by hepatitis B virus (HBV) DNA dot-hybridization. Presence or absence of HBV was then correlated with a biochemical profile of 20 common analytes obtained on these same specimens. We found that potentially infectious specimens could not be identified on the basis of any combination of simple biochemical tests; indeed, the infectious specimens were more "normal" in some tests of liver function than were the non-infectious specimens.

Although good laboratory practice requires that all specimens be treated as if they were potentially infectious, it is common laboratory folklore that more care should be taken with specimens that are grossly icteric or found to have markedly abnormal tests such as "raised liver enzymes."

Previous studies examining the prevalence of hepatitis B virus (HBV) in clinical specimens have made use of markers such as the surface antigen (HBsAg, Australia Antigen) to infer the presence of this virus. However, only a small proportion of all HBsAg-positive specimens may contain intact viral Dane particles and be potentially infectious (1). Recent developments in molecular biology now permit direct detection of the HBV viral genome in serum or plasma, using DNA hybridization with a radiolabeled, specific HBV probe. The presence of HBV-DNA correlates closely with other markers of infectivity, such as endogenous DNA polymerase (2), and the e-antigen (1, 3, 4). Thus, assay for the presence of HBV-DNA should identify those specimens that are potentially infectious.

We studied a large number of randomly selected clinical specimens and measured a number of common biochemical analytes in them. We then compared these results with those for the presence or absence of HBV-DNA in the specimens. In this way, we were able to answer the question whether specimens that are HBV positive can be identified by use of commonly done biochemical tests.

Materials and Methods

Patients' specimens. We analyzed 1129 plasma samples in this study: unselected, consecutive specimens received in the laboratory of a large metropolitan tertiary referral hospital on three randomly selected days covering a period of approximately three weeks. The specimens were coded and analyzed in a Technicon SMA analyzer. The coded samples were then frozen at -20 °C, and analyzed later for HBV-DNA, in batches.

Biochemical analyses. The following analytes were measured in the specimens: sodium, potassium, chloride, total CO₂, glucose, creatinine, urea, calcium, phosphate, uric acid, total protein, albumin, total bilirubin, alanine and aspartate aminotransferases, alkaline phosphatase, glutamyltransferase, iron, cholesterol, and triglyceride.

Hepatitis B virus DNA dot hybridization. The DNA diagnosis probe used was pHBV.CB, which consists of the entire genome of the hepatitis B virus (subtype adw), cloned in the bacterial plasmid pBR322 (5). This probe hybridizes to all the known serotypes of HBV. The HBV insert was excised from the plasmid vector prior to use, and was radiolabeled with ³²P to high specific activity.

Specimens were immobilized on nitrocellulose membranes by the method of Scotto et al. (3) and hybridized to the DNA probe. After removal of unbound probe, positive specimens were identified by autoradiography. We determined the detection limit for the assay by including known amounts of pHBV.CB DNA on the nitrocellulose membrane; the limit was always better than 40 fmol/L.

Statistical analysis. The SPSS program (6) was used for analysis of results.

Because of the skewed distributions of the results for the HBV-negative specimens and the uncertainty about the shape of the distributions for the HBV-positive specimens, we used a nonparametric test (the Mann-Whitney U test) for comparison of groups. Comparison of groups by use of a parametric test (t-test) was confined to the analysis of certain analytes only.

Discriminant function analysis was performed using the Wilks' lambda (u-statistic) and univariate F-ratio method.

Results

Our sample population consisted of 1129 randomly selected specimens, derived from 1025 patients. Of these specimens, six were subsequently found to be HBV-DNA positive. These six specimens consisted of four specimens from one patient and two from a second.

We compared the biochemical analyses of the HBV-DNA positive and negative populations, using the SPSS program. We found that the HBV-DNA positive population had statistically significantly higher values for the aminotransferases and glucose, and statistically significant lower values for total bilirubin, albumin, calcium, and iron (Table 1) than did the HBV-DNA-negative group. Although the HBV-DNA-positive group also had lower values for the

| Table 1. Comparison of Results from HBV-DNA-Positive and Negative Specimens* |
|-----------------------------|--------------|----------------|------------------|
|                            | HBV -ve (n = 1123) | HBV +ve (n = 6) | p                |
| HBV-DNA-positive group higher |                     |                |                  |
| Alanine aminotransferase, U/L | 19.6           | 65.0           | <.001            |
| Aspartate aminotransferase, U/L | 27.6           | 43.0           | <.01             |
| Glucose, mmol/L            | 5.8            | 8.9            | <.001            |
| HBV-DNA-positive group lower |                     |                |                  |
| Albumin, g/L              | 41.9           | 35.5           | <.05             |
| Bilirubin, μmol/L         | 8.1            | 5.5            | <.05             |
| Calcium, mmol/L          | 2.33           | 2.22           | <.05             |
| Iron, μmol/L             | 12.03          | 4.55           | <.05             |

*The Mann-Whitney U-test was used for comparison of medians. No significant differences were found for Na, K, Cl, total CO₂, creatinine, urea, total protein, alk. phosphatase, glutamyltransferase, uric acid, phosphate, cholesterol, or triglyceride.

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other two enzymes (alkaline phosphatase and glutamyltransferase), these differences were not statistically significant.

Discriminant function analysis was performed in an attempt to define a discriminant function that would distinguish the two groups. Although the discriminant function was able to correctly classify 99.20% of cases, classifying all samples as negative would have given 99.47% success. The discriminant function correctly classified three of the six HBV-DNA-positive samples (involving samples from both positive patients), including two high-titer HBV-DNA-positive samples. The function also incorrectly classified six HBV-DNA-negative patients, only one of whom had evidence of a hepatic illness—which was not hepatitis B.

Discussion

Using the newly developed HBV-DNA dot-hybridization assay, we have been able to assess the probable infectivity of a large number of plasma samples submitted to a routine clinical biochemistry service.

It would appear that the common practice of treating icteric samples, and also those with very high "liver enzymes," as being more infectious than usual is not only bad laboratory practice, it is also completely wrong. We have found that specimens that were HBV-DNA positive were significantly less icteric than those that were HBV-DNA negative.

The higher values for the aminotransferases in the HBV-DNA positive specimens are unlikely to be of predictive use. In spite of being statistically significant at the 0.001 level, the differences in mean and median were of small magnitude, and the range of values encountered in the HBV-DNA positive group encompassed the entire range of values of the negative group.

Similarly, although albumin was found to be slightly lower in the positive group than in the negative group, this is too nonspecific a finding to have predictive use. The other markers of liver disease measured, such as alkaline phosphatase, glutamyltransferase, and urea, were lower in the HBV-DNA positive group than in the negative group, but these differences were not statistically significant.

Other differences noted between the two groups were the values for calcium, iron, and glucose. The difference in the case of calcium can be accounted for entirely by the difference in albumin concentration already noted. The higher values for glucose in the positive group were caused by the development of a period of glucose intolerance during the convalescent phase of the illness of the positive patient from whom the four positive specimens were obtained. Glucose values for the other four HBV-DNA samples tested were entirely within the normal range, so this analyte also is not useful for predictive purposes. The lower value for iron noted in the HBV-DNA positive group probably represents a real difference between the two groups, and may be a reflection of the well-described decrease in iron concentra-

tions in disease. As such, it again represents a very nonspecific finding.

Discriminant function analysis failed to provide any useful function that could adequately distinguish the two groups by use of any combination of these 20 biochemical tests. This is not to say that there are no biochemical abnormalities present in HBV-DNA positive specimens in general. We have studied other specimens from patients selected for the presence of HBV-DNA and liver disease and these specimens frequently have gross derangements in their biochemical analyses. However, selection of patients on the basis of other than their HBV status (as in this study) avoids the biasing of the patient population in favor of selecting abnormalities of liver-function tests.

Thus, it would seem that the time-honored belief—that some specimens are safer than other specimens and that the very worst ones are those with the most abnormal biochemical tests—is indeed a myth, and that there are sound reasons for treating all specimens as if they were potentially infectious. Further to emphasize the silent biochemical state of the HBV-DNA positive patient, the four HBV-DNA positive samples arising from different venipunctures from the same patient were obtained from a patient previously documented as being HBeAg negative, who was undergoing plasmapheresis at the time of the study and who had apparently become infected with HBV in the interim since the original serology without sufficient change in his biochemical profile to warrant repeat hepatitis serology testing; indeed, his incidental detection in the course of this study was the first indication of his seroconversion.

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References