Significance of tests for HBeAg and anti-HBe in HBsAg positive blood donors

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SUMMARY A sensitive radioimmunoassay method for HBeAg and anti-HBe allowed 98.4% of HBsAg positive blood donors to be classified. HBeAg was more prevalent in blood donors under 30 years of age and anti-HBe was more prevalent in those over 30 years. The mean HBsAg concentration was four times greater in donors with HBeAg than in those with anti-HBe. No significant associations were found with sex, blood groups, or HBsAg subtypes. It is likely that this test system will be extremely useful to blood transfusion centres and hepatitis reference laboratories in the future.

Since the description in 1972 of hepatitis B e antigen (HBeAg) and its antibody (anti-HBe) by Magnus and Espmark¹ the system has attracted attention because of its apparent clinical significance. Most workers now consider HBeAg to be a marker of infectivity; conversely, in hepatitis B surface antigen (HBsAg) carriers, the presence of anti-HBe may indicate low infectivity.² Tests for these markers therefore may have important practical uses in transfusion centres for (a) counselling HBsAg positive blood donors, and (b) advising the medical, dental, and nursing professions on the care of these donors.

Previous large-scale reports have tended to describe communities with differing carrier rates of HBsAg from those seen in the United Kingdom. Many of these reports have been based on insensitive tests for HBeAg and anti-HBe with the result that a substantial proportion of donors could not be classified. A sensitive radioimmunoassay (RIA) has been shown to increase the proportion of HBsAg positive sera that may be classified in the HBeAg and anti-HBe system.^{3,4}

An evaluation of the Abbott HBe RIA test has given us the opportunity to survey in detail the distribution of HBeAg and its antibody in our donor population and to consider the possible practical value of tests for 'e' status in blood transfusion practice.

Donors, materials, and methods

The sera tested were obtained from 246 HBsAg positive volunteer blood donors in the West of Scotland.

HBeAg was detected by solid-phase radioimmunoassay (RIA, Abbott).

HBeAg and anti-HBe determinations were performed by solid-phase radioimmunoassay (RIA, Abbott). Repeat testing was performed to confirm all positive reactions.

HBeAg subtyping was performed by our micro solid-phase radioimmunoassay. Polystyrene beads of 3.2 mm diameter (Precision Plastic Ball Co, Chicago, Ill, USA) were coated with guinea-pig monospecific anti-d or anti-y serum. The coated beads were dispensed into the reaction wells of a V-shaped microtitre plate, and 0.1 ml of test serum or plasma was added. Each serum was tested in duplicate against both anti-d and anti-y beads. The plates were sealed and left overnight at 22°C. The tests were washed four times with 0.3 ml distilled water, and 0.1 ml human ¹²⁵I-labelled anti-HBs (Abbott) reacting with both *ad* and *ay* subtypes was added. After incubation at 45°C for 1 hour in a water bath, the tests were washed four times and the beads were transferred to counting tubes. Each tube was counted for 1 minute in a gamma counter, and the ratio of test cpm/negative mean cpm was determined. HBsAg subtype *ad* produced higher ratios with anti-d coated beads compared with anti-y

coated beads; the opposite was true for HBsAg subtype *ay*.

Radioimmunoassay was also used to measure the concentration of HBsAg in the samples. Ten four-fold dilutions were prepared for each HBsAg positive sample. The diluent consisted of 1 part group AB serum and 9 parts 150 mM sodium chloride. Ten standards of known HBsAg concentration (20 ng/ml to 2 ng/ml) were also prepared in this diluent. Radioimmunoassay for HBsAg was carried out on each test dilution and standard. Diluent was also used as the negative control. The test cpm/negative mean cpm ratio was calculated for each dilution, and estimation of HBsAg concentration was obtained by direct comparison with the ratios obtained for the standards.

Results

Sera from 246 HBsAg positive donors were tested. HBeAg was detected and confirmed in 45 (18.3%) and anti-HBe in 197 (80.1%). Only four (1.6%) sera failed to show the presence of either marker.

The percentage of carriers with HBeAg declined with increasing age, and there was a corresponding increase in the percentage with anti-HBe (Figure).

Percentage of HBsAg positive blood donors with HBeAg and anti-HBe according to age. Comparing the donors under 30 years of age with those over 30 for HBeAg $\chi^2 = 21.6192$; 1 df; $P < 0.001$ and for anti-HBe $\chi^2 = 22.9862$; 1 df; $P < 0.001$.

The higher prevalence of HBeAg among HBsAg carriers under the age of 30 is significant ($P < 0.001$), and the higher prevalence of anti-HBe among carriers over the age of 30 is significant ($P < 0.001$).

No association was found between blood group A and the presence or absence of HBeAg or its antibody (Table 1), although an earlier report by Barr *et al.*⁵ suggested that HBeAg might be less common among donors of blood group A than in the general carrier population. The 105 donor samples in the previous report were re-tested in the present study, and the performance of immunodiffusion is compared with that of RIA in Table 2. All positive immunodiffusion results were confirmed by RIA, but RIA detected both HBeAg and anti-HBe more frequently.

HBeAg was present in 19% of the 211 male donors and in 11% of the 35 female donors. Anti-HBe was found in 79% of the males and in 89% of the females (Table 3). The difference is not significant.

Table 1 Distribution of HBeAg and anti-HBe by blood group

Group	Total	HBeAg	Anti-HBe	Neither
O	130	24	104	2
A	70	14	54	2
B	34	5	29	0
AB	12	2	10	0
All	246	45	197	4
Rh(D) positive	212	42	166	4
Rh(D) negative	34	3	31	0

ABO for HBeAg $\chi^2 = 0.1719$; 2 df; $0.95 > P > 0.9$
and for anti-HBe $\chi^2 = 0.6013$; 2 df; $0.75 > P > 0.7$
Rhesus for HBeAg $\chi^2 = 1.6887$; 1 df; $0.2 > P > 0.1$
and for anti-HBe $\chi^2 = 2.2911$; 1 df; $0.2 > P > 0.1$

Table 2 Immunodiffusion and radioimmunoassay results on 105 sera

	HBeAg	Anti-HBe	Negative
RIA	24	79	2
ID	9	38	55

Table 3 Distribution of sex and HBsAg subtype in relation to the HBeAg/anti-HBe status of 246 carriers of HBsAg

	Total	HBeAg	Anti-HBe	Negative
Males	211	41	166	4
Females	35	4	31	0
HBsAg subtype				
ad	141	28	113	0
ay	99	16	80	3
Unclassified	6	1	4	1

Comparing HBeAg and sex, $\chi^2 = 0.8066$; 1 df; $0.5 > P > 0.3$ and anti-HBe and sex, $\chi^2 = 1.2756$; 1 df; $0.3 > P > 0.25$
Comparing HBeAg and subtypes *ad* and *ay*, $\chi^2 = 0.5308$; 1 df; $0.5 > P > 0.3$ and anti-HBe and subtypes, $\chi^2 = 0.0163$; 1 df; $0.9 > P > 0.8$

HBeAg was found in 20% of the 141 donors whose HBsAg subtype was *ad* and in 16% of the 99 donors of *ay* subtype. Anti-HBe was found in 80% of the *ad* and in 82% of the *ay* donors. All six unclassified subtyping results were from donors with very low levels of HBsAg.

Table 4 shows the results of the quantitative HBsAg assays. All 14 carriers with more than 75 µg/ml of HBsAg were HBeAg positive. The mean HBsAg concentration of samples found to be HBeAg positive was 72 µg/ml and anti-HBe positive 17 µg/ml, and neither detected 10 µg/ml. Although the mean HBsAg concentration in HBeAg positive individuals was four times greater than in those with anti-HBe, it should be noted that HBeAg was found at other levels of HBsAg concentration, one even as low as 4 ng/ml.

Table 4 Relationship of HBsAg concentration to HBe/anti-HBe status of 246 carriers of HBsAg

	HBsAg concentration (µg/ml)				Total
	<1	1-25	26-75	>75	
HBeAg	7	10	14	14	45
Anti-HBe	42	110	45	0	197
Neither	2	2	0	0	4
Total	51	122	59	14	246

Three samples in the panel were from donations that had been involved in post-transfusion hepatitis B cases. All three samples were found to be HBeAg positive.

A follow-up of eight HBeAg positive blood donors has revealed that two donors seroconverted from HBsAg HBeAg to anti-HBs anti-HBe, one donor seroconverted from HBeAg to anti-HBe while remaining HBsAg positive, and the remaining five were still positive for both HBsAg and HBeAg after one to four years.

A follow-up of one of the four donors giving unclassifiable results showed that this donor had seroconverted from HBsAg positive to anti-HBs anti-HBe positive. As the HBsAg concentration of the original sample was 10 ng/ml, it appears that the donor was in the early acute phase of viral hepatitis B at that time.

Discussion

In the whole survey, the percentages of HBeAg and anti-HBe were 18.3% and 80.1%, respectively, while 1.6% were unclassifiable. The unclassified results were in four donors, two of whom had very low levels of HBsAg (less than 10 ng/ml), and were most probably in the early acute phase of viral

hepatitis; the other two unclassified results were associated with higher levels of HBsAg and could be explained by these donors being in the process of seroconversion from HBeAg to anti-HBe.

In our population of HBsAg positive donors, HBeAg was present in 29.7% and anti-HBe in 68.0% of those under the age of 30 years, while in those over 30 years HBeAg was present in 5.9% and anti-HBe in 93.2%. This decreasing frequency of HBeAg with age and increasing frequency of anti-HBe with age confirmed earlier reports^{5,6} but was in contrast to a French study.⁷ The reduction in HBeAg with increasing age may be attributed to a tendency of HBeAg positive carriers to seroconvert to anti-HBe or to some progressive change in the clinical and epidemiological pattern of hepatitis B carriage. As such a change could be associated with an increase in hepatitis B among parenteral drug abusers, and as most of the drug-associated hepatitis in our region is of subtype *ay*,⁸ we examined markers according to the HBsAg subtype; no difference was found. Moreover, as our results show no difference in the relative numbers of *ad* and *ay* carriers between the younger and older age groups, it seems that the drug culture has had little or no impact on our HBsAg carrier blood donor population.

The similar distribution of HBe markers in *ad* and *ay* subtypes agrees with the report of Tachibana *et al.*⁹ but not with that of Ukkonen *et al.*,¹⁰ who found anti-HBe significantly more commonly among donors with subtype *ad* than among those with subtype *ay*.

Radioimmunoassay increased the HBeAg rate from 8.6% (by immunodiffusion) to 22.9% and the anti-HBe rate from 36.2% to 75.2% in a parallel study on 105 of the 246 HBsAg positive carriers. Biswas *et al.*³ reported a similar increase in sensitivity of radioimmunoassay over immunodiffusion.

The previously suspected reduction of HBeAg in blood group A donors⁵ has not been substantiated.

In this study males had HBeAg more often than did females and anti-HBe less often than females. A minimal sex difference was also reported in a Finnish study, but Courouce-Pauty and Plancon⁷ found a significant increase in HBeAg among male carriers.

The mean HBsAg concentration in HBeAg positive individuals was found to be four times greater than in those with anti-HBe. Other studies produced similar results.^{7,11} This cannot, however, be taken as an indication that blood donor screening may be undertaken using insensitive test systems. HBeAg can certainly be present with very low levels of HBsAg. A unit of HBeAg positive platelets containing HBsAg at a level of 40 ng/ml was transfused to an individual who subsequently had asymptomatic

HBs antigenaemia. In this particular case the outcome of the HBV infection was probably modified by the use of anti-HBs immunoglobulin.

Although only limited data have been presented on the infectivity of HBsAg, other reports have associated HBeAg with high infectivity and anti-HBe with low infectivity.¹²⁻¹⁴

Seroconversion from HBeAg to anti-HBe has been described during most acute HBV infections.^{4,15} From our age-related data and the one instance of HBe seroconversion in a chronic HBsAg carrier, it appears that seroconversion from HBeAg to anti-HBe can occur in the natural history of the carrier state. However, the data on this subject are limited and can be resolved only by an active follow-up programme on all HBsAg HBeAg positive blood donors.

Systematic testing of HBsAg positive donors for HBeAg and its antibody has practical advantages to regional transfusion centres. It should allow the transfusion services to offer realistic advice to the donor regarding his/her infectivity to others, while at the same time allowing medical, dental, and nursing professions to be accurately briefed on the level of precautions necessary for the safe management of the individual carrier. For example, in a recent case, it was possible to advise medical staff and other rescuers that a HBsAg positive donor involved in a serious accident had anti-HBe, so allowing a more rational view to be taken. Finally, when a sufficient number of donors has been followed up it may become possible to give a much more accurate prognosis to HBsAg positive carriers, and to select those who may benefit most from attempts to eradicate the virus.

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