

## Serum Hepatitis B virus DNA: relationship with the e-antigen and anti-e status in chronic carriers

S F YAP, FRCPATH, FRCPA, \*NW WONG, MRCP and \*KL GOH, MRCP.

*Departments of Pathology and "Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur.*

### Abstract

The relationship between serum Hepatitis B virus DNA (HBV-DNA) and the Hepatitis B e-antigen/anti-Hepatitis Be (HBeAg/anti-HBe) serological status in Malaysians was studied. 212 cases of asymptomatic HBV carriers were recruited for this study. 92 cases were positive for the HBeAg at the point of recruitment. 85 (92.4%) of these patients tested positive for HBV-DNA, of whom 55 (64.7%) had levels over 100pg/ml of serum. Three of the remaining 7 HBeAg positive cases who were negative for HBV-DNA subsequently seroconverted. The other 4 cases remained negative for HBV-DNA for periods of 6-12 months. Out of 113 cases who were anti-HBe positive, 12 (10.6%) gave a positive HBV-DNA result. 2 of these 12 patients were recent seroconverters; the remaining cases had transiently increased viral replicative activity which later subsided. 7 out of the 212 carriers were in the e-window period; all 7 tested negative for HBV-DNA. Our data confirm a high frequency of HBV-DNA in HBeAg positive carriers and a negative correlation between HBV-DNA and anti-HBe. An atypical profile of anti-HBe associated with HBV-DNA was observed in 10.6% of the carriers. An inverse relationship between serum HBV-DNA levels and age was also observed.

*Key words:* Hepatitis B virus, HBV-DNA, HBeAg, anti-HBe, chronic HBV carriers.

### INTRODUCTION

Replicative activity of the hepatitis B virus has traditionally been measured by serum HBeAg and/or HBV-DNA polymerase level. More recently, the use of molecular hybridization assays for the detection of the viral genome has become more widely available. Detection of the viral genome in serum provides a direct measure of viral replicative activity and thus the level of infectivity of a particular patient. Studies have pointed to a direct relationship between the HBeAg status and HBV-DNA positivity.<sup>1-4</sup> Conversely, subjects who are anti-HBe positive generally are negative for HBV-DNA. However, HBV-DNA has been reported to be persistently detectable in a proportion of anti-HBe positive carriers, particularly in patients of Mediterranean and Oriental extract.<sup>4-10</sup> This study describes the results of testing for the presence of serum HBV-DNA in our chronic HBV carrier patients who were regularly followed up in our "HBV carrier" clinic. The objectives were to determine the relationship between the HBeAg/anti-HBe status and HBV-DNA and to see whether there is, in the Malaysian carrier population, a subgroup who shows discordant results with the presence of HBV-DNA in anti-HBe positive sera, or its absence in HBeAg positive sera.

### MATERIALS AND METHODS

#### *Patients*

Chronic HBV carriers who were regularly seen at our follow-up clinic were recruited. These patients routinely have their serological profile and biochemical status monitored at each visit. In addition, we tested their serum for the presence of HBV-DNA as an additional marker of viral activity. Patients were followed up at about 6 monthly intervals. All cases studied were asymptomatic.

#### *Laboratory investigations*

Hepatitis B surface antigen (HBsAg), HBeAg and anti-HBe were tested by radioimmunoassay using commercial kits (Abbott Laboratories, Chicago, 11). Biochemical tests performed included the liver profile. Liver enzymes including aspartate and alanine transaminases were measured. The upper limits of the reference range for these enzymes are 40 and 54 IU/L respectively. HBV-DNA assay was performed using the dot-blot filter hybridization technique. The HBV DNA assay is an in-house method based on the indirect serum blot procedure. Briefly, the viral nucleic acid is isolated from sera by ultracentrifugation over a sucrose gradient

followed by proteinase K treatment. The nucleic acid is recovered by phenol extraction and ethanol precipitation. The ethanol precipitate is then resuspended in 5x SSC and applied onto a nylon membrane, dried, denatured and fixed by UV irradiation before prehybridization and hybridization. Hybridization is carried out using a <sup>32</sup>P-labelled HBV DNA derived from a recombinant plasmid, pHBV.<sup>11</sup> Assay sensitivity was about 1pg or less. Results for HBV-DNA were expressed as pg/ml of serum; the limit of detection was about 2-3pg/ml of serum.

**RESULTS**

A total of 212 consecutive HBV carriers were studied. 92 cases were HBeAg positive, 113 were anti-HBe positive and 7 were negative for both serological markers. 165 cases were males and 47 were females; their ages ranged from 9 to 64 years with a mean of 30 years. The results of HBV-DNA testing are shown in Table 1. Of the 92 HBeAg positive subjects, 85 (92.4%) had detectable HBV-DNA in their serum. HBV-DNA levels were greater than 100pg/ml in 55 out of 85 (64.7%) of these cases (Table 2). 3 out of the 7 cases who tested negative for HBV-DNA were in the period of seroconversion from HBeAg to anti-HBe. The remaining 4 subjects became HBV-DNA positive on subsequent follow-up. HBV-DNA was associated with the anti-HBe status in only 12/113 (10.6%) of the carriers. 2 of the 12 cases were seroconverting. Serum HBV-DNA was not detectable on subsequent testing in the other 10 carriers. The HBV-DNA levels were less than 100pg/ml in all except 1 subject (Table 2). Follow-up HBV-DNA analysis of this subject were all negative.

Serum transaminases were elevated in 42.4% (36/85) of patients in the HBeAg/HBV-DNA positive subgroup, and in 50.0% (6/12) of patients in the anti-HBe/HBV-DNA positive subgroup (Table 3). The distribution of HBV-DNA levels

**TABLE 1:** Relationship between HBe/anti-HBe status and serum HBV-DNA

HBe/anti-HBe	HBV-DNA		Total
	Positive (%)	Negative (%)	
+/-	85 (92.4)	7 (7.6)	92
-/+	12 (10.6)	101 (89.4)	113
-/-	0	7 (100.0)	7
<b>Total</b>	<b>97</b>	<b>115</b>	<b>212</b>

**TABLE 2:** Serum HBV-DNA in HBeAg and anti-HBe positive carriers

HBV-DNA (pg/ml)	HBe/anti-HBe status	
	HBeAg + (%)	anti-HBe + (%)
< 10	19 (22.4)	9 (75.0)
10 -	7 (8.2)	1 (8.3)
50 -	4 (4.7)	1 (8.3)
>100	55 (64.7)	1 (8.3)

among this group of carriers is shown in Figure 1. It appeared that cases with low levels of HBV-DNA had raised serum transaminases more frequently than those with higher levels of HBV-DNA. However, this trend was not statistically significant. There was also no definite relationship observed between the levels of serum transaminases and serum HBV-DNA. In comparison, only 14.9% (15/101) of anti-HBe positive/HBV-DNA negative carriers had raised serum enzymes. Similarly, among the 7 HBeAg positive carriers who were negative for HBV-DNA, all but one had normal serum enzymes. In the majority of cases who had raised enzymes, the levels were less than twice the upper limit of normal.

The inter-relationship between age and HBV-DNA status is demonstrated in Figure 2. The occurrence of HBV-DNA in sera declined with age falling from 64.0% in the 21-25 year age group to 18.2% in the over 40 year age group (chi-square test for trend: X<sup>2</sup>=24.809, p<0.05). High levels tended to occur more frequently in younger patients. Conversely, the frequency of anti-HBe positive carriers increased with age as illustrated in Figure 3. Despite the relatively young age of our HBV carriers, a definite trend is discernible.

**DISCUSSION**

The results of the present study conform to that

**TABLE 3:** Distribution of HBV carriers with elevated serum transaminases

HBe/anti-HBe status	HBV-DNA	
	+ (%)	- (%)
+/-	36/85 (42.4)	1/7 (14.3)
-/+	6/12 (50.0)	15/101 (14.8)
<b>Overall</b>	<b>42/97 (43.3)</b>	<b>16/108 (14.8)</b>

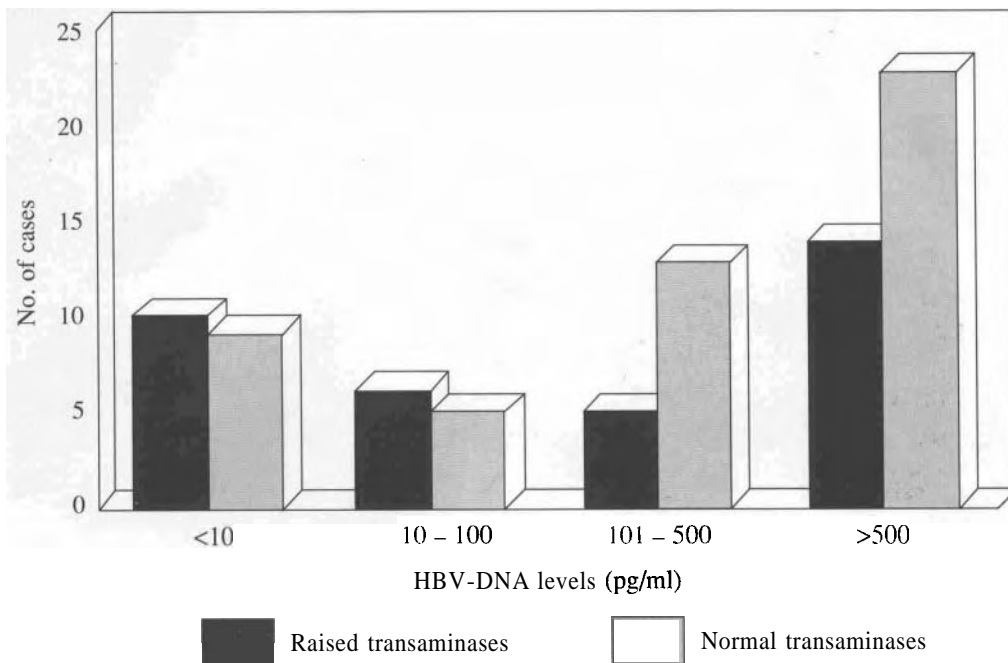


FIG. 1: Distribution of HBV-DNA levels among carriers with/without raised transaminases.

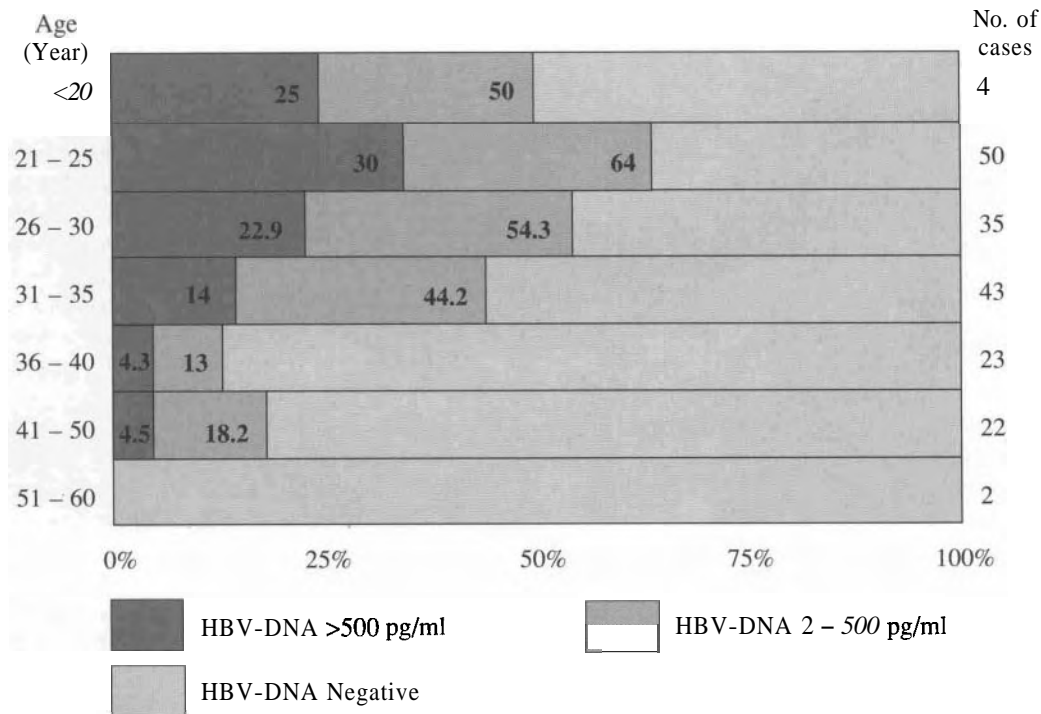


FIG. 2: Serum HBV-DNA in relation to age.

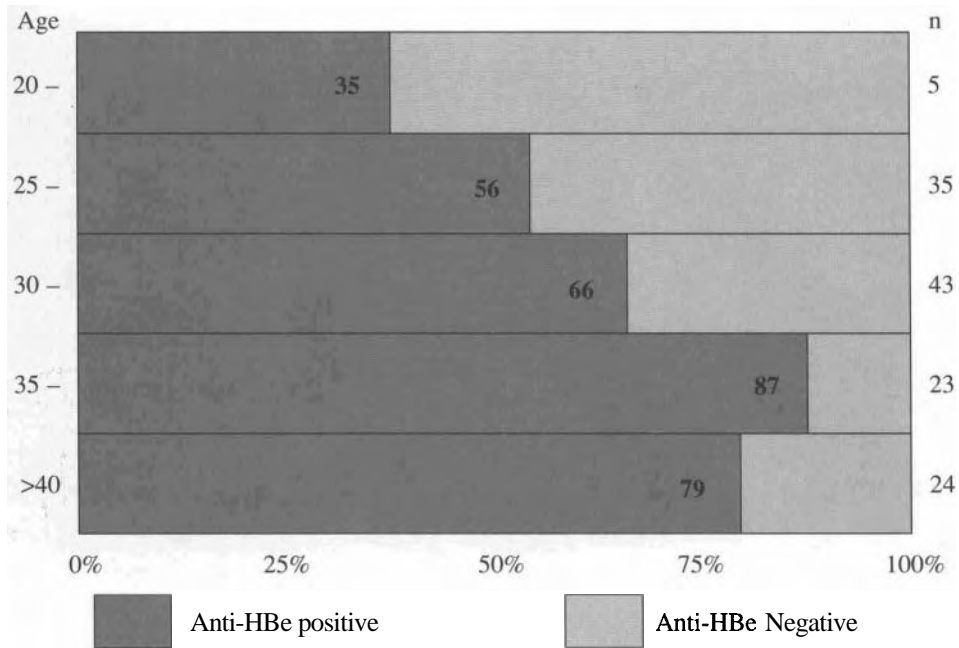


FIG. 3: Distribution of anti-HBe positive carriers by age.

of other workers who demonstrated that serum HBV-DNA is detectable in the majority of patients who are seropositive for the HBeAg.<sup>1-4</sup> The levels of HBV-DNA in the sera of the 85 HBeAg seropositive carriers were variable ranging from about 2pg/ml to over 1000pg/ml; the majority of subjects had levels above 100pg/ml. We also found detectable levels of serum HBV-DNA in about 11% of HBeAg negative subjects. Similar results have been reported, both in asymptomatic HBV carriers and in patients with HBV related chronic liver diseases,<sup>6-10</sup> supporting the premise that continued viral replication does occur despite seroconversion from HBeAg to anti-HBe. Recent studies have pointed to one possible explanation for this observation in some of the cases; the failure to produce HBeAg was reported to be related to **mutation(s)** in the pre-core region of the HBV genome.<sup>12,13</sup> The most commonly described mutation involves a base substitution in the second last codon of the pre-core genome resulting in the introduction of a stop codon and the failure of synthesis of the HBe protein. However, the replicative competence of the vims remains intact. The emergence of this HBeAg negative mutant (HBVe-) is reported to be dominant at the time of seroconversion. In a proportion of these cases, viral replication was observed to be persistent and associated with progressive and severe liver disease. In others, the HBVe- virus persists without associated inflammatory liver disease.

In our HBeAg negative carriers, the association with HBV-DNA was transient, lasting no longer than 12 months in all cases. We believe that the presence of serum HBV-DNA in these cases was due to temporary reactivation of viral replication, part of the natural course of the chronic carrier state. If at all there was acquisition of the mutant virus, it would appear to be a temporary phenomenon. Further studies is necessary to either confirm or refute this suggestion. There was no evidence of severe liver disease, either biochemically or clinically in this group of carriers who showed the atypical profile of anti-HBe positivity associated with HBV-DNA positivity. Further, the failure to detect HBV-DNA in the rest of the anti-HBe positive carriers do not necessarily mean non-replication of the virus. It may merely be a reflection of the limit of sensitivity of the assay used for the detection of the viral genome in sera. With the use of the polymerase chain reaction to amplify the viral DNA, it is highly likely that a larger proportion of anti-HBe positive carriers will be found to show viral replicative activity, albeit at a low level.

Seven cases showed a negative association between HBeAg and HBV-DNA. Serological follow-up showed that 3 of these cases later acquired anti-HBe, a finding also reported by other workers.<sup>4</sup> It appeared that declining viral replication heralded the onset of seroconversion in these 3 carriers. In the remaining 4 cases, this

was a transient phenomenon. Follow-up HBV-DNA monitoring of the carriers indicated that the latter was not an uncommon occurrence in our patients. It is not unreasonable to expect viral replicative activity to fluctuate during the course of the carrier state. That the HBeAg remained positive while replicative activity is low is intriguing; we are unclear as to the explanation for this observation. In the majority of anti-HBe seropositive cases, viral replicative activity remained undetectable by hybridization analysis. In the 12 cases who had detectable serum HBV-DNA, the levels were low with the exception of one case who subsequently became HBV-DNA negative. During the e-window period, HBV-DNA was undetectable in all 7 cases studied.

Serum transaminases were increased in over 40% of the carriers who had detectable HBV-DNA, irrespective of their HBeAg status. The association between HBV-DNA and liver disease in chronic HBV carriers has been noted in various studies.<sup>6,7,14</sup> It has been suggested that during the replicative, HBV-DNA positive phase of chronic HBV infection, the severity of liver cell inflammation varies inversely with serum levels of HBV-DNA. High levels of serum HBV-DNA would therefore be expected to be associated with minimal liver enzyme changes and low levels of HBV-DNA with increased transaminases. On the other hand, in the non-replicative, HBV-DNA negative phase, there appears to be only minor hepatic changes.<sup>15-17</sup> Our data is in agreement with the latter observation in that the majority of the anti-HBe seropositive/HBV-DNA negative carriers had normal transaminases. In contrast, among the anti-HBe positive/HBV-DNA positive carriers, 6/12 had raised enzymes. All 6 had low serum HBV-DNA levels. Raised transaminases was observed in 36/85 (42.4%) HBeAg positive/HBV-DNA positive carriers. Although there appeared to be an inverse relationship between the levels of HBV DNA and the frequency of raised transaminases, this trend was not statistically significant. Likewise, no relationship was observed between the levels of serum transaminases and HBV DNA. This would appear to contradict the suggestion that during the replicative phase of chronic HBV infection, liver cell inflammation varies inversely with serum HBV-DNA levels. However, we take note that all our cases were asymptomatic HBV carriers with no clinical evidence of chronic liver disease. In addition, the observation that the enzyme elevation in most instances were less than 2x the upper limit of normal suggests that hepatic

involvement, if any, was likely to be minimal. Nevertheless, we do not have histological data for confirming the presence or absence of liver disease or the extent of hepatic pathology, if any, in our cases.

The inverse relationship between HBV-DNA positivity and age of the carriers is not an unexpected finding. The natural course of chronic HBV infection is a decline of replicative activity with time. Using the anti-HBe status as an alternative indicator, a similar picture emerges.

## ACKNOWLEDGEMENTS

We are indebted to Mrs E Barton, Mr KC Lee and Mr HC Wong for technical assistance. This work was supported by the "R&D programme 3/07/04/022" funded by the Ministry of Science, Technology and Environment of Malaysia.

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