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# HBV-DNA, HBeAg/anti-HBe serological status in hepatitis B chronic individuals from central Italy

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SUMMARY

A population of 488 HBsAg carrier individuals, from central Italy, classified on the basis of biochemical, clinical and histological parameters, was analysed for the presence of HBV-DNA in serum and its relationship with HBeAg/anti-HBe markers. The prevalence of HBV-DNA was 32.8% in chronic patients with biopsy-proven liver disease, and 20 and 4·3% respectively in asymptomatic carriers with and without altered ALT levels. The values in chronic patients were correlated with the histological activity.

Concordance of HBV-DNA presence and HBeAg positivity was observed in only 61·4% of cases. However HBV-DNA prevalence in sera of anti-HBe positive individuals was very low in asymptomatic carriers with normal ALT levels (2·5%). Higher values were observed in anti-HBe positive chronic patients (15·8%) and in carriers occasionally found with changes in ALT without any other clinical sign of illness (16·7%). These data would indicate that HBV-DNA is the serological marker which is most closely related to liver disease.

## INTRODUCTION

The evolution of hepatitis B infection from the acute into the chronic and persistent phases is related to viral replication and its interaction with the host, and is associated with the presence of various serum markers such as HBeAg, anti-HBe, HBV-DNA, DNA-polymerase activity and with the presence of HBcAg in hepatocytes.

The prognostic value of persistence, and relative presence, of these markers particularly HBV-DNA, HBeAg and anti-HBe in the sera of HBsAg-positive carrier individuals is not completely defined. In this study differences were seen

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which were related to the stage of chronic infection, with severity of liver disease and with geographical origin of patients [1–3]. This work has been carried out on a large sample of HBV carriers from a well-defined area of central Italy (Latium) with an intermediate level of HBsAg endemicity and with a predominantly horizontal mechanism of transmission. The objective was to evaluate the prevalence of HBV-DNA and its correlation with the HBeAg/anti-HBe system in various groups of HBsAg-carriers classified according to clinical, biochemical and histopathological parameters.

## STUDY POPULATION

The study was based on a population of 488 HBsAg carrier individuals; 282 males and 206 females of mean age 36·1 years. Carriers were defined as those with steady levels of HBsAg in their serum for more than 6 months. Of these, 134 were patients with chronic hepatitis from departments of Infectious Diseases and Gastroenterology and 354 were individuals undertaking prevaccination screening or check-ups during pregnancy. We have classified as asymptomatic carriers the 354 individuals who showed no clinical symptoms ascribable to either acute or chronic hepatitis involvement, or who had gamma globulin values not exceeding the normal value by more than 10%, prothrombin activity not less than 75% and total bilirubinemia not exceeding 1·5 mg/ml. The population of asymptomatic carriers was mainly hospital staff (110), pregnant women (86), blood donors (46) and family relatives of carriers (36) while the remaining subjects were discovered in the course of routine blood testing. Most of the chronic patients underwent liver biopsy; some cases of cirrhosis were diagnosed on the basis of laparoscopic or laparotomy data. The time-span between the blood sample and the biopsy was always less than 2 months, or in the case of cirrhosis, less than 1 year.

## METHODS

Sera were tested for HBsAg, anti-HBc, HBeAg, anti-HBe and anti-delta (HD) either by commercial ELISA or radioimmunological assays (Abbott Lab. Chicago, USA or SORIN, Torino, Italy). Biochemical assays included the measurement of transaminases, alkaline phosphatase, glutamyl-transpeptidase, prothrombin time, and serum proteins by standard laboratory methods. The assay for HBV-DNA was performed by a rapid filtration hybridization assay. The virus particles were disrupted and the DNA molecules were denatured by treatment with 1 N-NaOH at 65 °C for 5 min. The samples were neutralized immediately before filtration by addition of 1·2 M Tris-HCl (pH 7·5) and 3 M-NaCl. $^{32}$P-nick-translated HBV-DNA (specific activity $2 \times 10^8$ c.p.m./$\mu$g), purified from pA01-HBV (a gift from Professor F. Deinhardt, Munich, FRG) was used as the probe. The filters were autoradiographed for 4 days. The sensitivity of the assay, measured by using a standard control serum [4], was in the range of 0·5–0·1 pg of DNA/20 $\mu$l of serum.

Needle liver biopsies were embedded in paraffin and 6 $\mu$m sections were stained with hematoxylin and eosin. The sections were examined for portal and parenchymal changes and graded for severity. The histologic interpretation was based on the criteria described by Bianchi and colleagues [5].

Table 1. *HBV DNA prevalence in sera of asymptomatic carriers and chronic patients with different histopathological diagnosis*

| Group | Subjects | HBV-DNA positive No. | (%) | | 95% confidence limits |
|---|---|---|---|---|---|
| CPH | 20 | 9 | (45·0) | | 23·2–66·8 |
| CAH | 38 | 16 | (42·1) | | 26·2–57·8 |
| CAH/Cirrhosis | 21 | 5 | (23·8) | | 5·6–42·0 |
| Cirrhosis | 43 | 11 | (25·6) | | 12·5–38·6 |
| HCC/Cirrhosis | 8 | 3 | (37·5) | | 3·9–71·0 |
| Lobular Hepatitis/other | 4* | 0 | | | |
| Total | 134 | 44 | (32·8) | | 26·4–42·5 |
| $\chi^2$ for trend | | 4·25 | | | |
| $P$ value | | 0·039 | | | |
| Asymptomatic carriers | | | | $P$ value*† | |
| Normal ALT levels | 324 | 14 | (4·3) | | 2·1– 6·5 |
| Altered ALT levels | 30 | 6 | (20·0) | 0·004 | 5·7–34·3 |
| Total | 354 | 20 | (5·6) | | 3·2– 8·0 |

*Two cases with normal histology.
†Fisher's exact test.

Table 2. *HBeAg/anti-HBe status in asymptomatic carriers and chronic patients*

| | Subjects | HBeAg positive No. | (%) | Anti-HBe No. | (%) | HBeAg anti-HBe negative No. | (%) |
|---|---|---|---|---|---|---|---|
| Asymptomatic carriers | | | | | | | |
| Normal ALT levels | 324 | 13 | (4·0) | 284 | (87·6) | 27 | (8·3) |
| Altered ALT levels | 30 | 3 | (10·0) | 24 | (80·0) | 3 | (10·0) |
| Chronic patients | 134 | 41 | (30·6) | 82 | (61·2) | 11 | (8·2) |
| Total | 488 | 57 | (11·7) | 390 | (79·9) | 41 | (8·4) |
| $\chi^2$ for trend | | 63·5 | | 40·7 | | < 0·1 | |
| $P$ values | | $< 10^{-6}$ | | $< 10^{-6}$ | | 0·99 | |

Analysis of data was performed by Fisher's exact test and by chi-squared ($\chi^2$) statistics for ordinary two by two tables and by chi-squared for trend (1 D.F.) to assess the significance of any trends.

## RESULTS

Table 1 reports the prevalence of HBV-DNA in the 134 chronic patients and the 354 asymptomatic carriers of which 30 had altered ALT levels. The prevalence of HBV-DNA was 32·8% in chronic patients, compared with 20 and 4·3% in asymptomatic carriers, with and without altered ALT levels respectively. In chronic patients the presence of viral DNA in serum correlated, in most cases, with

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Table 3. *HBV-DNA prevalence and HBeAg/anti-HBe status in asymptomatic carriers and chronic patients*

| | HBeAg positive | | Anti-HBe positive | | HBeAg negative anti-HBe negative | |
|---|---|---|---|---|---|---|
| | No. | HBV-DNA positive No. (%) | No. | HBV-DNA positive No. (%) | No. | HBV-DNA positive No. (%) |
| Asymptomatic carriers | | | | | | |
| Normal ALT levels | 13 | 5 (38·5) | 284 | 7 (2·5) | 27 | 2 (7·4) |
| Altered ALT levels | 3 | 2 (66·7) | 24 | 4 (16·7) | 3 | 0 |
| Chronic patients | 41 | 28 (68·3) | 82 | 13 (15·8) | 11 | 3 (27·3) |
| Total | 57 | 35 (61·4) | 390 | 24 (6·1) | 41 | 5 (12·2) |
| $\chi^2$ for trend | | 3·5 | | 22·4 | | 2·5 |
| $P$ values | | 0·062 | | $< 10^{-5}$ | | 0·11 |

Table 4. *HBV-DNA and anti-HD positivity in asymptomatic carriers and chronic patients*

| | | Anti-HD positive | | Anti-HD negative | | |
|---|---|---|---|---|---|---|
| | Subjects No. | No. | HBV-DNA positive No. (%) | No. | HBV-DNA positive No. (%) | $P$ value* |
| Asymptomatic carriers | 354 | 5 | 0 | 349 | 20 (5·7) | 0·746 |
| Chronic patients | 134 | 37 | 5 (13·5) | 97 | 39 (40·2) | 0·002 |
| Total | 488 | 42 | 5 (11·9) | 446 | 59 (13·2) | 0·518 |

*Fisher's exact test.

histological activity ($\chi^2$ for trend 4·25, $P < 0·05$). A significant trend ($\chi^2$ for trend 68·8, $P < 10^{-6}$) was found among the subgroups of asymptomatic carriers with normal ALT, asymptomatic carriers with altered ALT and patients with liver disease.

The HBeAg/anti-HBe serological status of these patients is reported in Table 2. In the study population most of the carriers were anti-HBe positive (61·2% of chronic patients and 87·6% of asymptomatic carriers). A significant correlation of HBeAg with presence of liver disease was observed ($\chi^2$ for trend 63·4, $P < 10^{-6}$). The relationship between HBV-DNA and HBeAg/anti-HBe presence is shown in Table 3. HBV-DNA was detected in only 38·5% of HBeAg-positive asymptomatic carriers with normal ALT but in 66·7% of those with altered ALT values. In addition HBV-DNA was present among anti-HBe-positive subjects in only 2·5% of healthy carriers with normal ALT levels. It increased to values similar to those observed for chronic patients in individuals who had only an alteration in transaminases without other clinical signs of illness (15·8 and 16·7% respectively; $\chi^2$ for trend 22·4, $P < 10^{-5}$).

Anti-HD prevalence is reported in Table 4. Among chronic liver patients 38

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were anti-HD positive and in this group HBV-DNA prevalence was lower (13·5%) than in anti-HD negative subjects (40·2%). In this context it is relevant to note that, out of 37 anti-HD positive individuals 6 (including the 5 HBV-DNA positive) (16·2%) were positive for HBeAg.

## DISCUSSION

The results of this study confirm that concordance between the presence of HBV-DNA and HBeAg in the sera of HBsAg carriers is not complete [6]. The absence of HBV-DNA in some HBeAg-positive individuals may be due to variations in the clearance time of HBV-DNA, which could be shortened by possible extensive lysis of hepatocytes, while HBeAg clearance is also influenced by the action of the reticuloendotelial system on this soluble antigen [7]. The HBV-DNA marker correlates more closely to the stage of liver illness than does HBeAg. The latter is detected more frequently if the asymptomatic carriers occasionally found with altered transaminase levels are included.

The prevalence of HBV-DNA observed in all the anti-HBe positive subjects is 6·1%. Studies by previous authors have shown that the HBV-DNA prevalence in anti-HBe-positive individuals varies with the stage of chronic disease [8–10] and is also related to the geographical origin of the patients, being more frequent in carriers from Southern Europe, the Middle East and Asia, than in those from Northern Europe and Africa [1, 2, 11–13].

This difference has been explained in terms of the mode of transmission and the duration of hepatitis B infection, as well as genetic or immunologic differences in the host. Geographical differences were also found for the relative presence of HBeAg or anti-HBe [3, 12, 14]. The population considered in the present study comes from a Mediterranean area with an intermediate level of HBV endemicity but has a high prevalence of anti-HBe positive individuals. In these individuals presence of HBV-DNA is more common in chronic patients and in asymptomatic carriers with alterations in ALT levels. In asymptomatic carriers with no alteration in ALT levels, prevalence of HBV-DNA is even lower than expected on the basis of previous studies in which transaminase value were not determined. The lower prevalence of HBV-DNA in chronic patients positive for anti-HD compared with patients negative for anti-HD could be explained by inhibition of HBV replication by HD superinfection, as others have reported [15]. Thus HD infection could have contributed to lowering the overall HBV-DNA prevalence in this group.

These findings show that the HBV-DNA marker is mostly associated with liver disease while its presence in asymptomatic carriers is rare, at least in this region of central Italy, and could be associated to the occurrence of reactivation and fluctuations of viral replication (also in asymptomatic carriers).

However, a consistent number of cases, though not positive for HBV-DNA, may still be positive for HBeAg and/or anti-HD and have markers of liver disease. It is therefore concluded that the combination of all three markers is a better indicator of chronic disease rather than one single marker.

More detailed long-term follow-up studies of HBV-DNA and ALT alteration in anti-HBe positive subjects are required. These would also contribute to the

understanding of the pathogenic mechanisms of hepatitis B-persistent infection and to the results of drug treatment of groups defined by these markers [16].

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