Antiviral Research, 23 (1994) 251–257

AVR 00681

Antiviral Research

Measurement of HBsAg to monitor hepatitis B viral replication in patients on α-interferon therapy

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(Received 6 January 1993; revised manuscript accepted 29 November 1993)

Summary

HBsAg was measured quantitatively in serum samples collected serially before and after the HBeAg seroconversion date from 69 patients with HBeAg seroconversion and 17 patients with both HBeAg and HBsAg seroconversion. In patients with only HBeAg seroconversion the median HBsAg level decreased from 8.39 μg/ml (range 0.01–57.51) before HBeAg seroconversion to 3.53 μg/ml (range 0.002–68.66) after seroconversion ($P<0.001$). No significant drop in HBsAg was found for the control group (18 HBeAg-positive patients without seroconversion). From 12 other patients on α-interferon therapy HBsAg was quantitatively assayed monthly during and after therapy; HBsAg levels were compared to the levels of HBV-DNA and HBeAg. We observed a good correlation between the HBsAg level and both the HBV-DNA ($r=0.76$; $P<0.001$) and the HBeAg ($r=0.70$; $P<0.001$) level, irrespective of the response to α-interferon. Quantified assessment of HBsAg appears promising as a simple and cheap method for monitoring viral replication in chronic hepatitis B in patients undergoing interferon therapy.

HBsAg; HBV-DNA; Chronic hepatitis B; α-Interferon

Chronic hepatitis B is a common liver disease which may progress to cirrhosis with complications as portal hypertension and hepatocellular carcinoma (Beasly et al., 1981; Hoofnagle et al., 1984). With the availability of effective antiviral agents for chronic hepatitis B, quantitative monitoring of hepatitis B virus (HBV) replication has become an important factor in patient management (Perrillo et al., 1990; Zoulim et al., 1992). The aim of antiviral

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SSDI 0166-3542(93)E0067-4

therapy is to induce a transition from active HBV replication to viral latency which is usually accompanied by arrest of disease progression (Fattovich et al., 1986). To monitor HBV replication measurement of HBV-DNA is widely used in research laboratories but not in routine clinical laboratories because of its time-consuming methods and its high cost in material and personnel. There is a need for a simple and cheap assay to assess changes in the level of HBV replication. Hepatitis B "surface" antigen (HBsAg) was the first hepatitis B-related antigen discovered and is by definition present in all chronic hepatitis B patients (Blumberg et al., 1965). HBsAg testing is therefore part of the routine diagnostic assessments in almost every hospital around the world. We investigated the level of HBsAg in patients who exhibited cessation of viral replication, as indicated by clearance of HBeAg and HBV-DNA. Furthermore we determined whether quantitative measurement of HBsAg can be used as a marker of viral replication in patients undergoing α-interferon therapy.

HBsAg was assessed quantitatively in sera collected from 4 different groups of chronic HBsAg carriers (total of 137 patients): Sixty-nine patients exhibited HBeAg seroconversion (group A), 39 after α-interferon therapy (5 MU per day for 4 to 6 months) and 30 spontaneously. Seventeen patients exhibited both HBeAg and HBsAg seroconversion within 6 months (group B), 8 after α-interferon treatment and 9 spontaneously. Thirty-nine HBsAg-positive patients (HBeAg positive $n=18$; HBeAg and HBV-DNA negative $n=21$) without α-interferon therapy and without HBeAg and/or HBsAg seroconversion served as controls (group C). Two serial samples were collected 6 to 12 months before and after the HBeAg seroconversion date; similarly in the control group two sera were collected 12 to 24 months apart. The remaining group (group D) consisted of 12 patients who were followed longitudinally during α-interferon therapy with different outcomes: nonresponse in 6, HBeAg seroconversion in 3 and HBeAg as well as HBsAg seroconversion in 3 patients. α-Interferon was given in a dose of 5 MU daily, in courses of 1 and 4 months duration, separated by 1 month of rest (Janssen et al., 1992). Serum samples of these 12 patients were collected every month during therapy and every 1–2 months afterwards (follow-up 1 year). In addition to quantitative HBsAg measurement, HBV-DNA and HBeAg were assessed quantitatively in the samples from group D. All patients receiving α-interferon therapy were HBeAg and HBV-DNA positive at the start of therapy. HBeAg seroconversion was always accompanied by clearance of serum HBV-DNA. All patients studied were negative for antibodies against the human immunodeficiency virus and hepatitis C and D virus. All control patients were serum HBV-DNA negative. HBsAg was detected by an enzyme immunoassay (EIA) employing enhanced luminescence (Amerlite HBsAg assay, Amersham, UK) according to the manufacturers prescription (Whitehead et al., 1983). The sensitivity of the assay was approximately 0.3 ng/ml of HBsAg (ad and ay). When appropriate, samples were tested undiluted or diluted 1:100 and 1:400 to remain within the detection range of the assay. HBsAg was quantified using a serial dilution curve of a reference sample from the Paul Ehrlich Institute (Langen, Germany)

containing both the ad and ay (mixture 1:1) subtypes of HBsAg. Results were, expressed in micrograms of HBsAg per ml of serum. HBV-DNA was measured by a liquid-phase hybridization assay using an ^{125}I-labelled HBV-DNA probe (Abbott HBV-DNA assay, Abbott, USA) and expressed in picograms per ml of serum; the cut-off of the assay was 1.7 pg/ml. HBeAg was measured by a radio-immunoassay (Abbott HBeAg test, Abbott, USA). For quantification the P/N ratio (counts patient sample/counts negative control sample) was determined. For each patient a fixed serum dilution (1:1, 1:5, 1:25, 1:125 or 1:625) was maintained. HBeAg seroconversion was defined as a P/N ratio below 2.1 (cut off level) for undiluted serum.

The levels of HBsAg before and after seroconversion for HBeAg and HBsAg are illustrated in Table 1. The HBsAg level decreased in 56 of the 69 patients who exhibited only HBeAg seroconversion (group A; median decrease of 59%; $P<0.001$). The median decrease in HBsAg was 59% for patients with α-interferon-induced HBeAg seroconversion and 55% for those with spontaneous HBeAg seroconversion. The difference between the HBsAg levels before and after HBeAg seroconversion was significant for both groups. The median HBsAg level in the initial samples was higher for the patients who underwent both HBeAg and HBsAg seroconversion (group B) – independent of α-interferon therapy – in comparison to those who only exhibited HBeAg seroconversion (group A); these differences were not statistically significant. For both the HBeAg-positive and HBeAg-negative control group (group C) we did not find significant differences between the HBsAg level in the initial and

TABLE 1

Median HBsAg level (range) before and after HBeAg seroconversion or HBeAg and HBsAg seroconversion

		n	HBsAg level (μg/ml)		*P* value
			month 0	month 12	
A					
HBeAg	IFN treatment	39	8.39 (0.01–57.51)	3.53 (0.002–68.66)	< 0.001
seroconversion:	No treatment	30	8.43 (0.02–52.12)	3.63 (0.002–65.99)	0.02
	Total	69	8.39 (0.01–57.51)	3.53 (0.002–68.66)	< 0.001
B					
HBeAg and	IFN treatment	8	32.58 (4.12–42.31)	< 0.001*	N.A.
HBsAg	No treatment	9	12.04 (0.02–61.69)	< 0.001*	N.A.
seroconversion:	Total	17	13.09 (0.02–61.69)	< 0.001*	N.A.
C					
HBeAg-positive controls:		18	25.55 (1.51–75.65)	21.70 (0.03–79.43)	0.87
HBeAg-negative controls:		21	4.96 (0.13–17.12)	6.01 (0.01–17.30)	0.18

*Cuf-off HBsAg assay: 0.3 ng/ml; N.A.: not applicable.

The two-sample Wilcoxon rank sum test was used to analyze unpaired observations and the Wilcoxon signed rank test to analyze paired observations. *P*-values were two-sided, the significance level was 0.05.

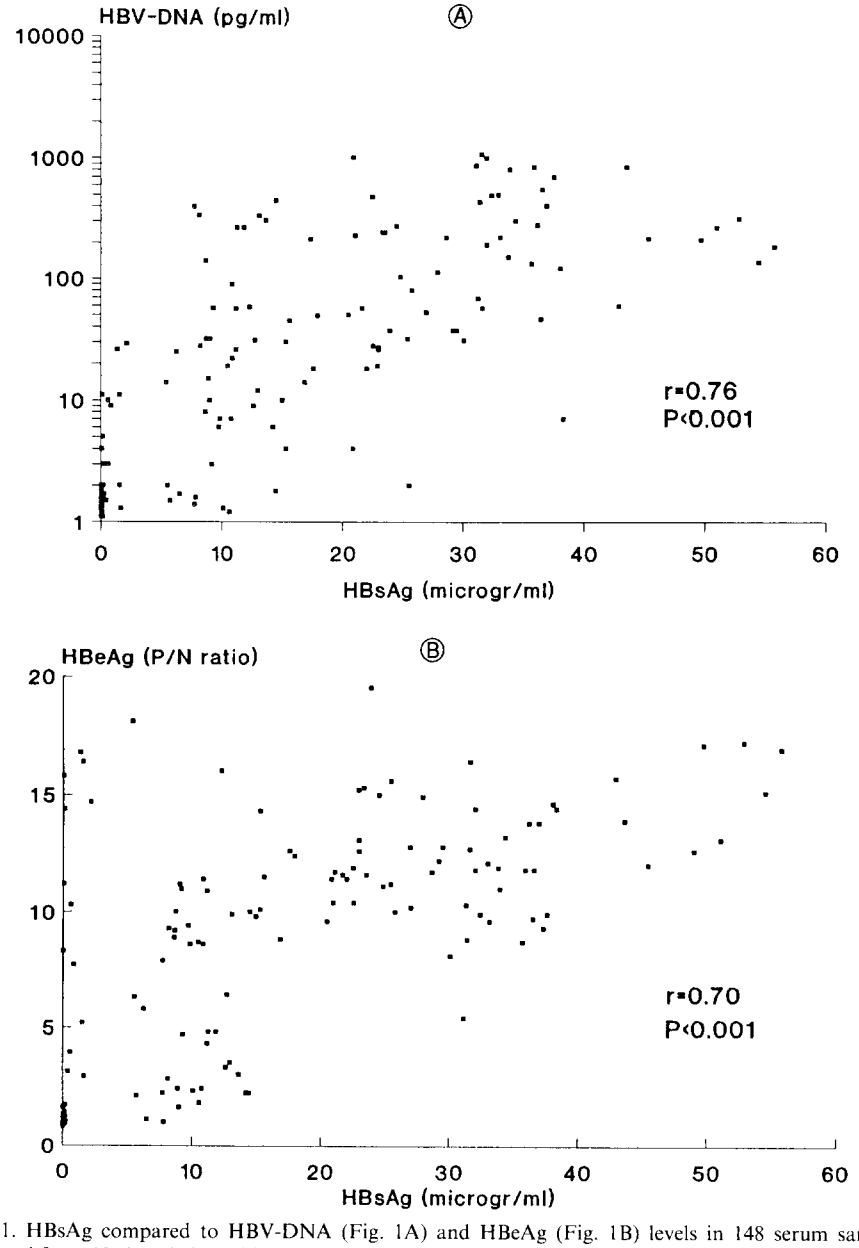

Fig. 1. HBsAg compared to HBV-DNA (Fig. 1A) and HBeAg (Fig. 1B) levels in 148 serum samples obtained from 12 chronic hepatitis B patients (group D) treated with α-interferon. Spearman's correlation coefficient (r) was calculated to assess the correlation between the levels of HBsAg and HBV-DNA or HBeAg.

follow-up serum samples (Table 1). The baseline HBsAg level for the 21 HBeAg-negative controls was lower compared to the initial HBsAg results of all other subgroups ($P<0.05$). A total of 148 serum samples were obtained from the 12 patients who were followed longitudinally during α-interferon therapy (group D). For this group we found co-linear results and a good correlation between the HBsAg level and both the HBV-DNA ($r=0.76$; $P<0.001$) and the HBeAg ($r=0.70$; $P<0.001$) level (Fig. 1). No difference in the correlation coefficients was found between patients with different response patterns (nonrespone $n=6$, HBeAg seroconversion $n=3$, HBeAg and HBsAg seroconversion $n=3$; Fig. 2).

In the present study we confirmed that a significant decrease in HBsAg level is related to loss of HBV replication as indicated by HBeAg seroconversion (Chien et al., 1978; Heijtink et al., 1978; Frosner et al., 1982; Hess et al., 1987). Furthermore, patients who underwent HBeAg seroconversion (no HBsAg seroconversion; group A) during α-interferon therapy exhibited a similar decrease in HBsAg level as patients who seroconverted spontaneously, suggesting that therapy-induced and spontaneous HBeAg seroconversion result in comparable reductions of the virus load. Eight patients exhibited

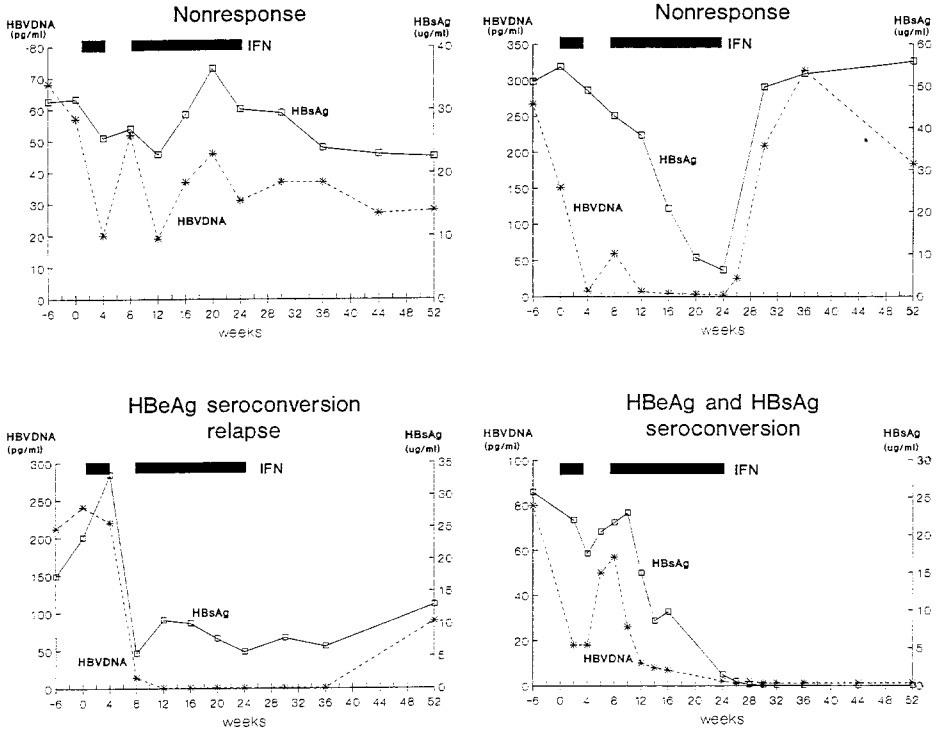

Fig. 2. HBV-DNA and HBsAg levels in 4 chronic hepatitis B patients with a different response to α-interferon therapy.

both HBeAg and HBsAg seroconversion after α-interferon treatment (group B). The initial HBsAg level found for these patients was similar to that for those who exhibited only HBeAg seroconversion (group A) during therapy, indicating that the amount of HBsAg in serum before therapy is not predictive for the occurrence of HBsAg seroconversion after α-interferon therapy. For patients who were followed closely during α-interferon therapy (group D) we found a good correlation between the HBsAg and the HBeAg or HBV-DNA levels. Interestingly, we found co-linear results for the HBsAg, HBeAg and HBV-DNA levels, irrespective of the response (nonresponse, HBeAg seroconversion, HBeAg and HBsAg seroconversion) to therapy. The interval between the start of the decrease in the HBV-DNA and HBsAg levels was short which suggests that the HBsAg level reflects changes in viral replication in a direct way. For the many laboratories which do not have the ability to measure HBV-DNA or HBeAg quantitatively, HBsAg measurement might be a good alternative to assess changes in HBV replication. In addition to HBsAg measurement a simple qualitative HBeAg/anti-HBe assay indicating seroconversion is most reliable for endpoint determination of α-interferon therapy.

Two disadvantages of the quantitative measurement of HBsAg should be mentioned. First, the basic levels of HBsAg vary widely between patients which needs the use of several dilution steps. However, in our studies more than 80% of the samples could be measured reliably after a 1:100 dilution step and the whole range of HBsAg could be covered with 3 dilution steps. In some cases HBeAg seroconversion was not accompanied by a dramatic drop in HBsAg level. Therefore we could not define an HBsAg cut-off level which could be indicative for HBeAg seroconversion. In comparison to quantitative assessment of HBeAg and HBV-DNA, HBsAg quantitation is less laborious and cheaper, especially when performed by assays designed for quantitative measurement (Amerlite assay, Abbott IMx) and might be of help in patients with delta infection or pre-core mutants (Carman et al., 1989) where the effect of treatment is often monitored by assessment of aminotransferase levels only.

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