Acta virol. **32**: 193–197, 1988

DIFFERENT LEVELS OF HEPATITIS B VIRUS REPLICATION AMONG HEPATITIS Be ANTIGEN-POSITIVE CHRONIC CARRIERS

J. BARTOLOMÉ*, I. CASTILLO**, I. MORA, J. GUTIEZ, J. C. PORRES, V. CARREÑO

Department of Gastroenterology, Fundación Jiménez Díaz, 2840 Madrid, Spain

Received June 6, 1987

Summary. — Detection of hepatitis B virus DNA polymerase (HBV DNA-pol) activity and of HBV DNA sequences in serum allowed to distinguish the different degrees of HBV replication in chronic HBsAg carriers. The amount of HBV DNA in the serum of 48 HBsAg and HBeAg positive patients in relation with the presence or absence of HBV DNA-pol was determined by dot-blot hybridization. The HBeAg positive cases with HBV DNA-pol activity had significantly higher HBV DNA levels than those which were DNA-pol negative ($p < 0.001$). However, no significant differences with respect to liver function tests (transaminase, albumin, gammaglobulin) or to the histological diagnosis were found between both groups. Quantitative detection of serum HBV DNA in HBsAg chronic carriers may be helpful for learning the natural history of HBV infection and monitoring the antiviral therapy.

Key words: HBeAg; HBV DNA polymerase; HBV DNA sequences; HBV replication; HBsAg carriers

Introduction

In the last years, several sensitive techniques have been developed to detect hepatitis B virus (HBV) replication in infected patients. These techniques can be divided into three main groups: detection in serum of HBV core antigen (HBcAg) (Quiroga *et al.*, 1985), of DNA polymerase activity associated with HBV (HBV DNA-pol) (Marion *et al.*, 1980), and of HBV DNA sequences (Bonino *et al.*, 1981). These techniques have allowed to establish two groups of patients among HBV "e" antigen (HBeAg) carriers and HBV DNA positive subjects. In the first, the HBV DNA-pol and HBV DNA sequences are simultaneously detected in the serum, while in the second group only HBV DNA can be detected in the absence of HBV DNA-pol activity.

* J. Bartolomé is a Research Fellow of the FIS, Insalud.
** I. Castillo is a Research Fellow of the Fundación Conchita Rábago.

The aim of the present study was to determine the possible differences in the level of HBV DNA replication between these two groups, by measuring the HBV DNA concentration in the serum.

Materials and Methods

Patients. Forty eight chronic HBsAg carriers with positive HBeAg and HBV DNA in the serum were included in the study. From these 73 serum samples were obtained and kept at -20 °C until tested.

The patients were divided in two groups with respect to the HBV DNA-pol activity. Into the first group belonged 30 patients (42 serum samples) which were positive for HBeAg, HBV DNA and HBV DNA-pol activity. Nineteen of them had chronic active hepatitis (CAH), 2 chronic persistent hepatitis (CPH), 7 hepatic cirrhosis (Ci) and 2 lacked histological diagnosis. Into the second group belonged 18 subjects (31 serum samples) which were positive for HBeAg and HBV DNA but negative for HBV DNA-pol activity (11 patients had CAH, 1 CPH, 1 Ci and in 5 cases no histological diagnosis was made).

Serological markers. The HBsAg, HBeAg and the anti-HBc were detected by comercial radioimmunoassay (RIA) (Abbott Laboratories, North Chicago, Ill).

The HBV DNA-pol activity was determined by the method of Marion *et al.* (1980), using ultracentrifugation and (^{3}H)-dTTP incorporation.

HBV DNA was detected by dot-blot hybridization (Weller *et al.*, 1982) using HBV DNA isolated from the recombinant plasmid pBH20-HBV. The cloned HBV DNA was nick translated with ^{32}P following the method described by Rigby *et al.* (1977). The specific activity obtained was between 1.3-2.5 $\times$ 10^8 cpm/μg. The sensitivity of the method, was evaluated using serial dilutions of known concentrations of the plasmid pBH20-HBV, was through to detect less than 1 pg HBV DNA. For semiquantitative scoring of the HBV DNA concentration in each serum, the nitrocellulose region corresponding to the radioactive spots were punched out and counted by liquid scintillation (Berninger *et al.*, 1982).

Liver function tests were done by standard methods using SMAC-20 (Technicon, NY).

Statistical test. The results obtained were analyzed by the Student's t-test.

Results

In order to avoid the possible interassay variations on HBV DNA quantitation, all samples included in the study were tested in the same run. When

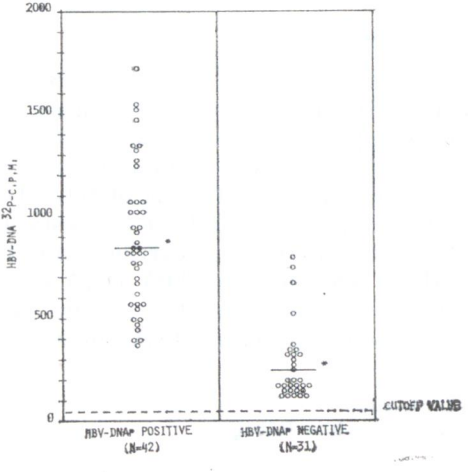

Fig. 1.

HBV DNA ^{32}P-cpm mean values in HBeAg positive chronic carriers with respect to the activity of HBV DNA-pol (*): $p < 0.001$.

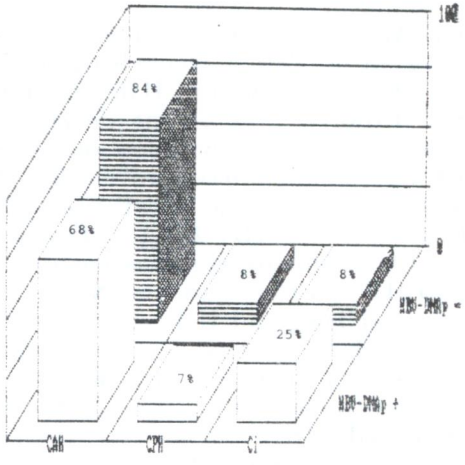

Fig. 2.
Histological diagnosis in HBV DNA-pol positive and negative patients
No significant differences between both groups were observed.

the serum HBV DNA was quantified, we found that this was significantly higher in the first group (HBeAg, HBV DNA and HBV DNA-pol positive) than in the second one (HBeAg, HBV DNA positive but HBV DNA-pol negative) (860.1 $\pm$ 350.3 cpm vs 250.6 $\pm$ 176.6 cpm, respectively, $p < 0.001$). (Fig. 1).

Although the transaminases were slightly more elevated in the HBV DNA--pol negative group than in the HBV DNA-pol positive one, we found no significant differences between them neither with respect to the albumin nor to gammaglobulin mean values (Table 1).

There were no differences with respect to the histological diagnosis between both groups (Fig. 2).

Table 1. Transaminase, albumin and gammaglobulin values among HBV DNA-pol positive and negative patients

Marker	N	GPT	Albumin	Gammaglobulin
HBeAg + HBV DNA-pol +	42	137.50 $\pm$ 95.01	3.26 $\pm$ 0.39	1.70 $\pm$ 0.50
HBeAg + HBV DNA-pol −	31	146.20 $\pm$ 64.80	3.23 $\pm$ 0.46	1.60 $\pm$ 0.30

No significant differences were observed.

Discussion

HBsAg chronic carriers have been divided in two groups according to the HBeAg status. Patients have been considered at a high or at a low replication level, whether or not anti-HBe antibody was detectable (Hadziyannis *et al.*, 1983). However, the recent development of more sensitive and specific techniques for the demonstration of virus replication, especially the HBV DNA determination have proven that the division by the "e" antigen system was not exact. There were patients who despite of being HBeAg positive, did not reveal serum HBV DNA, indicating that HBV did not replicate (Bonino *et al.*, 1981). Moreover, it was possible to detect circulating HBV DNA also in anti-HBe positive patients (Negro *et al.*, 1984). On the other hand, in the group of HBeAg and HBV DNA positive patients, there are two classes of chronic HBsAg carriers, depending on the activity of HBV DNA-pol.

It has been suggested that this fact could be due to different viral DNA structures (Cova *et al.*, 1985). In the patients with serum HBV DNA, but without HBV DNA-pol activity, the viral DNA plus strand could be in full length and the HBV DNA-pol would not incorporate nucleotides. If this hypothesis was true, the HBV DNA concentration would be similar in both groups. However, we have found a significant difference in the viral DNA concentration. This was higher in the group of patients with detectable HBV DNA-pol activity. In the HBV DNA-pol negative patients the level of viral DNA replication was lower, and so the number of circulating Dane particles was smaller. Because of the different sensitivities of both methods, the HBV DNA-pol activity may be undetectable although the viral DNA was present at low level.

The difference in the DNA replication rate among HBeAg positive patients could not be detected by testing the HBV DNA alone. It is also necessary to determine the HBV DNA-pol activity and/or to quantify the amount of HBV DNA.

Actually, in most of the antiviral therapy trials, the patients' selection is done according to the presence of HBV DNA (Mora *et al.*, 1987). Taking into account our results, it would be interesting to know if the differences in HBV DNA-pol activity and/or HBV DNA concentration influence the response to the treatment. In our study, the patients who were HBV DNA positive only, had slightly higher transaminases and they responded better to the treatment. Nevertheless, this must be confirmed in further trials.

References

Berninger, M., Hammer, M., Hoyer, B., and Gerin, J. (1982): An assay for the detection of the DNA genome of Hepatitis B virus in serum. *J. med. Virol.* **9**, 57–68.

Bonino, F., Hoyer, B., Nelson, J., Engle, R., Verme, G., and Gerin, J. (1981): Hepatitis B virus DNA in the sera of HBsAg carriers: A marker of active Hepatitis B virus replication. *Hepatology* **1**, 386–390.

Cova, L., Hantz, O., Arliaud-Gassin, M., Chevalier, A., Berthillon, P., Boulay, J., Jacquet, C., Chomel, B., Vitvitski, L., and Trepo, C. (1985): Comparative study of DHBV-DNA levels and endogenous DNA polymerase activity in naturally infected ducklings in France. *J. virol. Methods.* **10**, 251–260.

Hadziyannis, S. J., Lieberman, H. M., Karvountzis, G. B., and Shafritz, D. A. (1983): Analysis of liver disease, nuclear HBcAg, viral replication and HBV-DNA in liver and serum of HBeAg versus Anti-HBe positive carriers. *Hepatology* **3**, 656–662.

Marion, P. L., Oshiro, L. S., Regnery, D. C., Scullad, G. V., and Robinson, W. S. (1980): A virus in Beeckey Ground Squirrel that is related to Hepatitis B virus of human. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2941–2945.

Mora, I., Porres, J. C., Bartolomé, J., Quiroga, J. A., Gutiez, J., Hernández-Guío, C., Bas, C., and Carreño, V. (1987): Changes of Hepatitis B virus (HBV) markers during prolonged recombinant interferon alpha-2A treatment of chronic HBV infection. *J. Hepatol.* **4**, 29–36.

Negro, F., Chiaberge, E., Oliviero, S., Hammer, M., Berninger, M., Canese, M. G., and Bonino, F. (1984): Hepatitis B virus DNA (HBV-DNA) in Anti-HBe positive sera. *Liver* **4**, 177–183.

Quiroga, J. A., González, O., Carreño, V., Mora, I., Porres, J. C., Gutiez, J., and Hernández-Guío, C. (1985): Radioimmunoassay for detecting Hepatitis B Core antigen in serum from patients with chronic Hepatitis B infection. *Clin. Chem.* **31**, 831–834.

Rigby, P. W. J., Dieckman, M., Rhodes, C., and Berg, P. (1977): Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. mol. Biol.* **113**, 237–251.

Weller, I. V. D., Fowler, M. J. F., Nonjardino, J., and Thomas, H. C. (1982): The detection of HBV-DNA in serum by molecular hybridisation. A more sensitive method for the detection of complete HBV particles. *J. med. Virol.* **9**, 273–280.