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DYNAMICS OF SERUM MARKERS IN THE DIFFERENT COURSE OF HEPATITIS B VIRUS (HBV) INFECTION

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Summary. — The presence of serum markers of hepatitis B virus (HBV) infection in different stage of illness has a specific pathogenic and diagnostic significance. Based on the frequency of the appearance of HBV markers in patients' sera at different stages of the illness, we attempted a grouping possibly helpful for differentiation, epidemiologic and prognostic evaluation of hepatitis. The significance for chronic disease development of the dynamics of HBV marker levels in the serum is discussed.

Key words: hepatitis B virus; serum markers; chronic hepatitis

Introduction

HBV is a DNA-containing virus which replicates almost exclusively in hepatocytes (Summers, 1984). Replicative HBV-DNA was also shown to be present in peripheral blood mononuclear cells of chronic active hepatitis (CAH) patients with seropositive HBV markers (Gaull, 1985). The HBV contains three distinct immunoreactive antigens, i.e. HBsAg, HBeAg and HBcAg, which may stimulate corresponding antibody production during viral replication or shortly after its cessation (Neurath and Kent, 1985). This serves for sensitive and specific aetiological diagnosis of HBV infection (Aldershville and Nielsen, 1980; WHO Report, 1985). It was shown that in a variable proportion of acute hepatitis B (AHB) infections the HBV genome may be integrated into the host hepatocellular DNA resulting in chronic viral infection (Chakraborty *et al.*, 1980; Edman *et al.*, 1980; Deinhardt and Gust, 1982; Boender *et al.*, 1985; McMahon *et al.*, 1985). In our work we aim at an earlier recognition of the tendency for chronic development following AHB.

Materials and Methods

Serum samples were collected from the patients hospitalised for HBV infection in the Clinic for Infectious and Parasitic diseases, Faculty Hospital, Bratislava. Sera were taken at the begining of hospitalisation and then at different intervals during illness and also several months after the patients' release from the hospital.

Aetiological diagnosis: HBsAg, HBeAg, anti-HBe, anti-HBc and anti-HBs serum markers were tested by commercially available kits (Sevatest HBsAg RPHA, Immuna Š. Michal'any; Sevatest HBsAg ELISA, Sevac Praha; RIA or EIA sets for HBsAg, HBeAg, anti-HBe, anti-HBc or anti-HBs detection produced by Abbott Labs., U.S.A.; EIA sets for HBeAg or anti-HBe and anti-HBc detection produced by Sorin Biomedica, Italy; RIA sets for HBsAg and anti-HBs detection manufactured by Immuno AG, Austria). All tests were performed according to manufacturers' recommendations.

Biochemical tests for alanine aminotransferase (ALT), bilirubin and circulating immune complexes (CIC) blood levels were performed at the Clinic for Infectious and Parasitic diseases, Faculty Hospital, Bratislava or at the Department of Biochemistry, Faculty Hospital, Bratislava by commonly used methods employing commercially available tests.

Results

Biochemical and aetiological serum markers in the course of different forms of HBV infection

Serum levels of ALT and total bilirubin were compared in the group of patients with AHB, the patients showing a tendency for chronic development of AHB, with CAH or chronic persistent hepatitis (CPH) and finally, in HBsAg carriers. Table 1 summarizes the obtained values. From this data it is apparent that commonly used biochemical tests enabled us to reveal the damage of hepatic parenchyma not only during the acute form of hepatitis B (HB), but also during early stages of its chronic development. In CAH, the ALT levels were only slightly increased above the normal and the total bilirubin, as a rule, showed with few exceptions normal values. The values of these both biochemical markers were normal during CPH and in HBsAg carriers.

To determine the aetiologic markers in HB showing tendency for chronic development more reliably, the sera were taken from 6 to 12 weeks after hospitalisation and tested for the presence of serum markers such as HBsAg, HBeAg, anti-HBe and anti-HBc antibodies. As documented in Table 2, the most reliable marker of the early stage of chronic HBV infection was the anti-HBc level (present in 98% of the tested sera). Less frequent were the

Table 1. Biochemical markers in different forms of VHB

Serum marker	AHB	AHB→CAH	CAH	CPH	HBsAg carriership
Alanine aminotransferase (ALT)*	13.06 (44)***	2.70 (31)	0.49 (28)	0.27 (32)	0.26 (20)
Total bilirubin**	138. 20 (30)	46.47 (21)	9.72 (25)	11.72 (21)	7.65 (22)

* ALT values in μcat/ml (normal range 0.35—0.37)
** Total bilirubin values in μmol/l (normal range up to 25)
*** Number of sera examined (n)

HBsAg (85%) and the HBeAg (54%) presence while anti-HBe seroconversion could be demonstrated only in one fifth of the tested cases. In general, for reliable differentiation of the type of viral hepatitis in the stage of early development, the most convenient test was the anti-HBc detection. On the other hand, the persistence of HBeAg was a proof of continued replication of HBV, which consequently signalised the transition to CAH or CPH.

Different dynamics of serum markers in the course of AHB

Reliable and early determination of the aetiology of viral hepatitis, its stage and the prognose of its further development is important from both clinico-therapeutical as well as epidemiological points of view. The most valuable in this respect is the evaluation of the dynamics of serum levels of HBV markers. Fig. 1 represent four different examples of such dynamics obtained from four patients in different stages and with different forms of AHB development. In the case of 31 years old patient L.Š. a typical increase of HBsAg and anti-HBc blood levels was observed already in an early stage of AHB followed by decrease and eventually complete disappearance of HBsAg and HBeAg during convalescence, with anti-HBc persistence in patient's blood, seroconversion HBeAg — anti-HBe and a gradual increase of the protective anti-HBs immunoglobulin. An accompanying feature of the early stage of AHB in this case was the increased level of CIC which finally disappeared

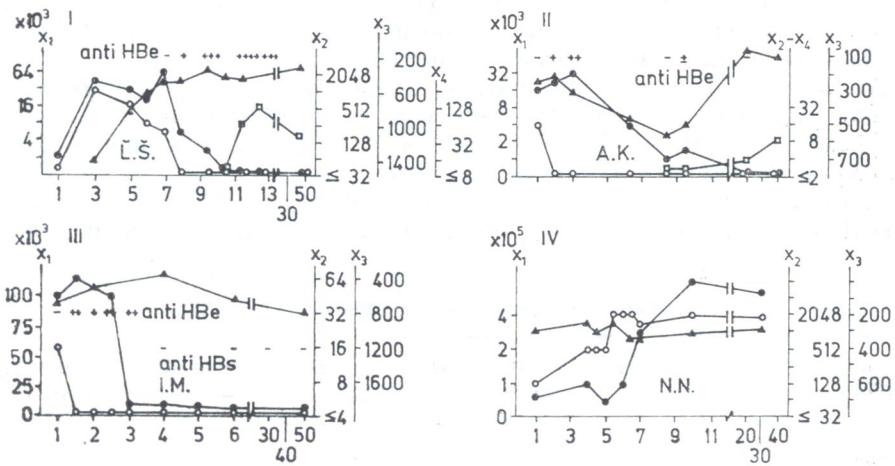

Fig. 1.

Dynamics of serum levels of HBV markers during different forms of AHB development
●——● HBsAg; ○——○ HBeAg; ▲——▲ anti-HBc; □——□ anti-HBs;
Abscissa: weeks after admission to hospital
Ordinates: X_1 (left) — HBsAg titres
X_2 — HBeAg titres (right)
X_3 — anti-HBc levels (inhibition of ^{125}I activity)*
X_4 — anti-HBs titres

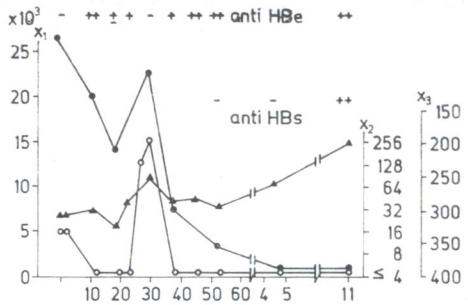

Fig. 2.
Cyclic character of HBeAg/anti-HBe appearance in serum during prolonged course of AHB
Abscissa: days (10—60) or months (4 to 11) after admission to hospital
Ordinates: as in Fig. 1
For symbols see Legend to Fig. 1.

during recovery from infection. A rather different dynamics of HBV markers during AHB infection was registered in 48 years old patient A.K. This involved namely the period between disappearance of detectable HBeAg serum levels and anti-HBs seroconversion which was more than 6 weeks as compared to the 3 weeks interval in the former patient (L. Š.). The dynamics of anti-HBc antibodies showed a cyclic rather than the gradually increasing character seen in previous patient.

In principle distinct was the dynamic of HBV markers seen in patient N.N. (46 years old man). During the first few weeks after hospitalisation the relatively high level of HBsAg has not changed significantly. Its sharp increase occured between the 5th and 10th weeks of the illness when HBsAg blood levels reached extreme values. The dynamic of HBsAg was characterised by increasing concentrations in two periods: between the first and the second week and then in the fifth week of illness followed by persistence at high level even 30 weeks after first check. The level of anti-HBc globulins was steadily high during this observation period. CIC were within the range of normal values at the beginning of the illness; by 11 weeks later, however, they increased several times above the normal. The diagnosis of CAH was confirmed also clinically and biochemically at checking after 30 weeks.

The dynamics of serum markers observed in patient I.M. (25 years old woman) reminded in principle of the situation seen in patient A. K. In this case, however, no evident anti-HBs seroconversion could be registered even

Table 2. HBV serum markers in acute hepatitis showing a tendency for chronic development

Result	HBV marker			
	HBsAg	HBeAg	anti-HBe	anti-HBc
Positive	186* (85%)	29 (54%)	6 (18%)	85 (98%)
Negative	34 (15%)	25 (46%)	27 (82%)	2 (2%)

* number of sera examined

Table 3. Identification of HB aetiology in chronic HBV patients* by "single" marker detection

HBV marker	positive/total	% of identified HBV infections
anti HBc	39/48**	81
HBsAg	69/91	76
anti HBe	40/75	53
anti HBs	13/91	14
HBeAg	9/91	10

* patients with CAH, CPH or HBsAg carriers
** number of sera examined

48 weeks following admission to the hospital. Changing increased CIC levels were present and "masked" HBsAg carriership was suspected to be involved. Figure 2 represents a diagram of a cyclic "masked" HBeAg presence during prolongued course of AHB.

Aetiological differentiation of viral hepatitis with respect to the tendency for chronic development of HBV infection

The above described followed-up of serum HBV markers is difficult to perform for practical diagnostic, prognostic or epidemiological purposes. We tried, therefore, to analyse the effectiveness of single marker detection on one hand and the combination of two or several markers on the other hand. It is

Table 4. Identification of HBV aetiology in chronic HBV patients* by combined marker detection

HBV marker combination	positive/total**	% of identified HBV infections
HBsAg/anti-HBs HBeAg/anti-HBe anti-HBc	88/91	97
HBsAg anti-HBc anti-HBe	45/48	94
HBsAg anti-HBc	44/48	92
anti-HBc anti-HBe	43/48	90
HBsAg anti-HBs	68/79	86

* patients with CAH, CPH and/or HBsAg carriers
** number of sera examined

Table 5. Serum markers during late convalescence and/or early stage of chronic VHB development

Group	HBV serum markers					Number of sera*	(%)
	HBsAg	anti-HBs	HBeAg	anti-HBe	anti-HBc		
A	+	−	+	−	+	3	4.6
B	−	−	+	−	−	1	1.5
C	+	−	+(±)	+(±)	+	1	1.5
D	+	−	−	+	+	31	47.7
E	−	−	−	+	+	1	1.5
F	+	−	−	−	+	9	13.8
G	−	−	−	+	−	1	1.5
H	−	−	−	−	+	5	7.7
I	+	−	−	−	−	2	3.1
K	+(±)	+(±)	−	−	−	3	4.6
L	−	+	−	+	+	2	3.1
M	−	−	−	−	−	1	1.5
O	−	+	−	−	−	3	4.6
P	−	+	−	−	+	2	3.1

* n = 65

evident from Tables 3 and 4 that only the combination of two or more markers gives satisfactory confirmation of HBV aetiology of hepatitis in the phase of late convalescence or in early stage of its chronic development. In the Table 5 we tried to summarise the presence of HBV serum markers, their grouping according to the frequency in particular tests, their relation to aggresivity of the disease and their pathogenetic significance. Most frequent (47.7%) was the simultaneous presence of HBsAg, anti-HBc and anti-HBe markers (group D). Substantially less frequent (13.8%) was the simultaneous presence of HBsAg and anti-HBc (group F). Complete seronegativity could also occur in a particular sample, however, in the repeated sample at least one positive marker confirmed the aetiology (group M). Quite rare but repeatedly confirmed were the findings of single markers (group B and G) present in patients' serum. The possible explanation of such situation is discussed. The Table 6 gives a more detailed interpretation of these results from the point of view of the infectivity (or aggressivity) of the disease, the prognosis of its further development as far as the cure or chronic conversion is concerned.

Discussion

Replication of HBV in hepatic tissue in individual cases may proceed for different periods of time, with different activity expression as far as aetiopathogenetic, immunopathogenetic or biochemical serum markers are concerned. There is no doubt that cell-mediated host response specific for HBV antigens plays a crucial role in HBV elimination or its persistance in infected organism (Frei *et al.*, 1978; Nicole *et al.*, 1985). Studies clarifying the molecular aspect of these processes were carried out (Jameel and Siddiqui, 1986). As

Table 6. Interpretation of HBV marker detection and their grouping

Observed seroconversion within groups	Interpretation	Possible conversion within groups	Interpretation
F to D	in F potentially infectious	arbitrary L, P, O, (K)	favourable development
E to L	reconvalescence	H → M	a) favourable development
C to D	in C infectious		b) "retreat" without detectable antibodies (possibility of reccurent HBV infection ?)
H to M/I	in M without immunity; in I HBsAg carrier		
I to F	in I no HBsAg carrier	F → D C → D	HBV infectious process continues
A to D	in A infectious	I → F A → D	
High aggressivity (decreasing)	A....H (AH-B, CAH)		
Low (no) aggressivity	I ....P (AH-B convalescent, CPH, CAH convalescent)		

shown in our work, at given time there was not always a clear-cut HBV marker expression in either the cured patient or in one with chronic development of disease. A dynamic balance between the immune potential and replication of HBV is often formed resulting in prolonged HBV presence in hepatic tissue. This may later terminate in seroconversion and complete clearance (Fig. 1 — patient A.K., Fig. 2) or continue in spontaneous exacerbations and regression of infectious process (Hoofnagle *et al.*, 1981; Perillo *et al.*, 1984).

The differences in the appearance of individual HBV serum markers demonstrated in our work might be, to a certain extent, attributed to the time differences between actual infection, hospitalisation and admission of serum samples. For more reliable confirmation of AHB in our four described patients (Fig. 1) the anti-HBc IgM test would be more suitable (Aldershville and Nielsen, 1980) to exclude exacerbations as may be suspected e.g. in patient N.N. with the high starting levels of anti-HBc. Early clearance of HBeAg from the sera of patient A.K. accompanied by normalisation of biochemical markers (personal communication) and HBsAg persistence signalised possible development of HBsAg carriership. Due to apparently increased levels of anti-HBc and finally due to the appearance of protective anti-HBs as a consequence of elevated immune reactions, the disease terminated in a complete recovery.

The "masked" carriership of HBsAg (see also Pintera, 1982) is a repeated finding of threshold serum levels of either HBsAg or anti-HBs with a random but definite proof of HBsAg positivity. In such cases steadily elevated or fluctuating levels of CIC could be detected (for more details see also Hořejší, 1985). The phenomenon of oscilating HBeAg/anti-HBe occurence in patient's serum expresses active HBV replication and consequent infectivity even in the stage when HBeAg is "covered" by temporally increased anti-HBe antibody. Both "masked" stages, e.i. HBsAg and HBeAg reversion in serum cease following an antigenic stimulus and/or an immune response leading to anti-HBe and/or protective anti-HBs seroconversion. The phenomenon of low oscilating and hardly detectable levels of HBsAg/anti HBs or HBeAg/ /anti-HBe together with pathologic CIC formation and clearance may explain the unusual and indeed controversial detection of certain HBV serum markers (Tables 5 and 6).

Anti-HBc detection is certainly convenient especially in chronic forms of HBV infection or in prolonged acute cases with a tendency for chronic development. The best results in aetiologic identification of chronic forms of viral hepatitis are achieved by testing the combinations of HBV serum markers. For more practical reasons the checking of two markers, e.g. HBsAg plus anti-HBc or HBsAg and HBeAg gives satisfactory results. The arrangement of HBV serum markers to several groups according to the frequency and mode of their appearance during early stages of a chronic development of HBV infection was designed for an earlier orientation in the aetiologic determination of a given viral hepatitis, more accurate prognosis of its further development and formation of a system convenient for deeper aetiopathogenetical studies of HBV infection. Our data signalise the need for more specialised and individually indicated examinations to explain the mechanism of chronic HB development with the final goal of more effective therapeutic means.

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