Hepatitis B surface antigen (HBsAg) in the liver of patients with hepatitis: a comparison with serological detection

M. B. RAY, V. J. DESMET, J. FEVERY, J. DE GROOTE, A. F. BRADBURNE, AND J. DESMYTER

From the Laboratory of Histochemistry and Cytochemistry and the Division of Hepatology (Department of Medical Investigation) and the Viral Diseases Laboratory (Department of Human Biology), Akademisch Ziekenhuis Sint Rafaël, University of Leuven, B-3000 Leuven, Belgium

SYNOPSIS Chronic hepatitis was diagnosed on liver biopsy of 76 patients; 52 (68%) had HBsAg. Of the 52 patients with HBsAg, 23% had HBsAg shown by immunofluorescence on the liver, while it could not be detected with radioimmunoassay on the serum; 77% had HBsAg detectable in liver and in serum, and none had HBsAg in serum only. HBsAg was detected more frequently in chronic aggressive hepatitis and active cirrhosis than in chronic persistent hepatitis and cirrhosis with little activity. No correlation was found in the different forms of chronic hepatitis between the HBsAg status on the one hand, and levels of transaminases, gammaglobulins, and auto-antibodies on the other.

Acute hepatitis was diagnosed on liver biopsy of 24 patients; 50% had HBsAg. Liver tissue positivity was very low in the fully developed stage compared to serum positivity. In 146 patients with other liver ailments, both liver and serum were negative for HBsAg.

The specific association between HBsAg (hepatitis B surface antigen) and viral hepatitis type B has been firmly established. Wide variations have been found in the incidence of HBsAg in the serum of patients with acute (Shulman and Barker, 1969; Prince *et al*, 1970; Edgington and Ritt, 1971; Mossor-Ostrowska *et al*, 1974) and chronic (Sutnick *et al*, 1969; Velasco and Katz, 1970; Wewalka *et al*, 1970) hepatitis. The frequency of HBsAg in chronic hepatitis as estimated in the blood varies from 3% in Australia (Cooksley *et al*, 1972) to 62% in Austria (Wewalka *et al*, 1970). The variation in the reported incidence of HBsAg may be due to differences in the geographical prevalence of HB virus infection (Cooksley *et al*, 1972) and to differences in the technique used for estimation (Prince, 1971).

In liver tissue, the antigen was consistently detected by immunofluorescence in various forms of chronic hepatitis with HBsAg (Hadziyannis *et al*, 1972; Krawczyński *et al*, 1972), suggesting that immunofluorescence on the liver may compete with serology in ability to detect HBsAg in those patients. There are conflicting reports regarding the detec-

tion of HBsAg in liver tissue of acute hepatitis with circulating HBsAg (Coyne *et al*, 1970; Cérat *et al*, 1973; Krawczyński *et al*, 1972; Mossor-Ostrowska *et al*, 1974; Gudat *et al*, 1975). In the present study, we compare immunofluorescent detection of HBsAg in the liver of biopsy-proven hepatitis with results of detection in the serum by radioimmunoassay, the most sensitive method available (Ling and Overby, 1972), and we compare the HBsAg status with the presence of various histological forms of hepatitis; in chronic hepatitis, we have also tried to correlate the HBsAg status and the histological type of disease with sex, gammaglobulin, and transaminase values, and the presence of auto-immune antibodies.

Material and Methods

PATIENTS AND DIAGNOSIS

Two hundred and forty-six liver biopsies were collected from patients submitted to needle biopsy during the last two years (December 1972 to December 1974) for various hepatic dysfunctions. The patients were mostly adults of Belgian origin. Biopsies were taken with a modified Vim-Silverman needle (Rake *et al*, 1969). The greater part of the

biopsy cylinder was used for routine histological diagnosis and the remaining part (approx. 5 mm) was processed for indirect immunofluorescence. One or more serum samples were taken during or within one week of the collection of the biopsy. Depending on the hepatic morphology, the patients were classified as acute or chronic hepatitis. Each main group was further subdivided into different subgroups on the basis of previously described morphological variations within the main groups (De Groote *et al*, 1968; Bianchi *et al*, 1971).

Chronic hepatitis without cirrhosis was subdivided into chronic persistent hepatitis, mainly characterized by portal inflammation, and chronic aggressive hepatitis, with periportal inflammation (piecemeal necrosis).

Cirrhosis was classified as cirrhosis with little activity when the degree of inflammation was histologically comparable to chronic persistent hepatitis, and as active cirrhosis when the histology displayed *inter alia* piecemeal necrosis as in chronic aggressive hepatitis. This group of patients does not always present clinically as what has been described under the headings 'active chronic hepatitis' or 'chronic active liver disease', which are mainly based on the serum levels of transaminases and γ globulins and the presence of clinical and laboratory signs of immunological disturbances.

Acute hepatitis was subdivided into four categories. The 'fully developed stage of acute hepatitis' (Bianchi *et al*, 1971) comprised seven patients, in whom the biopsy was taken one to six weeks (average five weeks) after the clinical onset of the disease. The 'later stage of acute hepatitis' (Bianchi *et al*, 1971) comprised nine patients; five of these had no apparent clinical symptoms of acute hepatitis and were submitted to liver biopsy because of the incidental discovery of raised transaminase levels (five times above the normal value); the remaining four patients had an acute disease lasting 8 to 20

weeks (average 16 weeks) before biopsy was taken. The 'residual stage of acute hepatitis' (Bianchi *et al*, 1971) comprised three patients biopsied 8 to 30 weeks (average 20 weeks) after the clinical onset of the disease.

'Acute hepatitis with signs of possible transition to chronicity' (Bianchi *et al*, 1971; Desmet, 1973) is a histological entity combining lobular features of acute hepatitis together with periportal hepatitis (piecemeal necrosis). This group comprised five patients biopsied 1 to 35 weeks (average 13 weeks) after the clinical onset.

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HBsAg was detected in the liver biopsies by the indirect immunofluorescence method. The technical aspects including the various immunochemicals used were described earlier (Ray *et al*, 1974).

SEROLOGY

In each serum sample HBsAg was determined by solid phase radioimmunoassay (Ausria); doubtful positives were checked with the RIA neutralization test (Bradburne and Desmyter, 1974). Sera from each patient were also assayed for auto-antibodies, ie, antinuclear factors (ANF), antimitochondrial antibodies (AMA), and smooth muscle antibodies (SMA) by indirect immunofluorescence.

Results

Of the 246 liver biopsies examined, 100 were from patients with histologically proven acute or chronic hepatitis, and 146 were from patients with a wide variety of other liver disorders. The histological diagnoses and results of immunofluorescence and circulating HBsAg are given in table I; further results on chronic hepatitis patients are given in table II. The biopsies in each subgroup of patients were divided into four categories: liver and serum positive,

Histological Diagnosis	Number	Liver + Serum +	Liver + Serum -	Liver - Serum +	Liver +	Serum +	Both Liver and Serum -
Chronic hepatitis							
Chronic persistent hepatitis	11	4	2	—	6 (55%)	4 (36%)	5 (45%)
Chronic aggressive hepatitis	20	12	2	—	14 (70%)	12 (60%)	6 (30%)
Cirrhosis with little activity	11	3	2	—	5 (45%)	3 (27%)	6 (55%)
Active cirrhosis	34	21	6	—	27 (79%)	21 (62%)	7 (21%)
Total	76	40	12	—	52 (68%)	40 (57%)	24 (32%)
Acute hepatitis							
Fully developed stage	7	1	—	4	1 (14%)	5 (71%)	2 (29%)
Later stage	9	4	—	—	4 (44%)	4 (44%)	5 (56%)
Residual stage	3	—	—	—	—	—	3 (100%)
Acute stage with signs of possible transition to chronicity	5	3	—	—	3 (60%)	3 (60%)	2 (40%)
Total	24	8	—	4	8 (33%)	12 (50%)	12 (50%)
Miscellaneous	146	—	—	—	—	—	146 (100%)

Table I Histological diagnosis and frequency of HBsAg obtained by serology and immunofluorescence

Diagnosis	IFT	Sero- logy	Number	Age		Sex		Gamma- globulins (g/100 ml)	SGOT ¹ (IU/l)	SGPT ¹ (IU/l)	Auto-antibodies			
				Mean	Range	M	F				ANF +	AMA +	SMA +	(No.) ²
Chronic persistent hepatitis	+	+	4	34	17-48	3	1	1.58 ± 0.34	37 ± 15	69 ± 47	—	—	—	(3)
	+	—	2	54	51-57	1	1	1.48 ± 0.52	9 ± 1	9 ± 1	—	—	—	(1)
	—	—	5	43	30-58	4	1	1.95 ± 0.57	21 ± 14	39 ± 32	2	—	—	(4)
Chronic aggressive hepatitis	+	+	12	46	15-58	7	5	1.66 ± 0.33	61 ± 44	117 ± 103	3	—	—	(7)
	+	—	2	64	61-66	1	1	2.01 ± 0.50	19 ± 1	35 ± 9	—	—	—	(2)
	—	—	6	43	16-53	3	3	1.84 ± 0.72	49 ± 60	81 ± 85	2	—	1	(5)
Cirrhosis with little activity	+	+	3	51	33-62	2	1	2.20 ± 0.93	27 ± 15	33 ± 18	—	—	—	(1)
	+	—	2	51	37-64	2	0	1.00 ± 0.08	17 ± 0	19 ³ ± 3	—	—	—	(1)
	—	—	6	55	44-66	3	3	1.16 ± 0.26	15 ± 8	13 ³ ± 2	—	—	—	(3)
Active cirrhosis	+	+	21	53	30-70	17	4	2.19 ± 0.95	72 ± 58	101 ± 74	1	—	—	(12)
	+	—	6	42	22-63	4	2	2.48 ± 1.33	76 ± 64	80 ± 71	2	1	—	(4)
	—	—	7	45	19-69	4	3	1.68 ± 0.64	84 ± 145	80 ± 116	—	2	1	(4)

Table II Comparison of age, sex, biochemical features, and auto-antibodies in HBsAg positive (in both liver and serum) and negative chronic hepatitis

¹Normal values for SGOT and SGPT are below 19 IU/l and 24 IU/l respectively.

²Numbers in brackets denote number of cases tested for auto-antibodies.

³Value significantly different from the group with positive serology ($P < 0.05$).

liver positive but serum negative, liver negative and serum positive, and liver and serum both negative. No consistent histological differences were found between HBsAg positive and negative hepatitis.

In chronic hepatitis, HBsAg was detected in 52 out of 76 patients (68%) with immunofluorescence, and in 40 with radioimmunoassay (57%). All HBsAg-positive cases could be detected with immunofluorescence; 12 out of 52 (23%) could not be detected by radioimmunoassay on the serum. The detection of HBsAg in the liver of patients devoid of demonstrable circulating antigen was specific, as shown by specificity tests reported elsewhere (Ray *et al*, 1974); also, HBsAg was absent from the liver of 146 patients without hepatitis. The highest incidence of HBsAg was seen in chronic aggressive hepatitis (70%) and in active cirrhosis (79%). A higher frequency of HBsAg in more active forms, compared to less active forms, was found in chronic hepatitis with and without cirrhosis, and attained statistical significance ($P \sim 0.05$). No significant correlation was found between the HBsAg status and levels of transaminases, gammaglobulins, and auto-immune antibodies (table II). In chronic hepatitis, 51 patients were males and 25 were females; 72% of males and 60% of females were HBsAg-positive.

In acute hepatitis, HBsAg was detected in 8 out of 24 patients (33%) with immunofluorescence, and in 12 with radioimmunoassay (50%). All HBsAg-positive cases could be detected with radioimmunoassay. In the fully developed stage, 4 out of 5 HBsAg-positive cases could be detected only by radioimmunoassay. The later stage of acute hepatitis,

and acute hepatitis with signs of possible transition to chronicity, although histologically distinct, resembled chronic hepatitis in that HBsAg was detectable both in liver and in serum (table I).

Discussion

In chronic hepatitis as a whole, the frequency of HBsAg as obtained with immunofluorescence (68%) and RIA (57%) is comparable with the percentages reported from other parts of this continent (Wewalka *et al*, 1970; Bianchi *et al*, 1972). The latter results were obtained by estimating the circulating antigen with methods as sensitive as the complement fixation test and immunoelectro-osmophoresis. Thus the differences in the sensitivity of the techniques used cannot be invoked as the only cause of variation in the frequency of HBsAg in chronic hepatitis (Cooksley *et al*, 1972; Reed *et al*, 1973). The frequency of HBsAg in Belgium, as determined by radioimmunoassay, is 0.4-0.5% (C. Vermylen and J. Desmyter, unpublished data) which is comparable to figures reported from Australia (Mason *et al*, 1972) and the USA (Ling and Overby, 1972). Therefore the high frequency of HBsAg obtained in chronic hepatitis in the population examined cannot be ascribed to a high prevalence of HB virus infection in this area. The variation in frequency may be due to differences in genetic make-up of the patients (Reed *et al*, 1973) or to other unknown factors. Whatever the explanation may be, our observations show a high frequency of HBsAg-positive cases in chronic hepatitis.

HBsAg-positive patients with chronic hepatitis

have been described as both younger (Bianchi *et al*, 1972) and older (Sherlock *et al*, 1970) than HBsAg-negative patients. Gammaglobulin levels have been reported to be lower in HBsAg-positive cases than in HBsAg-negative cases (Bulkley *et al*, 1970) and auto-antibodies to be absent in the blood of HBsAg-positive cases but not in HBsAg-negative cases (Bulkley *et al*, 1970; Wright, 1970; Cooksley *et al*, 1972). Such differences were not found in the present study.

There were no morphological differences between HBsAg-positive and HBsAg-negative biopsies. The highest incidence of HBsAg was seen in active cirrhosis, which was the prevalent type in this series. These findings contrast with those of Bianchi *et al* (1972), who have reported more frequent cirrhotic changes in antigen-negative groups. HBsAg was present more frequently in patients with chronic aggressive hepatitis or active cirrhosis than in patients with chronic persistent hepatitis or less active cirrhosis; yet, in HBsAg-positive patients, the amount of HBsAg in hepatocytes as seen by immunofluorescence was higher in less active forms than in aggressive forms (Ray *et al*, 1976).

Twelve patients had HBsAg which could be detected in the liver but not in their serum. These cases could not be sharply segregated from others on the basis of histological, epidemiological, and biochemical parameters.

We have used the most sensitive methods available to estimate HBsAg in tissue and serum; yet a number of cases of chronic hepatitis remain HBsAg-negative. This does not necessarily mean that HB virus infection is not associated with HBsAg-negative cases, since longitudinal studies and the detection of antibodies against HB virus may reveal additional associations.

In the 24 patients with the histological diagnosis of acute hepatitis, HBsAg was found in 50% in the serum, and in 33% in the liver. Our results with immunofluorescence and RIA in acute hepatitis are comparable with other observations (Edgington and Ritt, 1971; Mossor-Ostrowska *et al*, 1974). In particular, in fully developed acute hepatitis, HBsAg was much more readily detectable in the serum than in the liver, in contrast to the later stage of acute hepatitis and acute hepatitis with signs of longer duration, in which HBsAg was detected in tissue and in serum, and to chronic hepatitis, in which HBsAg was more readily detectable in tissue. These observations are consistent with the rare finding of HBsAg in acute hepatitis by immunofluorescence (Krawczyński *et al*, 1972; Gudat *et al*, 1975) and by electronmicroscopy (Nelson *et al*, 1970). This supports the hypothesis that HBsAg-positive cells are cleared at high efficiency in acute hepatitis by

normal immunological mechanisms (Dudley *et al*, 1972; Reed *et al*, 1974).

We are indebted to Dr L. Broeckert and Dr E. Stevens for valuable help and suggestion, and we thank Miss Rita Dereymaeker and A. De Boeck for technical assistance, and Mrs M. Veulemans-Weckx for typing the manuscript.

This study was supported by grants from Nationaal Fonds voor Wetenschappelijk Onderzoek and the Fonds voor Geneeskundig Wetenschappelijk Onderzoek of Belgium.

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