African Journal of Medicine and Medical Sciences

Editor: O.A. Ladipo
Assistant Editors:
B.O. Osotimehin and A.O. Uwaifo

Volume 18
1989

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Melbourne

Afr. J. Med. med. Sci. (1989) **18**, 21–24.

Hepatitis B surface antigen and immunoglobulin M complexes in chronic carriers and patients with acute and chronic liver diseases

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Summary

To determine the incidence and significance of circulating complexes between hepatitis B surface antigen (HBsAg) and immunoglobulin M (IgM), a total of 152 Nigerian adults with acute or chronic liver diseases were studied. They were compared with 100 chronic HBsAg carriers and 20 healthy volunteers. In HBsAg carriers HBsAg-IgM complexes persisted in 10% over a period of 6 months. In 53 cases of acute hepatitis B, 79% acute phase sera had detectable complexes. This initial incidence fell to 15% at 6 months. In the group of chronic liver diseases, the detection of HBsAg-IgM complexes occurred relatively infrequently. Circulating HBsAg-IgM complexes were associated with the presence of hepatitis B e antigen and a higher predisposition to chronicity after acute hepatitis. It is concluded that the presence of these complexes is specific to hepatitis B virus infections and may predict evolution to chronic liver diseases in Nigerians.

Résumé

Pour déterminer l'incidence et la signification des complexes circulantes entre l'antigène B extérieur de la hépatie de la séringue (HBsAg) et l'immunoglobuline (IgM) un total de 152 adultes Nigériens avec de maladies de foie soit aigues ou chroniques ont été étudiés. Ils étaient comparés avec 100 porteurs de l'antigène B extérieur de la hépatie de la séringue chroniques et 20 volontaires bien portants. Chez les porteum de HBsAg les complexes de HBsAg-IgM persistaient chez 10% pendant une période de 6 mois chez 53 cas de la hépatie de la séringue aigue, 79% de sérum pris les phases aigues avait des complexes discernables. L'incidence initiale a tombé à 15% à la fin de 6 mois chez le groupe avec la maladie de foie chronique, la découverte des complexes HBsAg-IgM se présentaient relativement rarement. Les complexes de HBs-IgM circulantes étaient associées avec la présence de l'antigène de la hépatie de la séringue et une haute prédisposition à la chronicité après la hépatie de la séringue aigue. Il est donc conclu que la présence de ces complexes est spécifique aux infections de la hépatie de la séringue et peut prédire l'évolution à la maladie de foie chronique chez les Nigériens.

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Introduction

The presence of circulating complexes between hepatitis B surface antigen (HBsAg) and immunoglobulin M (IgM) in sera of chronic HBsAg carriers was first described by Palla *et al.* [1]. Further studies suggested that the persistence of these complexes might predict the evolution of chronic liver diseases [2,3].

This study was therefore undertaken to determine the incidence and significance of HBsAg-IgM complexes in chronic carriers of HBsAg and patients with various acute and chronic liver diseases evaluated in the Liver unit of the University College Hospital (UCH), Ibadan, Nigeria between June 1981 and June 1983.

Subjects and methods

Subjects

The subjects comprised the following groups of Nigerians:

Group I. One hundred chronic HBsAg carriers who had no clinical or biochemical evidence of hepatobiliary diseases. Ages ranged from 14 to 60 years.

Group II. Fifty-three patients treated consecutively at the Liver unit for acute hepatitis B were diagnosed by clinical features (jaundice and hepatomegaly), elevated serum bilirubin, alanine aminotransferase (ALT) and positive HBsAg and/or antibody to hepatitis B core antigen (anti-HBc).

Group III. Seventy-seven patients with chronic liver diseases. In this group there were 13 cases of chronic active hepatitis, 28 patients with liver cirrhosis and 36 patients with primary liver cancer (PLC). The diagnoses were confirmed histologically.

Group IV. Thirty-two patients with acute non-B hepatitis. In 11 of these, Non-A, non-B hepatitis was diagnosed as previously described [4]. The remaining 21 patients had acute hepatitis A confirmed by positive IgM antibody to hepatitis A virus (anti-HAV) during the acute phase of the illness. Five of these were chronic carriers of HBsAg.

Group V. Twenty healthy volunteers served as controls. They were negative for markers of hepatitis B virus (HBV) and hepatitis A virus (HAV).

Serum specimens

Serial blood samples were obtained by venepuncture at entry to the study or admission into the hospital, and monthly thereafter for 3 months (Groups I and III) and for 6 months (Groups II and IV). After allowing clot formation, sera were separated by centrifugation. Aliquots of these were stored at −20°C until needed for testing.

Laboratory methods

Hepatitis B surface antigen, antibody to HBsAg (anti-HBs), anti-HBc, hepatitis B e antigen (HBeAg) and its antibody (anti-HBe) and IgM anti-HAV were all tested by radioimmunoassay (RIA) using commercial kits from Abbott Laboratories, Chicago (Ausria II, Ausab, Corab, HBe Kit and Havab-M respectively). Rheumatoid factor was detected by the latex agglutination method.

Detection of IgM-HBsAg complexes

Available reagents were limited and therefore only samples obtained at entry into the study, at 1, 3 and 6 months were tested for the presence of the complexes using the technique of enzyme-linked immunosorbent assay (ELISA).

The test is based on the sandwich principle [5]. Wells of microtitre plates were coated with anti-human IgM specific for all chain. Duplicate wells were incubated for 4 h at 37°C with 200 µl of test and control sera diluted 1:40 in 0.05 M phosphate-buffered saline (PBS) pH 7.4. After washing, 200 µl of horseradish peroxidase-conjugated antibody to HBsAg of a high specificity was added into the well after 1:4000 dilution in PBS with 1% human albumin and 1% HBV negative human sera. The wells were incubated for 1 h at room temperature (34–36°C) and then washed. Thereafter 100 µl of a solution containing *o*-phenylenediamine (0.04 mg/ml), 0.05 M sodium citrate, 0.15 M sodium phosphate and 0.32 µg/ml hydrogen peroxide was added into each well. The wells were incubated at room temperature for 30 min. To stop the reaction, 50 µl of 4 N sulphuric acid was added.

Colour intensities were read by a spectrophotometer (Dynatech). Results were obtained by comparison of test samples, negative controls and blanks.

Statistical comparison of proportions was performed using the Chi-square test with Yates' correction where necessary.

Results

Incidence rates

Table 1 summarizes the incidence of HBsAg-IgM complexes in the various groups. In the acute phase of hepatitis B (Group II) 42 (79.2%) had detectable complexes in their sera. However, the majority of subjects were cleared within 1 month. In chronic liver diseases, the incidence rates of detectable complexes were relatively low. Non-B hepatic diseases were not associated with circulating HBsAg-IgM complexes. The relationship between positive HBeAg and HBsAg-IgM complexes are summarized in Table 2. There was a positive correlation between HBeAg positivity and the

Table 1. Incidence of HBsAg-IgM complexes in Nigerian patients with various liver diseases

Groups	Category	No. studied	No. +ve for HBsAg	No. (%) with detectable complexes over time (months)			
				0	1	3	6
I	HBsAg carriers	100	100	18 (18)	14 (14)	10 (10)	—
II	Acute hepatitis B	53	53	42 (79.2)	9 (17)	8 (15.0)	8 (15.0)
III	Chronic liver diseases						
	Chronic hepatitis	13	9	3 (33.3)	3 (33.3)	—	—
	Liver cirrhosis	28	20	1 (5.0)	1 (5.0)	—	—
	Primary liver cancer	36	23	1 (4.3)	0 (0)	—	—
IV	Non-B hepatitis						
	Hepatitis A	21	0	0	—	—	—
	Hepatitis NANB	11	0	0	—	—	—
V	Healthy controls	20	0	0	—	—	—

Table 2. Relationship between HBeAg/anti-HBe system and detection of HBsAg-IgM complexes

Group	Condition	Total no.	No. with complexes	No. without complexes	*P*
I (100 HBsAg carriers)	HBeAg positive	33	18	15	< 0.001
	HBeAg negative/Anti-HBe positive	67	8	59	
II (53 patients with AVHB)	HBeAg positive	40	31	9	< 0.01
	HBeAg negative	13	4	9	

presence of complexes. However, the absence of HBeAg or anti-HBe did not exclude the occurrence of circulating complexes.

Chronic liver disease and HBsAg-IgM complexes

Persistence of complexes until 6 months after entry into the study, was observed in eight (15%) of the 53 subjects with hepatitis B. Of these, four had abnormal serum ALT (>40 IU/ml) at 6 months. Three subjects had persistent symptoms (jaundice/fatigue), abnormal signs including (hepatomegaly) a significant elevation of serum ALT (>80 IU/ml). They were subjected to liver biopsy. Histology revealed chronic acute hepatitis in two cases, and chronic persistent hepatitis in the third subject.

In two of 10 HBsAg carriers with detectable complexes at 3 months, serum ALT levels were abnormal (50 and 56 IU/ml respectively) but had returned to normal at 6 months.

Rheumatoid factor and HBsAg-IgM complexes

There was no relationship between HBsAg-IgM complexes and rheumatoid factor in the subjects studied. Only two subjects with chronic hepatitis were weakly positive for rheumatoid factor. Both were HBsAg negative and had no detectable circulating complexes.

Discussion

The findings in this study indicate that 10% of chronic carriers of HBsAg have detectable and

persistent HBsAg-IgM complexes in their sera. The incidence rate of this phenomenon varies with time and the complexes disappear in some carriers. The observation of the complexes in the majority of subjects with acute hepatitis B imply that this is a frequent occurrence in the acute phase of illness. While it is specific for hepatitis B, the detection of these complexes in single acute phase serum has no predictive value. However, Toti *et al.* [3] have suggested that the persistence of complexes in this category may indicate an evolution to chronicity. In three cases with sustained circulating HBsAg-IgM complexes, chronic hepatitis was subsequently confirmed to have evolved.

It was anticipated that the incidence of HBsAg-IgM complexes might be high in established chronic liver diseases. However, in only seven of 77 cases in this category were complexes detected. The reason is not clear. It is, however, likely that viral replication in established chronic liver diseases is relatively low. It has been previously shown that HBeAg, an index of viral replication, is detected infrequently in Nigerians with chronic liver diseases [6].

In the present study, circulating HBsAg-IgM complexes were closely associated with the presence of HBeAg. Of the seven subjects with chronic liver diseases and circulating complexes, five were positive for HBeAg. Furthermore, complexes were undetectable in subjects without markers of hepatitis B virus (HBV). This further confirms the specificity of HBsAg-IgM complexes for HBV infection.

The nature of the IgM component of these complexes is uncertain. It is, however, clear that this is unrelated to the rheumatoid factor. It has been suggested that the IgM within the complexes might represent antibody to denatured protein, such as polymerized human serum albumin (PHSA).

Receptors for PHSA have been shown to be higher in HBsAg positive sera containing HBeAg compared with those positive for anti-HBe [7,8]. This positive correlation between active viral replication and the expression of the receptors on HBV particles is supported by the findings in the present study where HBsAg-IgM correlated with the presence of HBeAg. In contrast, subjects who are positive for anti-HBe had low levels of complexes. Sero-conversion to anti-HBe has been associated with loss of receptors to PHSA on HBsAg particles [9].

Whether the detection of HBsAg-IgM complexes in asymptomatic HBsAg carriers involves similar implication of evolution to chronicity must await a longitudinal study of this sub-group of HBsAg carriers.

For now, it is suggested that HBsAg-IgM complexes determined about 3 months after recovery from acute hepatitis B may be of predictive value in determining predisposition to chronic liver diseases.

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(*Accepted* 24 *June* 1987)