Indian J Med Res 84, July 1986, pp 27-31

Significance of circulating HBsAg/IgM complexes in viral hepatitis type B

B.M. Gandhi, Hema Gupta, M. Irshad, Y.K. Joshi & B.N. Tandon

Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi

Accepted January 22, 1986

In sixty seven patients of viral hepatitis type B and 67 serum samples from healthy blood donors who were carriers of HBs Ag, circulating HBs Ag/IgM complexes were tested by an enzyme immunoassay based on the selective absorption of IgM on a solid phase and results were related to presence of HBe markers. HBsAg/IgM complexes were found to be more common in HBsAg positive blood donors (61·1%) in comparison with acute type hepatitis B *i.e.*, acute viral hepatitis (30·8%), fulminant hepatitis (35·5%) and sub-acute hepatitis (40·0%). The complexes were found to be present with HBe markers in 83 per cent of blood donors, suggesting that both HBsAg/IgM complexes and HBe-markers were associated with chronic carrier state in high proportion. In 82 per cent of FH and 50 per cent of SAH, these complexes were present in absence of any of HBe-marker. The absence of HBe-markers in these subjects was assumed to be due to sampling during 'e'-window period or the low sensitivity of the commercial HBe-EIA kit.

Hepatitis B surface antigen circulates in serum, complexed with IgM globulins in acute and chronic hepatitis[1,2]. Sequential serum testing for HBsAg/IgM has been shown to be of prognostic value in patients with acute viral hepatitis type B where the presence of these complexes have been shown in early phase of the disease[3]. The persistence of these complexes are independent of replication of hepatitis B virus and provide evidences related to transition of the disease to chronicity[2,4,5]. Such complexes occur together more frequently in the presence of HBeAg than anti-HBe or the absence of HBe markers[2,6,7]. More than 70 per cent of the healthy HBsAg positive blood donors carry such complexes[7]. The presence of high positivity of HBsAg/IgM complexes in blood donors and in viral hepatitis as evidence for HBV-induced liver diseases need further investigation. HBsAg/IgM complexes in healthy HBsAg carriers and patients of HBV hepatitis are reported here.

Material & Methods

Sixty seven patients of viral hepatitis type B, admitted to the Gastroenterology wards of All India Institute of Medical Sciences (AIIMS), New Delhi, were investigated for the presence of IgM/HBsAg complexes and markers of HBeAg.

This group included 26 patients of acute viral hepatitis (AVH), 31 acute fulminant hepatitis (FH) and 10 subacute hepatitis (SAH). The diagnosis of different conditions of hepatitis was based on conventional clinical, bio-chemical, histological and serological criteria described earlier[8]. In addition, 67 serum samples from healthy blood donors who were found to be carriers of HBsAg, were screened for markers of HBeAg and IgM/HBsAg complexes.

The patients with viral hepatitis type B and healthy HBV-carriers were screened by the micro-ELISA system[9]. HBeAg and anti-HBe test was carried out by the Abbott HBe-EIA obkit tained from Abbott Laboratories, Illinois Chicago, USA. The testing of IgM/HBsAg complexes was carried out by enzyme immunoassay based on the selective absorption of IgM on a solid phase. Briefly, the precoating of 96 well micro-titre plate was carried out with 1 : 25000 dilution of rabbit IgG antibody to human IgM (u-chain specific), in bicarbonate buffer (0·1 *M*) *p*H 9·2. Post-coating was carried out with 0·5 per cent gelatin in PBS, *p*H 7·2. The binding of IgM as well as IgM-complexes was attained by addition of test samples. The HBsAg moiety of HBsAg/IgM complex was revealed by addition of horse radish peroxidase conjugated with anti-HBs (HRPO-α-HBs) as detailed earlier[9] for detection of HBsAg.

Results & Discussion

HBsAg/IgM complexes were found to be more common (61%) in HBsAg positive healthy blood donors, in comparison with HBsAg positive patients with acute sporadic viral hepatitis; (30·8%; $P<0·01$), subacute hepatitis (40%; $P>0·05$) and fulminant hepatitis (35·5%; $P<0·05$).

HBe-markers in HBsAg/IgM complex positive blood donors and patients with liver diseases are shown in the Table. In healthy HBV-carriers, HBsAg/IgM complexes were found to be present with HBeAg in 37 per cent and anti-HBe in 46 per cent patients. These complexes in 50 per cent of AVH were present with HBeAg.

Table. Markers of HBe in relation to presence of HBsAg/IgM complexes in different group of subjects

Group	HBsAg/IgM complex positive	HBeAg positive	Anti-HBe positive	HBe-markers negative
Blood bank carriers	41	15 (37)	19 (46)	7 (17)
AVH	8	4 (50)	1 (13)	3 (37)
SAH	4	1 (25)	1 (25)	2 (50)
FH	11	1 (9)	1 (9)	9 (82)

Figures in parentheses indicate the percentages. AVH, acute viral hepatitis; SAH, sub-acute hepatitis; FH, fulminant hepatitis

In 82 per cent of fulminant hepatitis and 50 per cent of sub-acute hepatitis, these complexes were present in absence of any of the HBe-marker.

Our findings of high positivity rate of HBsAg/IgM complexes in carriers (61·1%) confirms the earlier reports[1,2,7]. It has already been suggested[2,5] that absence of these complexes from very beginning of illness or their disappearance after short period of positivity is associated with good recovery from viral hepatitis. The presence of HBsAg/IgM complexes in AVH (30·8%), SAH (40·0%) and FH (35·5%) in the present study show that these patients would have run the risk of poor prognosis while in the rest of patients in these groups, the HBsAg/IgM complexes were either absent or disappeared at the time of their admission to the hospital, indicating a good prognosis. The study of HBsAg/IgM complexes in serum can be a good marker for the long term sequalae of hepatitis. However, there is little relevance of such a study for the prognosis of acute and sub-acute illness *i.e.*, fulminant hepatitis and sub-acute hepatitis.

HBeAg status has been studied by several investigators for predicting the long term sequalae of acute hepatitis[10,11]. Persistence of HBeAg is known to be associated with chronic liver diseases[12]. The correlating study of the HBe markers and HBsAg/IgM complexes in different HBV infections show that the markers were associated with carrier state in high proportion and were responsible for continued liver injury. The asymptomatic HBsAg carriers have been reported to be potentially infective, regardless of the presence of HBeAg or anti-HBe and the HBsAg titer in the serum[7,13,14].

The results obtained in the present study contradict the earlier reports[2,6,7] that HBsAg/IgM complex were always present with HBeAg and they disappear immediately when HBeAg becomes seronegative. In AVH, the HBsAg/IgM complex was detected with HBeAg in 50 per cent of patients as compared to 13 per cent with anti-HBe. However, in FH and SAH, the complex was detected in equal proportion with both, HBeAg and anti-HBe. Since HBsAg/IgM complex has been shown to be associated with HBeAg in significantly large group of patients[7], the formation of these complexes may be used as a prognostic marker. In acute HBV infections *i.e.*, AVH, FH and SAH, therefore, the presence of complex is a sign of bad prognosis while absence of complex is a marker of good prognosis[2].

Among the asymptomatic blood donors, the complex was detected in 37 per cent subjects with HBeAg, 46 per cent with anti-HBe and 17 per cent in absence of HBe-markers. This is in agreement with the earlier report, where complex was present in high proportion with anti-HBe[14]. Since these blood donors were not followed up, it is difficult to predict the development of chronicity in them. However, all the blood donors in whom the HBsAg complex is present, are at a high risk of developing chronic liver disease in due course of time[7]. The presence of HBsAg/IgM complex in 17 per cent of subjects in absence of HBe-markers represents the complexes in 'e-window' and confirms the report of about 10 per cent asymptomatic HBV-carriers in absence of HBe-markers representing 'e-window'[15]. The integration of viral hepatitis sequences and persistant surface antigen production contributes to the development of chronic

liver disease, has already been demonstrated in clinically asymptomatic carriers[16]. Progression to chronicity has also been shown to occur in presence of anti-HBe[17,18] and HBsAg/IgM complex has also been found frequently in anti-HBe positive patients[13].

The presence of HBsAg/IgM complexes in acute HBV infections *i.e.*, AVH, FH and SAH when compared with blood donors, were found to be non-significant in presence of HBeAg. However, these complexes were found to be more with anti-HBe in blood donors ($P<0{\cdot}01$) and in the absence of HBe-markers in acute cases ($P<0{\cdot}001$). In the absence of HBe-markers, the complex was detected in quite a significant proportions of all the acute HBV infections *i.e.*, AVH (37%), SAH (50%) and FH (82%). The absence of HBeAg and anti-HBe in high proportion of FH cases is further supported by the data of Gimson *et al*[19] who reported HBeAg and anti-HBe each in only 12 per cent patients. The absence of HBe-markers in such high proportions of the acute HBV infections was assumed to be due to the sampling in either exactly the 'e-window' period or the inclining or declining phase of this window period, where the levels of HBe-markers are too low to be detected by the HBe-EIA kit supplied by Abbott Laboratories, USA[20]. The co-occurrence of sampling time and 'e-window' in these patients is not very unusual because this period is quite variable and the patients usually turn up to the hospitals at a latter stage of disease that corresponds to the 'e-window' period[21].

References

1. Palla, M., Toti, M., Recchia, S., Rizzi, R. and Bonino, F. Complexes between IgM and HBsAg in serum of HBsAg carriers. *Ital J Gast* **13** (1981) 223.
2. Careoda, F., Franchis, R., Monforts, A.D., Vecchi, M., Rossi, E., Primignani, M., Palla, M. and Dioguardi, N. Persistance of circulating HBsAg/IgM complexes in acute viral hepatitis, type B. An early marker of chronic evolution. *Lancet* **ii** (1982) 358.
3. Toti, M., Rizzi, R., Almi, P., Palla, M. and Bonino, F. Complexes between HBsAg and IgM in serum of patients with acute hepatitis. *J Med Virol* **11** (1983) 139.
4. Palla, M., Rizzi, R., Toti, M., Almi, P., Rizzetto, M., Bonino, F. and Purcell, R. Complexes of hepatitis B surface antigen and immunoglobulin M in the sera of patients with hepatitis B virus infection. *Infect Immunn* **41** (1983) 950.
5. Rizzi, R., Palla, M., Almi, P., Toti, M. and Bonino, F. HBsAg/IgM complexes in the serum of HBsAg carriers. *Il Fegato* **28** (1982) 121.
6. Coursaget, P., Barres, J.L., Yuonnet, B., Petal. E., Boup, S.M., Bocande, J.E., Diop-Mar, I. and Chiron, J.P. Detection of HBsAg/IgM complexes in sera from HBsAg carriers in Africa (Senegal). *Hep Sci Memo* (1983) 101.
7. Butler-Brunner, E., Price, P. and Butler, R. Detection of HBsAg/IgM complexes in sera of HBV infected persons. *Hep Sci Memo* (1983) 73.
8. Tandon, B.N., Gandhi, B.M. and Joshi, Y.K. Etiological spectrum of viral hepatitis and prevalence of markers of hepatitis A and B virus infection in north India. *Bull WHO* **62** (1984) 67.
9. Gandhi, B.M. and Tandon, B.N. A low cost micro-ELISA test for hepatitis B surface antigen. *Trop Gastroenterol* **5** (1984) 142.
10. Aldershville, J., Frosner, G.G., Nielsen, J.O., Hardt, F., Deinhardt, F. and Skinhoj, P. Hepatitis Be antigen and antibody measured by radioimmunoassay in acute hepatitis B surface antigen-positive hepatitis. *J Infect Dis* **141** (1980) 293.
11. Deinhardt, F. Predictive values of markers of hepatitis virus infection. *J Infect Dis* **141** (1980) 229.
12. Aldershville, J., Nielsen J.O., Dietrichson, O., Hardt, F., Juhi, E. and Tage-Jensen, U. Long term follow up of e-antigen (HBeAg) positive

acute viral hepatitis. *Scand J Gastroenterol* **14** (1979) 845.

13. Vranckx, R., Cole, J. and Peetermans. Correlation between concentration of HBsAg and the presence of HBeAg, anti-HBe and anti-HBc in chronic asymptomatic carriers of HBsAg. *Infection* **8** (1980) 279.
14. Musca, A., Cordova, C., Barnaba, V., Zaccari, C., Levreso, M., Van Dyke, A. and Balsano, F. Circulating HBsAg/IgM complexes in acute and chronic hepatitis B. *Hepato-gastroenterology* **31** (1984) 208.
15. Aldershvile, J., Skinhoj, P., Frosner, G G.. Black, F., Deinhardt, F., Hardt, F. and Nielsen, J.O. The expression pattern of hepatitis B e-antigen and antibody in different ethnic and clinical groups of hepatitis B surface antigen carriers. *J Infect Dis* **142** (1980) 18.
16. Sakuma, K., Takahara, T., Okuda, K., Tsuda, F. and Mayumi, F. Prognosis of hepatitis B virus surface antigen carriers in relation to routine liver function tests: A prospective study. *Gastroenterology* **83** (1982) 114.
17. Fay, O., Tanno, H., Roncoroni, M., Edwards, V.M., Mosley, J.W. and Redeker, A.G. Prognostic implications of the e-antigen of hepatitis B virus. *JAMA* **238** (1977) 2501.
18. Caredda, F., D' Aminio Monforte, A., Rossi, E., Cultraro, D., Mariscotti, C. and Moroni, M. HBeAg/anti-HBe system in the acute phase of HBsAg positive hepatitis as a predictive test of developing chronic hepatitis. *Boll Ist Sieroter Mil* **60** (1981) 468.
19. Gimson, A.E.S., Tedder, R.S., White, Y.S., Eddleston, A.E.W.F. and Williams, R. Serological marker in fulminant hepatitis B. *Gut* **24** (1983) 615.
20. Liaw, Y.F., Huang, M.J., Chu, C.M., Sheen, I.S. and Lin, D.Y. The window period between hepatitis B e-antigen and antibody in chronic type B hepatitis. *Hepatology* **4** (1984) 619.
21. Gvojic, B and Miling, M. HBeAg/anti-HBe system in acute viral hepatitis B. *Lijecnicki Vjesnik* **107** (1985) 191.

Reprint requests : Dr B.N. Tandon, Professor of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029