SEROLOGICAL RESPONSES TO HEPATITIS B VIRUS INFECTION IN MULTI-TRANSFUSED THALASSEMIC CHILDREN

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ABSTRACT

One hundred children with beta-thalassemia major were studied prospectively. A one time analysis of serum samples was carried out for a battery of hepatitis B viral markers viz., HBsAg, anti-HBs and anti-HBc.

Seven mutually different serological patterns were observed. The commonest profile seen in 49 patients was a combined seropositivity for anti-HBc and anti-HBs indicating past HBV infection with persisting immunity. Definite evidence of active HBV infection (seropositivity for HBsAg and/or HBeAg) was demonstrated in 10 cases, six of these were HBsAg positive. Anti-HBc positivity alone was detected in 17 patients. The remaining 24 children were seropositive for anti-HBs alone suggesting a possible passive transmission of anti-HBs through blood transfusion.

Key words: *Beta-thalassemia, Hepatitis B virus infection HBsAg, HBeAg, anti-HBs, Anti-HBc.*

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Hepatitis B virus (HBV) infection is an important cause of hepatitis not only because of its significant morbidity and mortality, but also because it is preventable(1). The prevalence of a carrier state in the general population is high(2). This problem is of particular significance in patients on regular transfusion of blood or blood products–thalassemics and hemophiliacs. HBV infection is characterized by several distinctive serologic markers. These consist of 3 different antigens and their antibodies, *viz.*, hepatitis B surface antigen (HBsAg), hepatitis Be antigen (HBeAg), hepatitis B core antigen (HBcAg), anti-HBs, anti-HBc and anti-HBc. The serologic and immunologic profiles provide a guideline for monitoring the course of infection and the degree of infectivity in such patients(3). Testing of sequential serum specimens is helpful in diagnosis but one is often required to decipher the significance of a single serological profile(3). In this study we determined the frequency of HBV infection in multitransfused children with beta-thalassemia and analysed the varied serological pattern observed in response to this infection.

Material and Methods

This study was carried out in 100 multitransfused children with beta-thalassemia major between the ages of 7 months to 12 years. All these patients were on a regular transfusion regimen receiving blood at intervals of 15-30 days. They had been receiving blood for a variable period of 6 months to 11 years. Blood was obtained from the hospital blood bank which functions on an entirely voluntary donation scheme. Routine screening for HBsAg has, however, been introduced only in recent years. None of the children had received

Hepatitis B vaccination or immunoglobulin. Serum specimens were collected and a one time analysis done for a battery of HBV markers, namely-HBsAg, HBeAg, anti-HBc and anti-HBs. The donor blood was seroanalysed for anti-HBs only.

Screening for HBsAg was done by the micro-ELISA technique using the sandwich principle described by Gandhi *et al.*(4). Commercially available anti-HBs HRPO antibody conjugate was used. The micro-ELISA technique of Gandhi *et al.*(5) was similarly used for detecting anti-HBs. The vaccine for HBsAg, a source of purified antigen and enzyme linked protein-A, served as the conjugate thus reducing the cost of each test without compromising on its specificity. Anti-HBc and HBeAg were detected by enzyme immunoassay (ELISA) technique as described by Krauldat *et al.*(6) using Enzygnost anti-HBc kits and Enzygnost Hbe kits (Behringwerke AG, Marburg, West Germany), respectively. Results were recorded by photometric evaluation using the ELISA processor.

Results

These children were subdivided for the purpose of analysis according to their age and the number of transfusions received. This is depicted in *Table I*. Twenty four cases received upto 20 transfusions while 49 had received more than 50 transfusions.

Seropositivity for one or more of the viral markers was detected in all 100 children. The observed serological profiles, with their frequencies and interpretations are dipicted in *Table II*. Seven mutually different serological patterns were observed in this study. Seventy-six children had definite evidence of exposure to HBV infection (Pattern I to VI–*Table II*). The largest number of patients (49) showed seropositivity for both anti-HBs and anti-HBc and these are referred to as 'true positive.' (pattern VI). Next in frequency was the presence of seropositivity to anti-HBs alone and these cases are categorized as 'false positive' (pattern VII). The distribution of both these subgroups in relation to the age and the number of blood transfusions is depicted in *Figs. 1 & 2*, respectively. The least common serological profile was presence of seropositivity to HBsAg and HBeAg (pattern I).

Discussion

Unlike many other viral diseases, HBV

TABLE I–*Distribution of Thalassemic Children According to the Age and the Number of Transfusions*

No. of transfusions	Age in (years)				Total
	<2	2-4	4-8	8-12	
0-20	14	8	2	–	24
21-50	2	17	8	–	27
51-75	–	1	12	4	17
>75	–	2	18	12	32
Total	16	28	40	16	100

TABLE II—*Observed Patterns of HBV Serological Markers and Interpretation*

Pattern	HBsAg	HBeAg	anti-HBc	anti-HBs	Number of patients	Interpretation
I	+	+	–	–	1	Active infection
II	+	+	+	–	3	Active infection
III	+	–	+	–	2	Active infection
IV	–	+	+	–	4	Active infection
V	–	–	+	–	17	Probable active infection
VI	–	–	+	+	49	Past infection
VII	–	–	–	+	24	False positive

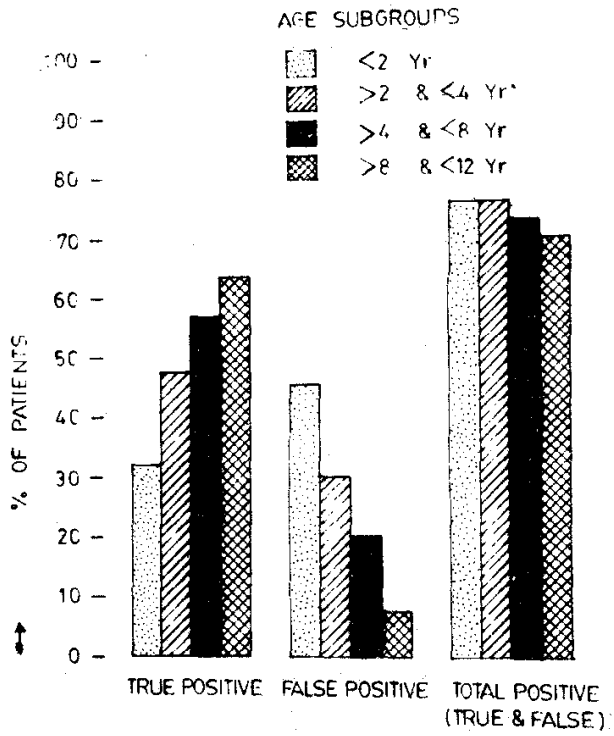

Fig. 1. Age distribution of true and false positivity in study group.

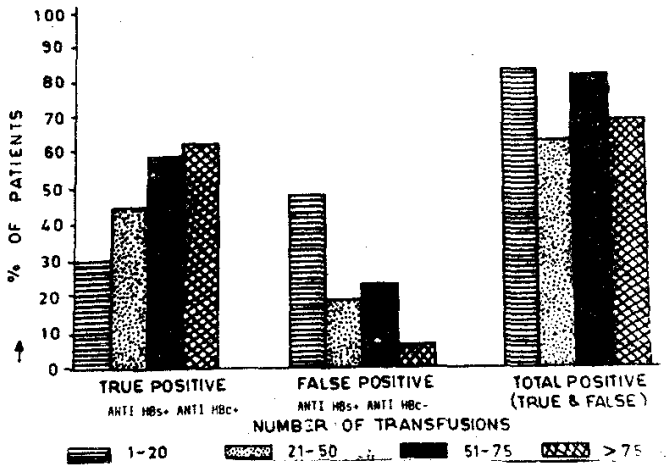

Fig. 2. Distribution of true and false positive cases based on number of transfusions in study group.

infection is characterized by several distinct serologic and immunologic responses. The temporal profile of the appearance of various antigens and antibodies can serve as a useful guide to monitor the course of the disease and to provide a serologic correlation with progress of the disease(7-9). When a serum sample is available only at one point, diagnostic sensitivity and accuracy are improved by reference to the complete battery of HBV markers. Analysis of the serological profile also provides useful clues to interpreting the course of the disease and level of infectivity.

Seven different serological patterns were observed in this study:

Pattern I (HBsAg +ve, HBeAg +ve): which represents an early acute HBV infection with a high likelihood of infectivity(7) was observed in only one case.

Pattern II (HBsAg +ve, HBeAg +ve and anti-HBc +ve): 3 of our patients showed this pattern, which indicates an

acute or chronic HBV infection. Since there is active viral replication during this phase the likelihood of infectivity is high(7).

Pattern III (HBsAg +ve, anti-HBc +ve): was observed in 2 children and indicates the presence of an acute or a chronic HBV infection(7). Testing for IgM anti-HBc can help to differentiate between these two entities(9).

Pattern IV (HBeAg +ve, anti-HBc +ve): is generally associated with the early phase of an acute HBV infection(10). This was seen in 4 patients. The presence of HBeAg implies continued viral replication and consequently a high degree of infectivity. In such cases, the HBsAg levels are very low and cannot be detected even by the current generation of highly sensitive immunoassays(7,10).

Pattern V (anti-HBc +ve): 17 cases belonged to this category. This serological profile may be observed in the following situations: (*i*) silent carriers of HBV where HBsAg is below the threshold of detectability, (*ii*) the gap period between the decay of HBsAg below detectability and prior to seroconversion to anti-HBs-such cases are potentially infectious, (*iii*) remote past infection with HBV, *i.e.,* long persistence of anti-HBc, and (*iv*) a cross reacting antibody (false positive)(3,7,9).

Pattern VI (anti-HBc +ve, anti-HBs +ve): was the commonest pattern and was seen in 49 children. It represents the recovery phase of HBV infection and persisting immunity. Anti HBe in these cases is short lived and hence absent(7).

Pattern VII (anti-HBs +ve): was present in 24 subjects. It may be observed under the following circumstances: (*i*) responder to active immunization, (*ii*) recipient of hepatitis B immunoglobulin, (*iii*) long after HBV infection, in the uncommon situation when anti HBc disappears before anti HBs and (*iv*) false positive antibody–passively transmitted through blood or a cross-reacting antibody(7,8,11-14). In the present study none of the children had received hepatitis B vaccination or immunoglobulin. Hence the latter two possibilities seem more plausible. It is our hypothesis that this anti-HBs was passively transfused through the donor blood. This is supported by the following facts:

(*i*) The overall anti-HBs seropositivity (regardless of anti-HBc positivity) is 73% and is significantly higher than that reported in similar studies(11,14). A 'true' positive anti-HBs sample should also demonstrate anti-HBc positivity(7,12,13) and this was the situation in 49 of our cases. The latter figure is in conformity with findings in other studies(11,14).

(*ii*) Anti-HBs positivity alone was higher in patients under the age of 2 years and in those who had received a lesser number of transfusions (*Figs. 1 & 2*). This is contrary to the expectation that older children and a larger number of transfusions would increase the risk of exposure to HBV infection. However, when the true positive (anti HBS +ve and anti HBc +ve) cases were separately analysed the expected pattern of a direct correlation of HBV infection with the age and the number of transfusions emerged.

(*iii*) Simultaneous screening of the donor blood revealed anti-HBs positivity in 44% of the samples tested.

Hepatitis B virus infection thus remains a significant transfusion related complication. A combination of serological markers is of much greater diagnostic and prognostic value in interpretation of response to HBV infection. In the present study serological estimation of HBsAg alone would

have detected just 6 children with HBV infection. The use of the total battery of markers increased the sensitivity and yielded evidence of HBV exposure in 76 children, with 10 of them having active infection and 17 others having possible active infection. These would have been missed had HBsAg been used as the sole marker. The use of the other markers further helps in determining the potential infectivity of a case.

The high prevalence of HBV infection in our study emphasizes the need for vaccinating all children at the time of induction into the thalassemia transfusion programme. Moreover, since a significant proportion have active HBV infection and constitute a potential source for horizontal transmission, the vaccination programme could be extended to the close family contacts and the health personnel involved in their care.

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