

A STUDY OF HEPATITIS B AND C PREVALENCE AND LIVER FUNCTION IN MULTIPLY TRANSFUSED THALASSEMIC AND THEIR PARENTS

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ABSTRACT

A series of clinics were conducted in Delhi, India, in January, 1990. Of 54 patients with beta thalassemia major (mean age 7.6 years), 11.1% (6 out of 54) tested positive for antibodies to hepatitis C virus (anti HCV antibodies) and 66.6% (36 out of 54) showed evidence of hepatitis B virus (HBV) infection. Only 7.4% (4 out of 54) were hepatitis B surface antigen (HBsAg) positive. Of their parents, 2.2% (2 out of 90) tested positive for anti HCV antibodies, 28.9% (26 out of 90) showed evidence of previous HBV infection and 11.1% (10 out of 90) were HBsAg positive. We argue that HCV constitutes a greater long term threat than HBV in these patients due to the higher incidence of chronic liver disease. We would advocate the introduction of HCV screening of donated blood as well as reinforcing the importance of HBV screening and immunization.

Key words: *Hepatitis C, Post transfusion non-A non-B hepatitis, Hepatitis B.*

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The thalassemias are one of the commonest inherited hematological disorders. They form a heterogeneous group of conditions resulting from a wide variety of mutations of the genes which code for hemoglobin synthesis. Beta thalassaemia major is the homozygous form, inherited recessively and resulting in reduced or absent beta chain production. It's distribution is concentrated in a belt stretching from the Mediterranean through the Middle East, India and South East Asia. It also occurs in parts of West Africa.

In India, regional carriage rates have been estimated at between 0.6 and 15%(1) being particularly common in Gujarat and Sind. In the Delhi area the carriage rate is 3.5%(1). More than 8,000 children with one of the major thalassemia syndromes are born annually in the country(2).

The ideal treatment of these patients involves regular, 2-4 weekly blood transfusions. The major complications of this treatment are the transmission of transfusion acquired infections such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), syphilis and malaria and transfusion acquired iron overload.

HCV, first identified in 1989(3), is now widely accepted as the main causal agent in blood born non-A non-B hepatitis (NANBH) (4-7). An assay based on the presence of antibodies to the virus is now available(8). Infection carries with it a high risk of developing chronic hepatitis. Of all those infected, 50% develop evidence of chronic liver disease and 10% develop chronic active hepatitis or cirrhosis(9,10). Hepatitis C, like hepatitis B, also predisposes to malignant liver disease(11).

It has been estimated that the prevalence of HCV positivity in Europe in the population of non high risk blood donors

lies between 0.13 and 1.2% (4,12) but that the figure is nearer 70% in high risk groups such as hemophiliacs and IV drug abusers (4). The prevalence in India in one group of 122 voluntary blood donors has been calculated at 1.6% (Agarwal MB *et al.*, unpublished data). It has been proposed that routine screening of donated blood could greatly reduce the risk of transfusion acquired HCV infection (5) and this is rapidly becoming standard practice in Europe and North America.

HBV infection is common in India. HBsAg positivity in blood donors has been variously estimated at between 0.4 and 22.1% (13-15). Chronic liver disease in HBV infection appears to be associated with the carrier state. Approximately, 5 to 10% of those infected as adults go on to become carriers. This figure may be nearer 40% in the pediatric population (16). Of these, most will develop chronic persistent hepatitis but only a small percentage will go on to develop progressive liver disease. It remains a major health risk in the regularly transfused but this risk can be reduced by routine screening of donated blood, careful selection of blood donors (preferably excluding the use of paid donors) (15), and by vaccination (17,18).

This study was designed to determine the prevalence of transfusion related viral hepatitis and chronic liver disease in a population of multiply transfused patients with beta thalassemia major and their parents in Delhi, India.

Materials and Methods

BW and TW conducted a series of thalassemia clinics in Delhi, India during a five-day period in January, 1990. All patients with beta thalassemia major were asked standard questions in an attempt to elicit a history of previous clinical hepatitis.

They were also asked about risk factors for hepatic disease including a transfusion history, alcohol consumption, a drug history and whether or not they had received hepatitis B vaccination. Blood was drawn from patients for assessment of hepatitis B and C status and serum aspartate transaminase (AST) and ferritin levels. Blood was drawn from one or both parents for hepatitis B and C status and estimation of serum AST levels.

All hepatitis B serology [HBsAg, hepatitis B surface antibody (anti HBs) and hepatitis B core antibody (anti HBc)] was determined by radioimmunoassay using kits from Organon Teknika, B.V., Boxtel, Holland. A diagnosis of previous acute HBC infection in non immunized subjects was made on the basis of positive serology for one or more of these markers. Anti HVC antibody was identified using an ELISA kit from Ortho-Diagnostics, Raritan, New Jersey, USA. Positive results were interpreted according to the manufacturer's recommendations, as described previously (19). Aspartate transaminase levels (AST) (normal range 8-40 IU/L) were measured using an SMA auto analyzer and continuous flow analysis. Ferritin levels were assayed by a radioimmunoassay kit from Becton and Dickinson, Orangeburg, New York, USA (normal range 20-300 ng/ml for males, 30-150 ng/ml for females).

Data was collected on 54 patients and 90 parents. In the patient population the age range was 18 months to 18 years (mean age 7.6 years). They included 32 males and 22 females. They had received between 10 and 450 units of transfused blood (mean 152). Only 8 out of 54 (14.8%) were vaccinated against hepatitis B while 12 out of 54 (22.2%) gave a positive history of previous clinical hepatitis.

In the parent population the age range

was 23-54 years (mean age 36.3 years). They included 44 males and 46 females. Only 6 (6.5%) gave a positive transfusion history while 1 (1%) was vaccinated against hepatitis B. Seventeen out of ninety (18.7%) gave a positive past history of clinical hepatitis. In none of these did the history suggest that the hepatitis was transfusion related.

All data were entered onto a micro-computer and analyzed statistically using the "Minitab" package (Minitab Inc., 3081 Enterprise Drive, State College, P.A. 16801, USA, Date 1987 version 7.1).

Results

In the patient sample, 6 out of 54 (11.1%) tested positive for HCV, 36 out of 54 (66.6%) showed serological evidence of HBV infection and 4 out of 54 (7.4%) were HBsAg positive. All those immunized against HBV tested negative for HBsAg and anti HBc. Only 18 out of 54 (33.3%) showed no evidence of HBV or HCV infection. In the parent sample only 2 out of 90 (2.2%) tested positive for HCV, 26 out of 90 (28.9%) showed evidence of HBV infection, 10 out of 90 (11.1%) were HBsAg positive and 63 out of 90 (70.0%) showed no evidence of HBV or HCV infection (Table I).

Of the 12 patients with a positive history of clinical hepatitis, 11 had serological evidence of HBV infection and 2 of HCV infection (10 showed evidence of only

HBV, 1 of only HCV and 1 showed evidence of both). Of the 17 parents with a positive history of clinical hepatitis, 9 had serological evidence of HBV infection and 1 of HCV infection. Only one of the seropositive parents had been transfused.

Routine histories failed to demonstrate obvious risk factors for the acquisition of HBV, HCV or chronic liver disease in patients or parents other than those related to the treatment of thalassemia or transfusion for other reasons. We divided the patient and parent groups into 4 subgroups according to their hepatitis serology (Table II).

There was no significant difference demonstrable in plasma AST levels when these groups were compared although all groups in the patient sample had mean plasma AST levels above the normal range. Multi-variate analysis of HBV and HCV status, ferritin values and plasma AST levels in the patient population demonstrated no convincing linear relationship between these variables. Standard statistical analysis demonstrated no statistically significant link between liver function as represented by plasma AST levels and either hepatitis B or C status or serum ferritin levels.

Discussion

Our study population includes some patients who receive optimum treatment and others who receive the bare minimum. Most receive a compromise treatment due to the financial and logistic constraints of desferrioxamine iron chelation therapy and regular blood transfusions.

The parent population is included as a convenient control. Although, far from random, it is roughly representative of the voluntary donor population. Indeed, most parents will regularly donate blood into the donor pool due to the scarcity of

TABLE I—Prevalence of HBV and HCV in Patients and Parents

	Patients		Parents	
	n	%	n	%
HCV +ve	6/54	11.1	2/90	2.2
HBV +ve	36/54	66.6	26/90	28.9

TABLE II —Results of AST and Ferritin Levels in Patients and Parents. Grouped According to Hepatitis Status

Status	Patients								Parents			
			AST (IU/L)		Ferritin (ng/ml)				AST (IU/L)			
	n	%	Range	Mean	Range	Mean	n	%	Range	Mean		
HCV-HBV-	17	31.5	26-372	96.40	793 - 3353	2227.5	63	70.0	13-69	31.69		
HCV+HBV+	4	7.4	36-200	97.75	1380 - 10224	4614.3	1	1.1	39	3900		
HCV+HBV-	2	3.7	36-188	112.00	980 - 4599	2789.5	1	1.1	21	2100		
HCV-HBV+	32	59.2	20-558	115.34	877 - 12325	4865.4	25	27.8	5-91	32.48		

safe available blood. These factors should be considered when interpreting the results.

Regarding the results of HCV investigations, we had anticipated that more patients would have been infected than our figures showed (11.1%). BW, in a previous study(19), found that of a group of thalassemics transfused mainly in the UK, 12.2% were anti HCV positive compared with 45.8% of a group transfused abroad. When comparing the populations, however, our group (mean age 7.6 years) was much younger than the UK population (mean age 19.4 years) or the patients transfused abroad (mean age 13 years) in the previous study. We suspect that the age of the patient (and therefore the number of transfusions received) will correlate with the likelihood of acquiring HCV infection. The mean age of anti HCV positive patients in our current study [11.8 years (range 5.5-17 years)] was greater than that of the group as a whole adding weight to this hypothesis.

In the parent population, the prevalence of anti HCV antibodies (2.2%) was higher than estimates in non high risk European blood donors (0.13-1.2%)(4,12) and in a similar parent population (1.6%) (Agarwal MB *et al.*, unpublished data).

The numbers, however, were very small (only 2 out of 90) and cannot be regarded as statistically significant. It is interesting to note that both anti HCV positive parents had HCV antibody positive children.

With regard to the hepatitis B findings, our results confirm that HBV is highly prevalent in India. A total of 28.9% of parents and 66.6% of patients were positive for one or more hepatitis markers. More worryingly, 7.4% of patients and 11.1% of parents were HBsAg positive (*Table II*). Despite this high figure, HCV is likely to constitute a greater threat to patients than HBV since chronicity is much more common in the former than the latter. From risk figures quoted earlier we predict that 10% of the anti HCV antibody positive patients (1.07% of the population) will develop severe chronic liver disease secondary to HCV infection compared to only a small percentage of the 7.4% with chronic HBV. With these factors in mind, we would strongly agree with other workers who advocate HCV screening of donated blood(5).

There is mounting evidence that alpha interferon represents an effective temporary therapy for chronic liver disease associated with HCV infection(20,21). This cannot, however, in any way be considered

a substitute for prevention, particularly when considering the high costs and lack of current data on long term outcome.

Alternative screening methods for transfusion associated non-A, non-B hepatitis have been suggested in the past including screening for the presence of raised plasma AST levels(22-24). Unfortunately, the number of HCV positive parents in our study (2 out of 90) was insufficient to support these observations although it is interesting to note that both parents had AST levels within the normal range. Of the patient sample, all groups had AST levels above the normal range irrespective of hepatitis serology. This is likely to be due to the effects of iron overload.

Ferritin levels are of limited value as a marker of iron status in patients with chronic liver disease as they are often disproportionately raised(25). They should, therefore, be interpreted with caution where hepatitis status is unknown. Obviously, the current emphasis in the management of thalassaemia major patients in India should be the provision of regular transfusion and adequate iron chelation. We would, however, advocate that wherever feasible, the routine screening of blood for HCV and HBV and routine vaccination against HBV would significantly reduce long term morbidity associated with chronic liver disease in these patients.

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