Original Research Article

Study of seroprevalence in thalassemic patients

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Abstract

Background: Thalassemias are a group of congenital anemias that have in common deficient synthesis of one or more of the globin subunits of normal human haemoglobins. They are one of the commonest inherited hemolytic disorders.

Materials and methods: Present study was done at a teaching hospital in Ahmedabad between August 2005 and September 2007. Blood samples of patients attending Thalassemia Clinic and Pediatric Department were tested. Total 93 patients were tested for HIV, HBsAg and HCV as and when they came for transfusion.

Results: 4 Patients were found HIV positive. All of them were male. The increased seropositivity coincided with increased no of transfusions. Incidence of HIV positivity is 4.3%. Two of them were also HCV reactive and one HBSAg reactive. Out of 93 thalassemic children 4 were found HBsAg positive. All of them were male and non - vaccinated. Incidence of HBsAg positivity is calculated as 4.356. The low incidence of HBsAg positivity can be correlated with high proportion of the children getting vaccinated. 72 patients (77.4%) are vaccinated or undergoing vaccination. 19 Patients were found anti - HCV antibody positive showing an incidence of 20.4%. 13 of them were male and 6 female

Conclusion: Thalassemic children receiving multiple transfusions are at high risk of acquiring transfusion transmitted infections (TTIs). Incidence of HIV positively has decreased due to mandatory screening of all blood bags. Ideally all patients should complete vaccination for hepatitis B before starting transfusion or as soon as possible thereafter. At present HCV infection has higher incidence in

thalassemics as there is no vaccination available. Now a thalassemic with optimum transfusion and chelation has life expectancy like non thalassemics.

Key words

Seroprevalence, Thalassemia, HIV, Hepatitis B, Hepatitis C.

Introduction

Thalassemias are a group of congenital anemias that have in common deficient synthesis of one or more of the globin subunits of normal human haemoglobins. They are one of the commonest inherited hemolytic disorders [1]. Beta thalassemia major is clinically the most significant homozygous form, resulting in reduced or absent beta chain production. In India, there is variable carriage rate in different parts of the country. It is more common in Sindhis, Lohanas, Bhansalis and some tribal communities. State-wise Punjab, Gujarat, West Bengal have higher incidence [2].

Mainstay of management of thalassemics is 2-4 weekly packed red cell transfusion. Major complications of this treatment are iron overload and chance of contracting transfusion transmitted infections. Most common among them are viral infection (HIV infection, hepatitis B and C) syphilis, malaria etc.

The first reported case of transfusion associated AIDS was in 18 month old infant who had been transfused repeatedly at birth [3]. Thalassemia with HIV is a terrible combination of hereditary and acquired disease. Most countries adopted universal screening of blood bags for HIV 1 and 2 since 1985. Still, there is a small risk because of window period donation i.e. donation after getting infected but before becoming seropositive.

The usual window period of about 45 days can be brought down to around 15 days by recently invented nucleic acid amplification technology (NAT) [4]. Proper screening and self exclusion of high risk donors and encouraging repeat donors is another way.

Hepatitis B is now preventable by vaccination. For detection of infection HBsAg is most commonly used. However failure to detect HBsAg does not necessarily indicate absence of infection. In such cases anti-HBc may be the only serological marker [5].

Hepatitis C is now widely accepted as the main causal agent in the blood borne non - A non B hepatitis. It may progress to cirrhosis and hepatocellular carcinoma after many years [5]. (As more and more thalassemics are living up to adulthood - these complications become significant)

Antibodies to circulating RNA of hepatitis-C can be detected by various immunoassay techniques.

Transfusion transmitted diseases add to the misery of multi-transfused thalassemia children and create additional burden to the health care system. So there should be proper assessment of the magnitude of the problem. This will help to provide an optimally safe blood transfusion service.

Aim and objectives

- To determine the prevalence of HIV, hepatitis B and hepatitis C in thalassemia major children.
- To detect antibodies to HIV 1 and 2, Hepatitis B surface antigen and anti-HCV antibodies in multi-transfused thalassemia children.
- To compare the results of the present study with other studies.
- To find some measures to reduce the risk of transfusion transmitted disease in thalassemia children.

- Advising thalassemics for Hepatitis B vaccination, receiving transfusion from government recognized blood banks.

Material and methods

Present study was done at a teaching hospital in Ahmedabad between August 2005 and September 2007. Blood samples of patients attending Thalassemia Clinic and Pediatric Department were tested. Total 93 patients were tested for HIV, HBsAg and HCV as and when they came for transfusion.

Collection of samples

Following details about the patients were noted before collection sample like name, age, sex, clinical diagnosis, history of previous transfusion, history of immunization against Hepatitis B, its schedule, last dose etc. The blood samples were collected in plain vial. In laboratory, serum was separated.

Routinely ELISA method was used for screening HIV. If result was positive for any serum sample by EL1SA that sample was retested by other the kits using Immuno assay principle. The patients whose serum came positive after tasting by 3 different kits are declared HIV positive and informed in a confidential report after counselling.

Screening for HBsAg was done by ELISA (Micro screen) direct non competitive solid phase enzyme immunoassay method. Screening of Anti-HCV was done by Anti HCV antibody test kit (Innova).

Results

A total of 93 thalassemic children were tested for anti HIV, HBsAg and anti-HCV. These patients attend thalassemia clinic which runs every Thursday in O.P.D. Our youngest thalassemic child was a 5 month old female and eldest thalassemic was 15 years old male as per **Table - 1**.

Table - 1: Distribution of Thalassemic children according to age and number of transfusions taken.

No. of Transfusion	Age groups (in years)					Total
	<2	2-5	>5-8	>8-12	>12	
0-50	7	28	6	0 '	0	41
51-100	0	2	17	2	0	21
101-150	0	0	10	9	0	19
150-200	0	0	0	3	2	5
>200	0	0	0	3	4	7
Total	**7**	**30**	**33**	**17**	**6**	**93**

There were 57 male child (61.3%) and 36 female child (38.7%) among the thalassemics. They got hepatitis B vaccination from this hospital. 72 patients (77.4%) of these patients of these patients were either completed 3 doses or were undergoing vaccination as per **Table - 2**.

Among 4 HIV positive patients, one was positive for HBSAG and two were positive for HCV also. So total no of patients who were reactive for any of these 3 viral diseases were 24 (25.8%) as per **Table - 3**. Total 69 patients (74.2%) were free from all of these 3 diseases. All 4 HIV positive patients in present study were male as per **Table - 4**.

One patient received around 40 transfusions after which he lost follow up. He was also HCV +ve. One patient had received more than 200 transfusions. He was also HBSAg +ve and also taking antiretroviral therapy. Unfortunately one patient expired in 2006. He was also HCV +ve and took more than 200 transfusions as per **Table - 5.** There was increase in positivity with

increase in no of transfusions. All 4 HBsAg patients in present study were male (**Table – 6**).

Table – 2: Distribution of Thalassemic children according to age, sex and immunization against hepatitis B.

	Age group (in years)					
	<2	**2- 5**	**> 5-8**	**>8- 12**	**> 12**	**Total**
Fully immunized						
Male	0	6	• 15	6	4	-31
Female	0	5	12	3	1	21
Undergoing immunization						
Male	1	8	1	0	0	10
Female	3	6	1	0	0	10
Non-immunized						
Male	1	3	4	7	1	16
Female	2	2	0	1	0	5
Total	7	30	33	17	6	93

Table – 3: Anti HIV, HBsAg, Anti HCV seropositivity among thalassemic children.

Total patients	**Anti HIV positivity**	**HBsAg positivity**	**Anti HCV positivity**
93	4	4	19
100%	4.3%	4.3%	20.4%

Table – 4: Incidence of anti HIV seropositivity in relation to age and sex.

	Age groups (in years)					
	<2	**2- 5**	**> 5-8**	**>8- 12**	**> 12**	**Total**
Male						
Total	2	17	20	13	5	57
Positivity	0	1	0	1	2	4
Female						
Total	5	13	13	4	1	36
Positivity	0	0	0	0	0	0

Table – 5: Incidence of anti - HIV seropositivity with relation to no. of blood transfusions.

No. of transfusion	**Total patient**	**Positive patient**
0-50	41	1 (2.4%)
51 - 100	21	0 (0%)
101 -150	19	0 (0%)
151 -200	5	1 (20%)
>200	7	2 (28.5%)

Table – 6: Incidence of HBsAg seropositivity in thalassemic children with relation to age and sex.

	Age group (in years)					
	<2	**2-5**	**> 5-8**	**>8- 12**	**> 12**	**Total**
Male						
Total	2	17	20	13	5	57
Positivity	0	0	1	2	1	04
Female						
Total	5	13	. 13	4	1	36
Positivity	0	0	0	0	0	0

Increase in no. of transfusion increased the risk of infection. All 4 patients found HBsAg positive had not taken vaccination. Till the end of the study they developed no complications due to hepatitis B as per **Table - 7**. Among male, 13 (22.8%) and among female, 6 (16.6%) was found anti - HCV antibody positive as per **Table - 8**. None of the patients had yet developed any HCV related symptoms. This data also showed that incidence of anti HCV seropositivity increaseed with no. of transfusions as per **Table - 9**.

Table – 7: Incidence of HBsAg seropositivity in thalassemic children with relation to no. of transfusions.

No. of transfusion	**Total patient**	**HBsAg +ve**
0-50	41	0 (0%)
51 - 100	21	1 (4.76%)
101 - 150	19	2 (10.5%)
151 -200	5	0 (0%)
>200	7	1 (14.3%)

Table – 8: Incidence of anti - HCV seropositivity in relation to age and sex.

	Age group (in years)					
	< 2	**2- 5**	**> 5- 8**	**>8- 12**	**> 12**	**Total**
Male						
Total	2	17	20	13	5	57
Positivity	0	6	2	5	0	13
Female						
Total	5	13	13	4	1	36
Positivity	0	1	1	4	0	6

Table – 9: Incidence of Anti HCV seropositivity in thalassemic children with relation to no. of blood transfusions.

No. of transfusion	**Total patient**	**HCV +ve**
0-50	41	6 (14.6%)
51 - 100	21	2 (9.5%)
101 - 150	19	6 (31.6%)
150-200	5	2 (40.00%)
>200	7	3 (42.8%)
Total	93	19 (20.4%)

Discussion

Transfusion transmitted infections (TTIS) had always been a major problem in multi-transfused patients (including thalassemics) in the past. So the magnitude of the problem was always a topic for various studies, with advent of improved technology and universal screening of blood the risk is now decreased but definitely present.

The situation in developed counties is like this- The new England Journal of Medicine in 1996 quantitated the risk of giving blood in window period as follows, for HIV, 1 in 4,93,000 (95% confidence interval) for HBV 1 in 63000 (31,000 to 1,47,000) for HCV 1 in 1,03,000 (28,000to 2,88,000) [6].

In 1994, the risk in USA was 1 in 5,000 for HCV, 1 in 2,00,000 for HBV, and 1 in 4,10,000 for HIV.

In contemporary period in UK it was 1 in 40,000 for HCV, in 2,00,000 for HBV and 1 in 50,00,000 for HIV [7].

In Germany the risk of an undetected infection in 2002 was 1 in 27,70,000 for HIV, 1 in 2,30,000 for HBV and 1 in 6,70,000 for HCV. They have farther reduced this risk by implementing Nucleic acid amplification (NAT) testing [4].

In Italy there is high prevalence of thalassemia. There the risk of TTI as found in a study between 1994 and 1999 was estimated to be 2.57 million donation for HIV, 15.8/ million donation for HBV and 4.57 million for HCV [8].

So we can sec that the chance of getting infected for a multi-transfused thalassemic is variable in different parts of the world according to prevalence among general population and also safety of blood transfusion policy.

HIV had become very rare after testing became mandatory for HIV - 1 on 1989 and HIV - 2 in1993 [9].

Testing for HBSAG, anti - HCV and syphilis also serves as "surrogate markers" of high risk donors whose chance of being in window period is more.

Prevalence of anti HIV antibodies as found by different workers [9-16] was as per **Table – 10**.

Table – 10: Prevalence of anti HIV antibodies as found by different workers.

Sr. No.	Authors	Place	Year of Publication	No. Total	%Positivity
1.	Amarapurkor D.N., et al. [10]	India	1992	40	2.5%
2.	S.K. Bichile, et al. [11]	India	1992	50	6%
3.	S. Chandra, et al. [12]	Mumbai, India	1993	22	0%
4.	Choudhary N., et al. [13]	India	1995	19	0%
5.	Jaju N., et al. [9]	Mumbai, India	1998*	402	21%
6.	M.AI- Sheyyab, et al. [14]	Jordon	1999	143	0%
7.	Shaharam M., et al. [15]	Iron	2006	732	0%
8.	Chakrabarti S, et al. [16]	Kolkata, India	2006	20	0%
9.	Present Study	Ahmedabad, India	2016	93	4.3%

In present study all four patients found HIV reactive received more than 40 transfusions. Other modes of HIV transmission were screened and found not feasible. They were transfused HIV non reactive blood from this Hospital and outside. So they are likely to be infected by window period transmission.

Hepatitis B infection is endemic in India. The prevalence of HBsAg positivity was much more when routine vaccination was not done in thalassemics. Till now, it is highly prevalent in states where there is no free vaccination in govt. Hospitals for thalassemics. Prevalence of HBsAg as found by different workers [10, 11, 14-17] was as per **Table – 11**.

Table – 11: Prevalence of HBsAg as found by different workers.

Sr. No.	Authors	Place	Year of Publication	No. Total	% Positivity
1.	Amarapurkar D.N., et al. [10]	India	1992	40	4.5%
2.	S.K. Bichile, et al. [11]	India	1992	50	56%
3.	M.Al- Sheyyab, et al. [14]	Jordon	1999	143	3.5%
4.	Shah SMA, et al. [17]	Pakistan	2005	250	8.4%
5.	Shaharam M, et al. [15]	Iron	2006	732	1.5%
6.	Chakrabarti S, et al. [16]	Kolkata, India	2006	20	5%
7.	Present Study	Ahmedabad, India	2016	93	4.3%

The data clearly showed that in the pre-vaccination era the prevalence of hepatitis B was much higher than as of today. The study by M. Al Sheyyab, et al. [14] in Jordon also measured anti HBS antibody in thalassemics during the study and found it in 80% of patients. In case of the study by Shaharam, et al. [15] in Iran HBsAb was found in 55.2%.

This indicates that good antibody titre after vaccination correlates with low rate of HBsAg infection. In present study, all 4 HBsAg positive patients did not receive vaccination. Actually they were found HBsAg reactive before starting free vaccination.

Ideally minimum two dose of vaccination should be given before starting transfusion in a newly diagnosed thalassemic. It is possible only if patient comes well in advance when there is no need of emergency transfusion due to severe anaemia.

Hepatitis C is emerging as the predominant transfusion transmitted infection nowadays. There is no. vaccination for HCV. Prevalence of anti - HCV antibodies in thalassemia children as discovered by different workers [10, 13-19] was as per **Table – 12**.

Table – 12: Prevalence of anti - HCV antibodies in thalassemia children as discovered by different workers.

Sr. No.	Authors	Place	Year of Publication	No. Total	% Positivity
1.	Williams TN, et al. [18]	Delhi, India	1992	54	11.156
2.	Amarpurkar, et al. [10]	India	1992	40	17.5%
3.	Choudhary N., et al. [13]	India	1995	19	63.8%
4.	Chitnis D. S., et al. [19]	India	1996	789	25.45%
5.	M. AI- Sheyaab, et al. [14]	Jordon	1999	143	40.5%
6.	Shah SMA, et al. [17]	Pakistan	2005	250	56.8%
7.	Shaharam M, et al. [15]	Iran	2006	732	19.6%
8.	Chakrabarti S, et al. [16]	Kolkata, India	2006	20	5%
9.	Present Study	Ahmedabad, India	-	93	20.4%

The prevalence is consistently high for anti - HCV all over the world. It is found to be very high when study population of thalassemic children consisted predominantly of older children receiving more no of transfusion.

The studies which comprised of large no of patients having thalassemics of all age groups showed anti - HCV prevalence of around 20% which is comparable with present study.

A major fraction of these anti - HCV positive children develop chronic liver disease they may progress to cirrhosis and hepatocellular carcinoma after many years. A small number of patients spontaneously clear the virus. They can be detected by negative HCV RNA by PCR rest.

Conclusion

Thalassmic children receiving multiple transfusions are at high risk of acquiring transfusion transmitted infections (TTIs). Incidence of HIV positively has decreased due to mandatory screening of all blood bags. Window period can be decreased by using improved technology. Ideally all patients should complete vaccination for hepatitis B before starting transfusion or as soon as possible thereafter. At present HCV infection has higher incidence in thalassemics as there is no vaccination available. HCV is a slow infection and causes chronic liver disease and hepatocellular carcinoma after many years. Now a thalassemic with optimum transfusion and chelation has life expectancy like non thalassemics. So these complications become significant.

Thalassemic are more prone to liver dysfunction due to hepatitis because their livers are already compromised due to iron overload. The focus is now on minimizing window period donation. Proper selection of donors is of utmost importance. Donor awareness programme and providing a good questionnaire before transfusion can lead to self exclusion of high - risk donors. Purely voluntary donors (and not relatives of patients who are persuaded for replacement donation) are ideal for donation. Our goal is to prevent thalassemic by prior testing of couples and also provide optimum care to the thalassemics already born. Proper health education can make a difference in achieving that goal.

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