



TO STUDY THE PREVALENCE OF TRANSFUSION TRANSMITTED INFECTIONS AMONGST MULTI-TRANSFUSED THALASSEMIC PATIENTS OF WESTERN RAJASTHAN

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ABSTRACT

Objective: -To determine the prevalence of Transfusion Transmitted Infections (TTI) amongst multi-transfused thalassemic patients of Western Rajasthan region.

Study: - Hospital based cross-sectional study

Study Group: - A total of 100 Thalassemic patients receiving (10) blood transfusions as part of their management were enrolled in this study.

Result: -Male to female ratio in the study group was 2.0:1. The mean age of the study group was 8.48 ± 4.78 years. Half of the cases were nutritionally wasted whereas more than half of the cases were stunted according to their age as per WHO standards. 40% cases were HCV positive of which 26 (65%) cases were males whereas 14 (35%) were females with a male to female ratio of 1.8:1. There was a linear relationship between HCV positivity and age and number of transfusions. Serum ferritin, SGOT, SGPT and total bilirubin were significantly higher in HCV positive cases. Three cases were co-infected with HCV and HIV.

Conclusion: -A high prevalence of TTI was there among the study group, of which HCV was the most common. Efforts must be made to cut down the risk by proper screening of every unit of blood to be transfused for common infective agents. NAT testing should be made mandatory in every center having transfusion services in a phased manner.

INTRODUCTION

Thalassemia is the most common monogenic disorder of the world, characterized by reduced or absent amount of hemoglobin, the oxygen-carrying component of red blood cells.¹ Thalassemia was first described by Thomas Cooley and Lee in the year 1925 in children of Mediterranean origin as anemia, splenomegaly and peculiar bony changes and named it as Cooley's anemia.² It is an autosomal recessive disorder. Absent production of α chain results in excessive production of β -globin chains. Excess of β -globin chains precipitate in red cell precursors resulting in intra-medullary destruction of red cell precursors and hence ineffective erythropoiesis. Ineffective erythropoiesis leads to anemia and tissue hypoxia requiring regular blood transfusion. The optimal transfusion regimen involves regular blood transfusions, usually administered every two to five weeks, to maintain the pretransfusion haemoglobin level of 9 to

10 g/dl and a post-transfusion level of 13 to 14 g/dl. This prevents growth impairment, organ damage and bone deformities, allowing normal activity and good quality of life. Different transfusion regimens are used among which moderate transfusion regimen is the one which is the most favored and used. In this regimen pre-transfusion hemoglobin of 9-10.5g/dl and a mean of more than 12g/dl is maintained.

The treatment of thalassemia has its own drawbacks, repeated blood transfusions leads to a buildup of iron in the body (Transfusion Siderosis) and there is risk of Transfusion Transmitted Infection (TTI). Infections are a frequent complication of thalassemia and they can be fatal. In an Italian multicenter study³, infections were the second cause of death after heart failure in thalassemia. The probability of acquiring TTIs is related to the probability of being exposed to the infected units of blood which in turn depends on the prevalence of carriers among the blood donors in the population and the number of units transfused. HIV, HBV, HCV, Syphilis, Malaria, Epstein - Barr virus, Cyto-megaloVirus, Human T Lymphocytic virus (HTLV-1 and HTLV-2) and bacterial infection are important causes of concern. Viral hepatitis in 7- 12% cases are caused by transfusion of blood and its products⁴. Post transfusion Hepatitis B and C is a major problem in India because of low viraemia and mutant strains undetectable by routine ELISA. In India, it is mandatory to screen donated blood for anti-HIV 1 and 2 (since 1991), anti-HCV (since 2000), HBsAg, syphilis, and malaria. TTI can still occur from blood donations negative for markers for these infections as reported by various Indian investigators⁵ and international studies.⁶ Prevalence studies have found that common infections occurring in thalassaemic patients are Hepatitis C (2.2%-44%), followed by Hepatitis B (1.2%-7.4%) and HIV (0%-9%)⁶. There is a paucity of data regarding the prevalence of TTI in multiple transfused thalassaemia patients in Western Rajasthan, so this study was undertaken to find out the prevalence of HCV and HIV in these patients in our region.

METHODS

The present study was a hospital based cross sectional study which was conducted in the Department of Pediatrics, Umaid Hospital, Dr. S.N. Medical College, Jodhpur. This study was approved by ethical committee of Dr. S. N. Medical College, Jodhpur. The duration of study was from November 2011 to November 2012.

Inclusion criteria

All known cases of Thalassaemia major who have taken multiple blood transfusions, as part of their management (at least ten blood transfusions) irrespective of their age were included in this study.

Exclusion criteria

1. Patients who had been given (administered) less than ten blood transfusions, as part of their management were not included in this study.
2. Patients with other hemoglobinopathies like Sickle cell anemia were excluded from the study.

100 Thalassaemia patients were included in the study after an informed consent by the guardians or parents of the patients. On admission, particulars of the patient (name, age, sex, religion, residence, etc.) were noted in a pre-designed proforma, for study. A complete detailed history was taken regarding age of diagnosis, age of first transfusion, average number of transfusion in a month and during last year, family history, if any so as to ascertain the pedigree. A thorough anthropometric examination was done with regards to height, weight, head circumference, mid arm circumference and body mass index of the patient.

INVESTIGATIONS

Collection and processing of samples: - Blood samples (5ml) were taken from the patient with all aseptic precautions which were processed as early as possible. If the samples were to be stored, they were stored at 2-8°C for a week or at -20°C (if longer). Serum was separated by centrifugation at 1000 rpm for 15 min at room temperature or if frozen were allowed to thaw and the clear supernatant was then centrifuged. All the cases were subjected to routine investigations including hemoglobin, liver function tests and renal function tests. Serum Ferritin estimation was done using Chemiluminescence Immunoassay (CIA). Hepatitis-C antibody detection was done using 4th Generation Anti-HCV Immunosorbent Assays. HIV1/2 antibodies were detected for screening purpose and positive samples were confirmed using ELISA.

Data was managed on Microsoft Excel spreadsheet, all the entries were double checked and analysis was done using Statistical software STAR PACK version 3.0 by Student t test, Chi-Square test and other appropriate tests were used. Continuous data of sample were summarized as mean \pm S.D. and categorical data of sample were presented as proportions. The appropriate p value was calculated and difference between the two values was considered to be significant if p value was <0.05 .

RESULTS

A total of 100 patients were enrolled in the study, there was no attrition during the study period. There were 67 (67%) males and 33 (33%) females with an overall male to female ratio of 2.0:1. The mean age of the study group was 8.48 ± 4.78 years (range 1.5-23 years). Among the study group 49 (49%) cases had weight for age < -3 S.D. (wasted) and 61 (61%) cases had height for age < 3 S.D. (stunted) while 37 (37%) cases had both weight for age and height for age < 3 S.D. i.e. both stunted and wasted according to the CDC 2000 standards. Hepatomegaly was observed in all the cases and splenomegaly was observed in 94 (94%) cases. 4 (4%) cases had undergone splenectomy because of hypersplenism and these cases had a mean age of 13.25 ± 2.21 years. Majority (91%) of the cases in our study were diagnosed as Thalassemia before the age of 12 months, among which 46 (46%) cases were diagnosed before 6 months of age, remaining 45 (45%) were diagnosed between 6-12 months of age. The mean hemoglobin of the study group was 7.62 ± 1.09 g/dl. Sixty one (61%) cases had pre-transfusion hemoglobin between 7-9 g/dl, 35 (35%) cases had hemoglobin < 7 g/dl and only 4 cases had hemoglobin between 9-10.5 g/dl. Average transfusion requirement in age group 0-5yr was 46.6 ± 22.4 ml/kg/year and for age group 16-20yr and > 20 yr were 264.5 ± 116.27 and 308 ± 5.65 respectively ($p < 0.001$). Among the study group 65 (65%) cases were already immunized against Hepatitis-B virus and the remaining were immunized during the study period to obtain 100% immunization coverage. Among the rural cases 44.18% cases were immunized as compared to 78.94% among urban cases ($p < 0.001$). At the time of our study 95 (95%) cases were on one or the other chelating agent whereas 5 (5%) cases were not taking any kind of chelation therapy. The mean serum ferritin of the study group was 2621.29 ± 1516.98 ng/ml.

In present study 40 (40%) cases tested positive for HCV and 3 (3%) cases were positive for HIV. Among HCV positive cases, 29 (72%) were from urban region and 11 (28%) cases from rural region ($p < 0.02$). 26 (65%) cases were male whereas 14 (35%) were females with a male to female ratio of 1.8:1 (Table II). Mean age of the HCV positive cases was higher than negative cases (10.26 ± 3.88 years as compared to 7.30 ± 4.99) ($p < 0.01$). Maximum HCV positivity 19 (47.5%) was in 5-10yr age group; followed by 11 (27.5%) in 10-15yr age group ($p < 0.001$) (Table-I). The number of blood transfusions received by anti-HCV positive children (Avg. Transfusion 166.7 ± 80.15 ml/kg/year) was significantly higher than that by anti-HCV negative patients (Avg. Transfusion 107.3 ± 86.48) ($p < 0.001$). Maximum HCV positive cases 15 (37.5%)

had total transfusions ranging from 101-150 in a year followed by 7 (17.5%) cases with 151-200 transfusions ($p < 0.001$) (Fig. 1). There was only one case with transfusions < 50 in a year. Average serum Ferritin of HCV positive cases was higher as compared to HCV negative cases (3170.15 ± 1823.27 vs. 2255.38 ± 1150.46 ng/ml, $p < 0.01$). Mean serum SGOT of HCV positive was significantly higher than those of negative cases (78.87 ± 25.08 IU/dl compared to 59.06 ± 18.17 IU/dl, $p < 0.001$). Mean serum SGPT of HCV positive cases was higher than HCV negative cases (64.3 ± 26.35 IU/dl compared to 50.58 ± 16.0 IU/dl, $p < 0.01$). The mean serum total Billirubin of HCV positive group was higher as compared to that of negative group (1.89 ± 0.96 mg/dl compared to 1.35 ± 0.82 mg/dl, $p < 0.01$). The average liver span of HCV positive cases (13.32 ± 1.46 cm) was significantly higher as compared to HCV negative cases (11.55 ± 2.20 cm) ($p < 0.001$). Jaundice was noted in 77% (31 cases) of positive cases and was the most common clinical examination finding noted followed by anorexia in 15 (37.5%), Weight loss in 4 (10%) and edema in 3 (7.5%). No case had any evidence of any coagulation abnormalities or autoimmune disorders (Table III). All the HIV positive cases (3%) were previously diagnosed as HIV positive before enrolling in the study, no seroconversion was noted during the study period. All the 3 cases were also positive for anti-HCV antibody. All the positive cases were males, 2 (66.6%) were of 10-15 years age group and 1 (33.3%) case was of 15-20 years age group ($p > 0.2$) (Table II). The mean serum Ferritin of seropositive group (971.66 ± 890.95 ng/ml) was lower as compared to that of sero-negative group (2672.30 ± 1506.30) ($p < 0.01$). This was because there were only 3 cases who were seropositive and one had very low ferritin levels.

DISCUSSION

-thalassemia major is one of the major public health problems in India. The general incidence of thalassemia trait in India varies between 3 and 17%.⁷ The overall prevalence of beta thalassemia major in Rajasthan State, Western India, is 3.79%.⁸ Whenever, there is a breach in safe blood transfusion, these patients are confronted by new challenges, particularly in the form of TTI, especially HCV, HBV and HIV. Fortunately HBV infection to a larger extent can be and has been prevented by HBV immunization.

Male to female ratio of the study group was 2.0:1. The preponderance of males over females in the present study is difficult to explain, thalassemia is autosomal recessively transmitted with no sex predilection. One possible reason is the fact that the people are more concerned with the health of male offspring and hence is more likely to seek medical care for them. Among the study group 49% cases were wasted and 61% cases were stunted, while 37% cases were both stunted and wasted. This was in correlation to a similar study with 53.7% cases having weight less than 5th percentile and 66.9% cases having height less than 5th percentile according to age related charts.⁹ A high proportion of thalassemia patients show growth failure. Pubertal growth spurt is either delayed or absent in thalassemia patients because of hypogonadotropic hypogonadism due to loss of gonadotropic function of pituitary. Majority of the patients in our study had their first transfusion before their first birthday; this was in accordance with the study by Prati D. et al¹⁰ with 75.8% of their cases diagnosed below 2 years of age. Thalassemia major manifests between 6 to 24 months of age due to switch from α - to β -chain production. Moderate transfusion regime is the one which is followed at our center but only 4 (4%) cases in our study were adequately transfused. Majority of the cases did not meet up with the transfusion requirements and were at times receiving transfusion from peripheral centers and did not have a regular follow up which explains their low pre-transfusion hemoglobin. Average transfusion requirement increased among the study group as the age increased with requirements going beyond 200ml/kg/yr in ages more than 10 years ($p < 0.001$). Such a relation is expected, as due to worsening of the disease with progression of age, the requirement for blood transfusions

will increase. Hepatomegaly and splenomegaly was observed in almost all the cases which occurs due to extramedullary hematopoiesis occurring in the patients due to ineffective erythropoiesis. 4% of the patients had undergone splenectomy due to hypersplenism. A high HBV immunization status among urban population can be explained due to lack of proper health education and facilities in rural areas. Ideally Hepatitis B immunization should be given before starting transfusion therapy to all thalassemia patients.

The prevalence of anti-HCV in multiple-transfused patients is confirmed to be high. A three-year prospective study by Choudhury *et al.*,¹¹ observed that anti-HCV prevalence in the same number of thalassemia major patients was 23%, 30.7%, and 35.9% each year, respectively.

In our study the prevalence of HCV and HIV were 40% and 3% respectively. These findings when compared with the prevalence data in voluntary blood donors and general population are high. The prevalence of HCV seropositivity observed by different studies varies greatly from 2.2% to 44%. This extreme degree of variability can be because of different sensitivity and specificity of tests used, different anti-HCV prevalence in donor populations and differing donor selection criteria.¹² The high HCV seropositivity in our study can be explained by the fact that we used 4th generation HCV tests that have a sensitivity of 100% and a specificity of 98.9%.¹² This correlated with the study by Mishra D. *et al.*¹² who used a similar test and reported a prevalence of 34.1% in their study group. 65% HCV positive cases had age >9 years which means they had transfusion before 2002 when mandatory HCV testing for blood donors was started in India. The blood requirements of blood banks are met by voluntary blood donation or replacement donors but no professional donors are accepted at our center. A pre-transfusion screening is always done at our center for prevalent TTI's including HIV, HCV and HBV using ELISA tests approved by NACO. Potential transmission of viruses during the "immunological window period" cannot be undermined. The implementation of NAT testing can reduce the residual risk of viral transmission during this window period.

In our study the mean age of the HCV positive cases was higher than HCV negative case ($p < 0.01$). This is shown in Table No. II by a linear relationship between age and HCV positivity which indicates that more the age of the patient, more is the chance of patient being HCV positive. With age the number of blood transfusions received increases and so does the risk of acquiring Transfusion Transmitted Infection (TTI); De Montalembert *et al.* further supported these findings in a study conducted in France.¹³ The number of blood transfusions received by HCV positive group in the study population was significantly higher than that by HCV negative group ($p < 0.001$). Figure No. 1 shows a linear relationship between HCV positivity and total number of blood transfusions received so far except for unexplained dips at 201-250 and 301-350. This linear relationship had been first demonstrated by el-Nanawy AA *et al.*¹⁴ Average serum Ferritin of HCV positive cases was significantly higher as compared to that of HCV negative cases ($p < 0.01$). This was in accordance to the observations by Shahram Mirmomen *et al.*¹⁵ who concluded serum Ferritin to be an independent predictor of HCV infection. Serum SGOT, SGPT, total bilirubin were significantly higher in HCV positive cases in comparison to HCV negative group and so these parameters can be used as a surrogate marker of HCV.¹⁶

The prevalence of HIV infection in thalassemia varies greatly worldwide, from less than 1% to more than 20%. With the use of standard procedures for prevention, it is possible to keep the risk of HIV transmission very low. The observations of our study were in accordance with these. All the positive cases were registered at our Anti Retro Viral center and were on regular therapy. All the 3 HIV positive cases were also positive for anti-HCV antibody. Both HCV and HIV share same route of transmission like blood and blood products; so, co-infection is seen in thalassemia patients.

CONCLUSION

In the present study, of the 100 multiple transfused thalassemia patients, 40 cases were reactive for TTI. HCV was the most commonly detected TTI followed by HIV in the study group. It was observed that no patient acquired TTI during the study period. Therefore it is concluded that HCV is a major problem in the management of Thalassemia patients. Serious attempts should be made to ensure a safe blood transfusion to every patient so as to cut down the prevalence of TTI's among multiple transfused -Thalassemia patients. Education regarding TTI's including HIV, HBV and HCV to these patients is of prime importance. NAT testing of every blood unit to be transfused should be there to bring down the window period. This is able to detect HIV RNA as early as 7-14 days after infection.¹⁷ NAT testing should be made mandatory in every center having transfusion services. Steps should be taken to help the patients in every respect so that they have a regular chelation and live a healthy life.

REFERENCES

1. Rodgers GP, Rachmilewitz EA. Novel treatment options in severe beta-globin disorders. *Br J Haematol.* 1995; 91: 263–268.
2. Cooley TB, Lee P. Series of cases of splenomegaly in children with anemia and peculiar bone changes. *Tr Am Pediatr Soc* 1925; 37: 19.
3. Ladis V, Chouliaras G, Bedousi H et al. Longitudinal study of survival and causes of death in patients with thalassemia major in Greece. *Ann N Y Acad Sci* 2005; 1054: 445-50.
4. Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion.* Aug 2002; 42(8): 975-9.
5. Choudhury N, Naik S, Ramesh V. High frequency of transfusion transmitted viral infection markers in thalassemia major patients. *Indian J Hematol Blood Transfus.* 1995; 13: 115–8.
6. The prevalence of hepatitis B, hepatitis C and human immune deficiency virus markers in multi-transfused patients. *Al-Sheyyab M, Batieha A, El-Khateeb M J Trop Pediatr.* 2001 Aug; 47(4): 239-42.
7. Balgir RS. The burden of hemoglobinopathies in India and the challenges ahead. *Curr Sci* 2000; 79: 1536-47.
8. Choubisa SL. Abnormal haemoglobins, thalassaemia and G-6-PD enzyme deficiency in Rajasthan (western-India). *Haematologia (Budap).* (1991) 24: 153-165.
9. Nabavizadeh SH et al, Evaluation of growth parameters in patients with thalassemia major; *Hematology.* 2007 Oct; 12(5): 445-7.
10. Prati D. Benefits and Complications of Regular Blood Transfusion in Patients with Beta-Thalassemia major. *Centro Trasfusionale e di Immunologia dei Trapianti, IRCCS Ospedale Maggiore di Milano, Italia. Vox Sang.* 2000; 79: 129–37.
11. Choudhury N, Phadke. Review Transfusion transmitted diseases. *S Indian J Pediatr.* 2001 Oct; 68(10): 951-8.
12. Devendar Mishra et al, Transfusion associated Hepatitis C in multi-transfused Thalassemic children; *Indian Pediatrics* 2004; 41: 287-288.
13. deMonthalembert M. Transfusion in patients with Hemoglobinopathies. *Transfus Clin Biol* 2000; 7: 553-8.
14. el-Nanawy AA, el Azzouni OF, Soliman AT, Amer AE, Demian RS, el-Sayed HM. Prevalence of hepatitis-C antibody seropositivity in healthy Egyptian children and four high risk groups. *J Trop Pediatr* 1995; 41: 341-3.

15. ShahramMirmomen et al, Epidemiology of Hepatitis B, Hepatitis C, and Human Immunodeficiency Virus Infections in Patients with Beta-Thalassemia in Iran: A Multicenter Study; Archives of Iranian Medicine, Volume 9, Number 4, 2006: 319- 323.
16. K.H. Katsanos et al, Epidemiological and clinical characteristics of HCV infection in transfusion-dependent thalassemia; Annals of Gastroenterology 2005, 18(1):56-64.
17. Stekler J, Maenza J, Stevens CE, Swenson PD, Coombs RW, Wood RW, Campbell MS, Nickle DC, Collier AC and Golden MR. Screening for acute HIV infection: lessons learned. Clinical Infectious Diseases. 2007. 44(1 February):459–461

Table I**Age Distribution of HCV Positive & Negative cases**

S. No.	Age	HCV Status		Total no of cases
		Positive n	Negative n	
1.	0-5yr	4	26	30
2.	5-10yr	19	20	39
3.	10-15yr	11	10	21
4.	15-20yr	6	2	8
5.	>20yr	0	2	2
Total		40	60	100

Table II**HCV & HIV Antibody Status of Thalassemic Children**

S. No.	Status		Rural		Urban		Total
			Male	Female	Male	Female	
1.	HCV	Positive	10 (10%)	1 (1%)	16 (16%)	13 (13%)	40 (40%)
		Negative	20 (20%)	12 (12%)	22 (22%)	7 (7%)	60 (60%)
2.	HIV	Positive	0 (0%)	0 (0%)	3 (3%)	0 (0%)	3 (3%)
		Negative	30 (30%)	13 (13%)	34 (34%)	20 (20%)	97 (97%)

Table III
Correlation between biochemical and physical parameters of HCV Antibody Positive & Negative cases

S. No.	Criteria	HCV Status		p Value
		Positive	Negative	
1.	Mean Age (yr)	10.26±3.88	7.30±4.99	<0.01
2.	Mean Height (cm.)	128.1±16.23	111.26±22.15	<0.001
3.	Mean Hb (g/dl)	7.33±0.86	7.80±1.10	<0.02
4.	Mean Wt (kg)	25.76±8.23	19.93±10.57	<0.01
5.	Mean SGOT (IU/dl)	78.87±25.08	59.06±18.17	<0.001
6.	Mean SGPT (IU/dl)	64.3±26.35	50.58±16.0	<0.01
7.	Mean S. Billirubin (g/dl)	1.89±0.96	1.35±0.82	<0.01
8.	Total Transfusion (no.)	166.7±80.15	107.3±86.48	<0.001
9.	Liver Span (cm.)	13.32±1.46	11.55±2.20	<0.001

Figure No. 1

