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ORIGINAL RESEARCH

SEROPREVALENCE OF HEPATITIS C VIRAL INFECTIONS IN THALASSEMIA PATIENTS UNDERGOING MULTIPLE BLOOD TRANSFUSIONS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Thalassemia is one of commonest hereditary disease worldwide, prevalent in humid climates and affects all races. The Transfusion Dependent Thalassemia require regular blood transfusion to survive. The thalassemia patients require lifelong blood transfusion on regular basis- usually administered every 2 to 5 weeks. Due to regular blood transfusion, transfusion transmitted disease e.g. HBV, HCV, HIV infections can occur.

Materials and Methods: Observational study conducted over a period of one year, a total of 284 thalassemia patients were studied. Patients were screened for Hepatitis C viral infections by rapid test kit Tridot and the conformation was done by ELISA.

Results: Out of 284 thalassemia patients, 176(61.9%) were male and 108(38%) were females. Rural population 223 (78.52%) was more affected than urban population 61 (21.47%). Maximum patients of thalassemia belong to B+ ve 109 (38.24%) and O+ ve 101 (35.56%) blood group. Maximum 120 (42.25%) patients belong to 5.1 to 10 years age group and 86 (30.28%) belong to 0-5 years age group. Out of 118 patients 85(29.92%) patients were anti–HCV reactive. Maximum anti-HCV positive patients belong to 5.1- 10 years (35.29%) age group and 10.1 -14 years (29.41%) age group. Anti-HCV positivity increase with number of transfusions, 38.8% patients had blood transfusion more than 100 times.

Conclusion: Multi transfused patients must be regularly tested and monitored to ensure safe blood transfusion practices. Stringent donor screening with modern advents such

as NAAT (nucleic acid amplification test) and PCR must be done. Bringing awareness in community will help in reducing the problem statement.
Keywords: Hepatitis C, Thalassemia, Multiple Blood Transfusion.

INTRODUCTION

Thalassemia is one of commonest hereditary disease worldwide, generally prevalent in humid climates but affects all races.[1] This disease causes morbidity, mortality, lot of financial and emotional miseries to the family.[2]Thalassemia refers to a group of blood disease characterised by decreased or absent synthesis of normal globin chain in blood due to defect in chromosome 11. According to the chain whose synthesis is impaired, thalassemia are called α, β, γ and δ thalassemia. β thalassemia is then divided into major and minor depending on severity of symptoms and requirement of blood transfusion.[3] Thalassemia major is inherited in an autosomal recessive pattern (chromosome 11) and thalassemia minor is inherited in autosomal dominant pattern. Regular blood transfusion is required in beta thalassemia major.[4] Thalassaemia constitute a major problem in the countries around the Mediterranean Sea, the Middle East and Trans-Caucasus, India, and the Far East.[5] India is second most populous nation in world and carry approx. 30 million cases of thalassemia.[6]
The highest carrier frequency of β thalassaemia is reported in Maldives (18%), Cyprus (14%), Sardinia (10.3%) and Southeast Asia (3-5%).[7] Autosomal recessive condition, heterozygotes of either α- or β-thalassaemia are usually asymptomatic and require no treatment. Homozygotes of thalassaemia alleles result in thalassaemia syndromes or diseases. Based on their clinical severity and transfusion requirement, these thalassaemia syndromes can be classified phenotypically into two main groups-
1. Transfusion Dependent Thalassaemias (TDTs)
2. Non-Transfusion Dependent Thalassaemias (NTDTs).

Fig 1: Effects of Thalassemia on body

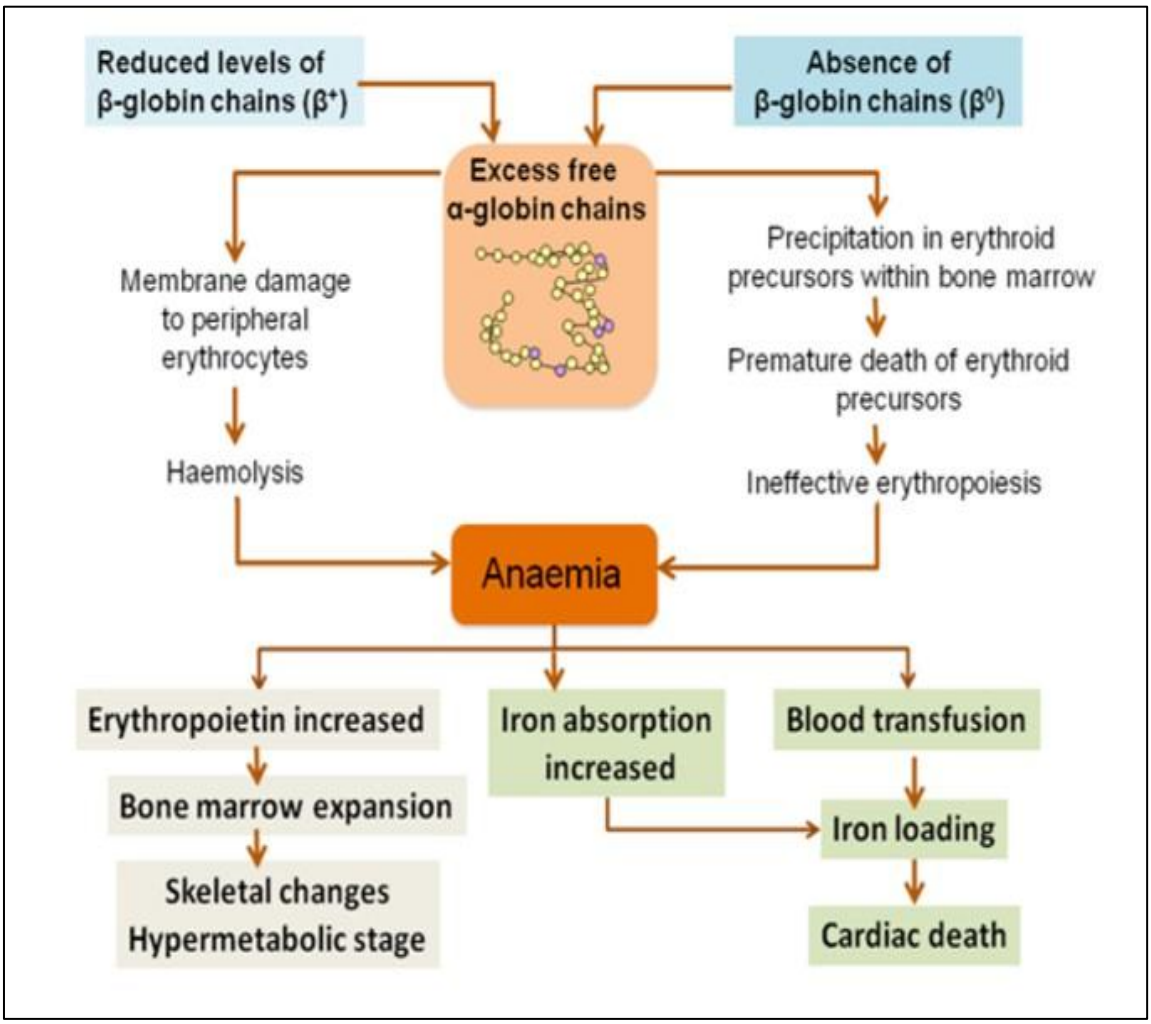
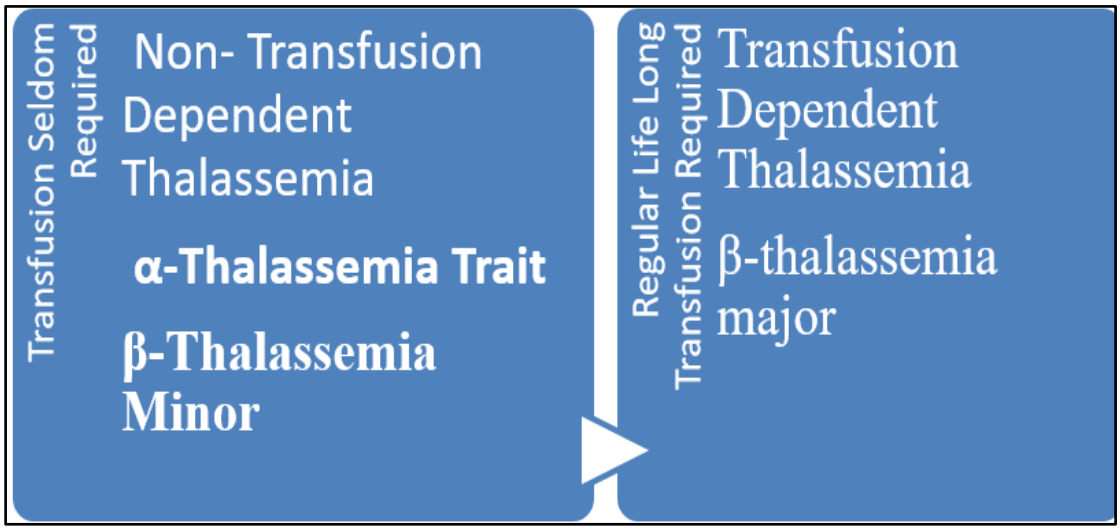


Fig 2: Transfusion requirement in NTDT and TDT



The TDTs require regular blood transfusion to survive and without adequate transfusion support, they would suffer several complications and a short life span.[3]

CRITERIA FOR BLOOD TRANSFUSION

LABORATORY CRITERIA

Hb -7g/dl on 2 consecutive occasions, >2week apart[6,7]

CLINICAL CRITERIA IRRESPECTIVE OF HB

>7g/dl, facial changes, poor growth, bone fracture, clinically significant extra-medullary haematopoiesis.[8]

The recommended transfusion programme for thalassemia requires lifelong blood transfusion on regular basis- usually administered every 2 to 5 weeks to maintain a pre – transfusion Hb level of 9-10.5g/dl and achieve a post -transfusion Hb level of 14-15g/dl.[9,10]

The β- thalassemia is the most common inherited hemoglobin disorder in the Indian subcontinent, with an uneven distribution among the different endogenous populations. They receive regular iron chelation therapy from early childhood, all of which improve the quality of life and survival of patients. On the other hand, blood transfusion exposes the patients to the risk of acquiring transfusion – transmissible infections (TTI). The possibility of acquiring TTI is associated with the number of units transfused; therefore, the infection rate of TTIs increases with age in subsequent years.

Due to regular blood transfusion, a series of complications like iron toxicity, hypersplenism, venous thrombosis, osteoporosis and transfusion transmitted disease e.g. Hepatitis B Virus (HBV), Hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) infections can occur.[7]

The viral agents transmitted by blood transfusion share certain characteristics and hall mark is persistence of infection:

(a) Long incubation period
(b) Carrier or latent state
(c) Asymptomatic sub clinical infection
(d) Viability in stored blood

These characteristics enable viruses such as HBV, HCV and HIV to be transmitted by blood transfusions.[9] Transfusion transmitted disease (TTD) is a major challenge to the transfusion services all over the world. Hepatitis B and Hepatitis C infection carrier rate is about 1-5% and 1% respectively, so post transfusion Hepatitis is a major problem in India.

Prevalence of HCV in beta thalassemia patients ranges from 3-67%.[12] Seropositivity of HCV increases with the number of transfusions.

Blood transfusion is responsible for majority of the cases of hepatitis C, it is a major source of preventable cause in post transfusion cases. Approximately 15million people are HCV positive in India. Seroprevalence of HCV in India is 0.9% and among blood donor is 0.7%. HCV infection has gained importance as one of major complications in multiple transfused patients, especially in countries where HCV is more prevalent in general population and also amongst blood donors.[13]Nearly 180 million people are infected with hepatitis C worldwide[10]. Out of 6 main groups of sequence variants (type 1-6), genotype 3 is most prevalent genotype in north and central India[11]. Hepatitis C virus is a major cause of post transfusion hepatitis

infection which leads to long term complications like cirrhosis and hepatocellular carcinoma. Since no vaccine is available against hepatitis C, the only effective measure against the virus is provision of HCV negative blood for transfusion in thalassemia patients. HCV hepatitis is more threatening than HBV hepatitis due to greater risk of chronic liver disease.[14]

MATERIALS AND METHODS

It was a retrospective study conducted over a period of one year (June 2019 to June 2020) in the Department of Microbiology, SMS Medical College and attached Hospitals. After getting appropriate institutional ethical committee approval and written informed consent from the patients, samples were collected from 118 thalassemia patients and processed. The study population included the thalassemia patients of age less than 18 years and received more than 6 blood transfusion. The blood from 118 multiple transfused Thalassemic patients is collected in plain vial and tested by rapid test and then confirmed by ELISA for antibodies to HCV and HBs Antigen.5ml blood sample of Thalassemia patients will be collected under aseptic precautions by a clean venipuncture. The blood will be allowed to clot. Serum will be separated by centrifugation at 2500rpm for 15min. Test for HCV antibodies will be performed in a batch along with two negative and two positive controls by Rapid test and by third generation ELISA kit. Tests will be performed according to the directions given in kit insert. The demographic variables were recorded on a predesigned proforma. It included name, age, sex, address, blood group, Rh factor, no. of transfusion, age of starting transfusion.

STATISTICAL ANALYSIS

The results were analyzed using descriptive statistics and making comparisons between two treatment procedures, with respect to various parameters. Discrete (categorical) data were summarized as in proportions and percentages (%) and Mean ± SD (standard deviation).

LEVEL OF SIGNIFICANCE

"p" is level of significance

- $p>0.05$ =Not significant
- $p<0.05$ =Significant at 5%
- $p<0.01$ =Significant at 1%
- $p<0.001$= Highly significant

STATISTICAL TOOLS

Categorical variables were presented in number and percentage (%) and continuous variables was presented as mean ± SD and median. Qualitative variables were compared using Chi-Square test /Fisher's exact test as appropriate. A p value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 16.0for Windows statistical software package (SPSS inc., Chicago, IL, USA). The Categorical data were presented as numbers (percent).

RESULTS

In our study, conducted on 284Thalassemia patients, we evaluated the seroprevalence of Hepatitis C in thalassemia patients. The important observations in the study were as follow:

- In our study males (61.97%) were affected more as compared to females (38%).
- Out of total 284 patients of thalassemia, 85(29.92%) patients were positive for anti-HCV. Out of 85 anti-HCV positive patients, 57 (67.06%) patients were males and 28(32.94%) patients were females. In 0 -5 year age group-17(20%) patients were anti-HCV positive. In5.1-10 years age group- 30(35.29%) patients were anti- HCV positive. In 10.1-14 years age group- 25 (29.4%) patients were anti-HCV positive. In 14.1-18 years age group-13(15.29%) patients were anti-HCV positive males. (P-value<0.001Highly significant)
- The mean number of blood transfusion in HCV positive patients=97.4±48.68 (P-value<0.001, highly significant). The mean age of anti-HCV positive patients 8.97±3.98. As the No. of transfusion increases, the positivity of HCV infection also increases as shown in table.
- Mean hemoglobin level of anti - HCV positive study population was 7.98±0.98 gm%.
- Rural population (78.2%) was more affected than urban population (21.47%).67.06% (n=57) anti- HCV positive patients were from rural area and 32.94% (n=28) from urban area with P-value 0.002, which is significant at 1%. Out of all anti-HCV positive patients 8.3%patients had splenomegaly
- Out of total 284 thalassemia patients, the majority of patients 38.24% belong to blood group B, followed by 35.56% blood group O, 19.71% blood group A, 6.33%blood group AB respectively. Out of 85 anti-HCV positive thalassemia patient, maximum patients were of O blood group (49.41%), 40% patients were of B blood group and 10.58% patients were of A blood group.
- P- value for anti – HCV positive patients of various blood group =0.0002, highly significant.

Fig 3:Preavalence of Hepatitis C among Thalassemia patients

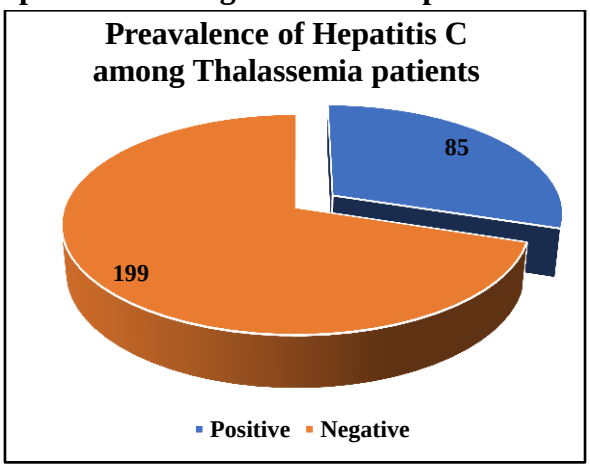


Table 1: Association of Age With HCV Status

Age	HCV By Tridot		P value
	Negative (199)	Positive (85)	
Mean	6.4683	8.9718	0.0000
SD	3.8647	3.9872	

Table 2: Association of HB With HCV Status

HB	HCV By Tridot		P Value
	Negative (199)	Positive (84)	
Mean	8.3372	7.9812	0.0301
SD	1.3625	0.9773	

Table 3: Association of No of Transfusion With HCV Status

No Of Transfusions	HCV By Tridot		P Value
	Negative (199)	Positive (85)	
Mean	57.8945	97.400	0.0000
SD	44.5755	48.6842	

Table 4: Association of Gender With HCV Status

Gender	HCV By Tridot		Total
	Negative	Positive	
Female	80 (40.2%)	28 (32.94%)	108
Male	119 (59.8%)	57 (67.06%)	176
TOTAL	199	85	284

Chi square= 1.3273, df=1, p value=0.24844

Table 5: Association of Blood Group With HCV Status

Blood Group	HCV By Tridot		Total
	Negative	Positive	
A	47 (23.61%)	9 (10.58%)	56 (19.71)
AB	18 (9.04%)	0 (0%)	18 (6.33%)
B	75 (37.68%)	34 (40%)	109 (38.24%)
0	59 (29.64%)	42 (49.41%)	101 (35.56%)
Total	199 (100%)	85 (100%)	284 (100%)

Chi square=19.4411, df=3, p value= 0.0002

Table 6: Association of Rh Factor With HCV Status

Rh Factor	HCV By Tridot		Total
	Negative	Positive	
Negative	7 (3.51%)	11 (12.94%)	18 (6.34%)
Positive	192 (96.48%)	74 (87.06%)	266 (93.66%)
Total	199 (100%)	85 (100%)	284 (100%)

Chi square=8.8784, df=1, p value=0.002

Table 7: Association of Area of Residence With HCV Status

Area Of Residence	HCV By Tridot		Total
	Negative	Positive	
Rural	166 (83.41%)	57 (67.06%)	223 (78.52%)
Urban	33 (16.58%)	28 (32.94%)	61 (21.47%)
Total	199 (100%)	85 (100%)	284 (100%)

Chi square=9.4167, df=1, p value=0.002

Table 8: Association of age and HCV status

AGE	HCV Tridot		Total
	Negative	Positive	
0-5	69	17	86 (30.28%)
5.1-10	90	30	120 (42.25%)
10.1-14	29	25	54 (19.01%)
14.1-18	11	13	24 (8.45%)
TOTAL	199	85	284 (100%)

Chi square=19.245, df= 3, p value=0.000243

Table 9: Association of no of transfusion with HCV status

No Of Transfusions	HCV by Tridot		Total
	Negative	Positive	
6-25	57	1	58
26-50	42	13	55
51-75	48	23	71
76-100	21	15	36
>100	31	33	64
TOTAL	199	85	284

Chi square=39.892, df=4, p value=0.00000

DISCUSSION

In this study out 284multiple transfused thalassemia patients, we found a slightly higher percentage of males (61.9%) as compared to females (38.0%). Study by Mukherjee K et al also reported similar finding, 58% were males and 42% were females[6]. In study by Chandani C. Surani et al 59.5% were males and 40.5% were females[15]. Similar findings were seen in the study by Neerja H Shah et al65.5% male and 34.5% females[16], Aritra Biswas et al 67.9% males and 32.1% females[17], Mahmood Iqbal M et al 82% males and 59% females[18], Muhammad Arif Ali et al 62% males and 38% females, Ahmed et al 55.5% males and 44.5% females[19], Mohamad D. Khaled et al 57% males and 43% females[20]. Study by Sumaira Khalil et al[21], H. Ansari et al[22], Hassan Raji et al and Mishra et al also reported similar findings 56% males and 44% females, 50.4% males and 49.6% females, 51.2% males and 48.8% females, 67.8% males and 31.2 females respectively[23].

On the other hand, study conducted by Al – Sharifi LM et al[24] and Ghafourian et al[25] had findings different than our study, 49% males and 51% females,47.1% males and 52.9% females respectively. Male to female ratio was 1.05:1 since the inheritance of thalassemia is autosomal which was agreed by Al-Naamani et al study[26].

In our study 78.5% patients were from rural area and only 21.5% were from urban area, which resembles with a similar study by Sattari M et al, in which only 25% patients were urban based.

The seroprevalence for anti- HCV was 29.92% in this study, which is quite comparable with Mukherjee et al study in anti- HCV seroprevalence is 24.64%.[6] Present study showed a very high seroprevalence of anti- HCV antibodies among the Thalassemia patients compared to the national average (2%). Studies conducted by Williams et al, have shown a seroprevalence of 11.1% for anti-HCV antibodies in multiple transfused thalassemia major patients[27]. Positive seroprevalence in present study findings were higher in comparison to results of similar studies conducted in India and other countries by Shaharam M. and Bhavsar H[28,29]. HCV seroprevalence ranges from 5% in Malaysian patients, 16.7% in Indian patients[70] to 63% in Iranian patients.

In this study maximum patients had blood transfusion more than 100, 38.8%anti-HCV seropositive patients had >100 blood transfusion. Similar results were shown in study by Ghafourian et al, where 28.4% anti-HCV positive patients had blood transfusion >100 and 32.3% had blood transfusion >200[25]. Similar findings were shown in study by Chandani C. Surani et al, out of 10 patients receiving >100 transfusion, 40% patients were anti-HCV positive.

In our study, maximum thalassemia patients belong to B+ ve (36.4%) and O+ ve (32.2%) blood group. Maximum anti- HCV positive patients belong to O+ ve blood group. Similar finding was found in study by Muhammad et al, where 33.77% thalassemia patients belong to O+ ve blood group[18]. In the study by H. Ansari et al, maximum patients 20.6% anti-HCV patients were of O+ve blood group[22].

SUMMERY

- In our study males (67.06%) were affected more as compared to females (32.94%). Mean age of HCV positive population was 8.97±3.98 years.
- The mean number of transfusions in HCV positive patients was 97.40±48.68 and in HCV negative patients was 57.89±44.57
- Mean hemoglobin level of HCV positive patients was 7.98±0.97 gm% and 8.33±1.36gm% with p value - 0.0301
- Maximum patients of study population belong to B blood group and maximum HCV positive patients belong to O blood group.

CONCLUSION

The decision to transfuse patients chronically should include a plan for blood administration and for evaluation of its efficacy and safety. Only in this way does the patients receive maximal benefit from the use of precious and limited human resource. In India the infections for which effective screening of blood products are currently mandatory are HIV, HBV, HCV, Syphilis and Malaria[8]. Despite blood screening of blood donors, post transfusion viral infections i.e HBV and HCV are still badly occurring. Patients with transfusion dependent Thalassemia are prone to HCV and possibility of developing liver disease is very high. Stringent donor screening, use of modern advents such as NAAT (nucleic acid amplification test) and PCR for screening of blood bags for HBV and HCV infection and bringing awareness in community will help in reducing the problem statement. Multi transfused patients must be regularly tested and monitored as a part to ensure safe blood transfusion practices. The patients should be encouraged to stick to one thalassemia management centre.

In our study high prevalence of HCV was observed. As there is no vaccine available for Hepatitis C, the only way of reducing the prevalence of HCV in multiple transfused patients is by effective and regular screening of blood by NAAT.

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