RESEARCH PAPER

Efficacy and Safety of Thalidomide in Patients With Transfusion-Dependent Thalassemia

JAGDISH CHANDRA,[1] NUPUR PARAKH,[1] SIDHARTH[1], NEHA SINGH,[1] SUNITA SHARMA,[2] MANISH GOEL,[3] HARISH PEMDE[1]

From the [1]Division of Pediatric Hematology, Department of Pediatrics; [2]Department of Pathology; and [3]Department of Community Medicine; Lady Hardinge Medical College and associated Kalawati Saran Children Hospital, New Delhi. Correspondence to: Dr Nupur Parakh, B-52, Ashoka Niketan, Opposite Vigyan Vihar, IP Extension II, Delhi 110 092. drnupurparakh@gmail.com

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Objective: To assess the efficacy and safety of thalidomide in children with transfusion-dependent thalassemia.

Methods: This prospective, single center, open-label study enrolled children aged 12-18 years, and who received thalidomide for a duration of 6 months at a starting dose of 2-3 mg/kg/day. Efficacy was assessed by reduction in transfusion requirement and rate of fall of hemoglobin. Efficacy was classified as major, moderate and minimal/no response depending on the reduction in transfusion requirement. Safety was assessed by adverse effects related to thalidomide.

Results: 37 children [mean (SD) age, 14.7 (1.8) years were included. Rate of fall of hemoglobin reduced from a mean of 1.0 (0.24) g/week pre-thalidomide therapy to 0.58 (0.26) g/week after 6 months of thalidomide (*P*<0.001). 19 children (51.3%) had major response and 12 (32.4%) had moderate response. In 13.5% and 32.4% children response was observed within the first and second month of therapy, respectively. 15 (40.5%) children remained transfusion - free for a median (IQR) time of 6 (3-10) weeks of thalidomide therapy. Mean serum ferritin (SD) decreased from 1758.9 (835.1) to 1549.6(1016.9) (*P*<0.001). Mean HbF (SD) showed an increase from 2.95(2.6) to 49.2(33.3) (*P*<0.001). In 32 children, 47 adverse events were observed. Common adverse events were constipation and neutropenia (mostly mild).

Conclusions: Thalidomide resulted in major/moderate response in majority of children with transfusion-dependent thalassemia with satisfactory adverse effect profile.

Keywords: *Hemoglobin F, Iron overload, Transfusion requirement*.

Coexistence of hereditary persistence of fetal hemoglobin (HbF) in patients with transfusion-dependent thalassemia (TDT) reduces the severity of the disease with several of them becoming non-transfusion dependent. This clinical benefit of increased HbF appears to be due to a decrease in the imbalance between β and non-β chains, resulting in reduction of ineffective erythropoiesis and hemolysis [1]. Based on these observations, many drugs including hydroxyurea, butyrate, 5-azacytidine etc have been studied as inducers of HbF for patients with thalassaemia and sickle cell disease (SCD) (2-6).

Thalidomide, a drug known for its immuno-modulating and anti-angiogenic properties, has recently been demonstrated to induce globin gene expression and to increase the proliferation of erythroid cells [7]. Experience with use in non-TDT (NTDT) and TDT is limited [8-10]. A recent study has shown major response (hemoglobin rise >2 g/dL) in 50% and 71 % at one month and three month of therapy, respectively in patients with NTDT [11].

In patients with TDT, a recent study showed mean hemoglobin increase from 8.9 g/dL to 10.5 (1.18) g/dL after 6 months of thalidomide treatment [12]. Ramanan and Kelkar from Pune have reported over 50% reduction in serum ferritin in 59 (50%) patients with thalassemia [13]. This study was thus undertaken to assess the efficacy of thalidomide in reducing transfusion requirement and iron overload and to assess its safety in patients with TDT.

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METHODS

This prospective single-center open-label study was conducted in a tertiary care public hospital of India from October, 2019 to April, 2020. The Study included children with TDT aged 12-18 years enrolled from the thalassemia day care center, after detailed counselling regarding the study and explaining the adverse effects of use of thalidomide. Out of 37 patients, 4 had HbE-β-thalassemia, but were clinically behaving as TDT. No patient in this study was on hydroxyurea. Those having HIV, hepatitis C

or hepatitis B infection, known neurological problems, known chronic systemic disease, hypersplenism, and patients with vitamin B12 or folate deficiency were excluded. Post- pubertal girls were enrolled immediately after menstrual period. Ethical clearance was obtained from institutional ethics committee and approval of Drug Controller General of India (DCGI) was obtained for use of thalidomide for a new indication. A written consent was obtained from the parents/ caregivers and assent was obtained from the participating children.

The sample size was calculated using Epi Info (*https://www.openepi.com/SampleSize/SSMean.htm*). A sample size of 32 children was calculated considering the current mean packed red blood cell (RBC) requirement of 220 mL/kg/year and likely minimum 10% reduction in annual packed RBC requirement when thalidomide is provided. The sample size was computed considering the two tailed test with an alpha error of 0.05 and power of 80%. Considering a drop out of 15%, a final sample size of 37 children was enrolled in the study.

Detailed history and examination was done at baseline and during each follow up visit at 2-4 weeks interval. At follow visits, enquiries were made specifically for constipation, sedation and neurological symptoms. Baseline investigations included complete blood counts, absolute reticulocyte count (ARC) (using XN-1000 automated hematology analyzer, Sysmex Corporation). Prothrom-bin time (PT), activated partial thromboplastin time (aPTT) and d-dimer levels were performed on STA compact Stago automated coagulo-meter (Diagnostica Stago). These investigations were repeated every four weeks. Hemoglobin F (HbF) levels were estimated at baseline using Bio Rad Variant II (BIO RAD, US) and was repeated at the end of study at 6 months.

The goal of transfusion therapy was to keep pre-transfusion hemoglobin level between 9-10.5 g/dL. Thalidomide was started at dose of 2-3 mg/kg for 24 weeks [12]. Thalidomide was used in rounded-off value and different strengths were also created using empty capsules containing 25 mg drug. If the patient was showing response and was free of adverse effect, the same dose was continued. The dose was increased upto 3-4 mg/kg in cases with no response with initial dose if the drug was well tolerated (maximum dose given to patients was 3.7 mg/kg/day). Ecosprin was not given to any patient enrolled for the study, irrespective of dose of thalidomide, except for the patient who were splenectomized or transiently for patients with increased D-dimer, during monitoring.

The response to thalidomide therapy was also assessed as mean change in rate of fall of hemoglobin and transfusion requirement during study period. The levels of pre-transfusion hemoglobin, fetal hemoglobin, ARC and serum ferritin were also compared. Subjects with more than 50% reduction in transfusion requirement as compared to pre-study transfusion requirement were classified as having major response (Group 1); those with 25-50% reduction in transfusion requirement were classified as moderate response (Group 2), and those with less than 25% decrease in transfusion requirement were classified as minimal/no response (Group 3).

Statistical analysis: The response was statistically analyzed, using paired t test. In the three groups, response was compared using ANOVA test. Post-hoc analysis was also performed for finding out the statistically significant differences. *P* value of less than 0.05 was considered as statistically significant.

RESULTS

The flow of the study is shown in **Fig. 1**. The study included 37 children (M: F-2.36:1) with a mean age of 14.7 (1.82) years. **Table I** describes baseline parameters of study subjects. Notably, only one child was splenecto-mized, and none had HIV, Hepatitis B or HCV infection. Mean (SD) HbF level of the subjects was 2.95% (2.6%). Of the 33 children for whom information on mutation study was available, seven children had variable combination of β^0/β^0 mutations, 9 patients had severe β^+/severe β^+ muta-tions, and 5 patients were compound heterozygous for β^0 and severe β^+ mutations. Seven patients had variable combination of either severe β^+/ mild β^+ or compound heterozygous for β^0 mutations with second mutation being

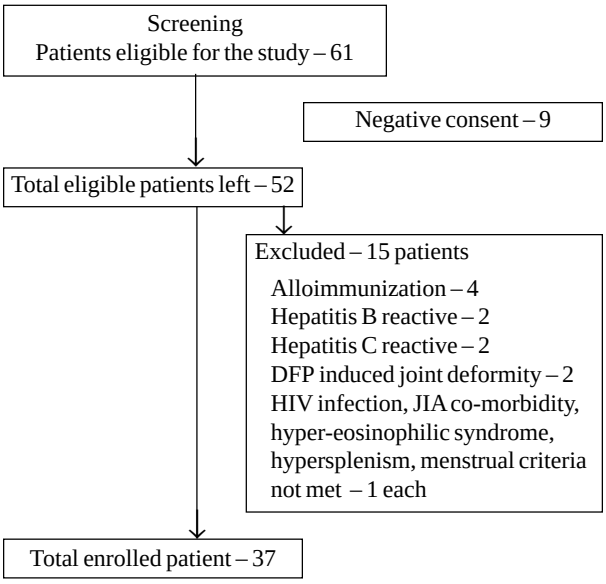

Fig. 1 Study flow chart.

Table I Baseline Characteristics of Children With Thalassemia Enrolled for the Study (*N*=37)

Characteristics	*Value*
Age (y)	14.7 (1.8)
M: F	26:11 (2.36:1)
Packed red cell received,[a] mL/kg	75.7 (12.3)
Pre-transfusion hemoglobin, mg/dL	9.45 (0.67)
Serum ferritin, ng/mL	1758.9 (835.1)
Absolute reticulocyte count	24076.8 (27781.3)
Fetal hemoglobin (%)	2.95 (2.6)
Mutations (*n*=33)[b]	
B^0/ Severe β^+	28
E β-double heterozygous	4
Thalidomide dose (mg/kg/day)	2.05 (0.35)
Chelation, no.	
Deferasirox	14
Deferasirox and deferiprone	23
Serum folate (ng/mL)	24.4 (14.9)
Serum vitamin B12 (pg/mL)	421.1 (147.2)

Data expressed as mean (SD) or as stated. [a]Six-month pre-study. [b]Mutation report of 4 patients were not available, and 1 patient did not have any of the common mutations.

uncommon Indian mutation which could not be detected. None of the subjects had low serum folate or vitamin B12 levels.

Table II describes changes observed in study parameters as compared to baseline parameters. Rate of fall of hemoglobin decreased from a mean of 1.0 (0.24) g/ week to 0.58 (0.26) g/week (*P*<0.001). Hemoglobin F levels showed a significant increase and serum ferritin decreased significantly (*P*<0.001). However, rise in mean ARC was statistically not significant. In 32/37 (86.5%) patients, the dose of drug was increased if they had tolerated the drug well without any evidence of adverse effects. In 27/37 (72.9%) patients, dose reduction was done for development of adverse effects on any follow up visit, but most of the adverse effects were either grade 1 or 2 [14].

Of the 37 patients recruited in the study, one child succumbed to dengue shock syndrome during second month of study period. In two children, therapy was discontinued due to withdrawal of consent and adverse effect in one case each. Nineteen children (51.3%) had major response while 12 children (32.4%) had moderate response; remaining 6 (16.2%) had minimal/no response. **Table III** shows that before intervention, the three groups were similar with respect to their mean pre-transfusion hemoglobin and mean packed RBC received in 6 months

Table II Patient Characteristics at Baseline and on follow-up in Children With Thalassemia Treated With Thalidomide

Characteristic	*Baseline*	*End of the study*
Hemoglobin, mg/dL (6 mo)	9.45 (0.67)	8.89 (0.6)
Transfusion requirement, mL/kg (for 6 mo)	75.7 (12.3)	38.9 (19.1)
ROF of hemoglobin, (g/wk)	1.0 (0.24)	0.58 (0.3)
Absolute reticulocyte count[a]	24076.8 (27781.3)	111518 (50236.8)
Serum ferritin, ng/mL	1758.9 (835.1)	1549.6 (1016.9)
Hemoglobin, F (%)	2.95 (2.6)	49.2 (33.3)

Data expressed as mean (SD). ROF- Rate of fall. All P<0.001 except [a]P=0.06.

preceding the study period. However, during the study period, the group with best response received 25.02 (10.37) mL/kg packed RBC compared to the group with minimal/ no response receiving 67.76 (16.31) mL/kg (*P*<0.001). Mean HbF in the group with best response was 66.9% (28.59%) while in group 3 it was only 16.62% (11.23%) (*P*<0.001). Although mean serum ferritin was significantly decreased, the fall in individual groups was not statistically significant.

In five children (13.5%) response was observed within first month of therapy, 12 more responded in the second month. Response was observed in third and fourth month of therapy in additional 5 (13.51%) and 6 (16.21%) patients, respectively. During the study period, 15 (40.5%) children remained free of transfusion for a median (IQR) time of 6 (3-10) weeks of thalidomide therapy. However, after stopping thalidomide therapy, all children have required transfusions after a median (IQR) of 24 (19-52) days.

A total of rest 32 children had 47 adverse events; constipation being the most common (14, 37.8%). Raised transaminases in two children were considered unrelated, as they were also receiving deferasirox. Other adverse effects included somnolence/sedation (*n*=3) and mild dizziness (*n*=5, in one child this necessitated discontinuation of therapy). One child developed acute kidney injury during study period. This child was also receiving deferasirox, but as renal injury occurred during the study period, thalidomide was discontinued. Neutropenia was observed in 10 children; however, only one child had absolute neutrophil count less than 500/mm^3, which required temporary cessation of thalidomide. D-dimer was elevated in 6 (16.2%) children but none had any features suggestive of thromboembolism. Infections occurred during study period in 8 subjects: pneumonia, 2; chicken-

Table III Study Parameters in Children With Thalassemia Based on Response to Thalidomide

Parameter	*Major response (n=19)*	*Moderate response (n=12)*	*Mild response (n=6)*
Pre-transfusion hemoglobin, mg/dL			
Pre-study	9.53 (0.61)	9.47 (0.67)	9.12 (0.87)
At the end of study[a]	9.2 (0.58)	8.65 (0.51)	8.39 (0.36)
pRBC received, mL/kg			
6 mo pre-study	73.96 (12.9)	77.8 (10.9)	77.1 (14.1)
6 mo during study[c]	25.0 (10.4)	48.8 (8.9)	67.8 (16.3)
Thalidomide, mg/kg/d	2.53 (0.36)	2.28 (0.49)	2.56 (0.35)
ROF of Hb (g/wk)[a]	0.45 (0.17)	0.67 (0.29)	0.8 (0.19)
Hemoglobin F (%)			
Pre-therapy	3.5 (2.2)	2.25 (3.0)	2.68 (3.0)
Post therapy[b]	66.9 (28.6)	39.74 (32.0)	16.62 (11.2)
Serum ferritin, ng/mL			
Initial	1648.8 (700.4)	1867.4 (1237.5)	1890.4 (640.6)
At the end of study	1314.4 (718.6)	1694.5 (1177.2)	1886.7 (1365.0)

Data expressed as mean (SD). Hb- Hemoglobin, pRBC-Pure red blood cell, ROF- Rate of fall. [a]*P<0.01,* [b]*P=0.001;* [c]*P<0.001.*

pox, 1; unclassified acute febrile illness, 3; and dengue infection in 2 (one of whom died of dengue shock syndrome). The patient who died of dengue shock syndrome, the starting dose of thalidomide was 1.6 mg/kg, upto maximum of 2.4 mg/kg in follow-up visit. That child was not splenectomized, never had neutropenia during the study period, and was on deferasirox alone. During the febrile period, thalidomide had been withheld.

All adverse events were grade1 to grade 2 except one episode of neutropenia (grade 3) and one episode of acute kidney injury (grade 4), necessitating temporary cessation of the drug. No female patient in our study population had any menstrual abnormality. One child withdrew from the study due to sedation interfering with his studies. This child was on starting dose of 2.4 mg/kg thalidomide which was reduced after grade 2 sedation.

Nerve conduction studies (NCV) were not performed routinely at baseline or after therapy. Only one child complained of mild tingling sensation, for which NCV was performed, and thalidomide was restarted as it was normal. His serum B12 and folate levels were normal.

DISCUSSION

Over the last decade, there has been an interest in use of thalidomide in patients with thalassemia syndromes. After initial isolated case reports, it was used with success in patients with NTDT. In TDT, the experience is limited and is now emerging. Jiskani and Memon [12] reported good response in 70 children but the extent of decrease in transfusion requirement was not commented upon. Yassin [15] described his results on 37 patients including adults and only 14 patients with TDT. He described response in over 75% cases. He also describes the fall in transfusion requirement in terms of 'units' of packed cells and not in mL/kg [15]. Other studies from India and China have also reported response in up to 70% patients [16-18].

The present study is exclusively on children with TDT. We included children above 12 years as FDA approval is restricted to 12 years or above [19]. We have demonstrated major and moderate response in 51% and 32 % patients with reduction in transfusion requirement coming up as early as first month of therapy. The response rates and timing of response are similar to earlier studies [15,16,20]. We assessed the weekly rate of fall of hemoglobin which decreased significantly, as well as a decrease in packed cell requirement. Of the responders, 15 patients remained transfusion free after a median (IQR) of 6 (3-10) weeks. However, all our patients have started requiring transfusions after stopping thalidomide.

The response to thalidomide is described to be by production of fetal Hb. There are experimental studies demonstrating increased HbF production with thalidomide and other related compounds [7,21,22]. Clinical studies have not looked at rise in HbF; though, we found a rise in HbF in those with major response. Thalidomide also seems to have effect on iron overload. We observed a modest but significant fall in mean serum ferritin; although, all the patients continued to receive chelation. This is in concordance with earlier observations [12,13,15].

Therapy with thalidomide was well-tolerated. Nag, et al. [16] observed constipation in over 40% cases. Shah,

WHAT IS ALREADY KNOWN

- Thalidomide induces globin gene expression and increases the proliferation of erythroid cells.

WHAT THIS STUDY ADDS?

- Thalidomide can be an effective drug to reduce transfusion requirement in children with transfusion-dependent thalassemia.

et al. [17] described thrombocytopenia in 66%, but their patients were also receiving hydroxyurea. Neutropenia was reported in 5% patients in another study [20]. However, we encountered neutropenia in 10 (27%) cases one of which was severe necessitating temporary cessation. Out of 10 study patients who developed low ANC during the study period, 6/23 patients were on DFX and DFP and 4/14 patients were on DFX. However, risk of development of neutropenia between the two group (on combined DFP and DFX and on DFX alone was not statistically significant (P=0.87). In a study by Naithani, et al. [23] on safety of deferiprone in children, neutropenia was observed only in 2/44 patients. However, as DFP can also cause neutropenia, children on deferiprone and thalidomide, they need a closer watch on their blood counts. One concern that we have is occurrence of infections in 8 subjects over the study period. It is unclear whether this is a chance occurrence or related to thalidomide administration. This is not described as a known adverse effect of thalidomide.

The study has certain limitations. We have not studied different doses. Moreover, follow up after stoppage of drug was not a part of the study.

Therapy with thalidomide is being looked at as an affordable alternative to transfusion therapy or at least to partially offset the transfusion needs [24]. Being relatively inexpensive and well tolerated also makes it a viable option. However, for a drug to be administered indefinitely there are certain questions which need to be addressed. First, studies are required to find out most effective and safe dose. Second, whether the drug should be continued in full doses or doses can be reduced after a response is obtained. Third, criteria for response need to be defined and applied uniformly. Role of intermittent therapy also needs to be explored. Safety under 12 years is also not been studied. Interactions with DFX and DFP- two commonly administered iron chelators also need to be studied. Larger studies to answer these issues are required before decision for long term routine use is taken. Till such time drug should be used under strict monitoring of the patients.

Ethics clearance: Institutional ethics committee, Lady Hardinge Medical College; No. LHMC/ECHR/2019/29 dated September 23, 2019. DCGI Clearance: F. No. 12-01/19-DC (Pt-208) dated November 20, 2019.

Contributors: JC: conceived and designed the study, drafted the manuscript; NP: reviewed the literature, collected the data, helped in drafting the manuscript; S and NS collected the data, SS supervised the laboratory work; MG: did statistical analysis: HP: helped in study design. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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