Transfusion of neocytes concentrate/pooled neocytes in β -thalassemic patients

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Abstract

Purpose of study Study was aimed to observe benefits of transfusion of neocytes/pooled neocytes over packed RBCs by super transfusion regimen in β -thalassemic patients and its comparison with related studies.

Materials and methods Twelve cases were selected and were equally divided into control and study group in which packed RBCs and neocytes were transfused respectively for one year. In study group, average 8 transfusions were required over 14 in control group.

Results A marked extension of transfusion interval was observed in study group patients receiving neocytes transfusion (avg. 45.8 days; range, 32–54) over control group patients i.e. those receiving conventional packed RBCs (avg.

26.1; range 22–30). There is an average 40.8% reduction in the volume of RBCs required in the study group compared to control group and this difference is statistically significant ($p = 0.00075$). In study group, 20.1% reduction in iron overload and increase of iron by 9.6% in control group was reported.

Conclusion We observed that neocytes transfusion is effective in extending the transfusion interval as well as in reducing the blood requirement and iron over load.

Keywords Neocytes · Pooled Neocytes · Iron overload · Thalassemia · Super transfusion regimen

Introduction

Thalassemias are widespread throughout the Mediterranean region, Africa, the Middle East, the Indian subcontinent and South East Asia. They occur due to decreased/absent synthesis of β globulin chains [1]. β -thalassemia major is an autosomal recessive genetic disorder, which is clinically; the most severe form and the affected child is dependent on regular blood transfusion for survival [2]. The current management of β -thalassemia major patient is based on regular transfusion of packed red cells and effective chelating therapy [3–6].

The aim of the transfusion therapy is to correct anemia and to maintain circulating level of hemoglobin (Hb) sufficient to suppress endogenous erythropoiesis [7]. Major complication in chronically transfused patients is iron overload [8]. It is not possible to manage and afford bone marrow transplantation in each and every thalassemic patient in Indian subcontinent. Packed RBCs are given

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generally 10–15 ml per kg body weight by hyper transfusion regimen [7]. Other transfusion products that can be used are leucodepleted RBCs, leuco-reduced RBCs, saline washed RBCs and neocytes. In hyper transfusion regimen the pre-transfusion Hb is maintained as >10 gm/dl with mean Hb of about 12 gm/dl, immediate post transfusion Hb rising to 14 gm/dl and returning to baseline after 3–4 weeks. In the Super transfusion regimen the pre-transfusion Hb is maintained at ≥ 11 gm/dl or a hematocrit of more than 35% with a mean Hb of about 14 gm/dl, while in moderate transfusion regimen the pre-transfusion Hb is maintained at the values between 9 and 10 gm/dl.

Neocytes are the young red cells. Concept of transfusion of younger RBCs i.e. neocytes was introduced by Proper et al. in 1980 in thalassemic patients. Neocytes survive in the circulation longer than older cells and may reduce the total blood requirement. These cells are harvested on the basis of density gradient centrifugation [9]. Usually 50% of original red cell volume is recovered as neocytes as they are less dense; they eventually position themselves in the uppermost layer, permitting their aspiration and collection. Neocytes have a higher survival rate in vivo than normal RBCs [10]. The transfusion of such cells lengthens the interval of transfusions in thalassemic patients. The program of super transfusion of neocytes produces a significant decrease in the rate of iron accumulation in patients with thalassemia [11].

Present study was aimed to observe advantages of leuco-reduced saline washed neocyte/pooled neocytes over conventional packed RBC unit transfusion by super transfusion regimen in thalassemic patients.

Material and methods

This study was carried out in year 2005–2006 in blood bank, department of Pathology, G. R. Medical College, Gwalior and Emergency blood bank Gwalior. Both centers were licensed and fully equipped for component preparation with the facilities of deep fridge centrifuge, laminar flow, plasma extractor, dielectric tube sealer, deep freezer of -40°C and -70°C , sterilized connecting device, cryo-water bath etc. Transfusion was carried out at different pediatric hospitals in Gwalior (M.P.). We selected 12 cases of β -thalassemia major, age group ranged from 3 years to 8 years. Parental consent was obtained in all cases. All patients were on iron chelating therapy (Cap. – Kelfer, Cipla®: drug – Deferiprone, dose 75 mg/kg body weight/ day) before and during the study period. Six patients were considered in control group and they were given conventional packed red cells, whereas remaining six patients were in study group and

were transfused with leuco-reduced saline washed neocyte concentrate/pooled neocytes. In both groups transfusions were given for 12 months and volume of single transfusion was between 120–300 mL as per body weight of child and transfused over a period of 3–4 hours.

Packed RBCs and Neocytes were separated from freshly collected whole blood (within 6 h of collection) by close method of preparation of component while pooling and saline washing of the products were done by open method under sterile conditions. Neocyte concentrate was prepared by using triple bag with slow centrifugation and separation method from fresh whole blood by recovering 50–60% of top of the packed red cells as neocytes. Wherever required pooling of packed cells and neocytes was done as per volume calculated and leuco-reduced saline washed units were prepared whenever necessary. Leuco-reduction was achieved by removing buffy coat layer from packed cell unit and isotonic saline was used to obtain saline washed, saline suspended neocytes.

Tests done on patient's blood were hemoglobin estimation, hematocrit, complete blood count, RBC indices, peripheral smear study and reticulocyte count before and after the transfusion. Serum ferritin level was done before starting, in between and after 12 months i.e. completion of the study. Hemoglobin of both group patients were maintained at more than or equal to 11 gm/dl (≥ 11 gm/dl) or a hematocrit of more than 35% with mean hemoglobin of about 12 gm/dl, according to super transfusion therapy. Before transfusion, ABO and Rh grouping of the patient and donor, pre-transfusion screening of unit for infectious diseases, antibody screening test of patient, and indirect coomb's cross match of the unit with patient's sample was done as recommended.

After one year i.e. completion of the study serum ferritin level, interval between transfusions, total number of transfusions, total volume of RBCs and adverse effects of transfusion in all the cases were observed. All the datas collected were analyzed manually. Proportion and student unpaired *t* test was applied to analyze the results.

Results

After at completion of the study by maintaining the hemoglobin more than or equal to 11 gm/dl in all the patients, the transfusion interval in control group was observed to be 22–30 days with an average of 26.1 days whereas it was 32–54 days with an average of 45.8 days in study group. An average number of transfusions given were 14 in control group whereas it was 8 in study group. A significant ($p = 0.00075$) average reduction in the total volume of

Table 1 Comparison of control and study group

Groups	Transfusion interval in days	No. of transfusion	Serum ferritin fall (%)	Serum ferritin Increase (%)
Control	22–30 (Avg. 26.15)	14	–	9.6
Study	32–54 (Avg. 45.86)	08	20.1	–

Avg. = Average

Table 2 Transfusion of conventional packed RBC units Group-I: control group

Sr. No.	Age in Years	Avg. transfusion interval in days	S. Ferritin level before study (ng/ml)	S. Ferritin level after study (ng/ml)	Increase in Ferritin level (%)	No. of transfusions	Total volume transfused in mL
1.	3½	24.33	2200	2420	9.1	15	2200
2.	5	26.07	3900	4280	8.08	14	2800
3.	7½	28.07	4012	4423	9.3	13	3120
4.	7	28.07	3982	4490	11.4	13	2660
5.	6	24.33	2850	3135	9.1	15	3750
6.	4	26.07	3100	3421	11.03	14	2530
Avg.		26.15	3340.66	3694.83	9.6	14	2843

Avg. =Average; S. = Serum.

Table 3 Transfusion of neocyte/pooled neocytes units Group-II: study group

Sr. No.	Age in years	Avg. transfusion interval in days	S. Ferritin level before study (ng/ml)	S. Ferritin level after study (ng/ml)	Decrease in Ferritin level (%)	No. of transfusions	Total volume transfused in ml.
1.	4½	45.62	2920	2328	20.27	8	1600
2.	6	52.14	4100	3275	20.12	7	1610
3.	8	45.62	4104	3278	20.12	8	2160
4.	3	40.55	2210	1760	20.36	9	1440
5.	5	45.62	3425	2745	19.85	8	1760
6.	4	45.62	3102	2482	19.99	8	1520
Avg.	45.86	3310.16	2644.66	20.11	8	1681	

Avg. =Average; S. = Serum.

RBCs by 40.8% was observed in study group patients over control group patients. In the study group, an average reduction in the serum ferritin level was 20.1% while average increase of 9.6% was observed in control group.

There were no transfusion discomfort and transfusion complications in study group while difficulties in transfusion, febrile and allergic reactions were observed in control group.

Discussion

Most of the pathological changes in β -thalassemic patients are thought to be secondary to both iron overload and chronic anemia. The prime source of iron deposition in thalassemic patient is the transfused red cells. Prolongation of the life span of transfused RBCs i.e. neocytes will result in decreased iron deposition per unit time [8, 12].

In our study marked reduction in number of transfusion was observed in neocyte transfused patients i.e. in study group as compared with conventional RBCs transfused patients i.e. control group (8 versus 14). This is because neocyte unit has half-life approximately twice than that of a conventional packed RBC unit and reduces the frequency of transfusion [13, 14]. Extension of transfusion interval observed in our study group patients were significant i.e. 32–54 (Average 45.8 days) over control group patients in which it was 22–30 days (Average 26.1 days). It is nearly the same as observed by Proper et al. and Collin AF et al. 43 $\pm$ 4.5 days and 35–44.5 days respectively in their studies of neocytes transfusion. In study group, we have also observed an average reduction of serum ferritin level by 20.1% i.e. reduction in iron overload (because iron over load is directly proportional to serum ferritin level) and it was better than the study done by Collin AF et al. in which they have

observed reduction of serum ferritin level by 19.05%. In the control group, we observed 9.6% increase in iron over load whereas 20.1% reduction in iron over load was reported in study group. This is because of significant ($p = 0.00075$) reduction in the volume of transfused RBCs by 40.8% in study group over control group.

In our study we have maintained normal hematocrit/ hemoglobin in transfusion dependent patients by super transfusion. By using super transfusion regimen, maintenance of physiological level of Hb% is beneficial in the management of patients with thalassemia major since it leads to better tissue oxygenation, reduction of blood volume and reduced intestinal absorption of iron [15]. Reduction in the rate of ineffective erythropoiesis by super transfusion therapy has an established benefit on bone development [16] and also decreases iron absorption from the gastrointestinal tract [17, 18] by markedly reducing plasma iron turnover [17].

We have used leuco-reduced saline washed neocytes/pooled neocytes in our study group, resulting in decreased viscosity of the unit and hence we have not observed difficulty during transfusion and transfusion reactions were also not observed due to removal of leucocytes and plasma from the unit. While in control group, occasional difficulties in transfusion as well as allergic and febrile reactions were observed due to donor's leukocytes and plasma. Leuco-reduction helps in preventing most of the febrile reactions, although it is doubtful that it can prevent post transfusion graft versus host disease, as the dose of lymphocytes needed for the same is not known [19]. Leuco-depleted RBCs (Irradiated RBCs) were not used in the study due to limited facilities. Leuco-reduction was not done in control group because we are trying to compare our study with conventional method of packed RBCs transfusion and it is also not affecting the rest of the parameters of the study. In the end of study Author(s) are of the opinion that leuco-reduction of the units should be done in chronically transfused patients. Sterile conditions were strictly followed during open method of preparation of components and this prevented any transfusion reaction due to bacterial contamination.

Neocyte aphaeresis is promising for hematological patients dependent on red cells transfusion [20] but centrifugation and separation method of obtaining neocytes used in our study is also effective.

Conclusion

Hereby we conclude that by maintaining normal hematocrit/ hemoglobin in transfusion dependent thalassemic patients

by super transfusion ($HCT \geq 35\%$ /Hb ≥ 11 gm/dl) with leuco-reduced saline washed neocytes/pooled neocytes along with effective chelating therapy, we are able to provide better quality of life and markedly altering the prognosis of thalassemic patients because this therapy benefits the patients in multiple ways as it extends the transfusion interval, reduces blood transfusion requirement, increases the survival of transfused cells in vivo, suppresses the ineffective erythropoiesis, reduces the plasma iron turnover, decreases iron absorption from gastrointestinal tract, corrects anemia in a better way and markedly reduces iron overload.

This therapeutic regimen resulted in normal growth, proper bone development and protection of vital organs from iron over load.

Our method of preparation of neocytes i.e. centrifugation and separation method is at par with results obtained with neocyte-aphaeresis and hence is very useful for places where aphaeresis facility is not available.

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