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IRON NEED DURING STIMULATED ERYTHROPOIESIS IN VERY LOW BIRTH WEIGHT PRETERMS: CAN SERUM CONCENTRATION OF TRANSFERRIN RECEPTOR BE USED AS AN ADDITIONAL MEASURE?

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SUMMARY

Serum transferrin receptor (sTfR) levels reflect both iron need and the rate of erythropoiesis. The objective of this study was to investigate iron requirements in simultaneously transfused very low birth weight (VLBW) preterms when erythropoiesis is stimulated by standard doses of human recombinant erytropoietin (rHuEPO). Included in the study were 23 VLBW infants. All of the infants received rHuEPO 200 IU/kg subcutaneously three times a week, starting by the end of the 1st week and continuing until the end of the 7th week. Fourteen of the infants were randomly selected to be supplemented with 3mg/kg/day of iron at the start of rHuEPO therapy (Group I). In the remaining nine infants, iron supplementation began after the end of the 6th week or when serum ferritin concentrations fell below 150 ng/ml (Group II). All of the infants were transfused according to the same transfusion policy. Haematocrit, reticulocyte, ferritin and sTfR levels were evaluated and compared at the beginning, during, and at the end of the study. Baseline parameters were similar in both groups. Haematocrit concentrations remained similar during and at the end of the study. Serum ferritin level was higher in Group I than in Group II during (day 28) and at the end of the study (day 42), but the differences were not significant (422.78(122.98 versus 227(33.47 and 204.5(61 versus 111.2(16.4 mg/dl, respectively). STfR concentrations were higher in Group I than in Group II during the study (Days 14 and 28) but lower at the end (Day 42) of the study, but the differences were again not significant (53.87(22.38 versus 23.17(1.09 on Day 14; 46.3(14.27 versus 24.84(2.66 on Day 28; and 26.1(3.01 versus 34.21(3.36 on Day 42, respectively). Reticulocyte counts in Group I were significantly higher than in Group II at the end of the study (6.1 (0.6 versus 2.9 (0.5; p=0.006).

Serum ferritin levels are high and remain stably high during rHuEPO treatment in VLBW infants, if they are transfused heavily, due to large volumes of phlebotomy during clinical follow-up; thus, the timing and dose of iron supplementation is questionable in this group. Erytropoiesis and iron need is slightly better met when iron supplementation is started simultaneously with rHuEPO. STfR levels are unpredictable, as the preterms were given heavy transfusions.

Key Words: *Anemia of Prematurity, Erythropoietin, Iron, Serum Transferrin Receptor.*

ÖZET

ÇOK DÜŞÜK DOĞUM AĞIRLIKLI PRETEMLERİN UYARILMIŞ ERİTROPOEZİNDE DEMİR GEREKSİNİMİ

Serum transferrin reseptör (sTfR) konsantrasyonu eritropoez ve doku demir gereksinimini göstermektedir. Bu çalışmanın amacı; sıklıkla kan transfüzyonu uygulanan çok düşük doğum ağırlıklı pretermlerde (ÇDDA) standart doz eritropoetin (rHuEPO) tedavisi ile uyarılmış eritropoez sırasında demir gereksiniminin belirlenmesidir. Çalışmaya alınan 23 ÇDDA'lı preterme 200 U/kg/doz, haftada 3 gün, subkütan postnatal 1. haftanın sonundan 7.hafta sonuna dek rHuEPO uygulandı. Grup I'i oluşturan 14 hastaya rHuEPO tedavisi ile eş zamanlı 3 mg/kg/gün dozunda demir verildi. Grup II'i oluşturan 9 hastaya ise serum ferritin düzeyleri 150 ng/ml'nin altına düştüğünde veya tedavi sonunda demir başlandı. Hastalara transfüzyon aynı kriterler göre uygulandı. Tedavi başlangıcı, tedavi sırasında ve sonunda hematokrit, retikülosit, ferritin, ve sTfR düzeyleri değerlendirildi ve gruplar karşılaştırıldı. Uygulanan benzer transfüzyon politikasına bağlı olarak her iki grupta hematokrit düzeyleri tedavi sırasında ve sonunda farklı değildi. Serum ferritin düzeyleri tedavi sırasında (28.gün) Grup I'de 441.78 (122.98 mg/ml Grup II'de 227(33.47 mg/ml, tedavi sonunda (42.gün) Grup I'de 204.5(61 Grup II'de 111.2(16.4 mg/ml bulundu. Grupların ferritin düzeyleri arasındaki fark anlamlı değildi. STfR düzeyleri Grup I'de 14.gün, 28.gün ve tedavi sonunda sırasıyla 53.87(22.8, 46.3 (14.27, 26.1(3.01 nmol/L; GrupII'de ise 14.gün, 28.gün ve tedavi sonunda sırasıyla 23.17(1.09, 24.84(2.66, 34.21(3.36 nmol/L idi. Gruplarda günlere göre sTfR düzeyleri arasındaki fark anlamlı değildi. Tedavi sonu retikülosit değerleri Grup I'de 6.1.(0.6 iken Grup II'de 2.9.(0.5 bulundu. Bu fark istatistiksel olarak anlamlı idi (p=0.0006). ÇDDA'lı pretermlerde aneminin eritropoetin ile tedavisi sırasında flebotomi kayıplarının yerine koymak ve klinik takip için uygulanan transfüzyonlara bağlı olarak serum ferritin düzeyleri yüksektir. Bu hastalarda demir suplemantasyonunun dozu ve zamanlaması tartışmalıdır. Eritropoetin tedavisi ile eş zamanlı demir başlanan Grup I'de demir gereksiniminin biraz daha iyi karşılandığı ve ferritin düzeylerinin yüksek bulunduğu erken dönemde sTfR'nin yükselmesinin uyarılmış eritropoezde artmış demir gereksinimine bağlı olabileceği düşünülse de yapılan çoklu transfüzyonlar nedeniyle sTfR düzeylerinin değerlendirilmesi güçtür.

Anahtar Kelimeler: *Demir, Eritropoetin, Preterm Anemisi, Serum Transferrin Resptörü*

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Abbreviations: sTfR: serum transferrin receptor

rHuEPO: recombinant human erytropoietin

VLBW: Very Low Birth Weight

AOP: Anemia of Prematurity

AGA: Appropriate for Gestational

Erythropoiesis in premature babies is limited by a relatively inadequate production of erytropoietin. This is partly due to dependence on the hepatic production of erytropoietin and an incomplete switchover to renal production. This model of neonatal erytropoiesis suggests that the use of exogenous erythropoietin should correct the early anemia developed as a result of frequent blood sampling and prevent the late anemia of prematurity (1,2). Investigations into the safety and efficacy of recombinant human erythropoietin treatment in anemia of prematurity have demonstrated a rise in haematocrit and reticulocyte counts, fewer blood transfusions, reduced transfused volume of blood per kilo of body weight and a decrease in bioavailable iron. RHuEPO treatment is more efficient when premature infants are older and in stable condition (3). Severity of illness and iron consumption represent the major limiting factors of the stimulated erytropoiesis of VLBW prematures (4).

STfR concentration is a recent sensitive measure of iron status, identifying iron deficient erythropoiesis. The serum levels increase soon after signs of iron deficiency appear, and this reflects the depletion of available tissue iron(4). Unlike serum ferritin concentrations sTfR concentrations are not influenced by infections and chronic inflammation. Not much information is available on the role of sTfR revealing iron depletion in stimulated erytropoiesis(5).

Timing of iron supplementation may theoretically create a problem in cases, where multiple transfusions cannot be withheld due to large volumes of phlebotomy during rHuEPO treatment. Packed red cells supply 0.75 mg/ml iron in each transfusion, whereas blood collected during phlebotomies contains less than half of this value per milliliter. As a result, serum ferritin levels are usually above normal in heavily transfused patients. The question is whether these levels suffice to meet the need of iron in stimulated erythropoiesis with rHuEPO in VLBW preterms.

This study was undertaken to assess the roles of sTfR and ferritin concentrations in the evaluation of iron need in transfused VLBW prematures when erythropoiesis is stimulated by standard doses of rHuEPO.

MATERIALS AND METHODS

Twenty-three VLBW infants were administered rHuEPO 200 IU/kg subcutaneously thrice a week, starting by the end of the first week until the end of the seventh week. All of the infants were AGA (birth weights within ±2 SD for gestational age). They received erythrocyte transfusions with a haematocrit of less than 0.30 and when sign and symptoms attributed to anemia - including persistent tachicardia, frequent apnea with bradicardia and weight gain less than 10g/kg/day, despite an optimal caloric and protein intake (120 kcal/kg/day and 3.5g/kg/day) - were present with a haemotocrit of less than 0.35. Fourteen of the infants were randomised to be supplemented with oral iron (ferroglycine sulphate) doses of 3mg/kg/day simultaneously with the start of rHuEPO treatment (Group I), while nine of them were started on oral iron supplementation after the end of the sixth week, when their serum concentration of ferritin fell below 150 ng/ml (Group II).

Blood samples were collected weekly from the first to sixth weeks of age for haemoglobin, haemotocrit, reticulocyte, white blood cell, ferritin, and sTfR measurements. The serum used for measuring sTfR concentrations were stored at -20°C until assayed.

Haemotocrit counts were measured with an automatic counter. Reticulocyte and granulocyte counts were determined via peripheral blood smear. Serum concentrations of ferritin were measured with RIA using commercial reagents (Ferritin Ria Kit, Kodak Clinical Diagnostics, UK)

ELISA method (Quantikine TM, IVD TM sTfR ELISA, R&D System Inc. Minneapolis MN 55413,

USA) was used to measure the serum concentration of TfR.

Mann-Whitney U test was used for statistical analysis. A p value of <0.05 was considered significant. (Mean (SEM values are used unless otherwise noted.)

RESULTS

There were no significant differences between the groups with regard to birth weight (1258.71 ± 16.05 versus 1244.44 ± 52.70 g), gestational age (30 ± 0.45 versus 30.55± 0.60 weeks), total phlebotomy (76.85 ±11.37 versus 54 ±6.82 ml) and transfusion volumes (48.14 ±13.18 versus 33.22 ±7.8 ml) (Table 1).

Haemotocrit, reticulocyte, ferritin and TfR were evaluated and compared between the groups at the beginning, during and at the end of the study (Tables 2 and 3).

All of the baseline parameters were similar in both groups. Haematocrit concentrations remained similar during and at the end of the study. In both groups, reticulocyte counts decreased following the first week of life and remained stably low until the end of erytropoietin therapy. By the completion of the study, the reticulocyte count in Group I was significantly higher than in Group II (6.1 ± 0.6 versus 2.9 ± 0.5; p=0.006) (Table 2).

Ferritin levels remained high in both groups through the first three weeks of erythropoietin therapy, probably due to heavy transfusions. However, from the end of the fourth week until the completion of the study, a decrease in ferritin levels was observed, and the serum ferritin level

Table 1: Clinical charachteristics of the infants*

	Group I	Group II
Number of the infants	14	9
Girls : Boys	7:7	4:5
Birth weight (g)	1258.71±16.05	1244.44±52.70
Gestational age (week)	30±0.45	30.55±0.60
Total phlebotomy (ml/kg)	76.85±11.37	54±6.82
Total transfusion (ml/kg)	48.14±13.18	33.22±7.8

*Mean ± SEM

Table 2: Comparison of haematocrit and reticulocyte counts at the beginning, during and at the end of the therapy

Age	Haematocrit (%)		Reticulocyte (%)	
(day)	Group 1	Group 2	Group 1	Group 2
0	48.70±1.22	49.28±1.94	5.35±1.36	3.65±0.44
7	37.58±1.24	40.25±1.75	2.22±0.40	1.86±0.45
14	34.95±1.47	36.61±1.33	2.25±0.25	3.21±0.61
28	34.03±1.47	34.48±1.29	2.80±0.28	4.32±0.97
42	30.55±0.72	34.65±2.37	6.07±0.64*	2.94±0.59*

*p<0.05

Table 3: Comparison of ferritin and sTfR levels at the beginning, during and at the end of the therapy

Age (day)	Ferritin (ng/ml)		sTfR (nmol/L)	
	Group I	Group II	Group I	Group II
7	339.28±31.91	305±11.45	26.17±2.47	20.91±1.21
14	380.21±92.07	304.55±33.77	53.87±22.38	23.17±1.09
28	422.78±122.98	227±33.47	46.30±14.27	24.84±2.66
42	204.46±61.3	111.22±16.4	26.10±3.01	34.21±3.36

was higher in Group I compared to Group II both during and at the end of the study (422.78±122.98 versus 227±33.47 and 204.46 ± 61.30 versus 111.22 ± 16.40 mg/dl, respectively). (Table 3 and Figure 1)

The concentrations of sTfR were higher in Group I compared to Group II during the study period (53.87 ± 22.38 versus 23.17±1.09 on Day 14 and 46.3±14.27 versus 24.84±2.66 nmol/L on Day 28, respectively). Serum TfR concentrations were lower in Group I compared to Group II at the end of the study (26.10 ±3.01 versus 34.21 ± 3.36 nmol/L, respectively (Table 3 and Figure 2). None of the differences was significant.

DISCUSSION

VLBW infants are likely to receive multiple blood transfusions in order to replace blood drawn during their medical course or to treat clinical symptoms attributed to AOP (1,2). Concerns over the large amounts of blood given to the average preterm infant lead to a search for an alternative therapy. Although rHuEPO levels are relatively low in infants with AOP compared to the degree of their anemia, it has been shown that there are sufficient erythroid precursor cells in the bone marrow to respond to rHuEPO stimulation (2,6). Several studies show that rHuEPO in doses of 300-1200 u/kg/week, with iron supplementation of 2-4mg/kg/day, induces erythropoiesis, resulting in an increase in the haematocrit and reticulocyte count and a reduction in the number of blood transfusions (7,3,8). In this study haematocrit concentrations remained similar in Groups I and II at the beginning, during and at the end of treatment because of the same trans-

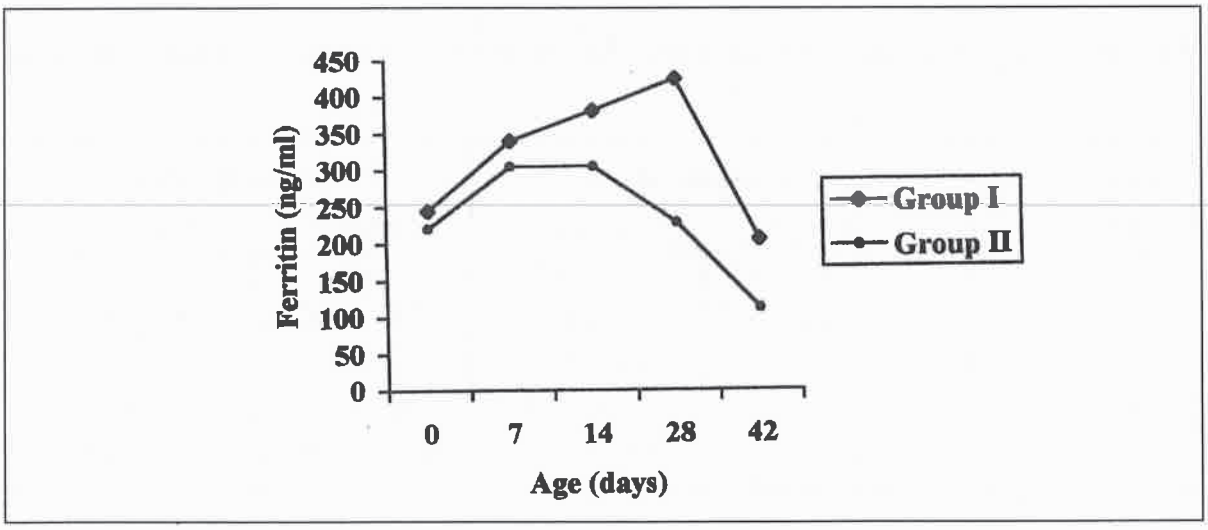

Figure 1: The concentrations of ferritin in group I and II

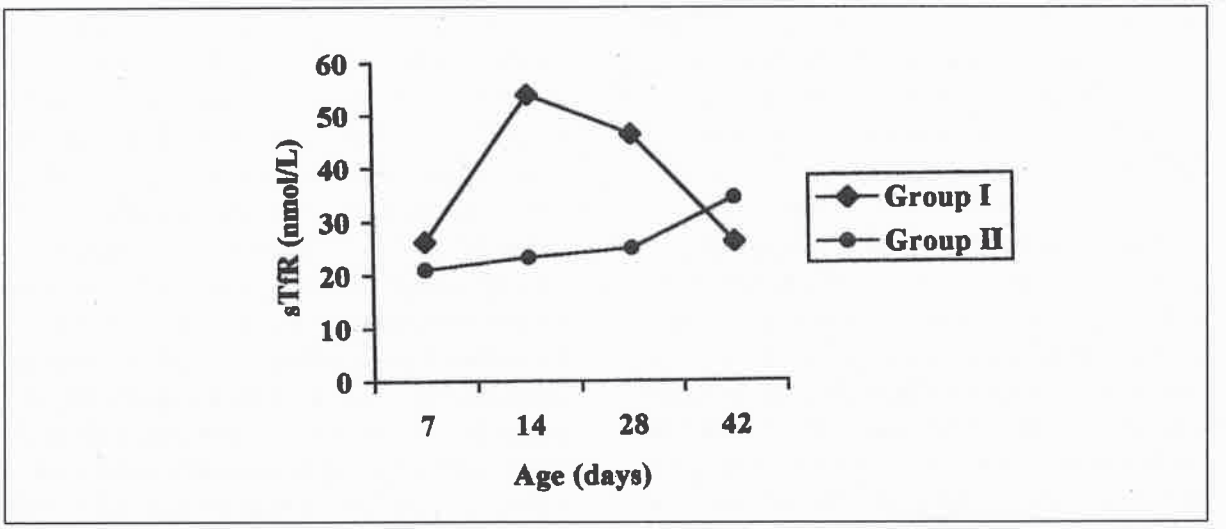

Figure 2: The concentrations of sTfR in group I and II

fusion policy. However, the significantly higher reticulocyte count in Group I compared to Group II (6.1 ± 0.6 versus 2.9 ± 0.5, p=0.006) at the end of the study may indicate a later but better-stimulated erytropoiesis in the simultaneously iron-supplemented group.

Severity of illness and iron consumption represent the major limiting factors of stimulated erytropoiesis (9,10,11). Preterm infants have low iron stores due to their short gestation and low iron intake during their prolonged medical course. Functional iron deficiency has been shown to develop in infants during rHuEPO therapy, placing limits on its efficacy. In order to prevent iron depletion during rHuEPO therapy, Carnielli et al, were among the first to supplement preterm infants with 20mg/kg/week of iron early in the course of rHuEPO therapy without complications (12). The European Multicenter Erytropoietin Study Group concluded that 2mg/kg/day caused depletion of iron stores, as reflected by decreased serum ferritin levels (13). Bader et al showed that iron at a dose of 6mg/kg/day in conjunction with rHuEPO 900 u/kg/week is effective in inducing erythropoiesis in stable growing preterms (2,13). Bechansteen et al reported an improved response to rHuEPO therapy by using even higher doses of iron (18-36mg/kg/day) with no apparent side effects. They also found that even with moderate doses of erythropoietin, higher doses of iron and protein intake improved the erythropoietic response to rHuEPo treatment (14,15). The decrease in serum ferritin levels and increase in sTfR levels in transfused preterms during the course of rHuEPO therapy raises the possibility that increased iron supplementation could further enhance the efficacy of rHuEPO(13,14,15). In our study, as a result of heavy transfusion, serum ferritin levels remained stably high in both of the groups until the end of the study. Although the iron stores were not depleted, functional iron deficiency may limit the erytropoiesis. The early rise of sTfR in Group I, when serum ferritin levels are relatively high, may be due to better-stimulated erytropoiesis with the simultaneous start of oral iron.

It should be taken into consideration that the amount of iron needed will depend on the volumes of blood removed and transfused (4). At collection, blood contains 3.4mg of iron per gram of haemoglobin (at a haemoglobin concentration of 10g%, this equals 0.34 mg /ml) whereas each milliliter of packed cells supplies 0.75mg iron (10). Most of our patients received transfusions during the study period, receiving extra iron simultaneously with the iron lost due to phle-

botomy. Thus, iron status of this group of infants were exposed to considerable changes. On the other hand, the use of rHuEPO therapy in more seriously ill preterm infants in the first weeks of life before enteral feedings are established makes oral iron supplementation even more difficult.

Functional iron deficiency during rHuEPO treatment has been frequently reported (16,5,17). It has been seen in the presence of increased as well as normal concentrations of stored iron. This may be because of the diminished rate of supply of available iron for the expansion of stimulated erythroid mass. This state in the human corresponds to what is seen in neonatal rabbit, in which parenteral iron increases red cell production to such an extent that the animals experience no early anemia. This effect of parenteral iron occurs even though the animals have abundant storage iron (14).

In our study, when rHuEPO therapy was initiated in unstable VLCBW infants at about seven days of age, despite the stimulation of erythropoiesis, the number of transfusions could not be reduced. Serum ferritin levels are high and remain stably high during rHuEPo treatment, due to heavy transfusions. Thus, the timing of iron supplementation in this group is questionable. Although serum ferritin levels of both groups were similar at the beginning of the study, they were lower in Group II compared to Group I during and at completion of the study. Due to the differences in iron intake among the groups, there was a notable decrease in serum ferritin level in Group II; however, the difference was not significant. Iatrogenic iron deficiency developed in Group II, raising the possibility that increasing iron supplementation irregardless of high serum ferritin levels could further enhance erythropoiesis. Erythropoiesis and iron need seem to be better met when iron supplementation is started simultaneously with rHuEPO.

Serum levels of TfR may reflect both iron status and rate of erytropoiesis. An early increase in TfR of more than 20% in adults has been regarded as an early indicator of successful rHuEPO treatment (15). High doses of rHuEPO in preterm infants also significantly induces elevated TfR levels. In our study, the early increase (Days14 and 28) in TfR in Group I, at a time when ferritin levels indicated no iron deficiency, may reflect stimulated erytropoiesis in the presence of orally supplemented iron. Similarly, the lack of oral supplementation in Group II may explain why this group showed no increase in TfR during the treatment. When iron supplementation was begun in Group II at the end of treatment, a slight increment of sTfR levels was also observed in this group.

The elevated sTfR levels may also be explained by an increase in the erythroid precursor cell mass. This relationship of transferrin receptor number to erythropoiesis exists only when there is sufficient iron-bearing transferrin to saturate receptors. When an iron deficiency exists, both erythroid and non-erythroid receptors increase, distorting the relationship between receptor number and erytropoiesis (16,5,18,19).

In conclusion, we suggest that early rHuEPO therapy given at a dose of 600 U/kg/week in conjunction with 3mg/kg/day of iron supplementation may stimulate erythropoiesis better in sick VLBW infants. In that unstable population, iatrogenic blood loss contributed to greater transfusion and a lower level of erytropoiesis. Based on this and other studies, if VLBW infants are at risk of greater phlebotomy losses, the use of vigorous iron supplementation is promising.

In stimulated erytropoiesis of VLBW infants, the rise in sTfR levels with a small decline in serum ferritin concentrations may explain a potential functional iron deficiency. Heavy transfusions lead to unpredictable sTfR levels, which should be further investigated.

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