Serum Erythropoietin Levels in Pediatric Hematologic Disorders and Impact of Recombinant Human Erythropoietin Use

Pediatrik hematolojik hastalıklarda serum eritropoetin düzeyleri ve rekombinan insan kaynaklı eritropoetin kullanımının olası etkileri

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Abstract

Objective: In anemic patients, the correlation between serum erythropoietin (sEpo) level and the severity of anemia has been reported previously. However, in different anemia groups, different sEpo levels are measured in patients with similar hemoglobin levels and the etiology of this situation could not be explained.
Methods: We evaluated hemoglobin and sEpo levels in 31 iron deficiency anemia, 26 Fanconi anemia (FA), 21 thallasemia intermedia (TI), 15 acute lymphoblastic leukemia (ALL) patients at presentation and 12 healthy controls.
Results: In all disease groups, an inverse linear correlation was shown between hemoglobin and logarhytmic sEpo level. The covariance analyses according to corrected hemoglobin levels exhibited the highest sEpo level in FA, followed by ALL, TI and iron deficiency anemia, sequentialy.
Conclusion: There was no statisticaly significant difference of sEpo levels in FA patients in terms of androgen treatment and this finding supports that androgen affects erythropoisis directly, and has no effect on erythropoietin. The results indicate that there is no erythropoietin deficiency in the anemia of these patients and the admnistration of exogenous erythropoietin offers no clinical benefit. *(Turk J Hematol 2009; 26: 72-6)*
Key words: Erythropoietin, thallasemia, Fanconi anemia, acute leukemia, iron deficiency anemia

Received: May 6, 2008 *Accepted: December 24, 2008*

Özet

Amaç: Anemik hastalarda, aneminin şiddeti ve serum eritropoetin (sEpo) düzeyleri arasındaki korelasyon bilinmektedir. Ancak, anemiye yol açan farklı hastalık gruplarında, benzer hemoglobin seviyelerinde farklı sEpo değerleri ölçülmektedir ve bu durumun nedeni bilinmemektedir.
Yöntemler: Bu çalışmada 31 demir eksikliği anemisi (DEA), 26 Fanconi anemi (FA), 21 talasemi intermedia (TI), 15 yeni tanı akut lenfoblastik lösemi hastası (ALL) ile 12 sağlıklı kontrolün hemoglobin ve sEpo değerleri ölçülmüştür.
Bulgular: Bütün hastalık gruplarında hemoglobin ve logaritmik sEpo seviyeleri arasında ters lineer bir ilişki gösterilmiştir. Kovaryans analizinde düzeltilmiş hemoglobine göre en yüksek sEpo değerleri FA grubunda ölçülürken, bunu sırasıyla ALL, TI ve DEA grupları takip etmiştir.

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Sonuç: Fanconi anemili hastaların sEpo seviyelerine androgen kullanımının istatistiksel olarak anlamlı bir etkisinin olmaması androjenin eritropoezi doğrudan uyardığına ve eritropoetin üzerinden gelişen eritropoeze etkisi olmadığına bir işaret etmektedir. Sonuçlar bu hastalık gruplarında eritropoetin eksikliğinin olmadığına işaret etmekte ve egzojen eritropoetin kullanımının yararsız olacağını göstermektedir. *(Turk J Hematol 2009; 26: 72-6)*

Anahtar kelimeler: Eritropoetin, talasemi, Fanconi anemi, akut lösemi, demir eksikliği anemisi

Geliş tarihi: 06 Mayıs 2008 Kabul tarihi: 24 Aralık 2008

Introduction

Erythropoietin is a glycoprotein hormone produced primarily by cells of the peritubular capillary endothelium of the kidney and in hepatocytes in lesser amounts. Erythropoietin is responsible for the proliferation and differantiation of erythroid progenitors and especially erythriod colony forming units [1,2]. Enzyme immunoassays which can readily quantitate serum erythropoietin levels are currently available [3-5]. In order to evaluate the differences in serum erythropoietin (sEpo) levels in different anemia groups among pediatric patients, we worked sEpo levels in iron deficiency anemia, acute lymphoblastic leukemia (ALL), fanconi anemia (FA), thallasemia intermedia (TI) and healthy controls. Recombinant human erythropoietin (rHuEpo) has become an essential part of the management of anemic patients with end-stage renal disease. It is also used to treat the anemia associated with cancer and other diseases, and it improves quality of life [6,7]. In recent years, studies have focused on the use of rHuEpo for other indications. Additionaly, we aimed to define the therapeutic role of rHuEpo in these disease groups, if any deficiency of sEpo was demonstrated.

Material and Methods

Thirthy-one iron deficiency anemia and 15 ALL patients at diagnosis and 26 FA, 21 TI patients, who were being followed in a single pediatric hematology unit were included in the study. The renal function tests and urinalysis were normal in all of the patients. None of the patients included in the study had clinical findings of acute or chronic hypoxia. Additionally, none of the patients in the study group had a transfusion history within six months. Of FA patients, seven had renal anomalies (ectopic kidney in three, unilateral renal agenesis in two, horseshoe kidney in one and right ureteric dilatation in one patient).

Hemoglobin, hematocrit, serum iron (SI), serum iron binding capacity (SIBC), ferritin and serum erythropoietin (sEpo) levels were measured. Taking the circardian rhythm of sEpo into consideration [8], the sampling was done between 10-12 AM. After clooting at room temperature for 2 to 4 hours, serum was obtained and stored at -20^{O}C. sEpo was quantitated by ELISA (Medac diagnostica, Germany) [9] for twice for each patient. Blood samples were obtained at diagnosis, before initiation of chemotherapy in ALL and before and by the second and fourth weeks of the onset of iron treatment in iron deficiency anemia patients. sEpo levels in a control group of 12 healthy children were also analyzed.

The logarithmic (log) value of sEpo level in each group was correlated with hemoglobin values. Statistical analyses were performed by SPSS version 11, Chicago, IL, USA. The comparisons of four disease and one control group were done by ANOVA test and in case of significance in ANOVA test, pairwise Tukey test was performed. In covariance analyses, hemoglobin was used as covarite variable tested. Correlation analyses were performed by Pearson correlation test.

Results

Means and ranges of age, hemoglobin concentration and sEpo levels in each group of patients are shown in Table 1. The data of iron deficiency anemia group has been reported previously [10]. The age of iron deficiency anemia and ALL patients were statisticaly indifferent (p=0.27), wheras FA and TI patients

Table 1. Clinical and laboratory data of the patients

Groups	n	Age(year)	Gender		Hemoglobin	sEpo	sEpo median	log sEpo
			M	F				
Fanconi anemia	26	10.1±5.6 (2-26)	14	12	10.6±3.1 (4.7-16.1)	3712±6640 (57.6-20480)	470	2.89±0.86 (1.76-4.3)
ALL	15	6.1±3.9 (1.5-13)	10	5	7.7±2.5 (3.3-11.5)	1510±1629 (132-4448)	485.6	2.92±0.50 (2.12-3.65)
Thalassemia intermedia	21	18.1±12.2 (5-60)	13	8	8.5±1.9 (4.8-11.1)	1885±4995 102-20480)	360	2.66±0.8 (2.0-4.3)
Iron deficiency anemia	30	4.7±5.0 (0.6-15)	19	12	6.7±1.7 (3.4-12.1)	2384±3177 (12.8-10240)	716.8	2.81±0.8 (1.76-4.3)
Healthy control	12	8.1±8.5 (0.8-23)	6	6	17.8±5.9 (12-22)	1.2±0.1 (1.08-1.54)	17	13.1±1.0 (11.8-15.1)

Values=mean± standart deviation, range in parenthesis
n=number, m=male, f=female

were elder than iron deficiency anemia and ALL patients (p<0.001). The hemoglobin concentrations were significanly lower in iron deficiency anemia and ALL groups, compared to others (p<0.001). In iron deficiency anemia, ALL, FA and TI groups, a statistically significant inverse linear correlation was shown between hemoglobin and log sEpo level (p=0.032), correlation coefficients: iron deficiency anemia -0.75, ALL -0.6, FA -0.83, TI -0.76). The covariance analyses according to corrected hemoglobin levels exhibited the highest sEpo level in FA, followed by ALL, TI and iron deficiency anemia, sequentialy.

The androgen treatment status of 26 FA patients are summarized in Table 2. There was no statisticaly significant difference between androgen administered (n=19) and androgen not administered (n=7) FA patients in terms of age, hemoglobin, hematocrit and log sEpo (p>0.05) (Table 3). Log sEpo value was higher in patients with hemoglobin less than 10 g/dl (U=139, p=0.001).

TI patients were also subgrouped as β^0 (n=7) and β^+ (n=14) and no statistically significant difference between β^0 and β^+ was observed in terms of age, hemoglobin, hematocrit, ferritin and log sEpo levels (Table 4). Of 21 TI patients, 13 (61.9%) were splenectomized. No statistically significant difference was found between splenectomized (n=13) and non-splenctomized (n=8) TI patients in terms of hemoglobin, hematocrit and log sEpo. Similar to FA patients, log sEpo values were significantly higher among TI patients with hemoglobin concentrations lesser than 10 g/dl (U=70.5, p=0.04).

Among the iron deficiency anemia group, sEpo level was significantly higher in patients with hemoglobin concentration lesser than 8 g/dl, when compared to the patients with those with hemolobin concentraion above 8. g/dl (U=128, p=0.002). Statistically, age, SI, SIBC and transferin saturations were similar in two subgroups.

Eight of the iron deficiency anemia patients were reevaluated by the second and fourth weeks of iron replacement treatment. Although hemoglobin concentraions were still very low, sEpo levels were seen to rapidly approximate to normal values by the second week and reach to normal ranges by the fourth week.

In ALL group, while hemoglobin concentrations were ranging between 3.3 and 11.4 g/dl, sEpo levels ranged between 132 and 4448 mu/ml.

Table 2. Androgen response status of Fanconi anemia patients

Groups	n
Using androgen when sEpo measured (1 month-7 years)	19
Complete response (Hb>10 g/dl)	12
Partial response (Hb 8.1-9.9 g/dl)	3
No response (Hb<8 g/dl)	4
No androgen treatment when sEpo measured	7
Never used androgen since Hb normal	4
Responded to previous androgen treatment and ceased	2
Newly diagnosed	1

Table 3. Hemoglobin and log sEpo values in FA patients according to androgen use

	Androgen(+) n=19	Androgen(-) n=9	p value
Age (year)	10.4±4.2 (5-21)	10.9±7.7 (2-26)	0.75
Hb (g/dl)	11.2±2.4 (6.7-14.3)	10.2±3.7 (4.7-16.8)	0.28
Log sEpo	2.7±0.7 (1.7-4.2)	2.6±0.5 (1.9-4.3)	0.65

Values=mean± standart deviation, range in parenthesis

Table 4. Laboratory values of β^0 and β^+ thallassemia internedia patients

	β^0 n=7	β^+ n=14	p value
Age (year)	16±5.7 (8.27)	19.2±14.5 (5-60)	0.94
Hb (g/dl)	8.9±2.2 (4.8-11.6)	8.3±1.8 (5-11.4)	0.37
Ferritin (ng/ml)	726.8±873.0 (138.7-2613)	548.5±353.0 (148-1177)	0.77
Log sEpo	2.8±0.7 (2.4-4.3)	2.6±0.5 (2.0-4.1)	0.3

Values=mean± standart deviation, range in parenthesis

Discussion

The inverse correlation between sEpo level and the severity of anemia is well-established [11,12]. As previously reported [13], in the presented study, the highest sEpo level was measured in FA patients whereas the lowest sEpo levels were among iron deficiency anemia patients by the covariance analyses according to corrected hemoglobin levels. The etiology of different erythropoietin responses in different disease groups at the same levels of hemoglobin concentrations is obscure. sEpo level is determined not only by erythropoietin production, but also its clereance, inhibitors and consumption.

A great proportion of FA patients lack or are defective of erytroid progenitors and approximately 50% of FA patients are responsive to androgen treatment [14]. Androgens are able to stimulate erythropoiesis in-vitro and in-vivo [15-17]. This effect can be directly on hematopoitic cells or through the stimulation of erythropoietin secretion. The higher hemoglobin concentrations of males are attributed to direct effect of androgens, since no gender difference exist in sEpo levels [18]. In the presented study, as previously reported [18], we found that there was no statistically significant effect of androgen application on sEpo levels in FA patients and this supports the direct effect of androgens on erythropoiesis. Furthermore, high sEpo level was present among FA patients with normal hemoglobin concentrations, supporting other factors affecting sEpo levels. In a previous study by Piedras et al [19], androgens at pharmacological doses were reported as not to have influence on non-anemic aplastic anemia patients' sEpo levels, whereas increase sEpo levels in anemic patients. Children with Fanconi anaemia have also been reported to have considerably higher serum

erythropoietin levels than children with haemolysis for the same degree of anaemia [20]. In-vitro and in-vivo studies have shown that rHuEpo has no impact on anemia correction in FA or acquired aplastic anemia patients [21,22]. In a contradicting study, non-severe aplastic anemia patients were reported to be more likely to respond to rHuEpo [23]. This may indicate the importance of the status of bone marrow on the response of rHuEpo treatment.

The mean serum EPO levels were reported as significantly higher in patients with both thallasemia major and TI compared with the control group, previously [24]. In our study, TI patients were found to have very much higher sEpo levels than that reported previously. In both thallasemia major and TI patients Manor et al, have reported a little increase in sEpo levels independent of gender, severity of anemia, ferritin level and splenectomy status. Furthermore, sEpo level was found higer in infancy and than declined to lower values in adolescence [25]. In our study group, the highest level was measured in 11-13 years of age patients. Additionally, we found no difference between β^0 and β^+ patients. This may indicate that although HbF and HbA have different affinities for oxygen, this may not have any influence on tissue hypoxia or alternatively, sEpo levels may be influenced by other factors than hypoxia in TI patients. The finding of higher sEpo levels in FA and TI patients may indicate that rHuEpo is unbeneficial in these diseases.

In iron deficiency anemia group of the presented study, we observed that sEpo levels approximated to normal values sooner than anemia recovery, supporting the previous data [26,27], this might be attributed to a direct feedback mechanism of control between marrow cellularity and sEpo levels [19,28]. However, at the hemoglobin levels achieved by the second week of iron treatment, in other groups we measured significantly higher sEpo levels. This may indicate that sEpo level is not only dependent on hemoglobin concentration, but also on the erythroid activity of bone marrow. It has been shown that erythropoietin gene regulation depends on heme-dependent oxygen sensing and assembly of interacting transcription factors [29]. This protein requires adequate amounts of iron for normal function and this may explain the lower sEpo levels in iron deficiency anemia patients.

The etiologies of anemia in malignancy patients during follow-up may be anemia of chronic disease, bone marrow infiltration by tumor cells, myelosuppression by the therapeutic agents, hemolysis or blood loss. In acute leukemia patients the leading cause of anemia is the infiltration of bone marrow by leukemic cells. In our study we observed sEpo levels consistently higher with the lower hemoglobin concentrations in these children.

Although the use of rHuEpo was shown to be effective for anemia seen in chronic renal failure patients and cancer patients and important for the improvement of quality of life, our study indicate that an expected benefit of the rHuEpo may be theoreticaly limited because of the high sEpo levels in FA, TI and ALL patients.

Acknowledgements: A. Gurgey is supported by TUBA.

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