APPLICATION OF RADIOIMMUNOASSAY DETERMINATION OF ERYTHROPOIETIN IN MONITORING OF THALASSEMIC PATIENTS

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ABSTRACT

Erythropoietin (EPO) is a glycoprotein produced primarily by the kidney in response to hypoxia and anemia. A number of kits are available for measuring EPO, using different immunoassay techniques. Analysis of serum EPO measurement variation between methods showed evidence of significant negative bias with ELISA when compared with RIA methods. Therefore, the RIA method seems better method for determination of EPO. In this article, the author reviewed the previous studies on using EPO determination in monitoring of the thalassemic patients.

Key words: EPO, RIA, thalassemia

WHAT IS ERYTHROPOIETIN ?[1-13]

Erythropoietin (EPO) is a glycoprotein produced primarily by the kidney in response to hypoxia and anemia.[1] It is the principal factor initiating and regulating red cell production.[2] EPO is responsible for the regulation of red blood cell production. Secondary amounts of this hormone are synthesized in liver hepatocytes of healthy adults. In premature as well as full term infants, the liver is the primary site of EPO production. The kidney becomes the primary site of EPO synthesis shortly after birth. EPO production is stimulated by reduced oxygen content in the renal arterial circulation. Circulating EPO binds to EPO receptors on the surface of erythroid progenitors resulting in replication and maturation to functional erythrocytes by an incompletely understood mechanism. Recent knowledge gained regarding the relationship between erythropoietin, iron, and erythropoiesis in the patients with anemia has implications for patient management.[1-3]

Clinical conditions that give rise to tissue hypoxia including anemia, lung disease, or cyanotic heart disease, lead to increased levels of serum EPO. In anemia, serum EPO levels do not rise above normal until hemoglobin levels fall below 110 g/L. As may be expected in patients with renal insufficiency, serum EPO levels remain inappropriately low despite the anemia. However, inappropriately low serum EPO levels may also be seen in anemic patients with cancer, as well as those with rheumatoid arthritis, HIV infection, ulcerative colitis, sickle cell anemia, and the anemia of prematurity. The mechanism of the inappropriate EPO response varies.[3-4]

Treatment with recombinant erythropoietin (rHuEPO) has been shown to be useful in disease states such as anaemia of chronic renal failure[5] inflammatory bowel disease,[6] multiple myeloma[7] and acquired immunodeficiency syndrome.[8] Other uses include treatment of anaemia of orthostatic hypotension[9] and anaemia

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of prematurity.[10] Monitoring serum EPO concentrations enables detection of toxicity and ensures adequate therapeutic concentrations are achieved.[11]

The availability of rHuEPO since the mid 1980s has led to the development of sensitive and specific immunoassay methods for measuring EPO and it has become easier to determine the role of abnormal circulating serum EPO concentrations in a variety of clinical states.[12]

DETERMINATION OF ERYTHROPOIETIN BY RADIOIMMUNOASSAY

A number of kits are available for measuring EPO, using different immunoassay techniques. Most are based on ELISA or RIA. Some of these kits are EPO-Trac RIA (Incstar Corporation, Stillwater, MN, USA), EPO RIA (Ramco Laboratories Inc. Houston, Texas, USA) and DSL RIA (Diagnostic Systems Laboratories Inc. Webster, Texas, USA), which are competitive binding disequilibrium radioimmunoassay (RIA) methods which use recombinant human EPO for both tracer (^{125}I) and standards. The other kits are EPO EIA (bioM?rieux, Marcy-I'Etiole, France), IBL ELISA (Immunobiological Labs.Hamburg, Germany) and Medac ELISA (Medac, Hamburg, Germany) are assays based on a sandwich technique using two monoclonal antibodies.[13]

According to a recent study of evaluated these kit,[13] analysis of serum EPO measurement variation between methods showed evidence of significant negative bias with ELISA when compared with RIA methods. Therefore, the RIA method seems better method for determination of EPO.

SOME STUDIES ON THE ERYTHROPOIETIN MONITORING IN THALASSEMIC PATIENTS[14-22]

In Thailand, thalassemia syndromes, which are a group of disorders resulted from inherited abnormality of globin chain production, is another hematologic disease presented with anemia. This diseases is interest about the serum EPO levels.[14] Presently, the radioimmunoassays for serum erythropoietin seem valuable tools for clinical research, but their roles in routine clinical practice remain undefined.[15-16] Because hyperstimulated ineffective erythropoiesis due to inappropriate transfusion regimen in the therapy of thalassaemia major may lead to complications, hence, serum EPO may be a useful test in follow up of these patients.

Presently, the only one classical tool for monitoring of erythropoiesis in the thalassemic patients is determination of reticulocyte count. Serum EPO is of interest for many research group focused on its possibility as alternative tool in monitoring of these patients. Of interest, recent study of Paritpokee et al, showed the high level of serum EPO in the beta thalassemia /Hb E patients despite polytransfusion.[17] However, it does not agree with those of Dore et al (1993)[18] and Nisli et al (1997),[19] which suggest that even a low transfusional regimen may cause a decrease in serum concentration of EPO, independent of the level of total Hb.

Paritpokee et al's observation in this regard agrees with those reported by Chen et al (1998),[20] who found a significant inverse correlation between serum EPO levels and Hct in polytransfused beta thalassemia major patients, but not with Dore et al (1993)[18] and Nisli et al (1997)[19] who found a significant inverse correlation between serum EPO levels and Hct only in the patients who had only a few or no transfusions. The conflicting data on the studies of EPO level in thalassemic patients may be due to the method of serum EPO evaluation, the few number of subjects and the time of drawing the sample at pre or post transfusion.

Nevertheless, an increase in circulating hematopoietic progenitor cells with an elevated serum EPO level in polytransfused beta thalassemia major was well described by Chen et al (1992).[21] This observation can suggest that physiologic suppression of erythropoiesis is not successful in thalassemic patients, who were oftenly transfused. Nevertheless, in 1999 Chaisiripoomkere et al reported that serum EPO levels increased in all thalassemia patients despite repeated transfusion.[22] The similar findings in our polytransfused beta thalassemia /Hb E pediatric patients can be demonstrated.

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