Review Article

The relation of parathyroid hormone and hematologic parameters under erythropoietin administration in hemodialysis patients

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Abstract

Hemodialysis has been the principal therapeutic tool for End Stage Renal Disease (ESRD) for decades. ESRD patients are defined as those having Glomerular Filtration Rate (GFR) under 15 ml/min. Loss of renal function can result either as acute kidney failure or gradually, following the natural course of a vascular disease such as hypertension and Diabetes mellitus. Metabolic bone disease is a very common situation for the majority of patients with chronic renal disease due to severe imbalance in bone remodeling as well as mineral homeostasis. The aim of the present study was to investigate the relation of Erythropoietin (Epo) administration in ESRD patients with hematologic factors as well as parathyroid hormone (PTH) levels. A retrospective observational study was conducted in an outpatient Chronic Hemodialysis Unit between January 2015 and December 2016 (24 months). Patients with ESRD were recruited, who had received hemodialysis for at least twelve months. Several hematologic factors as well as PTH levels were evaluated in ESRD patients and the results were compared in order to find the effect of Epo administration on those factors. We have found that Epo significantly affects hematologic factors, but it marginally affects PTH levels. Our study has confirmed that Epo administration has beneficial effects to hematologic parameters, in ESRD patients, yet with marginal effect on PTH and thus its role in bone metabolism needs to be further investigated.

Keywords: Hemodialysis, End Stage Renal Disease, Erythropoietin, Parathyroid hormone, Hematologic parameters

Introduction

Hemodialysis (HD) has been applied for End Stage Renal Disease (ESRD) treatment since 1960. ESRD patients are defined as those having Glomerular Filtration Rate (GFR) under 15 ml/min. Loss of renal function can result either as acute kidney failure or gradually, following the natural course of a vascular disease such as hypertension and Diabetes mellitus. Metabolic bone disease is a very common situation for the majority of patients with chronic renal disease due to severe imbalance in bone remodeling as well as mineral homeostasis. Secondary or tertiary hyperparathyroidism is a common condition in these patients and has been associated with bone marrow fibrosis, insufficient erythropoiesis and anemia^{1,2}.

Parathyroid Hormone (PTH) starts to rise very early

in the course of kidney disease. The release of PTH is normally stimulated by low calcium levels in the body. Increases of serum PTH induce bone resorption due to the activation of osteoclasts while at the same time increased reabsorption of calcium and increased excretion of

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phosphorus is promoted. Continued stimulation of PTH secretion leads to irreversible parathyroid gland hyperplasia. Though normal levels of PTH are between 10-65 pg/ml; however in patients with ESRD, due to skeletal resistance to PTH hormone, these values may increase 9-10 times in patients with ESRD³⁻⁷.

Erythropoietin (Epo) maintains red blood cell count by promoting the survival, proliferation and differentiation of erythrocytic progenitors. Circulating Epo originates mainly from fibroblasts in the renal cortex. Chronic Renal Disease (CRD) leads to decreased production of Epo to prevent and or treat anemia. Lack of Epo is replenished with exogenous administration. Several mechanisms are implicated in the development of anemia in ESRD⁴ and the mechanism of bone marrow fibrosis⁹ as a complication of secondary hyperparathyroidism, although is not a main factor of dysfunctional erythropoiesis, is actively involved in process of anemia⁸. The recording of blood values of patients with secondary hyperparathyroidism under hemodialysis treated with recombinant Epo is of great interest and the results are presented in our study.

Materials and methods

Patients

A retrospective observational study was conducted in an outpatient Chronic Hemodialysis Unit between January 2015 and December 2016 (24 months). We recruited patients with End Stage Renal Disease (ESRD) who had received hemodialysis for at least twelve months. Recombinant Epo was administered to patients in doses compliant with the KDIGO guidelines for anemia in CKD and we assessed the effects of Epo on hematological parameters and PTH.

Inclusion and exclusion criteria

The inclusion criteria were hemodialysis (HD) for at least twelve months, for four hours three times per week. Adequacy of HD treatment, as expressed by KT/V was larger or equal to 1.3 based on National's Kidney Foundation Guidelines⁹.

Patients were excluded if they have been hospitalized more than twice in a year. Further on, patients with tertiary parathyroidism were excluded due to the presence of malignancy and in some cases subsequent parathyroidectomy. Finally, patients with more than 10 years under hemodialysis were also excluded.

Blood sampling

Blood sampling was performed in the morning of the second weekly session from peripheral vein pre-dialysis. Serial blood samples were obtained after overnight fasting. PTH tests were obtained on the second treatment day of the week immediately prior to HD. Phlebotomy was performed by a trained health professional, while all precautions were undertaken for patient safety and well-being. Blood samples were allowed to clot in heparin-free vial for 30-40 minutes after phlebotomy and samples were centrifuged for 10

minutes at 2000 rpm. Obtained serum samples were stored at -80°C until further processing.

Hematologic and biochemical factors estimation

Blood test parameters estimation included: a) Parathyroid Hormone (PTH) estimated every three months by Enzyme Linked Immuno Sorbent Assay (ELISA) method, b) HD adequacy expressed by standardized $Kt/V(StdKt/V)$ equation was $\geq$ (larger or equal) to 1.3, c) Hematocrit (Ht) (%), d) Hemoglobin (Hb)(gr/dl), e) Mean Cell Volume (mean volume of red blood cells) (MCV) (fl), f) Mean Corpuscular Hemoglobin (MCH)(pg), g) *Mean Corpuscular Hemoglobin Concentration* (MCHC)(gr/100ml), h) Red Blood Cells (RBC) (cells $\times 10^6$ /ul), i) Platelets (PLT) (cells $\times 10^3$ /ul).

Ethics statement

All experiments were conducted in compliance with the international biomedical studies stipulations, with reference to the Declaration of Helsinki of the World Medical Association. No personal data of patients were kept, while it was impossible to trace back any personal data from the data collected for the present study. The present retrospective observational study, and data acquisition was approved by the dialysis unit Medical Board.

Results

One hundred ($n=100$) Caucasian End Stage Renal Disease (ESRD) patients were included in the analysis. Of them, seventy-two ($n=72$) were males and twenty-seven ($n=27$) females. Mean age of patients was 65.25 ± 12.52 years for men and 63.1 ± 17.35 years for women. One patient underwent parathyroidectomy. All patient received Fe supplementation and paracalcitol. From our patient cohort 22% received calcium supplementation, while 78% did not receive any calcium supplementation. In addition, 20% received 30mg cinacalcet, 18% received 60mg cinacalcet and 57% did not receive any cinacalcet supplementation. For the remaining 5% there were no available data concerning cinacalcet administration. Finally, four patients received denosumab treatment throughout the hemodialysis time course. The patients underwent a four hour hemodialysis (HD) session, 3 times a week.

Erythropoietin administration

Throughout the complete treatment course of our patient sample, 14% of the total time course did not receive erythropoietin supplementation, while 86% of the time course did receive (Figure 1). In particular, for the female population they did not receive any erythropoietin supplementation for the 4% of the complete time course (Figure 1). Respectively, male patients did not receive erythropoietin supplementation for 10% of the complete treatment time course (Figure 1). Hence, for the female population, 23% did receive erythropoietin supplementation

Figure 1. Prevalence of erythropoietin throughout the complete treatment course. Results concern the percentage of months throughout which patients did or did not receive erythropoietin supplementation for the total population (left) and with respect to gender (right).

Figure 2. Time course of parathyroid hormone (PTH) development.

for the complete time course and the respective percentage for males was 63% (Figure 1).

Parathyroid hormone (PTH) levels of the patient sample

A time series analysis of our patient sample has demonstrated a quasi-linear trend in PTH levels with respect to time (Figure 2). It appeared that PTH levels remained unchanged throughout the complete course of treatment in hemodialysis patients.

Descriptive statistics of hematological factors with respect to erythropoietin supplementation

Further on, we have attempted to identify possible differences between the cohort of time points on which patients received erythropoietin and those that did not. Time series analyses manifested possible differences with respect to hematocrit (Figure 3A), hemoglobin (Figure 3B), Mean Corpuscular Volume (MCV) (Figure 3C), Mean

Figure 3. Descriptive statistics of hematological parameters with respect to erythropoietin supplementation. In particular, the time-dependent effects of erythropoietin on hematocrit (A), hemoglobin (B), Mean Corpuscular Volume (MPV) (C), Mean Concentration of Hemoglobin (MCH) (D), mean corpuscular hemoglobin concentration (MCHC) (E), Red Blood Cells (F), White Blood Cells (G), Platelets (PLT) (H), Mean Platelet Volume (MPV) (I) and parathyroid hormone (PTH) (J) (**Legend: YES:** Implies the time points on which patients received erythropoietin supplementation, **NO:** Implies the time points on which patients did not receive erythropoietin supplementation).

Concentration of Hemoglobin (MCH) (Figure 3D), Mean Corpuscular Hemoglobin Concentration (MCHC) (Figure 3E), red blood cells (Figure 3F), white blood cells (Figure 3G), platelets (Figure 3H), Mean Platelet Volume (MPV) (Figure 3I) and parathyroid hormone (PTH) (Figure 3J).

The effects of erythropoietin on clinical and hematological factors

Patients who received erythropoietin supplementation, manifested several significant differences with respect to

clinical and hematological factors. In particular, it appeared that significant differences were observed between those patients who received erythropoietin supplementation for the specified time period and those that did not with respect to PTH in the total population (Figure 4A). Further on, significant differences were observed between gender-related erythropoietin supplementation groups with respect to hematocrit (Figure 4B). In addition, similar behavior was observed with respect to hemoglobin and gender (Figure 4C). It also appeared that cell populations were affected by

Figure 4. Effects of erythropoietin. The effects of erythropoietin are presented and in particular with respect to parathyroid hormone (PTH) (A), Hematocrit (Ht) (%) (B), Hemoglobin (Hb) (gr/dl) (C), Red Blood Cell count (RBC) (D), White Blood Cell count (WBC) (E), Neutrophils (%) (F), Lymphocytes (%) (G), Platelets (PLT) (H) and Mean Platelet Volume (MPV) (I) (* depicts a $p < 0.05$ significance and ** depicts a $p < 0.01$ significance).

the administration of erythropoietin such as red blood cells (Figure 4D), white blood cells (Figure 4E), neutrophils (Figure 4F), lymphocytes (Figure 4G), platelets (Figure 4H) and the mean platelet volume (MPV) (Figure 4I).

ROC analysis of erythropoietin with respect to hematological parameters and parathyroid hormone

Previous results have been confirmed by ROC analysis of hematological parameters with respect to erythropoietin administration. In particular, hematocrit could be considered as a significant factor that can discriminate between the time points on which patients received erythropoietin and did not ($AUC=0.95$, $p=0.001$) (Figure 5A). Similarly, hemoglobin could be also considered as a factor that discriminates between the time points on which patients received erythropoietin and did not ($AUC=0.95$, $p=0.001$) (Figure 5B). Further on, the number of red blood cells (RBC) could be also considered as a factor that discriminates between the time points on which patients received erythropoietin

and did not ($AUC=0.78$, $p=0.03$) (Figure 5C). Finally, the levels of PTH could discriminate patients with respect to the supplementation of erythropoietin ($AUC=0.62$, $p=0.045$) (Figure 5D).

Discussion

Patients under HD treatment suffer from major different bone metabolic entities as a result of renal impairment. Kidneys, apart from their role in homeostasis and fluid management also have an important role in endocrine and exocrine function. Kidneys excrete erythropoietin, a hormone that stimulates bone marrow to produce red blood cells and maintain hematocrit levels within normal limits securing tissue-oxygenation.

Erythropoietin's production is reduced or eliminated in renal disease resulting in Anemia of Chronic Disease (ACD). Anemia of chronic disease is the second most common anemia (most frequent is Iron Deficiency Anemia (IDA)) can be also called Inflammation Anemia. In ACD, MCV is normal

Figure 5. Effects of erythropoietin. The Area under the Curve (AUC) of erythropoietin with respect to hematological parameters. In particular, significant AUC were manifested with respect to hematocrit ($AUC=0.95$, $p=0.001$) (**A**), hemoglobin ($AUC=0.95$, $p=0.001$) (**B**), red blood cells (RBC) ($AUC=0.78$, $p=0.03$) (**C**) and parathyroid hormone (PTH) ($AUC=0.62$, $p=0.04$) (**D**).

(between 80-100 fL), Red Blood Cells (RBC) have a normal mean corpuscular volume (normocytic anemia) and the mean RBC mass is decreased. Iron values are also usually low while ferritin values may be normal or high. Prior to EPO administration, iron deficiency needs to be corrected. EPO must be administered intravenously or subcutaneously in doses which are defined according to the patients' needs and the target values.

In our study 86% of the total time course did receive EPO. This observation proves the critical need of EPO supplementation in HD patient's therapy. Furthermore is not only the direct effect in hematocrit and hemoglobin improvement that was found, but also it was identified that values of MCV, MCHC, RBC, WBC, PLTs, MPV, PTH were affected.

Severe secondary hyperparathyroidism, when untreated, causes the development of osteitis fibrosa cystica. It is presented radiographically as subperiosteal bone resorption mostly seen in the middle phalanges of the hands, proximal ends of the tibia and distal ends of clavicles. The characteristic of this situation is rapid bone turnover with increased osteoclastic resorption and osteoblastic bone formation.

The rapid bone turnover is associated with marrow fibrosis and creation of haphazard arrangement of collagen fibers known as 'woven osteoid'. Woven bone can be mineralized but hydroxiapatite is replaced by phosphate and amorphous calcium^{9,10}.

This disorder is not limited in bone structure but it also affects marrow. Inflammation factors such as cytokines are released causing marrow fibrosis that worsen or provoke anemia¹¹⁻¹⁵. This complication starts at early stages of kidney failure but only when in hemodialysis can give clinical manifestations.

Tetracycline double labeling of bone biopsy puts the diagnosis of osteitis fibrosacystica and laboratory blood tests can direct the renal physician to include in the investigation of anemia marrow fibrosis. Resistance in treatment of anemia despite the administration of iron and erythropoietin in patients with no other obvious reason of response delay, probably is because of incorrect functionality of erythropoiesis process. Patients in our study have shown that the need of erythropoietin administration was necessary in various doses in all of them in order to maintain hemoglobin levels in the normal values.

In our study, no bone biopsy specimens were analyzed. It is thus out of the scope of this paper to discuss the role of EPO in the development of osteitis *Fibrosa Cystica* (FC). However, our observations that marginally significant differences in PTH levels were accounted as a result of EPO administration, provide evidence that there is no protective effect of EPO in the pathogenesis of the disorder.

Conclusions

The aforementioned study and the register of patients does not confirm a causal relationship between secondary hyperparathyroidism and erythropoiesis in ESRD patients under HD. Promising would be an innovative medical treatment aiming in erythropoiesis as well as in secondary hyperparathyroidism. Simultaneous therapy could ensure a lower medical total cost and it could improve patient's life quality reducing the consumption of several different medication. When SH is well treated we expect an unbearable response in Anemia medical treatment. Further studies have to be done to interpret possible beneficial effect of EPO administration in WBC and PLTs. The above expresses the need of an innovative medical treatment aiming in erythropoiesis as well as in secondary hyperparathyroidism. It is also important to mention that cornerstone in the elimination of metabolic's bone disorders manifestations is kidney transplantation and should be preferred whenever is possible. Kidney transplantation can restores PTH levels in normal values and can delay the whole range of renal disease complications. The research in gene therapies might be a challenge but not known steps have been taken in this direction.

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