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Effect of Recombinant Human Erythropoietin on Hematological Parameters among Patients complaining from Chronic Kidney Diseases (CKD) - Jeddah KSA

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Abstract:

***Background**: Recombinant human erythropoietin (rHuEpo) was highly effective as a therapeutic agent to treat anemia of chronic kidney disease CKD. It's produced by recombinant DNA technology in mammalian cell culture. **Aim and objective**: This study aimed to evaluate the effect of rHuEpo on hematological parameters in anemic patient diagnosed with chronic renal failure and to evaluate the effect of erythropoietin antibody on hematological parameter. **Material and methods**: Prospective case control study conducted in different dialysis*

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kidney centers in Jeddah state - Saudi Arabia, during the period of Feb. 2013- Feb. 2015. 150 subjects were enrolled in this study, 100 patients with CKD under hemodialysis classified as test group and 50 apparently healthy volunteers classified as control group. ***Results****: Present study revealed that diabetes mellitus has highest prevalence of chronic diseases associate with CKD. Results of this study observed significant increase in Red Blood Cells RBCs, hemoglobin Hb and hematocrit HCT compared to baseline values (P > 0.001), (P > 0.001) and (P > 0.001) respectively. Results also showed no clinical significant of anti-erythropoietin antibody titer in all patients (P < 0.05).* ***Conclusion:*** *Anemic patients with CKD were responding to rHuEpo and their anemia was corrected without any adverse effect of erythropoietin antibody.*

Key words: Hematological parameters, hemodialysis, erythropoietin antibodies

INTRODUCTION

CKD is a pathological condition that results from a gradual, permanent loss of kidney function over time, usually, months to years. CKD can result from primary diseases of the kidneys. However, diabetic nephropathy and hypertension have been considered as the main causes of CKD. Anemia is a common complication in hemodialysis (HD) patients, mainly due to the insufficient production of erythropoietin (EPO) by the failing kidneys [(1)].

EPO binds and activates the EPO receptor by EPO binding induces a cascade of signaling events that support both erythroid proliferation and differentiation [(2)]. EPO, is a heavily glycosylated polypeptide (40% of total molecular weight) of 165 amino acids with molecular weight of 34-kDa with half-life in blood around five hours [(3)].

Anemia can lead to other organ complications like affecting cardiac function, cognitive function, exercise capacity and quality of life, and it has been independently associated with increased mortality and progression of renal disease [4,5] .

A successful management of anemia is, therefore, crucial, as it may improve clinical outcome. The introduction of recombinant human EPO (rhEPO) therapy to treat anemia of chronic kidney disease (CKD) patients reduced anemia, improving patients' quality of life [3]. There is, however, a marked variability in the response to this therapy and 5-10% of patients develop resistance to rhEPO therapy [6].

EPO presents also an important protective role in other tissues, outside of the erythropoietic system. Actually, a biological response to EPO and the expression of EPO receptors, have been observed in many different cells, namely, in endothelial, neural and cardiac cells. However, HD patients requiring high rhEPO doses present an increased risk of death [7]. Recently, randomized controlled trials showed no benefit, or even increased risk of mortality and/or cardiovascular complications, in HD patients with hemoglobin (Hb) concentration higher than the target levels [8].

Occasional case reports described patients with CKD who developed anti-erythropoietin antibody while on treatment with rHuEpo and finally developed anti-erythropoietin antibody-positive pure red cell aplasia (Ab^+ PRCA). Several hypotheses have been proposed to explain the increased incidence of Ab^+ PRCA. Most patients who developed Ab^+ PRCA received epoetin (Eprex) subcutaneously [9-11].

In this study, we aimed to evaluate the effect of rHuEpo therapy on hematological parameters among anemic patients with CKD, furthermore to study adverse immunological effect of rHuEpo on these parameters.

MATERIALS AND METHODS

Study approach, design, population, and area:

A cross-sectional prospective case control study was conducted at Saudi Arabia, Jeddah state, in different dialysis kidney centers from February 2013 to February 2015. The study population consisted of 150 male and female subjects divided into two groups, 100 subjects diagnosed with CKD were enrolled in this study, and they regularly visit dialysis center for hemodialysis. Totally 50 healthy volunteers (age- and sex-matched) were included as control group.

Ethical consideration:

All participants were told about the study aims and benefits during interview and all of them agreed to participate and all samples were taken from the participants after their agreement and filling the questionnaire and health education was provided to all participants. A written consent was obtained from each participates in this study.

Uses of recombinant erythropoietin:

Recombinant human erythropoietin dose was calculated for every patient according to his hemoglobin level and was administered intravenously in a regular basis 1-3 times per week. Monthly dose of rHuEpo followed by observation hematological parameters like RBCs, Hb, PCV and reticulocyte production index and their responsiveness to the study drug was assessed on a continuing basis every 8, 16 and 24 weeks. Screening and titration of anti-erythropoietin antibodies was done after every 8 weeks.

Sample collection, separation and preservation

With all aseptic precautions 6 ml of venous blood had been collected from each patient and controls using a disposable

sterile plastic syringe, 3.5 ml voided was into container with EDTA anti-coagulant for measurement of RBCs, Hb, PCV and reticulocyte production index immediately and 2.5 ml was voided into plain container without anticoagulant to obtain serum for measurement of titration of anti-erythropoietin antibodies, the serum was collected and kept at -20◦c in different vials till used for analysis.

Analytical procedure:

Hematological parameters (RBCs, Hb and PCV) was measured us fully automated blood cells counter - Sysmex Kx-21, RBCs count done by direct current (DC) detection method, Hb by Non-cyanide method and PCV by cumulative pulse height detection method (HTC).

Reticulocytes count performed by mixing equal volume of blood collected in EDTA with methylene blue and thin blood film made to assess reticulocytes count and after certain calculations we were found out the reticulocytes production index.

Double antigen bridging ELISA assay was used for the detection and quantification of anti-EPO antibodies

Quality control:

The precision and accuracy of all methods used in this study were checked each time a batch was analysis by including known concentration blood sample.

Statistical analysis:

Statistical analysis was done using SPSS 20, *P* value less than ≤0.05was considered significant, values were expressed as mean ± SD, paired t. test was used to compare results indices before and after having rhEPO therapy and Pearson Correlation measure the strength of a linear association between variables.

RESULT:

The study involves 100 patients with CKD doing regular hemodialysis, 69 patients (69%) of them were male and 31 patients (31%) were female, whereas the control group was composed of 30 males (60%), and 20 females (40%) as indicated in table (1).Frequency of age for patients ranging from 18 to 75 years as indicated in figure (1). Frequency of diseases that cause CKD, 35 patients (35%) diabetes mellitus, 28 patients (28%) hypertension, 20 patients (20%) diabetes mellitus and hypertension, 17 patients (17%) other diseases as indicated in figure (2).

In reference to paired *t* test, significant increase in the levels of RBCs count, Hb, PCV and reticulocyte index was observed in patients after receiving rhEPO when compared with the level of base line ($P < 0.001$) as indicated in table (2,3,4,5) respectively.

Table (6) shows the levels of RBCs count, Hb, PCV and reticulocyte index, 12 and 24 weeks week following receiving rhEPO.

Table (7) shows mean values of erythropoietin antibody in patients and control.

Table (1): Frequency of sex among patient and control group

Variable	Number of Male	Number of Female
Test group	69	31
Control group	30	20

Table: 2 Comparison between means of RBCs count, base line with 12 and 24 weeks after treatment

Parameter	Mean±SD	N	*P*- Value
RBCs baseline	3.02±.39	100	
RBCs 12 weeks	3.55±.28	100	< 0.001
RBCs 24 weeks	3.99±0.24	100	< 0.001

- The table shows the mean ± Std. deviation and probability (P).
- Paired t- test was used for comparison.

- P- value ≤ 0.05 is considered significant.

Table: 3 Comparison between means of Hb, base line with 12 and 24 weeks after treatment

Parameter	Mean±SD	N	*P*- Value
Hb baseline	9.0±1.1	100	
Hb 12 weeks	10.4± .8	100	< 0.001
Hb 24 weeks	11.7± .7	100	< 0.001

- The table shows the mean ± Std. deviation and probability (P).
- Paired t- test was used for comparison.
- P- value ≤ 0.05 is considered significant

Table: 4 Comparison between means of HCT, base line with 12 and 24 weeks after treatment

Parameter	Mean±SD	N	*P* Value
HCT baseline	26.3± 3.17	100	
HCT 12 weeks	30.9± 2.13	100	< 0.001
HCT 24 weeks	34.7±1.95	100	< 0.001

- The table shows the mean ± Std. deviation and probability (P).
- Paired t- test was used for comparison.
- P- value ≤ 0.05 is considered significant

Table: (5) Comparison between means of reticulocyte index, base line with 12 and 24 weeks after treatment

Parameter	Mean±SD	N	*P* Value
Reticulocyte index baseline	0.29±0.09	100	
Reticulocyte index 12 weeks	2.28±0.36	100	< 0.001
Reticulocyte index 24 weeks	3.34±0.41	100	< 0.001

- The table shows the mean ± Std. deviation and probability (P).
- Paired t- test was used for comparison.
- P- value ≤ 0.05 is considered significant

Table: 6 Comparative mean values of haemoglobin, RBC count, reticulocyte count and hematocrit of patients who completed treatment with recombinant human erythropoietin.

Parameter	Baseline (N=100)	12 week treatment (N=95)	24week follow-up (N=91)
RBC count (10^6/cmm)	3.02±0.38	3.55±0.28	3.98±0.24
Hemoglobin (g/dl)	8.8 ± 1.1	10.3±0.8	11.5±0.7
Hematocrit (%)	26.3±3.15	30.8±2.15	34.5±1.79
Reticulocyte index (RI)	0.3±0.09	2.3±0.36	3.32±0.39

Table: 7 Comparison of mean values of erythropoietin antibody level between patients and control

Parameter	Control group (N=50) Mean±SD	24week follow-up (N=91) Mean±SD
Erythropoietin antibody	0.206±0.013	3.15±0.296

Figure (1): Frequency of age among patients

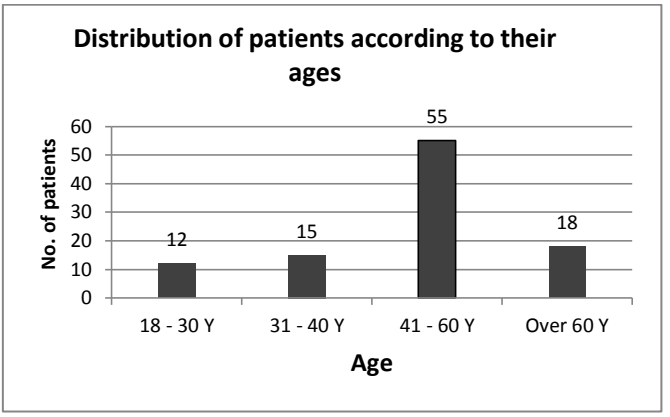
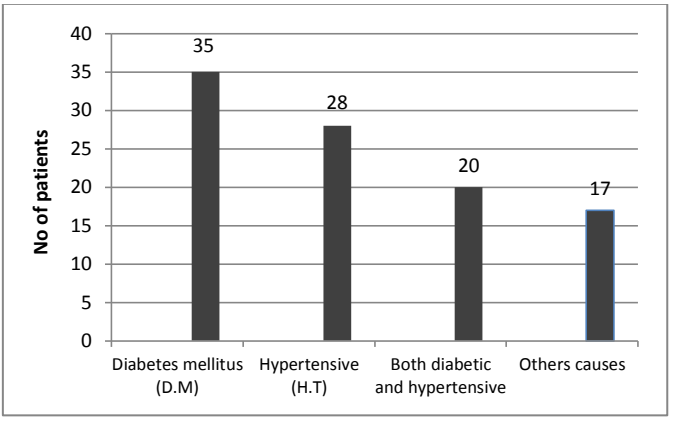


Fig. 2 Prevalence of chronic diseases among patients with CKD



DISCUSSION:

Since anemia is a well-known complication of chronic kidney disease that is mostly due to diminished production of

erythropoietin, previous study reported that the use of erythropoietin in anemia of CKD has been reported to reduce the risk of cardiovascular disease, improve patient well-being, exercise tolerance, and reduce the need for blood transfusion which also contributes to the decrease in transmission of hepatitis virus infection [12,13].

Recombinant human erythropoietin is the single most important advance in the management of ESRD (end stage renal disease) in the last 20 years. Different experimental data support the hypothesis that correction of anemia with erythropoietin may slow the rate of progression of kidney disease [14].

Accordingly the results of present study showed significant increase in RBCs count, Hb, PCV and reticulocyte production index among patients group 12&24 weeks after receiving rhEPO when compared with base line (p-value< 0.000). Furthermore our results were confirmed by previous studies which find an increase in the hemoglobin level by 1 gm/dl is noted every 4 weeks following rhEPO treatment. After week 8, rhEPO maintained the Hb level of 10 - 12 gm/dl in addition to an increase in the hematocrit level following rhEPO treatment [15].

In recent years a new complication, PRCA has been recognized as occurring in response to therapy with the development of neutralizing anti-EPO antibodies. These antibodies probably cross react with the patient's endogenous erythropoietin as well and lead to anemia that is more severe than even before the onset of erythropoietin therapy, the presence of anti-EPO antibodies leads to the absence of erythroblasts from the bone marrow [13,16]. In addition to previous study suggested that up to 67% of patients treated with rHuEPO to correct anemia of CRF developed anti erythropoietin antibodies [17]. Accordingly the results of present study showed increase in anti-erythropoietin antibodies in

hemodialyzed patient when compared normal subject. Since all hematological parameters not affected by these antibodies, we suggest that the titer of these antibodies is not sufficient to cause adverse effect. Furthermore no effect of anti-EPO antibodies in patients in our study compared to others may be explained by the relatively lower doses and short duration of injected EPO used to treat our patients relative to the doses used in these studies. Also recent report justify the different effect of anti-EPO antibodies, that commercial preparations of EPO could affect the immune system differently as different populations and ethnicities have different immune responses [(18)].

CONCLUSION:

This study concludes that recombinant human erythropoietin was found to be effective in correction of anemia in chronic kidney diseases. Furthermore, use of recombinant human erythropoietin look safe and without adverse resistance effect at least during period (24 weeks). Therefor this study recommend that patient on rHuEpo therapy should be screened regularly during treatment for the presence of anti-erythropoietin antibodies to decide the course of treatment.

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