Scientific Paper Titled:

Factors affecting insufficient response to erythropoietin therapy in end stage renal disease patients undergoing hemodialysis in a dialysis center in Khartoum

العوامل المؤثرة على عدم الاستجابة لعلاج هرمون اروثيروبيوتن في مرضى الفشل الكلوي

في مراكز غسيل الكلى بالخرطوم

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ملخص الدراسة

خلفية: المرضى الذين يخضعون لغسيل الدم المنتظم (HD) غالبًا ما يظلون مصابين بفقر الدم على الرغم من كفاءة .HD يعتبر الإريثروبويتين (EPO) علاجًا فعالًا ولكنه مكلف لمرضى فقر الدم المصابين بمرض الكلى في مراحله الأخيرة (الداء الكلوي بمراحله الأخيرة). هدفت هذه الدراسة لتقييم حالة الحديد والاستجابة ل EPOفي مرضى الداء الكلوي بمراحله الأخيرة على HD في مركز غسيل كلوي في الخرطوم، السودان.

الطريقة: شارك سبعة وخمسون مريضا مع الداء الكلوي بمراحله الأخيرة في هذه الدراسة. تم الحصول على البيانات من خلال استبيانا لمقابلة والتحقيقات المعملية. تم حساب مؤشر مقاومة EPO كجرعة أسبوعية معدلة للوزن من EPO مقسومة على تركيز الهيموجلوبين.

النتائج: كان حوالي 58٪ من المرضى مستجيبين وحوالي 42٪ لم يستجيبوا الـ .EPO علاوة على ذلك، أظهر المرضى المستجيبون أعلى في جميع الإلكتروليتات+ Ca2) ،+ K، (+ Naباستثناء الفوسفور. أظهر مصل الحديد و TIBC و TSAT و Hbو PCV زيادة معنوية في المرضى المستجيبين مقارنة بالمرضى غير المستجيبين .(P <0.01) كان فيريتين أعلى في المرضى غير المستجيبين من المرضى المستجيبين. تم العثور على ارتباط كبير $\chi 2 = 1.952$) ،قيمة ($P = 0.162$ بين استجابة ESA وعدد الجلسات في الأسبوع.

الخلاصة: السبب الرئيس لضعف الاستجابة لاستخدام EPO هو نقص الحديد. تم زيادة مستوى مؤشرات المظهر الجانبي للحديد (الحديد في الدم، TIBCوالتشبع) بشكل ملحوظ في مرضى الاستجابة مقارنة بالمرضى غير المستجيبين..

Abstract

Background: The regular dialysis patient remains anemic in spite of efficient hemodialysis. EPO is an effective but expensive treatment for anemic patients with end stage renal disease (ESRD). The study aimed to evaluate iron status and responsiveness to EPO in ESRD patients on hemodialysis.

Methods: Fifty seven patients with ESRD were participated in this study. Data was obtained by an interview questionnaire and laboratory investigations. The EPO resistance index was calculated as the weekly weight-adjusted dose of EPO divided by the hemoglobin concentration.

Results: Around 58% of patients were responsive and about 42% were non-responsive to ESA. Furthermore, responsive patients showed higher in all electrolytes (Ca^{2+}, K^{+}, Na^{+}) with exception of phosphorus. Serum iron, TIBC, TSAT, Hb and PCV showed significant increase in responsive patients as compared to non-responsive patients ($P<0.01$). Ferritin was higher in the non-responsive patients than responsive patients. Significant association ($\chi 2= 1.952$, P-value= 0.162) was found between the responsiveness of ESA and number of sessions per week and route of administration.

Conclusion: The main reason for poor response to the use of EPO is iron deficiency. The level of iron profile indices (serum iron, TIBC and saturation) was increased significantly in responsiveness patients as compared to non-responsive patients.

Key words: Hemodialysis, CKD, EPO, TSAT, Anemia, Sudan

Introduction:

The regular dialysis patient remains anemic in spite of efficient hemodialysis. A major etiological feature of the anemia is blood losses of which the residual blood volume of the dialyzer is an extremely important part [1]. Inadequate iron availability, either because of absolute or functional iron deficiency, is now the most common, yet easily treatable cause of a sub-optimal response to EPO. Two recent studies illustrate the clinician's problem with maintaining adequate available iron in dialysis patients. According to end stage renal disease (ESRD), The cause of a hematocrit of less than 25% in a group of hemodialysis patients receiving EPO, 67% of patients were found to be iron deficient and 36% of patients had a ferritin level of less than 100 ng/ml [2]. Iron-deficiency anemia results from the absence of sufficient stores of iron within the body to sustain hematopoiesis. Iron is present in circulating RBCs and myoglobin, in the erythroid marrow, and in longer term storage sites, such as the liver and macrophages within the reticuloendothelial system. Thus, although iron deficiency anemia is independent, in terms of etiology, from the anemia that results from EPO deficiency, the two causes of anemia frequently coexist because of the effects of EPO on iron store mobilization [3]. Administration of EPO, therefore, may further expose or exacerbate underlying iron deficiency, particularly in the hemodialysis population, which suffers ongoing substantial blood loss [4].

In ESRD patients on hemodialysis other than impaired EPO production, iron deficiency significantly contributes to anemia. Iron deficiency anemia has been shown to occur in 40% of HD patients with ESRD. In addition , adequate iron stores are vital for anemic patients to achieve maximum benefit from Erythropoiesis Stimulating Agents (ESA) and decrease iron stores or reduced availability of iron are the most common reasons for poor response to these agents. Besides increased demand for iron, driven by the accelerated erythropoiesis that occurs with exogenous ESA administration, iron deficiency and eventually Iron Deficiency Anemia (IDA) was occur in mostly in patients on HD because of the on-going blood losses from dialysis and tubing, frequent blood sampling and gastrointestinal blood losses as well as blood lost at time of needle placement and removal [5, 6]. However, among patients with renal failure, nephritic patients have higher plasma EPO and hematocrit levels than nephritic patients, indicating that the diseased kidneys achieve residual EPO production [7].

Treatment of patients on hemodialysis with subcutaneous epoetin is associated with more favorable clinical outcomes than those associated with intravenous EPO treatment [8]. The recent studies suggest the importance of measures of erythropoietin responsiveness association between clinical outcomes and Epoetin alpha dose or hematocrit values [9]. Hb was dependent upon ferritin levels in patients with ferritin levels <50 ng/mL but not in patients with ferritin levels ≥50 ng/mL. Patients with hypo-responsiveness to ESA had a greater risk of composite events, but ERI was unrelated to iron storage [10]. These findings reinforce the importance of defining valid measures of erythropoietin responsiveness when evaluating associations of clinical outcomes with factors, such as Epoetin alpha dose or hematocrit/hemoglobin values in clinical trials and observational studies. Therefore, the study aimed to evaluate iron status and responsiveness of EPO in ESRD patients undergoing hemodialysis.

Methods:

An observational cross-sectional descriptive, analytical study was conducted through an interview schedule with ESRD patients attended in Renal Dialysis unit in Asbab Specialized Hospital, Bahri state, Sudan from March to May 2017. A total of 57 patients were drawn as samples from renal unit. The questionnaires were pretest before finalization. Data for the study were collected through an interview schedule comprising both open and close-ended questions. The study has been approved by the Research Ethics Committee, Faculty of Medicine, International University of

Africa. An informed verbal consent was taken from dialysis center and health provider. Furthermore, written informed consent was taken from each patient before blood collection.

Interview Schedule:

Questionnaire on socio-demographic data (age, sex, occupation and education), pathological and biological data (date of starting hemodialysis, number of sessions per week, flow rate and number of EPO administered) were recorded. Blood pressure, and calibrated weight and height were measured then BMI was calculated.

Blood samples collection:

Five ml of venous blood was drawn just before commencing dialysis from the fistula site (to minimize punctures) and prior to being connected to the hemodialysis machine (heparin was run after samples taken). Blood was momentarily drawn into 5 ml Ethylene Diamine Tetra Acetic Acid (EDTA) containers and was taken to the lab within 1-6 hours and kept in standard cool temperature.

Iron profile measurement:

Iron level was measured by using a spectrophotometer. Serum iron was measured in samples obtained after blood drawn into red-stoppered tubes, the samples were allowed to clot, and centrifuged to separate the serum and read at 560 NM. Total iron binding capacity (TIBC) was measured by the chemistry auto analyzer. Ferritin was measured by using immunoassays ELISA. TSAT was calculated by using formulae; serum iron /total iron binding capacity x 100%.

Hematological parameters and electrolytes measurement:

Hematological indices (Hb, Hct and RBCs count) were measured by using hematological auto analyzer. Total calcium was evaluated by spectrophotometric method. Serum Phosphorus and Magnesium were evaluated by colorimetric method.

Erythropoietin responsiveness assessment:

EPO resistance index (ERI) was used to assess EPO responsiveness. ERI was calculated as follows; the weekly weight-adjusted dose of ESA (U/kg/week) divided by the Hb concentration (g/dL) [11, 12].

Statistical analysis:

The data has been analyzed using the Statistical Package for Social Science (SPSS). Descriptive of the results had been carried out using descriptive charts and graphs. Moreover, qualitative statistical tests such as an independent T-Test and Chi-squared had been used in our continuing

response. Multiple regression models have been further used in order to determine different covariates on the outcome variables.

Result:

Respondents of the study, 52.6% were male and 47.4% were female. Around 70% patients ages were 34 -65 years, about 21% was 18-33 years and remaining 9% was 66+ ages. The iron indices of study patients showed the following values: serum iron 95.60 ± 11.28 ng/ml, ferritin 63.27 ± 83.32 ng/ml, TIBC 222.13 ± 18.58 ng/ml and saturation 42.01 ± 3.45 %. Around 49.1 % had normal iron status, and about 38.6 % had iron deficiency (low level). Contrary, around 78.9% had high ferritin, and about 3.6 % had low level of ferritin. For TIBC, about 52.6 % had low levels, and around 40.4 % had normal. While almost half of the patients had normal (49.1%), 26.3 % had high and, 24.6 % had low saturation respectively (Table-1).

Table-1: Percentage Distribution of Iron Profile of the study respondents

	Variable	**Percent (%)**
Serum Iron	High	12.3
	Normal	49.1
	Low	38.6
Ferritin	High	78.9
	Normal	17.5
	Low	3.6
TIBC	High	3.6
	Normal	7.0
	Low	40.4
Saturation	High	26.3
	Normal	49.1
	Low	24.6
x^2= 19.20; Cramer's V= .22, df = 4; Sig; P= < .001		

According to responsive to ESA around 57.9 % were responsive and 42.1 % non-responsive to ESA. There was no significant difference in the plasma level of electrolytes between responsiveness and non-responsive patients. However the responsive patients showed higher

values in all electrolytes with exception of phosphorus. Results of comparison of iron profile between responsiveness and non-responsive patients shown a highly significant increase in serum iron, TIBC, saturation, Hb and PCV in responsiveness patients as compared to the non-responsive patients (P<0.001). Whether, serum ferritin was higher in non-responsive patients than responsiveness patients and it was not significant compared to the responsiveness group.

Table 2. Comparison of iron profile between responsiveness and non-responsive patients

	Responsive to ESA			
Blood parameters	Responsive (n=33)	Non responsive (n=24)	SEM	Significant
Serum iron	138.84	36.15	10.537**	**
Ferrtin	614.06	730.94	120.239	NS
TIBC	270.00	156.30	24.650**	**
Saturation	51.56	28.89	4.360	**
HB	10.99	9.34	0.219	**
PCV	33.12	28.53	0.755	**
SEM= Standard error of mean				
NS= No significant differences, **= significant at P<0.01				

Three potential risk factors include: number of session per week, route of administration, symptoms of anemia were found to be significantly associated with responsiveness of ESA in the Univariate analysis (P<0.025). Significant association ($\chi 2$= 1.952, P-value=0.162) was found between responsiveness of ESA number of session per week. out of 39 patients who did session two times per week, 25 (64.1%) were found to have positive response to ESA compare with 8 out 18 patients who done session three times per week were found to have positive to ESA. Moreover the majority of patients (56) take their drugs subcutaneously and more than 50% of them had positive response to the ESA. Highly significant association ($\chi 2$= 11.055, P-value=0.001) was found between anemic patients and responsiveness of ESA where more than 45 % of positive response of ESA patients were suffer from anemic symptoms while all non-anemic patients (12)

had positive response to ESA. Other risk factors showed no significant differences (P>0.05) with responsiveness to ESA.

Table 3. Univariate analysis of association of potential risk factors with responsiveness of ESA Chi-squared test

Factor	No. of patients	No. positive (%)	df	X^2	P. value
No. of session per week					
Two times	39	25 (64.1)	1	1.952	0.162
Three times	18	8 (44.4)			
Route of administration					
Inter vinous	1	0 (0.0)	1	1.400	0.237
Subcutaneous	56	33 (58.9)			
Causes of renal failure					
Renal stones	9	5 (55.6)			
Nephritis	10	4 (40.0)			
Hemorrhage	1	0 (0.0)	5	4.149	0.528
PCKD	7	5 (71.4)			
Hypertension nephropathy	11	6 (54.5)			
Unknown	19	13 (68.4)			
Duration of dialysis					
3 months to one year	5	3 (60.0)			
1-3 years	12	8 (66.7)	3	2.687	0.442
3-6 years	29	18 (62.1)			
6 years and above	11	4 (36.4)			
Body mass index					
Underweight	6	4 (66.7)			
Normal	25	17 (68.0)	3	3.194	0.363
Overweight	21	9 (42.9)			
Class I obesity	5	2 (40.0)			
Flow rate					
150 to 200	12	7 (58.3)			
200 to 250	15	7 (46.7)	2	1.141	0.565
250 to 300	30	19 (63.3)			
Eprex Injection (per/week)					
1	5	2 (40.0)			
2	47	29 (61.7)	2	1.593	0.451
None	5	2 (40.0)			
Oral supplement					
Yes	49	29 (59.2)	1	0.238	0.626
NO	8	4 (50.0)			

Discussion:

This was an observational descriptive cross sectional study and patients were drawn from a specialized hospital of renal unit. Our present study demonstrated that around 57.9 % of ESRD patients on HD were responsiveness and 42.1 % non-response to ESA. Same studies were conducted [13, 14] and found that the responsiveness was higher for ESRD patients. Our present study revealed high level of iron profile indices (serum iron, TIBC and saturation) in responsiveness patients as compared to non-responsive patients. In addition, we demonstrated that the mean serum iron, ferritin, TIBC and saturation in ESRD patients on HD were 95.60 ± 11.28 ng/ml, 663.27 ± 83.32 ng/ml, 222.13 ± 18.58 ng/ml and 42.01 ± 3.45 % respectively. We have found the relationship between bodyweight-adjusted dose of EPO and hemoglobin concentration, as described in earlier studies (15, 16). The usual approach to evaluate the clinical course in patients who have undergone a therapeutic procedure often involves two variables: ESA dose and haemoglobin level. To analyse the response to treatment, one variable should remain unchanged whereas changes in the other variable are observed; however, in actual clinical practice this set of circumstances is unlikely to occur. The ERI is useful in that it allows us to compare the response to EPO treatment in a straight-forward manner by observing a single variable. In addition, this variable allows us to compare groups of patients under different circumstances and evaluate the effect of different treatments in a single patient.

Current guidelines recommend initiation of ESA therapy when serum haemoglobin drops below 10 g/dl, but should not be used to intentionally increase Hb concentration above 13 g/dl as higher Hb concentrations raise the risk for stroke, hypertension, vascular access thrombosis and may perhaps also increase risk for death or serious cardiovascular events [17, 18, 19, 20]. Among our study population, a significant proportion was receiving ESA (91.2 %). The mean Hb concentration was 10.17 ± 1.23 g/dl, with mean Hb concentration for responsive patients 10.99 ± 0.98 g/dl and non-responsive patients 9.34 ± 1.63 g/dl.

It is widely accepted that iron deficiency in HD patients is a strong risk factor for the development of ESA resistance [21, 22]. The investigators of the "European Survey on Anemia Management" (ESAM) study found inadequate iron stores in more than fifty percent of all patients treated with ESAs [23]. Moreover, in a study by DeVita and his colleagues, lower doses of ESAs were required

to reach target Hb levels, if patients had higher-than-average ferritin levels [24]. Present study demonstrated that the levels of iron profile indices (serum iron, TIBC and saturation) were increased significantly in responsiveness patients as compared to non-responsive patients.

Previous study revealed that higher potassium levels were associated with resistance to ESAs. Patients in whom it is difficult to maintain potassium levels within the physiological range are often inadequately dialyzed. This group of patients is frequently found to suffer from malnutrition and an increased inflammatory state, which are also, associated with ESA resistance [25]. In contrast our present study revealed that high level of plasma Ca^{2+}, K^{+} and Na^{+} in responsive patients as compared to non-responsive patients so its level was not significantly different to non-responsive patients.

Iron is critical for hemoglobin synthesis; consequently patients should be carefully evaluated for the availability of iron, by measuring the serum iron and the total iron binding capacity. The SI and the percentage of TSAT reflect the amount of iron immediately available for Hb synthesis, serum ferritin level reflects total body iron stores, and low level of either of these indices may indicate the need for supplement iron to support erythropoiesis. Iron deficiency has been shown to be present in as many as 25% to 37.5% of patients presenting with the anemia of chronic kidney disease, and if treated, can at least temporarily improve or correct the anemia. Adequate iron stores are essential for achieving maximum benefit from EPO. Decreased iron stores or decreased the availability of iron is the most common reason to the resistance to the effect of EPO. In our present study the mean iron profile indices iron, ferritin, TIBC and saturation were 95.60 ± 11.28 ng/ml, 663.27 ± 83.32 ng/ml, 222.13 ± 18.58 ng/ml and42.01 ± 3.45 % respectively.When compare with previous study that done in India, showed that mean of serum iron was (84.31µg\dl) and total patients were iron deficiency [26], and another result in New Delha showed that renal failure patient developed a significant decline in serum iron [27]. In patients with CKD the values of these markers are higher and the diagnosis of functional Iron deficiency will be difficult. The majority of studies setup a cutoff values for serum ferritin and TSAT as being important in the diagnosis of functional iron deficiency when correlated either to bone marrow iron stores or the response to the administration of iron [28]. In the present study, we were unable to perform bone marrow iron stores or the response of serum ferritin and TSAT to iron replacement. We measured these two parameters in our patients and the level of Hb. Overall serum ferritin levels reflect iron stores but levels are well known to increase in the inflammatory conditions.

Several clinical studies have demonstrated that lower albumin levels, decreased BMI as well as increased C Reactive Protein (CRP) level are associated with ESA resistance in HD patients [29, 30]. The joint occurrence of malnutrition and inflammation in HD patients is consistent with protein energy wasting. Present study revealed that low potential risk factors include: number of session per week and route of administration were found to be significantly associated with responsiveness of ESA in the Univariate analysis. Significant association was found between responsiveness of ESA and number of session per week. out of 39 patients who done two session per week, 25 (64.1%) were found to have positive response to ESA compared with 8 out 18 patients who done session three times per week were found to have positive to ESA. Moreover the majority of patients take their drugs subcutaneously and more than 50% of them had positive response to the ESA.

Conclusion:

The study results suggest that treatment of anemia with ESA in patients with CKD number increasing significantly. Furthermore, TSAT is the best predicator of the initial week's responsiveness to ESA. The main reason for poor response to the use of EPO is iron deficiency. The level of iron profile indices (serum iron, TIBC and saturation) were increased significantly in responsiveness patients as compared to non-responsive patients.

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Competing Interests

The authors have declared that no competing
interest exists.

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