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Factors Predicting Erythropoietin Responsiveness among Maintenance Hemodialysis Patients: Prospective Longitudinal Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AIM and AME put the study aim, wrote the protocol, designed the research plan, analyzed and justified the results, wrote the first draft of the manuscript and follow up the publication. Author AME collected patients' data under the supervision of author WAT. All authors contributed in the revision of the final form manuscript.

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ABSTRACT

Aim: To characterize the longitudinal hemoglobin (Hgb) variability in response to erythropoietin (Epo) and to identify the influence of clinical factors on Hgb level and Epo responsiveness in a sample of hemodialysis (HD) patients using longitudinal statistical techniques.
Study Design: Prospective longitudinal study.
Place and Duration of Study: The study was conducted at the dialysis units in the Nephrology Hospital of the Armed Forces Medical Center at Cairo, Egypt, during one year duration.
Patients and Methods: The study was conducted on patients on maintenance HD who were subjected to an erythropoiesis stimulating agents (ESA) treatment. The time course of Hgb response to Epo therapy was analyzed in relationship to patients' demographics, clinical and laboratory factors using individual growth curve modeling.

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Results: Hgb levels of 89 studied patients regressed to a mean of 10.77 g/dl. The average slope of Hgb explained 12.6% of the variance in Hgb whereas an additional 8.66% was explained with the interaction of Epo dose with time. An Hgb change of -.116g/dl (*P* =.003) and -.124 (*P* =.000) was associated with non Epo use and non iron administration, respectively. Epo use was associated with a rate of Hgb change of .011g/dl per month per 10,000 IU (*P* =.042). An average Hgb change of .394(*P* =.040), .007(*P* =.000) and .601(*P* =.007) g/dl was associated with each unit increase in albumin, cholesterol and alkaline phosphates (ALP) concentrations respectively, while the rate of Hgb change was increased by .025(*P*=.046), .001(*P*=.005), .062(*P*=.024) and .024(*P*=.007) g/dl per month for each unit per month increase in albumin, cholesterol, ALP and calcium concentrations, respectively. Baseline Hgb was .128 higher (*P* =.000) and .449 lower (*P* =.000) for each unit increase in phosphorus and iPTH levels, respectively. Hospitalization lowered both baseline Hgb level by1.304 g/dl (*P* =.000) and the rate of change of Hgb by 1.022 g/dl per month (*P* =.000).

Conclusion: Laboratory values routinely measured at monthly intervals in HD patients could provide clinicians with a tool guide to predict Hgb response to Epo therapy for better anemia management in such population.

Keywords: Anemia; erythropoietin responsiveness; hemoglobin; hemodialysis; growth curve model.

1. INTRODUCTION

Erythropoiesis stimulating agents (ESAs) is the corner stone to correct renal anemia in patients on hemodialysis (HD). Previous studies have found a considerable variability of hemoglobin (Hgb) response over time; in one study, only 5% of patients staying in the target range of 11- 12 g/dl during a 6-months period [1]. It has been reported that more than 90% of patients on HD experience Hgb fluctuations [2–4]. Fishbane and Berns described (in 2005) Hgb levels cycling up and down and reported (in 2007) that changes in ESA dosing, hospitalization, and iron dosing were the primary factors responsible for Hgb cycling phenomenon [2,3]. Other studies characterized the Hgb variability patterns and examined their relationship with intercurrent events, hospitalizations, mortality and other specific patient characteristics e.g. sex, age, duration of dialysis and comorbid conditions [5–7]. A recent study reported that the risks of cerebrovascular and cardiovascular disease, infection, and hospitalization were higher among patients who failed to maintain a target range Hgb level and who exhibited high-amplitude fluctuations in Hgb compared with those who maintained a target range Hgb level of 10- 11 g/dl [8]. On the other hand, several studies investigated Hgb stability and maintenance in Hgb target range [9–11].

Erythropoietin (Epo) hyporesponsiveness is widely investigated [12–15]; however, data are limited by the cross-sectional nature of these studies. Analyzing the dynamic nature or the time-dependent changes in Hgb and Epo responsiveness, by allowing individuals patients to have their own Hgb trajectories, would determine the relationship of substantive predictors of Hgb trajectories. Individual growth curve (IGC) model approach, a type of mixed regression modelling, provide a method for modelling change which explicitly accounts for inter- and intra-individual change simultaneously in a single model [16].

The aim of this study was to characterise the trend of Hgb variability in response to Epo and to identify the influence of clinical factors on Hgb level and Epo responsiveness among HD patients, treated with ESAs through using longitudinal follow up study.

2. PATIENTS AND METHODS

2.1 Patients

The study subjects were recruited from outpatients who received HD for 3.5 to 4 hours three times weekly in the dialysis units in the Nephrology Hospital of the Armed Forces Medical Center at Maadi; Cairo, Egypt. All patients received conventional HD with bicarbonate as a dialysate. Patients were eligible for the study if they fulfilled the following criteria; aged ≥18 years, on stable HD for at least 3 months or longer, and who subjected to an ESA treatment. Eligible patients agreed to sign an informed consent were followed prospectively for 12 months from February 01, 2015 to January 31, 2016. The patients were excluded for one or more of the following criteria: (1) had active or recent neoplasia, (2) received red blood cells

(RBCs) transfusion one month before the start or during study period, (3) had major surgical surgery or bleeding one month before the start or during study period, and (4) failed to be followed during one year study period either due to death, discontinuation of HD or transfer out of the unit.

2.2 Anemia Management

All patients received ESA treatment according to routine clinical practices in the unit which followed the standard guidelines at the time of the study[17]. According to the hospital suppliers, Epoetin alfa (Eprex® 4000 IU), Epoetin beta (NeoRecormon® 5000 IU) and darbepoetin alfa (Aranesp® 20, 30 and 60 µg) were the available ESA during one year study period. The dose was monthly determined on the basis of the target Hgb level that amounted to 10-12 g/dl. Patients received Epo subcutaneously or intravenously at the end of the HD section. A dose conversion ratio of 1:200 was used to convert darbepoetin to that of epoetin (1 mg of darbepoetin alfa=200 IU of epoetin alfa or beta) [18,19]. Intravenous iron sucrose (Ferosac®) was given to achieve serum ferritin levels between 200 - 500 mg/l and individualized based on the patient's Hgb concentration at the physician's clinical discretion. Iron therapy was interrupted when serum ferritin levels exceeded 800 mg/l.

2.3 Data Collection and Blood Sampling

Data on demographic characteristics (gender and age), pre and post dialysis body weight, height, etiology of chronic kidney disease (CKD), dialysis duration, vascular access, co-medications and various co-morbidities were recorded at the start of the study. During the follow up period, data pertinent to treatment of anemia including ESA type and dose, iron doses and blood transfusion administration, were monthly collected. Patients' adequacy of dialysis dose measured by single pool Kt/V (spKt/V) was monthly calculated according to the second-generation equation of Daugirdas [20]. K stands for the dialyzer clearance, the rate at which blood passes through the dialyzer, expressed in ml/min. t stands for time of HD session. Kt, the top part of the fraction, is clearance multiplied by time, representing the volume of fluid completely cleared of urea during a single treatment. V, the bottom part of the fraction, is the volume of water a patient's body contains. For patients who required to be hospitalized, dates and causes of hospitalization were recorded.

Blood was drawn in the third week of each month, after an overnight fast, before the start of the HD session. Patients' blood samples were taken for laboratory analyses. Complete blood count, serum albumin, iron, calcium, inorganic phosphorus, alkaline phosphatise (ALP) and lipid profile (total cholesterol and triglyceride) were measured at monthly intervals for one year duration, while serum ferritin and intact parathyroid hormone (iPTH) were performed on quarterly basis in February, May, August and November 2015. Laboratory analyses were measured according to widely used techniques.

2.4 Statistical Analysis

The data were analyzed with SPSS V. 22. Results are presented as means and SD for continuous variables and as frequencies and percentages for categorical data. Significance was set at two-sided *P* value of <.05. Change in Hgb during one year study period was analyzed with an IGC modelling. This model allowed individual patients to have their own Hgb trajectories, accounted for the correlation among repeated measurements in the same patient, and is unaffected by randomly missing data. It helped to discover both intra- and inter-individual differences in the studied growth parameters (e.g., intercepts and slopes) [21].

According to the nature of the collected data, IGC involved 2 levels; Level 1 model (repeated measurements over time) focused on the individual and described how each patient changed over time (i.e., the variation within individual over time) and the Level 2 model described how the growth parameters (i.e., the within-subjects intercepts and slopes differ across people (inter-individual change)) [21]. The intraclass correlation coefficient (ICC) (formula 1) is an estimate of the serial correlation, the correlation of the repeated measures within an individual. It measures directly the closeness of observations within patient relative to observations between patients [21].

$$ICC=\sigma_{between} / (\sigma_{between}+ \sigma_{within}) \quad (1)$$

The general IGC with predictors is presented in formulas 2- 4 [21].This IGC approach was able to capture non linearity in Hgb level changes, since it considers multiple longitudinal responses simultaneously. The longitudinal response was studied along with other measures treated as time varying covariates (TVCs). That is useful in characterizing change in a variable after

accounting for variation in the response which is due to other longitudinal variables [22].

$$Y_{it} = \pi_{0i} + \pi_{1i}(\text{Time})_{it} + \pi_{2i} X_{it} + e_{it} \quad (2)$$

$$\pi_{0i} = \beta_{00} + \beta_{01} Z_i + r_{0i} \quad (3)$$

$$\pi_{1i} = \beta_{10} + \beta_{11} Z_i + r_{1i} \quad (4)$$

Where; Y_{it} is the Hgb level for the *i*th individual (*i* = 1,..., N individual) at the *t*th measurement occasion (*t* = 1,..., Time), π_{0i}, is the baseline Hgb level for the *i*th individual (intercept), $\pi_{1i,}$ is the linear rate of change in Hgb level for the *i*th individual(slope), $(\text{Time})_{it}$, represents the value of time for the *i*th individual at the *t*th measurement occasion, and e_{it} ,it is the error for the *i*th individual at the *t*th measurement occasion (i.e. the within person residual), β_{00} is the overall intercept or baseline Hgb level for the whole sample at Time *t*, β_{10} is the linear slope of change relating to the Hgb level for the whole sample at Time *t*, r_{0i} and r_{1i} represented the deviation of person *i*'s intercept and slope from the overall intercept and slope (Between person's errors). π_{2i} represents the effect of the TVC, X_{it} while β_{01} and β_{11} are the effects of the time-invariant covariate, Z_i on the intercept and linear slope.

As suggested by Singer and Willet [23], these models for Hgb variability were tested:

1. An unconditional mean model (Model 1): This model served as a baseline model to assess the differences between the observed Hgb mean value of each person and the overall Hgb mean from the population.
2. An unconditional linear change model (Model 2): In this model, time is the only independent variable that is analyzed for its influence. This is a baseline growth curve model that examined individual variability of the change rates (i.e., any significant variations in individual trajectory changes over time).
3. A conditional model (Model 3) that was formed by incorporating Epo dose as TVC to investigate its effect on the intercept and slope (i.e., baseline Hgb level and its linear change, respectively).
4. The interaction of Epo dose with time (Model 4) was performed. It represented a model of Epo sensitivity because it accounted for the time varying relationship of Epo dose and Hgb response. An unstructured covariance matrix was used in Models 2, 3 and 4 to allow for intercepts and slopes determined by the data to vary independently from each other [23].
5. All subsequent models used Model 4 to explore the effects of predictors. The tested TVCs were iron dose, post dialysis weight, spKt/V, serum albumin, serum iron, ferritin, calcium, phosphorus, iPTH, total cholesterol, triglycerides, ALP and hospitalization status. All tested covariates were centred around their means. Serum triglyceride was skewed distributions, so it was log transformed. To select the best model, likelihood ratio test/deviance test, Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used. Generally, the smaller the statistical values, the better the model fit to the data.

3. RESULTS

A total of 101 patients were identified by inclusion criteria. Out of them, 12 were defaulters during the follow up study period because 7 were died, 2 had blood transfusion, and 3 were transferred out of unit. The final sample included 89 patients. Baseline demographics and clinical characteristics of the studied patients are shown in Table 1. The relevant laboratory tests averaged over 12 months are shown in Table 2. The average (±SD) Epo dose was 22,566.48 ± 14,178.61 U/month. The within SD of monthly Epo dose was 10,258.42 while between SD was 9,707.88. The average (±SD) dose of iron was 140.26 ±159.19 mg/month and with ICC of 0.59. Hgb level had slightly lower SD between subjects than within patients, this yielded ICC of 0.47.

Causes of patients' hospitalization during the follow up period were as the following: 8 patients (9%) were hospitalized for infections of different origin, 6 patients (6.7%) for vascular access problems, 3 patients (3.4%) for cardiovascular events (arrhythmia and hypertension crisis) and 6 patients (6.8%) for other causes including hypoglycaemia, hypercalcemia and liver coma.

Fig. 1 showed the variability in Hgb as a function of increasing mean Hgb over 12 months. A considerable variability in Hgb level within and between the studied patients during 12 months follow up was observed. It appears that patients with lower or higher levels of Hgb have had a greater variability compared with those with average Hgb between 10 and 11 g/dl.

Development of Hgb variability models from model 1 to model 4 are presented in Table 3. In Model 1, the unconditional means model, mean Hgb level was 10.77 g/dl with SE of .087. The ICC was .40, suggesting that about 40% of the total variation the Hgb level was due to inter-individual differences.

Table 1. Baseline demographics and clinical characteristics of the studied patients

Characteristic		Population study Mean ± SD or n (%)
Number of patient (n)		89
Gender (Male)		36 (40.45)
Age (years)		54.16 ± 15.68
Baseline dry body weight (kg)		76.05 ± 19.37
Height (cm)		164.25 ±10.22
Body mass index, BMI (kg/m^2)		28.16 ±7.08
Time on Hemodialysis (Months)		94.47± 76.71
Baseline spKt/V		1.16 ± .37
	Hypertension	35 (39.33)
	Diabetes mellitus	11 (12.36)
	Chronic interstitial nephritis	12 (13.48)
Etiology of CKD	Polycystic kidney	8 (8.99)
	Glomerular disease	4 (4.49)
	Others	17(19.10)
	Unknown	2 (2.25)
Dialyzers'	Low flux (FX 10)	27 (30.34)
membrane	High flux (F70S)	62 (69.66)
	Arterial hypertension	62 (69.66)
	Diabetes mellitus	20 (22.47)
Comorbidity factor	Congestive heart failure	14 (15.73)
	Ischemic heart disease	6 (6.74)
	Positive HCV Abs	50 (57.47)
ACEI/ARB use		19 (42.22)
Statin use		14 (15.73)

CKD: Chronic kidney disease, spKt/V: Single-pool Kt/V (index of adequacy of dialysis dose), FX 10, Fresenius Helixone® low-flux dialysis membrane, F70S: Fresenius Polysulfone® High-Flux dialysis membrane. HCV Abs: Hepatitis C virus antibodies, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin II receptor blocker

Table 2. Laboratory parameters measured during one-year follow up

Laboratory parameters for the whole year	Overall mean ± SD	Between SD	Within SD	ICC
Hemoglobin, g/dl	10.77 ±1.12	.83	.88	.47
spKt/V	1.19 ±.37	.23	.26	.44
Calcium, mg/dl	8.35 ±.94	.64	.67	.48
Phosphorus, mg/dl	4.69 ±1.32	1.00	.84	.59
ALP, U/l	215.48 ±245.69	232.14	50.31	.96
Albumin, g/dl	3.98 ±.48	.24	.30	.39
Total cholesterol, mg/dl	170.55 ±43.26	38.41	19.31	.80
Triglyceride, mg/dl	157.76 ±78.77	67.90	37.05	.77
Serum iron, µg/dl	74.95 ±26.43	18.71	18.14	.52
Ferritin, ng/ml	426.90 ±358.80	352.52	171.39	.81
iPTH, pg/ml	571.31 ±446.81	445.66	193.21	.84

spKt/V: Single-pool Kt/V (index of adequacy of dialysis dose), ALP: Alkaline phosphatase. SD: standard deviation, iPTH: intact-parathyriod hormone, Between SD: standard deviation between patients, Within SD: standard deviation within individual patients, ICC: intraclass correlation coefficient or within-patient correlation, ICC directly measures the closeness of observations within patient relative to observations between patients.

In Model 2, the unconditional linear change model, the average baseline Hgb level was 10.77 g/dl and there was a raise in Hgb level approximately .04 units per month (β = .04, SE = .012, *P* =.000). The relationship between the intercept and the slope was negative and significant (β = -.028, SE = .013, *P* = .031). This means that patients with high Hgb level had a slower linear increase over time, while patients with low Hgb level had a greater linear increase over time. To support this, slopes and intercept for each patient was calculated by ordinary linear regression. An inverse relationship between the individual Hgb trajectories and intercepts was observed, which may be due to regression to the mean phenomenon, as shown in Fig. 2. The decline in the residual variance between Model 1 and Model 2 was .126 (.891 to .779) suggesting that the factor time explained about 12.6% of the within-individual variation in Hgb levels.

Adding Epo dose as TVC in Model 3 improved model fit and explained an additional 3.72% of the total variation in Hgb level (over and above the linear change model). Furthermore, this model improved model fit over the previous model (χ^2 (1) = 3043.410– 3017.518= 25.892, P =.000).

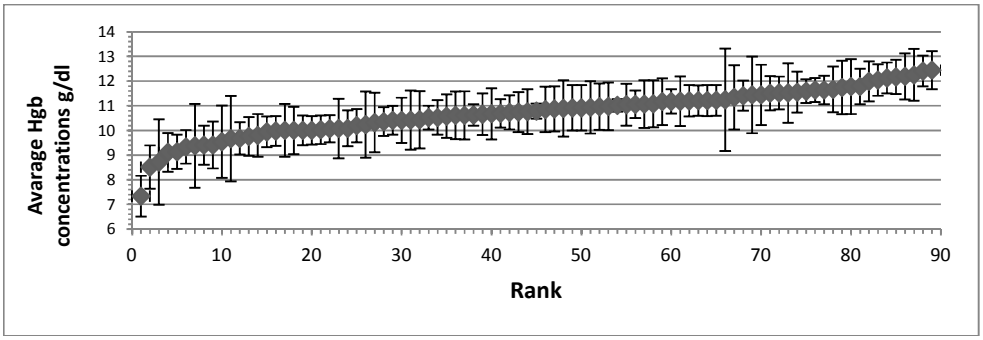

Fig. 1. Rank ordered variability in Hgb within individuals

A plot of average Hgb level for each patient, measured over 12 months, sorted in rank order. The error bars indicated standard deviation within individuals. A greater variability appeared in patients outside average Hgb range between 10 and 11 g/dl

Table 3. Development of models of Hgb variations

Model description	Model 1	Model 2	Model 3	Model 4
	Coefficient [SE]	Coefficient [SE]	Coefficient [SE]	Coefficient [SE]
Fixed effect parameters[a]:				
Intercept	10.77*[0.087]	10.77*[.087]	10.86*[.112]	11.06*[.139]
Time		.04*[.012]	.04*[.011]	.004 [.019]
Epo dose[#]			-.14*[.026]	-.22 *[.045]
Epo dose[#] ×Time				.02 **[.007]
Random effect parameters[b]:				
Intercept	.60*[.102]	.61*[.102]	.55*[.122]	.49*[.115]
Time		.007*[.002]	.005**[.002]	.006**[.002]
Correlation (intercept, time)		-.028***[.013]	-.023***[.011]	-.018 [.011]
Residual	.89*[.040]	.78*[.037]	.79*[.038]	.78*[.038]
Goodness of fit				
-2 Log Likelihood	3103.818	3043.410	3017.518	3013.125
AIC	3109.818	3055.410	3031.518	3029.125
BIC	3124.739	3085.251	3066.333	3068.913
No. of parameters	3	6	7	8
Model comparison		2 vs.1*	3 vs. 2*	4 vs. 3*
				4 vs. 2*

a: independent t test, b: Wald test, [#]Epo dose in 10,000 units/month.
**P <0.001, **P <0.01 and ***P <0.05*

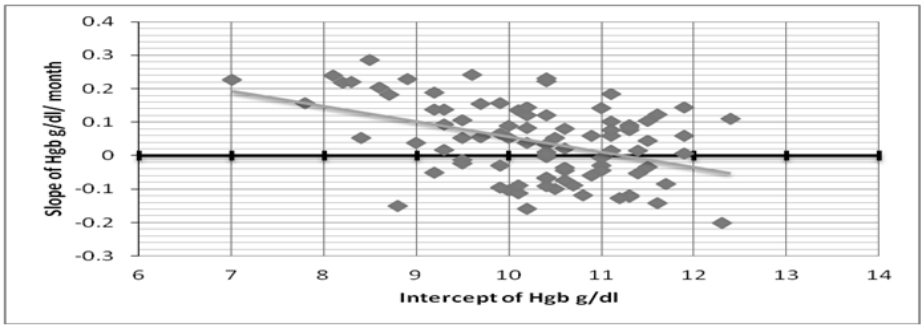

Fig. 2. Relationship between individual Hgb trajectories and intercepts
Slopes and intercept for each patient was calculated by ordinary linear regression. An inverse relationship between the individual Hgb trajectories and intercepts was observed

In Model 4, a model of Epo sensitivity, the interaction of Epo dose with time explained an additional 8.66% of the total variation in Hgb concentrations (over and above the linear growth model). Given that Epo sensitivity model improved model fit over model 3 (χ^2 (1) = 3017.518–3013.125= 4.393, P =.036) and over model 2 (χ^2 (2) = 3043.410– 3013.125= 30.285, P =.000). The random error terms associated with the intercept and linear change were significant (*P* =.000), inspiring that the variability in these parameters could be explained by between individual predictors. All subsequent models used model 4 to explore the effects of predictors.

In a model with all TVC parameters incorporated (Model 5), only a few predictors were statistically significant. The interaction effect of Epo × iron dose was tested and found no statistical significance. TVC parameters with significance of 0.1 or less were retained in the next model (Model 6) after controlling with time invariant covariates which were gender, initial age and type of dialysis membrane. Then Final model was created to incorporate the relevant significant predictors from the previous model. Hgb variation models (Models 5, 6 and final model) were showed in Table 4.

According to results of the final model (Table 4), the average baseline Hgb level was 10.06 g/dl and increased by .126 g/dl per month (*P* =.002). It should be noted that this estimate only applies to patients who have a 0 value on all the covariates in the model; in other words, at the centered values of the covariates.

An Hgb change of -.116 g/dl (*P* =.003) and -.124 (*P* =.000) was associated with non Epo use and non iron administration, respectively while Epo use was associated with rate of change of 0.011g/dl per month per 10,000 IU (*P* =.042). The average Hgb level was 1.304 g/dl significantly lower if patient was hospitalizated (*P* =.000). Hospitalization lowered the rate of change of Hgb by 1.022 g/dl per month (*P* =.000).

The mean Hgb concentration was .394 g/dl higher with each 1 g/dl increase in albumin concentration (*P* = .040). The rate of change in Hgb concentration was elevated by .025 g/dl per month for each 1 g/dl per month increase in albumin concentration (*P* = .046). Hgb concentration was .007 g/dl higher with each 1 mg/dl increase in total cholesterol concentration (*P* =.000) and its rate of change was increased by .001 g/dl per month for each 1 mg/dl per month increase in total cholesterol concentration (*P* =.005). A raise of .601 g/dl in Hgb concentration was associated with each 1 U/l increase in ALP (*P* = .007). An increase of .062 g/dl in the rate of Hgb change was associated with each 1 U/l per month increase in ALP (*P* = .024). The mean Hgb concentration was .128 g/dl significantly higher with each 1 mg/dl increase in phosphorus concentration and .449 g/dl significantly lower with each 1 pg/ml increase in iPTH (*P* =.000). Calcium concentration wasn't associated to the baseline Hgb but related to its change over time since the rate of change in Hgb was increased by .024 g/dl per month for each 1 g/dl per month increase in calcium concentration (*P* = .007). Post dialysis weight, spKt/V as well as serum iron, ferritin and triglycerides concentrations in addition to gender, initial age and type of dialysis membrane had no independent role in predicting the baseline Hgb or its change over time as shown in Table 4.

Table 4. Continuation of development of Hgb variation models

Parameters	Model 5			Model 6			Final model		
	Coefficient	SE	P	Coefficient	SE	*P*	Coefficient	SE	*P*
Fixed effect parameters[a]:									
Intercept	9.95	.757	.000	9.97	.323	.000	10.06	.292	.000
TIME	.155	.097	.110	.104	.043	.016	.126	.041	.002
Epo dose[#]	-.162	.046	.000	-.122	.039	.002	-.116	.039	.003
Epo dose[#] × Time	.014	.006	.009	.011	.006	.026	.011	.006	.042
Iron dose*	-.139	.044	.002	-.001	.022	.000	-.124	.022	.000
Iron dose* × Time	-.007	.006	.263						
Epo dose × Iron dose	.018	.013	.155						
Albumin	.446	.213	.007	.426	.211	.021	.394	.207	.040
Albumin × Time	.037	.029	.025	.032	.029	.027	.025	.028	.046
Calcium	-.034	.060	.568	-.058	.061	.341	-.040	.059	.496
Calcium × Time	.023	.009	.009	.027	.009	.003	.024	.009	.007
Phosphorus	.092	.046	.048	.127	.027	.000	.128	.027	.000
Phosphorus × Time	.006	.007	.344						
iPTH	-.445	.159	.006	-.462	.109	.000	-.449	.109	.000
iPTH × Time	-.003	.022	.892						
Post dialysis body weight	.002	.005	.751						
Post dialysis body weight × Time	-.001	.001	.193						
spKt/V	-.050	.161	.755						
spKt/V× Time	.010	.023	.655						
Serum iron	.004	.446	.994						
Serum iron × Time	-.012	.060	.848						
Ferritin	.125	.134	.354						
Ferritin × Time	-.021	.017	.224						
Cholesterol	.006	.002	.000	.006	.002	.000	.007	.002	.000
Cholesterol × Time	.001	.000	.046	.001	.000	.034	.001	.000	.005
Log Triglycerides	.109	.378	.774						
Log Triglycerides × Time	-.022	.049	.648						
ALP	.598	.249	.017	.659	.228	.004	.601	.221	.007
ALP × Time	.060	.034	.078	.056	.028	.044	.062	.027	.024
Hospitalization	-1.169	.151	.000	-1.292	.092	.000	-1.304	.092	.000
Hospitalization × Time	-.921	.023	.000	-.985	.046	.000	-1.022	.071	.000
Gender				.048	.189	.802			
Gender× Time				-.032	.022	.143			
Baseline Age				.001	.006	.813			

Parameters	Model 5			Model 6			Final model		
	Coefficient	SE	P	Coefficient	SE	*P*	Coefficient	SE	*P*
Baseline				-.001	.001	.311			
Age× Time									
Dialysis membrane				.199	.191	.300			
Dialysis membrane × Time				.028	.021	.202			
Random effect parameters[b]:									
Intercept	.483	.110	.000	.470	.105	.000	.489	.107	.000
Time	.004	.001	.002	.004	.001	.001	.004	.001	.000
Correlation (intercept, time)	-.011	.009	.220	-.012	.009	.163	-.012	.009	.171
Residual	.534	.027	.000	.536	.027	.000	.536	.027	.000
Goodness of fit									
-2 Log Likelihood	2518.613			2520.395			2528.659		
AIC	2588.613			2572.395			2568.659		
BIC	2760.940			2700.410			2667.132		
No. of parameters	31			23			17		

*a: independent t test, b: Wald test, #Epo dose in 10,000 units/month, * iron dose in 100 mg/month*

4. DISSCUSION

Using IGC modeling, longitudinal data of Hgb level and Epo responsiveness were examined across a period of one year among a representative sample of HD patients and it was evident significant inter-individual variations in Hgb level at baseline and over time. The rate of change of Hgb was inversely related to baseline Hgb level; this phenomenon of regression to the mean to a set point of about 10.8 g/dl probably indicates appropriate management of anemia to maintain Hgb levels within the target range of 10-12 g/dl.

The major finding is that serum albumin, total cholesterol, ALP and hospitalization were related to the Hgb level and Epo responsiveness. In addition, iron administration, serum phosphorus and iPTH were strongly related to baseline Hgb level but not to time dependant change in Hgb. Serum calcium had no independent role on baseline Hgb level but it was associated to its change over time and Epo responsiveness.

In this study, intravenous iron administration associated strongly with baseline Hgb level and Epo responsiveness. It has been demonstrated that administration of iron is required to maintain effective anemia control in patients who receive Epo [24]. Somewhat surprising, there was no independent effect of intravenous iron administration on Hgb change and Epo responsiveness over time. This may be explained as it have been evident that continuous iron delivery through the dialysate has resulted in reduction of Epo needs and enhancement of Hgb synthesis [25–27] and therefore good anemia management and less Hgb fluctuation.

In the present study, although serum iron and ferritin levels were within the recommended range, they were poor markers of Hgb change. A similar poor relationship between markers of iron stores and Hgb response was found [28]. Results of the Dialysis Patients' Response to IV Iron with Elevated Ferritin (DRIVE study) were reported that serum ferritin is not a good indicator to assess iron requirements [29]. Also, in another study which compared intravenous iron administration to no iron use in HD patients treated with Epo, an increase in Hgb occurred despite substantially elevated serum ferritin levels. Iron stores markers were useless to guide anemia management in HD patients [30].

Previous studies have concluded that inflammatory biomarkers particularly serum albumin and C- reactive protein (CRP) are strongly associated with Epo responsiveness in HD patients [13,29–31]. In consistent to those

studies, our results showed that albumin was a predictor of baseline Hgb and Epo sensitivity over time. Albumin level could be considered a marker of nutritional status as well as inflammation [29,32]. Nutritional status plays a fundamental role in the clinical course of patients on HD because it is closely related to inflammation through common mediators such as interleukin (IL) 6 or tumor necrosis factor (TNF) α [33–35]. Inflammation can cause hypoalbuminemia and suppress erythropoeisis since the elevated levels of cytokines particularly IL -1, mediates acute phase response, enhances CRP production and lowers serum albumin and transferrin synthesis [31,36]. Low level of transferrin prevents transportation of iron to the hematopoietic sites and leads to suppression of Hgb synthesis as well as hyporesponsiveness to ESA [12].

To our knowledge, no study investigated the effect of cholesterol on anemia management in HD population. Several studies have been found a consistently higher mortality with lower cholesterol level in HD population which contrasts finding in general population [37–39]. This supports our finding that poor anemia management, which impairs quality of life and increases mortality, is associated with reduced cholesterol level. The possible explanation of such association may relate to systemic inflammation which is common in HD population. Induction of IL 6, may lead to hypocholesterolemia by enhancing catabolism and decreasing appetite [40]. IL-6 affects lipid metabolism by stimulating lipid uptake via very-low-density lipoprotein receptors (VLDLR) induction, increasing hepatic and adipose tissue lipolysis and decreasing hepatic lipid synthesis [41].

Our results have revealed a significant direct association of ALP concentrations with baseline Hgb level and its change over time. ALP levels could correlate with production of hematopoietic stem cells because bone-type ALP expression evaluates the activity of osteoblasts [42]. Therefore, elevated ALP levels may be associated with anemia improvement by signifying higher production of RBCs. This comes in agreement with a study on Japanese anemic patients [43].

This study and other [44], have shown that elevated iPTH was closely associated with lower Hgb level and reduced Epo sensitivity. It was evident that hyperparathyroidism inhibited the activity of Na/K/ATP and affected the energy metabolism resulting in shorten the lifespan of RBCs [45]. In addition, elevated iPTH suppressed the calcitriol receptor activity on the surface of RBCs leading to deficiency of active vitamin D causing suppression of endogenous erythropoietin release and inhibition of eryth-ropoiesis [46]. Also, higher iPTH enhanced the osteoclasts activity resulting in the bone marrow fibrosis [47].

Calcium-phosphorus metabolism disturbances are common in HD patients and found to be related to renal anemia [48]. Epo is thought to stimulate proliferation and differentiation of RBCs by increasing the influx of calcium into them. Epo modulates the activity of voltage-independent, calcium-permeable channels to stimulate calcium influx [49]. Phosphorus level may affect this multistep process of proliferation and differentiation of RBCs cells [48] since calcium combines with phosphorus, lowering calcium level in the blood.

In the present study, hospitalization was the strongest predictor associated with Hgb change since hospitalized patients showed a significant sharp Hgb decrease. This comes in agreement with others studies conducted over different follow up periods [5,8,50,51]. Withholding of Epo therapy, increasing Epo resistance and/or repeated phlebotomy due to multiple vein punctures are the main causes of Hgb decline during the hospitalization[51].

The strengths of this study are the prospective collection of the data that are repeatedly assessed over relative long time and the use of a mixed modelling approach to account for intra- and inter-patients variability. Also, studying the effect of several clinical and laboratory parameters associated with patient outcome. A limitation of our study is the relative small number of patients due to single center nature of the study.

5. CONCLUSION AND RECOMMENDA-TIONS

In conclusion provides evidence that serum albumin, calcium, ALP and total cholesterol levels as well as hospitalization are effective indicators of Hgb level and Epo responsiveness in HD population. These laboratory parameters are routinely measured at monthly intervals. Therefore they could provide clinicians, month by month, with a tool guide to expect Hgb response to Epo therapy for better management of anemia

in that population. Further studies are needed to emphasize our results and to investigate the possible effect of longitudinal measures of other inflammatory markers such as CRP on Epo responsiveness.

CONSENT

Informed consent was obtained from the participating patients in adherence with the guidelines of the ethical committee of the Nephrology Hospital of the Armed Forces Medical Center at Cairo, Egypt. Participation in the study was voluntary and nobody was coerced into participation. The data will be confidential and used only in this research.

ETHICAL APPROVAL

The study protocol was approved by the research and ethics committee of the Faculty of Pharmacy, Helwan University and the Ethical Committee of the Nephrology Hospital of the Armed Forces Medical Center at Cairo, Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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