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ORIGINAL ARTICLE

MODERATE HYPOXIC CONDITIONS IMPROVE ANEMIA AND IRON PROFILE IN END STAGE RENAL DISEASE PATIENTS

HESSIEN M.
Department of Chemistry, Section of Biochemistry, Faculty of Science, Tanta University, Egypt
(Presently) Department of Clinical Biochemistry, College of Medicine, King Khalid University, KSA

ABSTRACT

The objective of this study was to investigate anemia and iron profile of hemodialysis patients permanently living at moderate altitude (2400m) and to monitor their response to erythropoietin (*Epo) treatment.*

Materials and Methods: The study included 80 patients; their glomerular filtration rate (GFR) was estimated according to the Modification of Diet in Renal Disease formula. Hematocrit *(HCT) percent and iron profile were estimated in moderate altitude patients receiving or not receiving Epo therapy, and compared to the corresponding patients living at low altitude.*

Results: The results indicated that altitude patients gained higher HCT% and showed better response to Epo treatment. Their eGFR was improved in Epo – treated patients. Significant improvement in iron profile was observed, where iron level was significantly increased, at hypoxic conditions. However, iron was extensively consumed, for erythropoiesis in Epo – treated patients. Total iron binding capacity was lower than normal, but significantly better than low altitude patients. Serum ferritin showed a wide inter-individual variability, where it varied from normal to high normal and showed a significant increase compared to healthy patients. The study demonstrates that anemia, eGFR and iron indices in end stage renal disease patients were improved due to their exposure to moderate hypoxia and Epo thereby.

Keywords: Hypoxia, Hemodialysis, Anemia, Iron profile, Glomerular filtration rate.

INTRODUCTION

About 2% of the world's population is chronically exposed to hypoxic conditions due to their residence at high altitudes.[1] This hypoxia leads to fatal complications like pulmonary edema[2] and cerebral edema.[3] Also, anemia begins early in the development of kidney disease and aggravates with the progressive destruction of kidney tissue.[4] The hematocrit (HCT) generally begins to fall when serum creatinine exceeds 2 mg/dl and progressively decreases as glomerular filtration rate (GFR) declines.[5] The inadequate production of erythropoietin (Epo) is the main etiology of CRF – related anemia, other contributing factors include shortened erythrocyte survival, hemodialysis associated blood loss and abnormalities of iron metabolism.[6] Anemia management in CRF usually includes administration of Epo stimulating agents (ESA) and iron supplements. Although Epo stimulates the proliferation of erythroid precursor cells,[7] adequate iron store is important for management of iron deficiency[8] and for inducing response to Epo.[9] Serum iron levels depend on the intestinal absorption, transport capacity in the blood and recycling of iron released from phagocytosed erythrocytes, where most of iron used for normal erythropoiesis is derived from the recycled iron.[10]

In CRF patients permanently exposed to hypoxic conditions, at moderate altitude, erythropoiesis is stimulated by the low oxygen and Epo treatment. Consequently, the availability of sufficient iron in such hypoxic conditions is critically important for stress induced erythropoiesis. Also, the physiological effect of hypoxia depends upon the height and the duration of exposure.

Accordingly, the objective of this work was to investigate anemia and iron profile in hemodialysis patients permanently living at moderate altitude and to monitor their response to Epo treatment.

MATERIALS AND METHODS

Patients Grouping

The study included 120 CRF patients and control subjects (110 males and 10 females) categorized into 6 groups. The study protocol was approved by the ethical committee, where written consents were obtained from participants. Patients were undergoing hemodialysis in the dialysis units of El-Namas Hospital (2400m above the sea level) and El-Magarda Hospital (300m). The two hemodialysis facilities were observing the same hemodialysis protocol, supplies, the diag-

Corresponding author: Dr. Mohamed Hessien. Department of Chemistry, Faculty of Science, Tanta University, Egypt. Current Address: Department of Clinical Biochemistry, College of Medicine, King Khalid University, KSA. Email: mohamed.hessien@fulbrightmail.org

nostic protocols and anemia management. Anemic patients were treated with 51 ± 23 U recombinant human Epo / kilogram of body weight, starting with. 50 U/kg 3 times/week for 4 weeks, and then the dose was gradually increased by 25 U/kg until a satisfactory HCT% was reached.

According to their residence and Epo treatment, patients were categorized into four groups (20 patients each). Group I included moderate altitude patients receiving Epo treatment, Group II included moderate

Table 1: *Demographic and dialysis related clinical data.*

		Moderate Altitude			*Low Altitude*		
		EPO – Treated HD	*No Epo HD*	*Control*	*Epo –Treated HD*	*No Epo HD*	*Control*
Subject Number(n)		20	20	20	20	20	20
Gender	Male	18 (90.0%)	19 (95.0%)	17 (85.0%)	18 (90.0%)	19 (95.0%)	19 (95.0%)
	Female	2 (10.0%)	1 (5.0%)	3 (15.0%)	2 (10.0%)	1 (5.0%)	1 (5.0%)
Age (yrs)		56.35 ± 5.77	56.85 ± 5.13	53.45 ± 7.32	54.85 ± 5.48	55.75 ± 5.46	52.05 ± 4.19
Etiology of CRF:							
Glomerulonephritis		8	7	0.0	6	8	0.0
DM		5	6	0.0	5	6	0.0
Hypertension		6	3	0.0	6	3	0.0
Not known		1	4	0.0	3	3	0.0

Epo: Erythropoietin, HD: Hemodialysis, DM: Diabetes mellitus, HA: high altitude, LA: low altitude.

Table 2: *Blood urean nitrogen, creatinine, estimated glomerular filtration rate and electrolytes in hemodialysis and healthy groups at hypoxic and normoxic conditions.*

	Moderate Altitude			*Low Altitude*		
	EPO – Treated HD	*No EPO HD*	*HA Control*	*EPO – Treated HD*	*No EPO HD*	*LA Control*
BUN (N.V.: 4.66 – 23.3 mg/dl)	49.9 ± 3.9[a,b]	53.9 ± 3.7[a]	11.9 ± 1.8	52.7 ± 4.8[a]	55.8 ± 4.8[a]	11.1 ± 1.5
Creatinine (N.V:0.7 – 1.4 mg/dl)	5.7 ± 0.4[a,b,c]	6.9 ± 0.4[a,]	1.1 ± 0.1	7.0 ± 0.6[a,b]	7. 7± 0.5[a]	1.1 ± 0.1
eGFR (N.V: >60 mL/min)	13.5 ± 1.1[a,b,c]	10.6 ± 0.7[a,c]	74.3 ± 2.6	9.0 ± 1.0[a]	7.7 ± 0.74[a]	76.4 ± 3.7
Sodium (135 – 155 mmol/L)	139.5 ± 3.6	139.8 ± 4.3[a]	136.8 ± 1.7	139.8 ± 2.8	140.1 ± 3.9[a]	136.0 ± 2.5
Potassium (N.V: 3.6 – 5.5 mmol/L)	5.5 ± 0.2[a]	5.6 ± 0.2[a]	4.0 ± 0.3	5.5 ± 0.2[a]	5.6 ± 0.2[a]	3.9 ± 0.3
Phosphorus (N.V.:2.5 – 5.0 mg/dl)	6.00 ± 0.5[a]	5.8 ± 0.5[a]	4.0 ± 0.3	5.9 ± 0.3[a]	5.8 ± 0.3[a]	4.0 ± 0.3
Calcium (N.V: 8.1 – 10.4 mg/dl)	7.9 ± 0.3[a]	7.9 ± 0.2[a]	9.5 ± 0.4	8.0 ± 0.4[a]	7.9 ± 0.4[a]	9.7 ± 0.5

(a): Significant difference compared to the corresponding healthy subjects at the same altitude.
(b): Significant difference compared to patients at the same altitude.
(c): Significant difference compared to patients at the low altitude.

Table 3: *Hemoglobin, hematocrit and iron profile in patients at hypoxic and normoxic conditions.*

	Moderate Altitude			*Low Altitude*		
	EPO – Treated HD	*No EPO HD*	*HA Control*	*EPO – Treated HD*	*No EPO HD*	*LA Control*
Hemoglobin (g/dl)	11.4 ± 0.8[a,b,c]	9.7 ± 0.7	16.8 ± 1.3	9.7 ± 1.8[a,b]	10.0 ±	15.9 ± 1.1
HCT (%)	35.4 ± 1.7[a,b,c]	33.4 ± 2.0	50.7 ± 1.6	32.1 ± 1.8[a,b]	30.8 ± 2.9	47.1 ± 1.5
Serum Iron	73.8 ± 5.2[a,b,c]	97.8 ± 10.7 [a,c]	129.5 ± 9.8[c]	57.49 ± 9.5[a,b]	68.44 ± 7.4 [a]	119.3 ± 12.1[a,b,c]
TIBC	269.8 ± 12.07[a,b,c]	237.6 ± 10.76[a,c]	333.4 ± 9.8	236.4 ± 13.2[a,b]	208.2 ± 14.7 [a]	305.7 ± 13.8
TSAT (%)	26.8 ± 5.6[a,b]	39.0 ± 3.4 [c]	38.6 ± 3.7	23.8 ± 3.1[a,b]	27.4 ± 3.1 [a]	39.9 ± 4.0
Serum Ferritin	268.0 ± 27.8 [a,b]	302.0 ± 25.6[a,]	198.0 ± 18.3	291.0 ± 47.2[a,b]	358.0 ± 51.8 [a]	226.8 ± 16.4

(a): Significant difference compared to the corresponding healthy subjects at the same altitude.
(b): Significant difference compared to patients at the same altitude.
(c): Significant difference compared to patients at the low altitude.
Reference range of iron: 59 – 148 ug/dl (male) 37 – 145 ug/dl (female)
TIBC: 274 – 385 ug/dl (male) 180 – 260 ug/dl (female)
TSAT: (N.V: 15 – 50% (male) 12 – 45% (female)
Ferritin: Male: 12 – 300 ng. Female: 12 – 150 ng/ml

altitude Epo – untreated patients. Groups IV and V included lower altitude patients treated or not treated with Epo, respectively. Also, the study included 40 healthy subjects enrolled in two control groups for moderate altitude and low altitude, respectively). Basic clinical and demographic data are shown in table 1.

Biochemical and hematological investigations
Blood samples were assigned to investigate blood urea nitrogen (BUN), serum creatinine, GFR and iron profile. The GFR was estimated according to the Modification of Diet in Renal Disease formula:[11] eGFR = 186.3× serum creatinine$^{-1.154}$ × age$^{-0.203}$ (× = 1 if male and 0.742 if female). Iron profile included serum iron, total iron binding capacity (TIBC), ferritin, and transferrin saturation (TSAT). Iron was measured according to the method described by Garcic,[12] and TIBC was estimated according to Starr.[13] Serum ferritin was measured using Ferritin (FTL) Human ELISA Kit (Abcam, MA, USA) following the manufacture instructions. Transferrin saturation (TSAT), that represents the percent of transferrin sites occupied by iron, was calculated as the ratio between iron and TIBC multiplied by 100.

Measurements were determined using a Hitachi 7050 analyzer (Hitachi Corp., Japan). Hemoglobin and HCT% were estimated using (HumaCount Plus, Human Diagnostics, Wiesbaden, Germany).

Statistical Analyses
Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), version18.0, Inc, Chicago, III, USA). Data was represented as mean (± standard deviation). Tukey's test was used for multiple comparisons between variables. "*P*" values equal to or less than 0.05 indicate a significant difference between variables.

RESULTS
The age of patients included ranged from 36 to 73 years (mean: 56.1 ± 7.97 years). The differences in ages among Epo – treated and untreated groups at the same height were statistically insignificant (gps: I *vs.* II and IV *vs.* V; P > 0.05). Similarly, no significant age differences were observed among moderate altitude groups and the corresponding groups at lower altitude (I *vs.* IV and II *vs.* V; P > 0.05). The etiologies of CRF were: chronic glomeriolonepheritis (in 29, 36.3%), diabetes mellitus (in 22, 27.5%), hypertension (in 18, 22.5%), and other unknown causes (in 11, 13.75%) patients (Table 1).

Patients have demonstrated the expected abnormal pattern of kidney functions, including significant increase in BUN, creatinine, potassium and phosphorrus, associated with significant decrease in serum calcium the eGFR (Table 2). Altitude Epo – treated patients had lower BUN compared to the untreated group at moderate altitude (P < 0.05). However, the difference between lower altitude patients was insignificant (P > 0.05). Also, the altitude change did not induce significant difference in BUN levels among Epo – untreated groups (gp II *vs.* V; P > 0.05) or untreated groups (gps: II *vs.* V, P > 0.05) (Table 2). Serum createnine showed significant improvement due to both altitude change and Epo treatment, where moderate alti-

tude – Epo – treated patients had lower creatinine compared to: the untreated patients at the same altitude (P < 0.001), low altitude Epo – treated patients (P < 0.001) and low altitude Epo – untreated patients (P < 0.001). Serum electrolytes did not show any significant changes due to the height difference and Epo treatment (Table 2). All patients showed a pronounced reduction in the eGFR compared to healthy subjects (gps: I and II *vs.* III, P < 0.001 and VI and V *vs.* VI, P < 0.001). The eGFRs of altitude Epo – treated patients, however, was higher than Epo – untreated group at the same hight (P < 0.001) and Epo – treated group at lower altitude (P < 0.001). Also, it was significantly higher in moderate altitude groups compared to the corresponding low altitude groups (Table 2).

The means HCT of altitude groups were variably higher than the corresponding lower altitude groups (Table 3). In general, altitude patients had higher HCT% compared to the corresponding lower altitude groups (P < 0.001, P < 0.001). Moreover, Epo treatment led to a significant improvement in HCT% (gp: I *vs.* II, P < 0.05 and gp IV vs. V, P < 0.001) (Table 3). Anemia was observed in 2 (10%) and 7 (35%) patients at moderate altitude treated or untreated with Epo, respectively. Lower altitude groups, however included more anemic patients: 5 (25%) and 11 (55%) in Epo treated or untreated groups, respectively (Fig. 1).

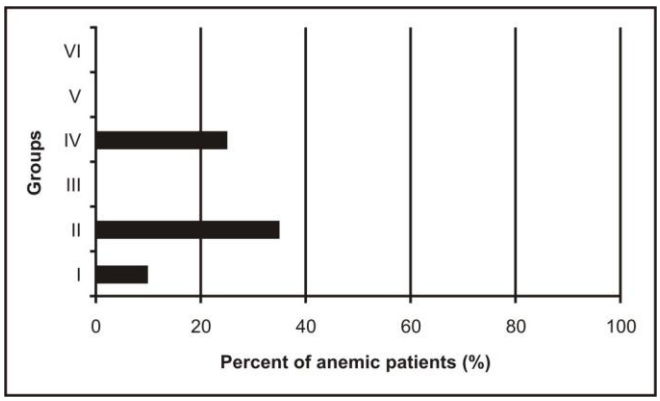

Fig. 1: *Absolute iron deficiency in hemodialysis groups at both hypoxic and normoxic conditions.*

Serum iron was lower in all hemodialysis groups compared to the corresponding healthy subjects (gps: I and II *vs.* III P < 0.001 and gps: IV and V *vs.* VI, P < 0.001). Also, the altitude difference led to significant changes in iron levels (gp I *vs.* IV, P < 0.001and gp II *vs.* V, P < 0.001). Moreover, Epo treatment was accompanied with a decrease in iron levels, where the change was highly significant among moderate patients (I *vs.* II, P < 0.001) and significant in lower altitude groups (IV *vs.* V, P < 0.01) (Table 3). A borderline level and mild decreased levels TIBC were observed in altitude patients, treated or untreated with Epo, respectively (gps: I and II). TIBC, has recorded the highest level in altitude patients treated with Epo, however the lowest level was seen in low altitude patients untreated with Epo. TSAT ratios exhibited the same changing pattern of iron, where it was higher in Epo – untreated groups than the corresponding Epo – treated groups at the same altitude (Table 3). Patients ferritin showed a wide inter – individual variability. In altitude patients it varied from normal to high normal and showed a significant increase compared to healthy group (I *vs.* III; II *vs.* III: P < 0.001). In lower altitude groups, however, ferritin was higher than both healthy subjects (P < 0.00.1) and the corresponding altitude groups (IV *vs.* I and V *vs.* II).

Absolute iron deficiency (AID) (ferritin < 150 ng/ml and TSAT < 20%), was observed in 1 (5%); 3 (15%), 4 (20%) and 5 (25%) in patients groups I, II, IV and V, respectively. Normal iron profile (TSAT range: 20 – 50%) was detected more in moderate altitude groups (gps: I and II) compared to the corresponding low altitude patients (gps: IV and V). Also, at both altitudes Epo treatment was accompanied with less percent of patients with absolute iron deficiency.

DISCUSSION

The obtained data suggests a possible ameliorative effect of hypoxia and Epo treatment on the renal performance. Exposure to moderate altitude has improved some kidney function markers. Although it did not affect BUN, Creatinine was improved relative to patients at normoxic conditions. Moreover, Epo treatment enhanced the ameliorative effect of hypoxia, where it reduced BUN levels. Although eGFR was markedly decreased in all patients. Epo – treated altitude groups demonstrated a better eGFR, which was improved by 27.7%, compared to Epo untreated group, and by 52%, compared to the corresponding patients at lower altitude. Similar retrospective study has suggested that moderate altitude may induce a renal protective effect.[14] Other prospective studies, conducted at altitudes > 3500m, did not report such an improvement, however the deterioration of eGFR was found in a limited percent (2.1%) of their patients.[15] The observed changes in renal functions in altitude patients may be attributed to the direct effect of hypoxia on the kidney, especially at 2400m blood oxygen (PaO_2) decreased to 91% compared to 98% in those living at 300m above the sea level. These changes may be induced through some compensatory adaptations including changes in ventilation, cardiac output and erythropoiesis.[16]

Although iron, TIBC, ferritin and TSAT are widely used to access iron deficiency anemia, they exhibit large variability in the context of underlying inflammation of chronic kidney disease (CKD). Also, iron disorder is complex in hemodialysis patients because they are in a state of continuous iron loss and supplementation. Also, hypoxia plays an impotent role in regulation of various pathways involved in iron metabolism.[17]

As the data shows, serum iron in HD patients was lower than healthy subjects, however hypoxic condition has improved serum iron level, where moderate altitude groups showed a higher serum iron compared to the corresponding lower altitude groups. Epo treatment, in contrast, was accompanied with a significant decrease in serum iron. In agreement with previous reports,[18] the results indicated that Epo treatment caused a significant increase of iron utilization. The relative improvements in both iron and the saturation of its carrier (TSAT) may be explained by the role played by hypoxia inducible factors (HIFs). HIFs are oxygen-sensitive transcription factors and they are essential for cell adaptation to low oxygen.[19] They target the divalent metal transporter-1, which mediate iron absorption.[20] Also, HIF is known to induce the expression of trsnafirrin, its receptor (transferrin receptor-1, TfR1)[21] and hepcidin peptide, which affect iron mobilization[21]

Ferritin stores iron that is not required for immediate metabolic needs. The concomitant decrease of ferritin in Epo – treated patients and the amelioration of their HCT% and TSAT may indicate the improvement of iron utilization in the erythropoiesis process. In agreement with other studies,[23] ferritin levels were higher in patients, compared to the corresponding healthy subjects, due to inflammation.

Treatment of anemia in haemodialysis with iron and ESA does not always lead to adequate anemia control.[24] Absolute iron deficiency (AID) was observed in some Epo – treated patients. However both hypoxia and Epo treatment decreased the prevalence of iron deficiency anemia, especially in altitude patients, indicating the enhanced mobilization of iron from both internal stores and dietary source. Although some proteins involved in iron absorption and metabolism are oxygen regulated, the literature did not confirm effect of hypoxia on Epo gene expression. Some data, however suggested that hypoxia is the driving force, through the role of Epo receptor, HIF-1, HIF-2 and some co-activators.[25]

It is ***concluded*** that this study suggests a possible ameliorative effect of mild hypoxia and Epo treatment on both anemia and iron profile in hemodialysis patients, chronically exposed to moderate hypoxic conditions. These improvements were relative to the corresponding patients at normoxic conditions.

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