Haemoglobin Variability in Patients with Chronic Kidney Disease Stage 5 under Haemodialysis.

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Abstract: Background: Hemoglobin variability is the fluctuation of hemoglobin above or below the target range over time. Haemoglobin variability had found to be a frequent finding in haemodialysis patients treated with rHuEPO. **Methods:** In this study, we studied the Contributors and Consequences of Haemoglobin Variability in 60 Patients who are stage 5 CKD under haemodialysis in haemodialysis unit at Benha University Hospital who were prospectively studied over successive six months period**. Results:** Iron treatment, iron deficiency, secondary hyperparathyroidism, HCV virus infection, interfering drugs with haematopoiesis e.g., ACEI or ARBs and male gender were significant contributing factors for haemoglobin variability. Other infections e.g., UTI, chest infections, gum and catheter related infections might play a role as a contributing factor for haemoglobin variability. Cardiac diseases were significant consequences of haemoglobin variability. Weakness, fatigue and autonomic dysfunction might be consequences of haemoglobin variability. **Conclusions:** We have found haemoglobin variability to be a frequent finding in haemodialysis patients treated with rHuEPO. We need to do every effort to minimize haemoglobin variability in haemodialysis patients by reducing incidence of contributing factors of haemoglobin variability.

1. Introduction

Hemoglobin variability is the fluctuation of hemoglobin above or below the target range over time. Hence, hemoglobin variability is the extent to which multiple measured hemoglobin values differ from each other within a given time span, whereas the calculated mean of all hemoglobin levels may still remain within the target range **(1)**.

Factors Affecting Hemoglobin Variability:
Drug-Related Factors

Drug-related factors such as differences in pharmacokinetic and bioavailability parameters among ESA and different routes of administration (intravenous *versus* subcutaneous (**2**), may affect hemoglobin stability in patients with CKD.

Patient-Related Factors

Hemoglobin levels vary by age, gender, and race. In general, lower hemoglobin levels have been observed with increasing age, in women compared with men, and in black patients compared with white patients **(3).**

Factors Related to Medical Care and Reimbursement Policies

Hemoglobin variability is also affected by anemia management practice patterns, which in turn are influenced by clinical practice guidelines, treatment protocols, and, in particular, reimbursement policies (**4**).

Additional reasons for hemoglobin variability include acute or chronic comorbidities(**5**); alteration in iron stores(**6**); infection or inflammation(**7**); blood loss or transfusion(**8**); dialysis treatment features such as dialysis adequacy(**9**) or water quality(**10**); stage of CKD and residual renal function(**11**); level of parathyroid hormone(**12**); vitamin and mineral status such as vitamin D, B12, or folate deficiencies (**13**); and seasonal effects(**10**).Hemoglobin variability is more prominent in patients who are younger, have lower albumin or higher serum ferritin levels, or have changes in appetite(**14**), possibly related to alterations in nutritional or inflammatory status(**15**), and higher mean corpuscular hemoglobin (**16**), Iron supplementation strategies, for example, intravenous iron maintenance *versus* repletion, may cause different patterns of variations in hemoglobin level.

Consequences of Hemoglobin Variability

In maintenance dialysis patients, hemoglobin variability seems to be associated with increased risk for death according to at least two studies **(17).**

In addition to associations of blood hemoglobin with mortality and hospitalizations in the CKD population, anemia is associated with fatigue, weakness, shortness of breath, and a decreased health-related quality of life (**18).** Furthermore, hemoglobin overshoot may be associated with various safety concerns, including the development of elevated BP with risk for hypertensive encephalopathy **(19)**, iron deficiency **(20)**, high platelet count **(21)**, thrombotic

events **(22),** and accelerated left ventricular dysfunction and hypertrophy (**23**).

2. Patients and Methods

60 Patients who are stage 5 CKD under haemodialysis in haemodialysis unit at Benha University Hospital were prospectively studied over successive six months period from July 2014 to January 2015.

Inclusioncriteria

- Male and female patients.
- Age more than 17 years.
- Patients who are stage 5 CKD under haemodialysis

Exclusion criteria

- Age less than 17 years.
- Patients with acute kidney injury.
- Patients with history of bleeding and bleeding tendencies.
- Patients with TB.
- Patients with concurrent haematological disorders (e.g., sickle cell anemia).
- Patients receiving cancer chemotherapeutic agents and HIV medications.

All of them were subjected to the following after taking their informed written consents:

- Complete history and physical examination.

History of :

➢ **Factors related to patient demographics:**
Age/ yr.
Causes and duration of ESRD/yr.
Gender.
Diabetes mellitus.

➢ **Drug-related factors:**

- ESA dosing/frequency and administration route (intravenous, subcutaneous).
- Type of iron supplements (PO vs. IV) and dose and frequency of administration.
- Medications that can interfere with erythropoiesis, such as ACEI, ARB, HCV medications.

➢ **Iron deficiency:**

- Iron loss as a result of comorbid conditions (*e.g.*, gastrointestinal bleeding).
- Ongoing iron loss related to hemodialysis treatment and frequent blood testing.
- Iron deficiency as a result of ESA treatment–related increase in hematopoiesis.

➢ **Infections:**

- Overt infections such as dialysis catheter–related infections, pneumonia, and urinary tract infections.
- Latent infections such as mild peritonitis, hepatitis C, and chronic gum infection.

➢ **Inflammation:**

- Chronic inflammation (*e.g.*, malnutrition-inflammation complex syndrome).

Clinical examination of:

- Vital signs (blood pressure, pulse, temperature and respiratory rate)
- CVS regarding (heart failure, LVH, hypertension, pericardial effusion, IHD, valvular heart diseases).
- CNS regarding (cerebrovascular diseases and stroke).
- Chest examination regarding (pleural effusion, pneumonia).
- Abdominal examination regarding (ascites, peritonitis).
- Genito-urinary examination.
- Malnutrition assessment with anthropometric measures.

Investigations:

- Blood Hemoglobin, gm/dl.**(24)**
- Serum Creatinine mg/dl. (2**5)**
- Blood urea mg/dl. (**26)**
- Kt/V. **(27)**
- Serum Albumin, g/dl.**(28)**
- Blood Bicarbonate, mEq/L.**(29)**
- Aspartate aminotransferase, IU/L. **(30)**
- Serum total Calcium, mg/dl. (**31**)
- Serum Phosphate, mg/dl. (**32**)
- Serum Intact PTH, pg/ml**.(33**)
- Iron profile (serum iron µg/dL, ferritin level ng/ml, and transferrin saturation%)(**34**)
- HCV Antibodies **(ELISA) (35),(36)**
- Resting ECG
- Transthoracic Echocardiography
- Doppler Ultrasound

Patients were classified into the following:

Classification of all patients into one of three groups:

Group A: mean haemoglobin measurements in haemoglobin variability are less than11 gm/dL.

Group B: mean haemoglobin measurements in haemoglobin variability are between 11 and 13 gm/dL.

Group C: mean haemoglobin measurements in haemoglobin variability are more than13 gm/dL.

Measurements of haemoglobin variability and Statistical analysis:

Descriptive statistics are presented as mean with SD

Coefficient of hemoglobin variation: Ratio of the SD to the mean hemoglobin (**5).**

Using the first, third quartiles and the interquartile ranges.

The collected data were tabulated and analyzed using SPSS version 16 soft ware (SpssInc, Chicago,

ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean ±standard deviation. Chi square test (X^2), Fisher's exact test, z test for proportions, Anova test (f) were used as tests of significance. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant).

3. Results
In our study we found that:
Group A: Mean haemoglobin in haemoglobin variability less than 11 gm/dl.includes 41 patients of the sixty patients of the study population with percentage 68%.

80% of patients in group A who started with mean haemoglobin less than 11 gm/dl in the second month of the study remain in the same group A in the sixth month of the study.

14.5% of patients in group A who started with mean haemoglobin less than 11 gm/dl in the second month of the study crossed to group B in the sixth month of the study.

5.5% of patients in group A who started with mean haemoglobin less than 11 gm/dl in the second month of the study crossed to group C in the sixth month of the study. 73.3% of total population of the study was in group A in the sixth month of the study

Group B: Mean haemoglobin in haemoglobin variability between 11 and 13 gm/dl. Includes 16 patients of the sixty patients of the study population with percentage 27 %.

62.5% of patients in group B who started with mean haemoglobin between 11and 13 gm/dl in the second month of the study crossed to group A in the sixth month of the study.

18.75% of patients in group B who started with mean haemoglobin between 11and 13 gm/dl in the second month of the study remain in the same group B in the sixth month of the study.

18.75% of patients in group B who started with mean haemoglobin between 11and 13 gm/dl in the second month of the study crossed to group C in the sixth month of the study. 18.3% of total population of the study was in group B in the sixth month of the study.

Group C: Mean haemoglobin in haemoglobin variability more than 13gm/dl. Includes 3 patients of the sixty patients of the study population with percentage 5 %.

33% of patients in group C who started with mean haemoglobin more than 13 gm/dl in the second month of the study crossed to group A in the sixth month of the study.

67% of patients in group C who started with mean haemoglobin more than 13 gm/dl in the second month of the study crossed to group B in the sixth month of the study.

0% of patients in group C who started with mean haemoglobin more than 13 gm/dl in the second month of the study remain in the same group C in the sixth month of the study. 8.4% of total population of the study was in group C in the sixth month of the study.

Table (1): Fluctuations in haemoglobin levels between the second and the sixth months

		HB 6th month			Total
		Group A	Group B	Group C	
HB 2nd month	Group A (41 patients)	33	6	2	41
	Group B (16 patients)	10	3	3	16
	Group C (3 patients)	1	2	0	3
Total		44	11	5	60

Figure (1): Description of haemoglobin variability inside the same group and between the three groups of the study throughout the study time.

The mean haemoglobin of the group A varied from one month to another but didn't cross to any other group in any month throughout the study time.

The mean haemoglobin of the group B varied from one month to another and crossed to group A in two months throughout the study time.

The mean haemoglobin of the group C varied from one month to another and crossed to group A in one month and crossed to group B in three months through the study time and overshoot in two months through the study time.

In Group (A)
There was significant statistical difference toward presence of HCV infection as there were 66% of patients infected with HCV virus.

Secondary hyperparathyroidism was present in group A (334.15±100.83) pg/ml.

There was highly significant statistical difference toward presence of iron deficiency as there were 95% of patients suffering from iron deficiency

There was highly significant statistical difference toward presence of cardiac diseases as there were 92.5% of patients suffering from cardiac diseases.

There was significant statistical difference toward the absence of autonomic dysfunction as there were 36.5% of patients suffering from autonomic dysfunction.

There was highly significant statistical difference toward the absence of thrombotic events as there were 2.4% of patients suffering from thrombotic events only.

There was highly significant statistical difference in the last three months of the study toward the absence of hospitalization as there were less than 5% of patients hospitalized.

There was highly significant statistical difference toward the absence of mortality as there was 2.4% mortality.

In Group (B)

There was significant statistical difference toward male genderas there were 62.5% males and 37.5% females.

There was significant statistical difference toward presence of HCV infection as there were 62.5% of patients infected with HCV virus. Secondary hyperparathyroidism was present in group B (383.44±125.27) pg/ml.

There was 100% presence of cardiac diseases.

There was significant statistical difference toward absence of interfering drugs with haematopoiesis as there were 37.5% of patients receiving interfering drugs with haematopoiesis**.**

There was significant statistical difference toward the absence of other infections in month one, three and four as there were less than 25% of patients suffering from infections.

There was highly significant statistical difference toward the absence of thrombotic events as there were 6.25% of patients suffering from thrombotic events.

There was highly significant statistical difference in the last three months of the study toward the absence of hospitalization as there were less than 12.5% of patients hospitalized.

In Group (C)

100% of the group was males

100% of the group was HCV infected

There was significant statistical difference toward presence of interfering drugs with haematopoiesis as there were 66.5% of patients receiving interfering drugs with haematopoiesis.

There was 100% presence of cardiac diseases.

There was significant statistical difference toward the absence of other infections as there were less than 33% of patients suffering from infections.

There was significant statistical difference toward the absence of weakness and fatigue as there were less than 33% of patients suffering from weakness and fatigue.

Comparison between the three groups

There was significant statistical difference between the three groups according to iron dose per month.

There was highly significant statistical difference between the three groups according to serum iron level, ferritin level and transferrin saturation level.

There was highly significant statistical difference between the three groups according to iron deficiency.

Comparison between all patients of the study throughout the different quartiles

There was statistical difference between all patients in the study throughout the different quartiles according to serum creatinine levels and this difference was statistically significant. In the first quartile (8.41±3.44 mg/dl), in the second quartile (7.08±3.07 mg/dl) and in the third quartile (6.24±2.80 mg/dl).

Table (2): Comparison between the three groups according to gender.

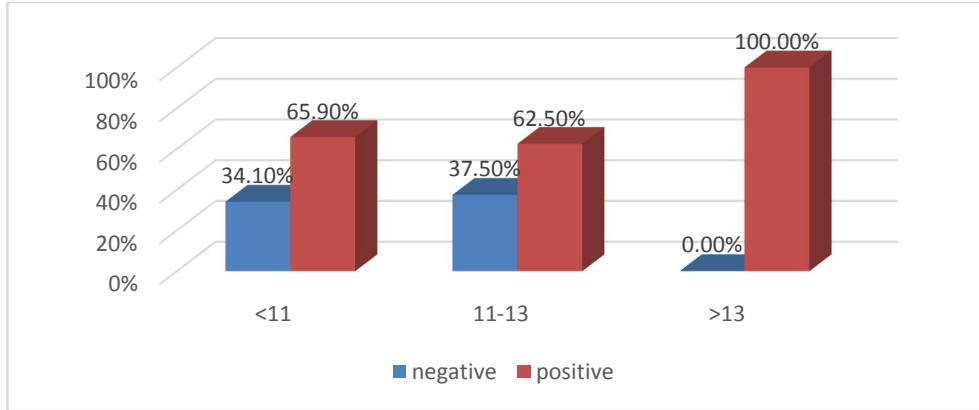|	Female		Male		Total		X^2	p-value
	No.	%	No.	%	No.	%		
Group A	19	46.3%	22	53.7%	41	100.0%	2.6	>0.05
Group B	6	37.5%	10	62.5%	16	100.0%		
Group C	0	0.0%	3	100.0%	3	100.0%		
Total	25	41.7%	35	58.3%	60	100.0%		

Figure (2): Comparison between the three groups according to presence or absence of HCV infection.
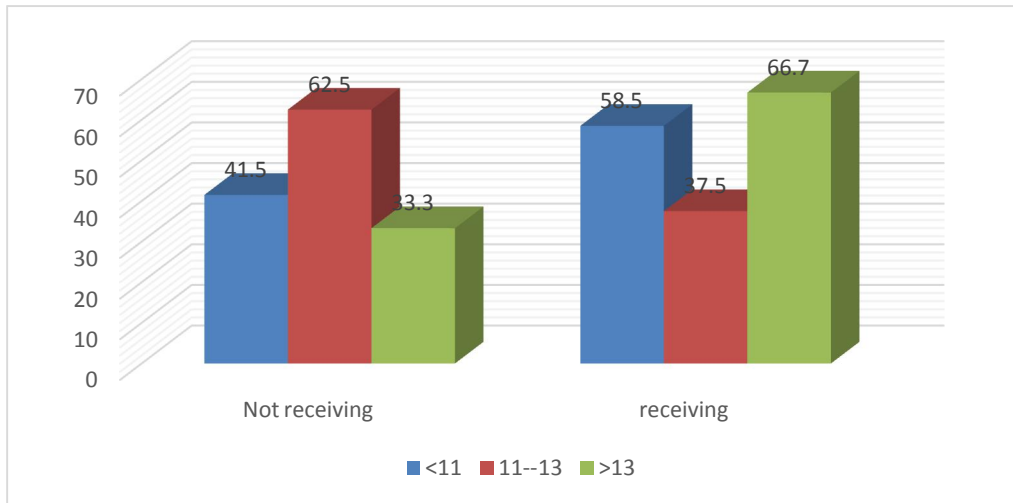

Figure (3): Comparison between the three groups according to presence or absence of interfering drugs with haematopoiesis e.g. ACEI or ARBs.
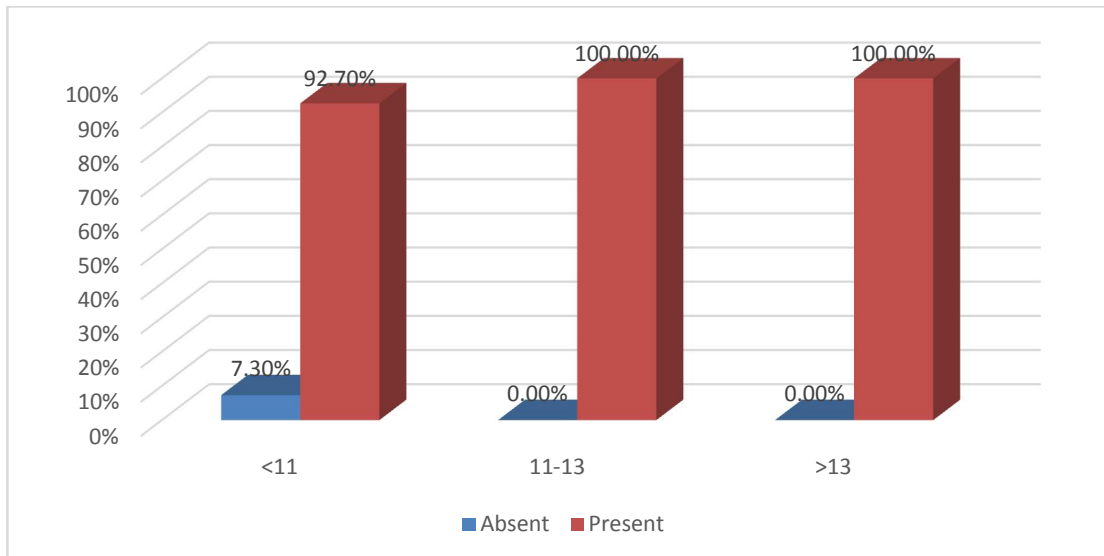

Figure (4): Comparison between the three groups according to presence of cardiac diseases.

Table (3): Comparison between the three groups according to serum iron, ferritin levels and transferrin saturation.

		N	Mean	±Std. Deviation	F	*p*-value
Fe µg/dL	Group A	41	40	20.42		
	Group B	16	70.49	36.90	9.1	<0.001
	Group C	3	79.44	49.96		
Ferritin ng/ml	Group A	41	89.85	58.09		
	Group B	16	206.81	65.08	32.5	<0.001
	Group C	3	323.67	142.39		
Transferrin saturation%	Group A	41	11.9	6.1		
	Group B	16	22.7	8.5	18.7	<0.001
	Group C	3	27.6	9.3		

There was significant statistical difference between the three groups according to duration of haemodialysis

Table (4): Comparison between the three groups according to duration of haemodialysis.

		N	Mean	±Std. Deviation	f	*p*-value
Duration of HD	Group A	41	5.29	4.11		
	Group B	16	3	2.02	4.3	<0.05
	Group C	3	9.33	6.43		

There was statistical difference between all patients in the study throughout the different quartiles according to blood Bicarbonate level levels and this difference was statistically significant in the first quartile (20.12±1.66 meq/l), in the second quartile (21.11±1.95 meq/l) and in the third quartile (21.11±1.42 meq/l).

There was statistical difference between all patients in the study throughout the different quartiles according to serum phosphorus levels and this difference was statistically significant. In the first quartile (5.54±2.63 mg/dl), in the second quartile (6.34±1.71 mg/dl) and in the third quartile (5.1±1.15 mg/dl).

4. Discussion
Group A.

There was comparison inside group A according to presence or absence of iron deficiency and there was highly significant statistical difference toward presence of iron deficiency as there were 95% of patients suffering from iron deficiency and this coincides with Kalantar-Zadeh *et al.* Who said that medications that modulate hemoglobin synthesis such as iron preparations may lead to hemoglobin variability, especially at the initiation, discontinuation, and dosage titration. **(37)** And this coincides also with (Coyne) who said that reasons for hemoglobin variability include iron deficiency. **(20)**

There was comparison inside group A according to presence or absence of HCV infection and there was significant statistical difference toward presence of HCV infection as there were 66% of patients infected with HCV virus and this coincides with (Priyadarshi *et al.*, who said thatadditional reasons for hemoglobin variability include infection or inflammation.**(7)**

There was secondary hyperparathyroidism of the group A and this coincides with **(Rao et al., 1993)** who said that level of parathyroid hormone is one of the contributing factors of haemoglobin variability.

There was comparison inside group A according to presence or absence of other infections e.g., UTI, chest infections, gum and catheter related infections and this difference was statistically insignificant but there were 39% of patients suffering from infectionsand this coincides with Priyadarshi *et al.*, who said that additional reasons for hemoglobin variability include infection or inflammation.**(7)**

There was comparison inside group A according to presence or absence of cardiac diseases and there was highly significant statistical difference toward presence of cardiac diseases as there were 92.5% of patients suffering from cardiac diseases and this coincides with (Habler and Messme) who said that the myocardium may be particularly vulnerable to hemoglobin variability, as it compensates for periods of reduced oxygen delivery with increased output and myocardial cell growth.**(38)** And coincides also with Parfrey *et al.*, who said that Accelerated left ventricular dysfunction and hypertrophy may be associated with hemoglobin variability.**(23)**

There was comparison inside group A according to presence or absence of weakness and fatigue and

this difference was statistically insignificant but there were 51% of patients suffering from weakness and fatigue and this coincides with (Macdougall) who said thathemoglobin variability may be associated with fatigue and weakness. **(18)**

Group B

There was comparison inside group B according to gender and there was significant statistical difference toward male genderas there were 62.5% males and 37.5% females.

There was comparison inside group B according to presence or absence of HCV infection and there was significant statistical difference toward presence of HCV infection as there were 62.5% of patients infected with HCV virus and this coincides with (Priyadarshi *et al.*) who said that additional reasons for hemoglobin variability include infection or inflammation **(7).**

There was secondary hyperparathyroidism of the group and this coincides with **(Rao et al., 1993)** who said that level of parathyroid hormone is one of the contributing factors of haemoglobin variability.

There was comparison inside group B according to presence or absence of cardiac diseases and there was 100% presence of cardiac diseases.

There was comparison inside group B according to presence or absence of autonomic dysfunction and there was 50% presence of autonomic dysfunctionand this coincides with(Romero et al) who said thatthe autonomic nervous system may also be vulnerable to hemoglobin variability, as autonomic dysfunction has been observed in other conditions that predispose patients to fluctuating hemoglobin levels, such as sickle cell anemia In this population, autonomic dysfunction has been implicated as a putative risk factor for sudden death**.(39).**

Group C

There was comparison inside group C according to gender and 100% of the group was males.

There was comparison inside group C according to presence or absence of HCV infection and 100% of the group was HCV infectedand this coincides with (Priyadarshi *et al.*) who said thatadditional reasons for hemoglobin variability include infection or inflammation.**(7)**

There was comparison inside group C according to presence or absence of interfering drugs with haematopoiesis and there was significant statistical difference toward presence of interfering drugs with haematopoiesis as there were 66.5% of patients receiving interfering drugs with haematopoiesis and this coincides with (Ishani *et al.*) who said that cardiovascular medications such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers that are commonly used in this patient population, may lead to hemoglobin variability,

especially at the initiation, discontinuation, and dosage titration. **(40)**

There was comparison inside group C according to presence or absence of cardiac diseases and there was 100% presence of cardiac diseasesand this coincides with (Habler and Messme) who said that the myocardium may be particularly vulnerable to hemoglobin variability, as it compensates for periods of reduced oxygen delivery with increased output and myocardial cell growth. **(38)** And coincides also with (Parfrey *et al.*) who said that Accelerated left ventricular dysfunction and hypertrophy may be associated with hemoglobin variability**.(23)**

The Three Groups

There was statistical difference between the three groups according to duration of haemodialysis and this difference was statistically significant.

There was statistical difference between the three groups according to mean ESA dose per month and this difference was statistically insignificant. Darbiboetin was used subcutaneously every week according to individualized anemia management protocol which may make it out of the factors causing Hb variability here and this coincides with (Besarab *et al.*) who said thatlonger dosing intervals may lead to less variability in hemoglobin levels over time by producing fewer peaks and troughs and thereby requiring fewer dosage adjustments). **(41)**

There was statistical difference between the three groups according to mean iron dose per month and this difference was statistically significant and this coincides with (Kalantar-Zadeh *et al.*). Who said that medications that modulate hemoglobin synthesis such as iron preparations may lead to hemoglobin variability, especially at the initiation, discontinuation, and dosage titration. **(37)**

There was statistical difference between the three groups according to serum iron, ferritin levels and transferrin saturation and this difference was statistically highly significant and this coincides with that (Gotloib *et al.*) who said that additional reasons for hemoglobin variability include alteration in iron stores**.(6)** And iron deficiency(Coyne) **(20).**

There was statistical difference between the three groups according to presence of iron deficiency and this difference was statistically highly significant and this coincides with (Kalantar-Zadeh *et al.*). Who said that medications that modulate hemoglobin synthesis such as iron preparations may lead to hemoglobin variability, especially at the initiation, discontinuation, and dosage titration. (**37).** And this coincides also with (Coyne) who said that reasons for hemoglobin variability include iron deficiency.**(20)**

All Patients throughout Different Quartiles

There was statistical difference between all patients in the study throughout the different quartiles

according to serum creatinine levels, blood bicarbonate levels and serum phosphorus levels and this difference was statistically significant.

4. Conclusion

We have found haemoglobin variability to be a frequent finding in haemodialysis patients treated with rHuEPO. Iron treatment, iron deficiency, secondary hyperparathyroidism, HCV virus infection, interfering drugs with haematopoiesis e.g., ACEI or ARBs and male gender were significant contributing factors for haemoglobin variability. Other infections e.g., UTI, chest infections, gum and catheter related infections might play a role as a contributing factor for haemoglobin variability. Cardiac diseases and were significant consequences of haemoglobin variability. Weakness, fatigue and autonomic dysfunction might be consequences of haemoglobin variability.

Recommendations

We need to do every effort to minimize haemoglobin variability in haemodialysis patients treated with rHuEPO by reducing incidence of contributing factors of haemoglobin variability by: Applying smart anemia management protocol or individualized anemia management protocol with avoidance of drastic changes in ESA dose and better management of iron status, proper management of hyperparathyroidism, prophylaxis and treatment of HCV infection and other infections and Reducing the use of drugs that interfere with haemtopiesis unless indicated. We need a study with large sample size, multicenter design and longer duration to make the results be easily generalizable.

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