Anaemia profile in children with ESRD on haemodialysis: a descriptive study from the Central Teaching Hospital of paediatrics in Baghdad

Layla Qasim H. Ali[1]

ABSTRACT

INTRODUCTION: Anaemia is a recognized complication of chronic kidney disease. Reduction of erythropoietin production is a major cause; however, many other factors may play a role. Accurate search for the cause with proper treatment is essential to have a good response.

OBJECTIVE: To determine the mean haemoglobin level and type of Iron deficiency anaemia in anaemic paediatric patients with ESRD who are on regular haemodialysis and erythropoietin And to find any correlation between the mean Hb level and some demographic, haematologic and biochemical factors.

METHODS: Analytical cross-sectional study conducted at Central Teaching Hospital of Paediatrics in Baghdad/Iraq from the 1st of October 2018 to the 30th of May 2019. Patients involved were those diagnosed as end stage renal disease with anaemia, and on regular haemodialysis and used of erythropoietin injections. Mean Hb level and other haematologic an biochemical indices were measured and tested for any correlation between them.

RESULTS: The mean age was 9.54 ± 3.67 years and the mean of Hb was 7.99 ± 1.48 g/dl. The mean Hb level for males was 7.87 ± 1.63 g/dl, and for female was 8.13 ± 1.30 g/dl. The mean serum iron level was 24.12 ±19.57 micromol/L, the mean serum ferritin was 617.87 ±439.83 ng/ml. The commonest type of anaemia found within the sample was the normochromic normocytic in 23 (46%) and the least was the macrocytic in 2 (4%). Hyperferritinaemia present in 30 patients (60 %), while functional iron deficiency anaemia in 14 patients (7 %) and absolute iron deficiency anaemia in 10 patients (5%).

CONCLUSION: The mean Hb level in our paediatric patients with ESRD who were on haemodialysis was still low despite using erythropoietin injections. The majority of anaemia is an absolute iron deficiency. Dose of the erythropoietin used, s. creatinine, parathyroid hormone, cause of renal failure, type of vascular access, s. ferritin level, and transferrin saturation were found to have a significant correlation with the mean Hb level.

Key words: Anaemia, Haemodialysis, Iron deficiency, Haemoglobin, ESRD.

INTRODUCTION

Anaemia is a frequent complication of chronic kidney diseases (CKD) in children and adults.[1] The major cause of anaemia in patients with chronic kidney disease and end-stage renal disease (ESRD) is the reduction of erythropoietin (EPO) production from the Kidneys.[2] However, among patients on haemodialysis (HD), several other factors may also contribute to anaemia such as increased destruction of red blood cells due to chemical effects of uremic toxins, blood loss (usually occult) due to platelet dysfunction, blood loss due to clotting inside haemodialyzers and sets during HD sessions, haemolysis associated with contamination of dialysate water, and water-soluble losses of folate and vitamin B12 through hemodialyzer membranes affecting red blood cell production.[3]

Anaemia, partially of wholly, may be responsible for many symptoms that have been historically attributed to uraemia. Its manifestations occur due to the effects of decreasing oxygen delivery to tissues and to the consequent compensatory changes of the heart.[4] Cardiovascular problems are the main causes of death among patient on HD, and anaemia imposes an overload on cardiac function, ultimately provoking left ventricular hypertrophy (LVH): a well-recognized marker of morbidity

1 MBCHB, FICMS (Paediatrics), FICMS (Nephrology). Senior Paediatric Nephrologist at the Child Central Teaching Hospital, Baghdad, Iraq. E mail: Layla.aloka@gmail.com, Layla_qasim@yahoo.com.

and mortality.[5] Effect of anaemia and its early treatment on development or regression of LVH is well documented.[6,7]

The cutoff point for diagnosing anaemia in child patient with CKD is vary among the guidelines. K/DOQI anaemia guidelines states that anaemia is present in the paediatric patient with CKD when the observed haemoglobin is less than the fifth percentile of the normal, adjusted for age and sex.[5] While KDIGO Clinical Practice Guideline for anaemia in CKD and the European Best Practice Guidelines (EPBG) report that anaemia is diagnosed if HB is < 12 g/dl in prepubertal age and < 11 g/dl in post puberty about age of 12 years.[8,9] The National Health and Nutrition Examination Survey (NHANES) III Study showed that the fifth percentile for haemoglobin (Hb) may vary by as much as 2.8 gm/dl from younger to older boys and by 2 gm/dl for girls and boys of a similar age group.[10]

The choice of Hb targets for treating patients with renal anaemia is still a matter of great debate.[11,12] The current practice aims at only partial correction of anaemia. The concern is that complete correction of anaemia in CRF patients may increase risk of adverse events such as arterial hypertension and vascular access thrombosis.[12] For patients with cardiovascular disease, an Hb concentration limited to 11-12 g/dl is suggested.[13,14]

The majority of patients who have CKD and anaemia respond to erythropoietin therapy, although a minority show a more sluggish response, which may be due to iron insufficiency, inflammation, or a number of other minor factors.[15,16]

Children with CRF are especially prone to iron deficiency due to increased iron requirements for growth and rHuEPO–facilitated erythropoiesis, which is associated with decreased iron intake and absorption, chronic blood loss, and iron sequestration secondary to chronic inflammatory processes.[17] Approximately 43-90% of paediatric patients undergoing HD experience iron deficiency.[18] Uraemia is a chronic inflammatory state, and increased hepcidin production under this condition may explain iron deficiency in the majority of patients with ESRD.[19]

Iron deficiency may be absolute that needs iron replacement for adequate Epo response[20] or functional when there is a failure to release iron rapidly enough to keep pace with the demands of bone marrow for erythropoiesis, despite adequate or even increased body iron stores. It is confirmed and resolved by administering iron intravenously in a readily available form.[21]

Iron status is evaluated by both the serum ferritin and transferrin saturation levels. While serum ferritin levels reflect total iron body stores, it is also an acute phase reactant and may be elevated in the face of systemic inflammation or malnutrition, compromising its ability to serve as a reliable measure of iron status when its value is elevated. The target serum ferritin level in the absence of inflammation is >100 ng/ml and a low serum ferritin level has been shown to be a specific predictor of iron deficiency in paediatric CKD.[22] Transferrin saturation (TSAT) is a measure of iron immediately available for haemoglobin synthesis. It is typically expressed as a percentage and is calculated as the serum iron divided by total iron binding capacity multiplied by 100%, with a therapeutic target of greater than 20% in patients with CKD.[23]

Other tests of iron status, such as percentage of hypochromic red blood cells and reticulocyte Hb content may be used instead of, or in addition to, TSAT and ferritin levels if available. Measurement of hepcidin levels has not been shown to be clinically useful or superior to more standard iron status tests in patients with CKD.[24] Iron can be supplemented orally or intravenously. Intravenously (IV) administered iron is well tolerated by the majority of patients. However, experience with IV iron therapy in children with CRF is based on a few small paediatric trials.[25]

Although the role of anaemia and its treatment in CKD is widely studied in adults, studies in children are relatively scarce. And in Iraq it is rather very rare. So, this study was designed to describe the mean HB level and types of Iron deficiency anaemia reported in children with CKD who are on regular haemodialysis and using erythropoietin therapy at the Al-Zohoor Haemodialysis centre in the Central Teaching Hospital of Paediatric in Baghdad. And to find the association between mean Hb level and some demographic, Haematologic and biochemical factors.

METHODS

Settings and study design: An analytic cross-sectional study conducted at Al- Zohoor Haemodialysis Centre at the Central Teaching Hospital of Paediatrics in Baghdad from the 1st October 2018 to 30th of May 2019. The Central Teaching Hospital of paediatric is the major paediatric hospital in Al-Karkh District in Baghdad, Iraq. It provides haemodialysis services to children from Baghdad and nearby provinces.

Ethical consideration: The research protocol was approved by institutional ethical committee and agreement was taken from the administration of the Central Teaching Hospital of paediatrics. The study has been conduced in accordance to the terms of the code of ethics in research of Ministry of Health in Iraq.

Definition of the cases, inclusion and exclusion criteria: Paediatric patients of both genders who were diagnosed by senior nephrologists in our centre as having ESRD and on chronic regular haemodialysis and having anaemia were included in this study. Paediatric age was defined in our inclusion criteria as age less than eighteen years. Chronic regular dialysis was defined as three-hour session three times a week for at least three months. All children on HD were receiving regular doses of erythropoietin (rHuEPO) to stimulate erythropoiesis, according to KDOQI guidelines for the treatment of anaemia in haemodialysis subjects.[23] Patient who had incomplete investigations, irregular sessions of dialysis, acute kidney injury needed short term dialysis, or normal Hb level according to K/DOQI guidelines were excluded from the study.

Sampling: We enrolled all registered patients who fulfil the inclusion criteria in were visiting our centre to undergo scheduled haemodialysis sessions during the studies period.

Data collection and procedure: Patients' data were taken from their monthly records. The data have included age, gender, cause of ESRD, Hb level, creatinine level, dose of rHuEPO (unit/kg/week). Serum iron, serum ferritin, total iron binding capacity (TIBC), transferrin saturation (TSAT) were done for all patients at least once during the study period due to shortage of resources. Transferrin saturation percentage was calculated by the formula TSAT (%) = Iron/TIBC x 100.[23] All routine laboratories measurements were performed by using automated methods in the central laboratories of our hospital. Serum intact parathyroid hormone (PTH) was measured by a chemiluminescence immunoassay which were done in a private reference laboratory because they were not available in our hospital at the time of the study.

In accordance with the National Kidney Foundation of America NKF-K/DOQI Guideline, anaemia was diagnosed in our study as a haemoglobin level less than 11g/ld. Ferritin greater than 200 ng/ml was considered as hyperferritinemia. Functional iron deficiency anaemia (IDA) was diagnosed when TSAT % was less than 20% and serum ferritin level was more than 100 ng/ml.23 While absolute iron deficiency anaemia was diagnosed as TSAT % of less than 20% and serum ferritin of less than 200 ng/m.[23]

In our hospital, the reference value of the mean corpuscular volume (MCV) is 80-94 fl, and the mean corpuscular haemoglobin concentration (MCHC) is 32-36 %. Blood films were recommended routinely for all patients for definition of the types of anaemia as normochromic normocytic, hypochromic microcytic, dimorphic and macrocytic anaemia. All blood films were read by an experienced haematologist at our institution. Inflammatory markers like CRP was not included in our study despite its importance on analysis the response to erythropoietin because it was not universally available at the time of the study. Also, we could not include the type, dose and way of administration of Iron in our study because the data were inconsistently available for variety of reasons.

Mean HB levels were correlated with gender, cause of ESRD, type of vascular access whether intravenous (IV) line or Arteriovenous fistula (AVF), and types of anaemia; normocytic, microcytic dysmorphic or macrocytic.

Statistical analysis: The data were analysed by version 21 SPSS software. Analysis of variance (ANOVA) used to find the significance of mean difference of the continuous variables of more than two categories, with a p value of less than or equal to 0.05 regarded as significant. Bivariate correlation between 2 continuous variables, with a p value of less than or equal to 0.05 is regarded as significant. Pearson test

Table 1| Means of some variables of the sample with their correlation to the mean of haemoglobin.

Variable	Mean	± SD	Statistics
Hb (g/dl)	7.99	1.48	
Age (years)	9.54	3.67	Pearson r =+ 0.121 P = 0.403 (NS)
EPO dose U / Kg / Week	114.44	39.34	P= 0.043*
S. Creatinine (mmol)	565.09	214.88	Pearson r = - 0.358 P = 0.011*
PTH (pg / ml)	548.24	411.29	Pearson r = - 0.364 P = 0.009**
MCV (fl)	72.53	17.19	P = 0.079 (NS)
MCHC (g/L)	38.77	15.09	P = 0.160 (NS)
S. Iron (micromole/L)	24.12	19.57	P = 0.927 (NS)
S. Ferritin (ng/ml)	617.87	439.83	P = 0.004**
TSAT%	34.67	18.79	P = 0.023*
TIBC	41.99	20.58	P = 0.121 (NS)

* Statistically significant at alpha level of less than 0.05 (NS) Not significant
** Statistically significant at alpha level of less than 0.01

was used to measure correlation coefficient (r) in order to find any correlation between Hb level of other factors.

RESULTS

The study analyses the records of 50 children who were on regular haemodialysis at the Central Teaching Hospital of Paediatrics in Baghdad/Iraq. **Table 1** shows the mean levels of some numerical variables with their correlation to the mean Hb level. The mean of haemoglobin ± standard deviation (SD) was 7.99±1.48 g/dl. The mean age ± SD of patients in the study was 9.54 ± 3.67 years. The mean (SD) rhEPO dose was 114.44 ±39.34 IU/kg/wk. The mean (SD) serum creatinine was 565.09 ± 214.88 mmol and the mean PTH level was 548.24 ± 411.29 pg/ml. For other variables see **table 1**.

Table 1 also shows that the dose of EPO and s. ferritin have a significant positive correlation, while s. creatinine, level of parathyroid hormone, TSAT % have a negative statistically significant correlation.

Table 2 shows the distribution of our sample among some categorical variables like gender, cause of ESRD, vascular access, and types of anaemia. Then the correlation of these variables with the mean Hb level. Males were 27 patients (54%) with a mean Hb of 7.87 ± 1.63 g/dl and 23 patients (46 %) were females with their mean Hb level of 8.13 ± 1.30 g/dl. This difference of the mean Hb level with gender was statistically non-significant. Tubular causes, CV line as a vascular access, and normochromic normocytic anaemia were the commonest among our sample 21 patients (42 %), 39 (78%), and 23 (46%) respectively. Tubular causes has the least Hb level 7.14 ± 1.26, while the highest mean Hb level was associated with anatomical causes and this was statistically significant correlation (p value 0.001). IV access was associated with lower mean Hb level compared to AVF but this correlation was insignificant for IV line but significant for AVF (p value 0.028).

Hyperferritinaemia found in 30 patients (60%), functional iron deficiency anaemia in 14 patients (7 %), and absolute iron deficiency anaemia in 10 patients (5%). **See table 3**

Table 2| Frequency distribution of the sample and correlation with Hb (total number is 50)

Variable		No (%)	Mean Hb in g/dl	± SD g/dl	Statistics
Gender	Male	27(54)	7.87	1.63	P=0.057(NS)
	Female	23 (46)	8.13	1.30	
Aetiology of renal failure	Anatomical	18 (36)	8.83	1.37	P= 0.001**
	Glomerular	11 (22)	8.24	1.21	
	Tubular	21 (42)	7.14	1.26	
Vascular access	AVF	11 (22)	8.21	1.44	P= 0.028*
	CV line	39 (78)	7.22	1.39	P= 0.051 (NS)
Type of anaemia	Normochromic normocytic	23(46)	8.26	1.58	
	Hypochromic Microcytic	19 (39)	7.80	1.41	
	Dimorphic	6 (12)	7.68	1.56	
	Macrocytic	2 (4)	7.65	0.91	

* Statistically significant at alpha level of less than 0.05 (NS) Not significant
** Statistically significant at alpha level of less than 0.01

Table 3| Frequency distribution according to type of IDA. (Total number is 50 Patients)

Variable		Frequency	%
Hyperferritinemia	Yes	30	60
	No	20	40
Functional IDA	Yes	7	14
	No	43	86
Absolute IDA	Yes	5	10
	No	45	90

DISCUSSION

Our study was conducted on a small sample of patients in which data were collected retrospectively making it rather difficult to include and analyse all necessary parameters for this topic. Studies on anaemia in children with ESRD on haemodialysis are scarce and most of our comparisons were made with data of the adults. These limitations should draw our attention towards conducting many high quality and large sample studies on paediatric age group in the future.

The mean Hb level in our sample was 7.99 g/dl. A higher level of Hb was found in a study from Iran where the mean Hb was 9.6 ± 1.9 g/dl.[26] Frankenfield et al have found that over one third of children ages 12 -18 maintained on chronic haemodialysis have a mean haemoglobin level of less than 11 g/dl.[9] Our lower level of HB may be due to the fact that our sample has included only patients with abnormal HB level. Poor adherence or unavailability of essential medications, in addition to racial and socioeconomic differences could explain this result.

The mean age of patients in our study was 9.54 ± 3.67 years, and it has no significant association with the mean Hb level. A result which is comparable to a study in Brazil.[27] The mean rhEPO dose was 114.44 ± 39.34 IU/kg/wk and it has a statistically significant association with the mean Hb level (p=0.043). This agrees with Azhir et al's results.[28]

Also, a statistically significant negative correlation was seen between mean Hb level and serum creatinine (P value 0.011). The more the serum creatinine level was, the less Hb. Madonre has similarly found that serum creatinine is inversely correlated with Hb levels.[29]

Few studies on the correlation between PTH and Hb have been conducted in children with CKD on regular dialysis, and those that exist have been limited by small sample size as in our study where we found that the mean PTH level was 548.24(± 411.29) pg/ml and it has a statistically significant inverse correlation with the mean level of Hb. Seeherunvong found that the children who required higher doses of erythropoietin stimulating agents (ESAs) due to less Hb level had higher serum iPTH levels.[30] This high level of PTH in our study indicates high turnover bone disease secondary to hyperparathyroidism which in turn impairs erythropoiesis either by higher degree of bone marrow fibrosis or deficiency of calcitriol with increase in red blood cells haemolysis.[31] This is an important issue that must be evaluated addressed in our daily practice because it is a major cause of erythropoietin hyporesponsivness.[28]

Most of the patients were males 27 (54%), and higher Hb level was found in females (8.13 g/dl) compared to that in males (7.87g/dl). There was no significant correlation of the mean Hb level and gender of the study population. A corresponding study in Korea found that the rate in males is 54.2% which is slightly higher than that in females 45.8 %; However, the association between Hb and gender was not significant.[32] Frankenfield et al[9] found that female had higher prevalence of anemia than males. While Mitra Mahdavi-Mazdeh et al found that there was negligible difference of Hb level according to gender and no significant correlation between them.[33]

The most common aetiology of ESRD in the study was the tubular 21 (42 %) followed by anatomical causes 18 (36 %). Patients with tubular causes has less Hb level an association which was statistically significant. (P value 0.001)

This is may be due to the fact that tubular diseases with tubularglomerular feedback injury is a major contributor to loss of renal function.[34] Tubular diseases may be overlooked and not fully understood and managed with the lack of genetic diagnosis in our country. Besides, most of tubular diseases are inherited in nature and expected to be more common in countries like ours where positive consan-

guinity marriages occur frequently. Contrary to ours, Frankenfield has found that anatomical causes were the prevalent cause of ESRD and that the causes have not shown a statistically significant correlation with Hb level.[9]

In our study, 39 patients (78 %) were using the central venous (CV) line, and the other 11 (22 %) were using arteriovenous fistula (AVF). This may be due to technical and surgical challenges in creating AVF especially in children with low body weight, and the families' desire to avoid surgical intervention. The mean Hb level in those dialysed through a catheter access was 7.22 ± 1.39 g/ dl, a level which is slightly lower than the mean in those using AVF which was 8.21 ± 1.44 g/ dl. However, this difference has not shown a statistically significant correlation. Similarly, Frankenfield[9] has found that the HB level is lower in patients using vascular access than using AVF, 9.8 versus 10.6. This can be explained by the association of the anaemic state with inflammatory states with the use of vascular access and the presence of co-morbidities in many HD patients. Patients with inflammatory states would have EPO resistance and, thus, lower Hb levels.[28]

The analysis of red blood cell indices shows that there was no statistically significant correlation for Hb level with the MCV and MCHC. The commonest type of anaemia found within the sample was the normochromic normocytic anemia, and there was no statistically significant correlation between type of anemia and level of Hb. Azhir et al found that MCV levels remained stable during follow-up, and MCHC increase more than (20%) of normal,[28] while Hayder et al, in a study at Hawler Teaching Hospital, has found that MCV stay unchanged while MCHC decrease with the predominance of microcytic anaemia.[35] The unchanged MCV in some patients is explained by the concomitant occurrence of both iron deficiency anaemia and EPO deficiency. The correlation between Hb level and RBC indices and type of anaemia failed to be significant and the cause was unclear.[28] The measurements of MCV and MCHC are hopelessly insensitive in diagnosing iron deficiency in that by the time their values fall below normal, iron deficiency is well advanced.[21]

Regarding the correlation of Iron indices to the mean Hb level, mean serum Iron and TIBC levels did not show a statistically significant correlation, while mean serum ferritin showed a positive correlation and TSAT % showed a negative correlation both with a statistical significance.

Madore et al[29] in their study also have found a significant correlation between Hb level, and S. Ferritin and TSAT %, but he, in contrary to what we found, has shown that S. Iron is significantly correlated to the Hb level tresses on the importance of Iron to optimise the response to erythropoietin.[29] Our different result regarding S. Iron may be due to the small sample that we have in our study or the difference in the target because his target was adults.

Hyperferritinemia was found in 30 patients of our sample (60%). Biniaz from Iran has found it 80.4 % of his sample.36 High S. Ferritin despite low levels of Iron indices may be due to impaired iron metabolism or being a response to nonspecific systemic inflammatory process rather than a real increase in iron store.[29] Due to lack of data, we could not identify the presence of chronic co-morbid inflammatory diseases that their presence could adversely influence the response to erythropoietin. Thus, the serum ferritin in this context may not only be unhelpful, but may even be misleading.

On the other hand, we found functional iron deficiency anaemia in 7 patients (14 %) and absolute iron deficiency anaemia in 5 patients only (10%). Relative iron deficiency anaemia in this study could be a manifestation of poor nutrition, poor and irregular iron supplementation, patient's intolerance to iron and lack of full investigations to cover causes of iron deficiency. A study in Spain on haemodialysis patients finds that 39 % of them had an absolute iron deficiency and 21 % had a functional iron deficiency.[26] A study in Iran has reported the prevalence of absolute and functional iron deficiency anaemia were 11 and 24 % respectively.[37]

CONCLUSION

The mean Hb level in our paediatric patients with ESRD who were on haemodialysis

was still low despite using erythropoietin injections. The majority of anaemia is an absolute iron deficiency. Dose of the erythropoietin used, s. creatinine level, level of PTH, cause of renal failure, type of vascular access, s. ferritin level, and TSAT % were found to have a significant correlation with the mean Hb level. .

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Abbreviations list: Analysis of variance (**ANOVA**), Arteriovenous fistula (**AVF**), Central venous (**CV**), Chronic kidney diseases (**CKD**), Correlation coefficient (**r**), End-stage renal disease (**ESRD**), Erythropoietin (**EPO**), Erythropoietin stimulating agents (**ESAs**), Femtolitre (**fl**), Gram per decilitre (**gm/dl**), Haemodialysis (**HD**), Haemoglobin (**Hb**), Intravenously (**IV**), Iron deficiency anaemia (**IDA**), Left ventricular hypertrophy (**LVH**), Mean corpuscular haemoglobin concentration (**MCHC**), Mean corpuscular volume (**MCV**), Millimole (**mmol**), Nanogram per millilitre (**ng/ml**), Non-significant (**NS**), parathyroid hormone (**PTH**), Picogram per millilitre (**pg/ml**), Red blood cells (**RBC**), Recombinant human erythropoietin (**rHuEPO**), Serum (**s**), Standard deviation (**SD**), Statistical package for social sciences (**SPSS**), Total Iron Binding capacity (**TIBC**), Transferrin saturation (**TSAT**).

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