

Original Article

Erythropoietic Response to Anaemia of Dialysis Naïve Patients with Chronic Kidney Disease in Zaria, North-West Nigeria

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ABSTRACT

Introduction: Chronic kidney disease (CKD) is a global health problem with an increasing prevalence worldwide. Anemia is one of its consistent and severe hematological complications although its mechanism is not fully elucidated. The primary defect could manifest as serum erythropoietin (sEPO) deficiency or EPO resistance. We set out to determine the erythropoietic response to anemia of patients with CKD and its relationship with their iron status in a cross-sectional descriptive study of 91 patients in various stages of CKD.

Materials and Methods: Soluble transferrin receptor (sTfR), sEpo, and serum ferritin levels were determined using ELISA method (Diagnostic Automation Inc and WKEA med supplies corp.). Data generated were analyzed using Epi Info version 3.5.3 and level of statistical significance was set at ≤ 0.05 .

Results: Participants comprised of 50 females (54.9%) and 41 (45.1%) males with an overall mean age of 47 ± 15 years. The major causes of CKD were hypertension (HTN) (50.54%), diabetes mellitus (DM) (6.59%), and HTN + DM (19.78%). The mean hemoglobin (Hb) concentration of the participants was 10.97 ± 2.28 g/dl; the red cell indices were within normal ranges except for Red cell distribution width-Coefficient of variation (%) which was elevated (16.29%). The mean serum ferritin, sTfR, and sEpo were 70.58 ± 46.44 ng/ml (interquartile range [IQR] 82.00), 22.9 ± 49.7 ng/ml (IQR 15.00), and 12.49 ± 33.47 IU/L (IQR 6.00), respectively, with a high variance. Serum ferritin and sTfR are consistently low across the stages of CKD (range between 54.54 ng/ml and 88.64 ng/ml), but sEPO for stage 3 and 4 showed a 2-fold increase when compared to normal level at Hb 10.97 g/dl (29.54 IU/L and 38.83 IU/L, respectively). Correlation between sTfR and sEpo ($r^2 = 0.96$, $P = 0.001$), while between sEpo and serum ferritin ($r^2 = 0.02$, $P = 0.185$), and between Hb and stage of CKD undulating ($r^2 = 0.41$, $P = 0.001$).

Conclusion: In contrast to some existing literature, this study has demonstrated that EPO resistance and iron deficiency contributes to anemia in CKD and serum ferritin can be used to assess the iron level of dialysis naïve CKD patients at every stage of the disease.

KEYWORDS: Chronic kidney disease, erythropoietin, serum erythropoietin, serum ferritin, serum transferrin receptor

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INTRODUCTION

Erythropoietin (EPO) deficiency has long been attributed to the incapacity of the kidney to make EPO as renal failure progresses.^[1] Normal response to anemia is an exponential increase in EPO production to about a 100-fold through a process that up-regulates

transcription. In patients with chronic kidney disease (CKD) this normal response is disturbed with

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the level of EPO reducing and even receptor response is blighted.^[2,3] These lead to hyporegenerative anemia due to EPO deficiency^[3] even though EPO level is difficult to interpret in the context of renal failure.^[3]

A number of studies have shown a correlation between EPO and Hb level in the setting of worsening renal failure. Anemia is observed when creatinine clearance falls below 60 ml/min/1.73 m² and inadequate synthesis of EPO is found when creatinine clearance falls below 40 ml/min.^[2-5] In some situations, there is a “relative EPO deficiency” a situation whereby either there is a low set point for EPO production in relation to tissue oxygenation or the production capacity of the kidney is reduced due to tissue damage by the underlying disease.^[3] In view of this, the measured EPO level appears within the normal range but not commensurate with the level of anemia.

The CKD patient is in a state of chronic inflammation. CKD and inflammatory milieu coexist. This inflammatory state results in release and dysregulation of cytokines, such as interleukins-I and TNF which blunts the effect of EPO on the bone marrow.^[6] Although the anemia in CKD has some cytokine dysregulation, it is not the same as the anemia of chronic disorder which is basically inflammation related, suppressing endogenous EPO production and erythropoiesis. There are reports by the National Kidney Foundation Kidney disease outcome quality initiative (NKF-KDOQI) of situations where stable cytokine level is seen in some stable dialysis patients.^[7]

Anemia (renal anemia) is one of the consistent and severe hematological complications in this population group.^[8,9] It is found in nearly all patients with severe renal failure and its severity increases along with the severity of disease.^[4,10] It is caused by relative EPO deficiency, and it is to be suspected in a patient with CKD who presents with normochromic anemia after exclusion of other causes such as hemodialysis, deficiency of iron,^[9] vitamin B12 or folate or malignant hematological disease.^[2]

With worsening renal condition, there is increasing uremia complicated by platelet dysfunction and subsequent or associated increasing risk of gastrointestinal bleeding, shortened erythrocyte survival and hemolysis due to accumulation of uremic toxins.^[10,11]

There are several mechanisms of anemia in CKD which makes having a specific laboratory test for diagnosing renal anemia lacking.^[2] There has not been uniformity in the indicators used to assess renal anemia, especially in developing nations like Nigeria.^[10] In view of this, we set out to determine the level of serum erythropoietin (sEpo), its relationship with soluble transferrin receptor (sTfR) and serum ferritin levels among patients with CKD in

Zaria as well as the hematologic indices at various stages of CKD among patients attending nephrology clinic in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, to find their role in diagnosis and management of renal anemia.

MATERIALS AND METHODS

Following ethical clearance by the Health and Research Ethics Committee of ABUTH Zaria, a total of 91 consenting adult patients with established CKD attending the Nephrology clinic of ABUTH Zaria were recruited using a convenience sampling technique. In this study, two indicators (sEpo and soluble transferrin receptor) that are fundamental for the purpose of evaluating the pattern and type of anemia were considered for calculating the sample size. Two previous studies by Quick *et al.* in Zurich, Switzerland,^[12] and Gupta *et al.* in India,^[13] reported the mean level of sEPO to be 11.4 ± 4.6 U/L and sTfR to be 3.23 ± 2.07 , respectively. The choice of these studies was based on the fact that one nation is a developing nation with related demography and disease pattern as the country of this study while the other is an advanced nation. This will give a figure that will be cross-sectional. Furthermore, the deviation 4.6 is larger than 2.07, and this will give a larger sample size and a better choice for this calculation.^[14]

A structured, interviewer-administered questionnaire was used. Samples for complete blood count, reticulocyte count, erythrocyte sedimentation rates, serum ferritin, sEPO, and sTfR levels were collected.

Complete blood count was done using the BioMaxima BM HEM 3 hematology analyzer^[15] while reticulocyte count was done by incubating equal volumes of blood and new methylene blue (double dilution for anemic samples) at 37°C for 20 min before counting at x1000 magnification with light microscopy. Erythrocyte sedimentation rate was by Westergren method using Sedivette® tubes as recommended by the International Council for Standardization of Haematology (ICSH).^[16]

Determination of serum ferritin (Diagnostic automation Inc^[17]), sTfR receptor, and sEPO (WKEA Med Supplies Corp^[17,18]) was carried out by double-antibody sandwich ELISA techniques according to manufacturers' instructions. Staging of renal failure was done using Cockcroft and Gault formula.

All data were collated and analyzed using Epi Info Version 3.5.3. (CDC, Atlanta, Georgia). $P \leq 0.05$ was taken to be statistically significant.

RESULTS

Participants comprised of 50 females (54.9%)

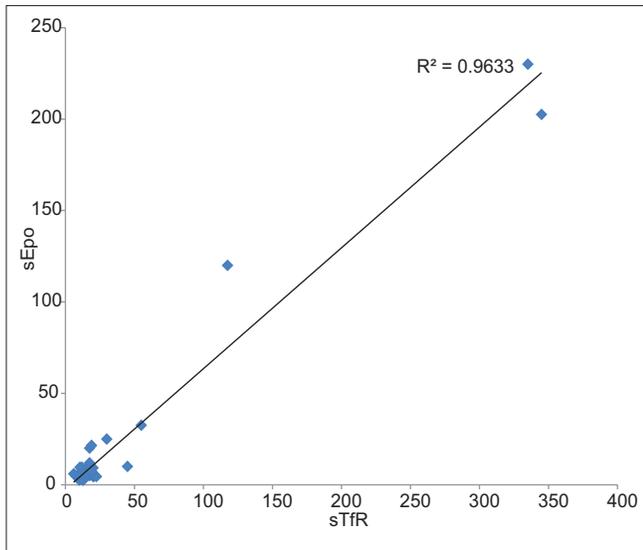


Figure 1: Relationship between serum erythropoietin and soluble transferrin receptor, shown as scatter chart ($r^2 = 0.96$, $P = 0.001$)

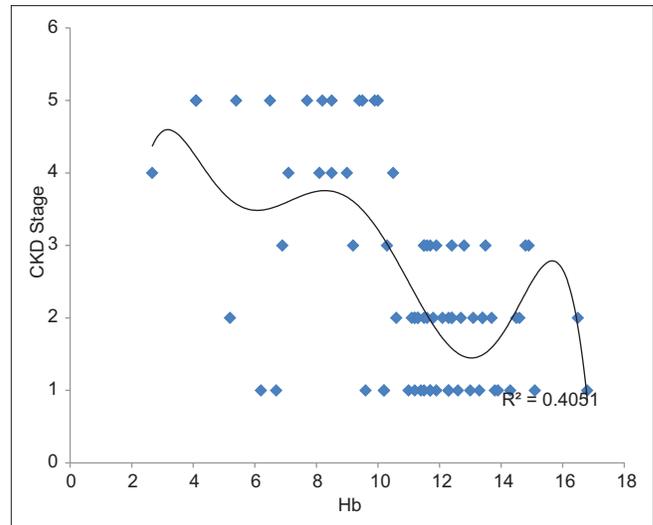


Figure 2: Polynomial scatter plot of the relationship between haemoglobin and Stage of chronic kidney disease in the Study Participants ($r^2 = 0.41$, $P = 0.001$)

Table 1: Summary of group analysis of mean haematological parameters of the participants by stage of chronic kidney disease

| Variable | Stage 1 (n=22) | Stage 2 (n=31) | Stage 3 (n=19) | Stage 4 (n=7) | Stage 5 (n=12) | P | | |
|-------------------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------|------------|----------------|
| | | | | | | ANOVA | Bartlett's | Kruskal-Wallis |
| ESR (mm in 1 st h) | 28.64 | 28.64 | 46.69 | 56 | 72.27 | 0.027 | 0.019 | 0.012 |
| Platelet ($\times 10^9/L$) | 280.55 | 247.05 | 236.31 | 306.83 | 275.55 | 0.491 | 0.119 | 0.869 |
| TWBC ($\times 10^9/L$) | 6.53 | 7.28 | 5.03 | 7.72 | 7.35 | 0.130 | 0.001 | 0.018 |
| Retic count (%) | 3.05 | 2.76 | 3.13 | 4.37 | 3.96 | 0.909 | 0.394 | 0.397 |
| Serum ferritin (ng/ml) | 81.16 | 53.48 | 54.54 | 64.83 | 88.64 | 0.068 | 0.001 | 0.108 |
| sTfR (ng/ml) | 15.64 | 14.43 | 42.79 | 67.42 | 13.91 | 0.128 | 0.001 | 0.924 |
| sEpo (IU/L) | 8.59 | 6.26 | 29.54 | 38.83 | 5.32 | 0.114 | 0.001 | 0.511 |
| Age at diagnosis (year) | 37.96 | 48.64 | 43.93 | 43.83 | 52.46 | 0.054 | 0.111 | 0.059 |

CKD=Chronic kidney disease; ESR=Erythrocyte sedimentation rate; TWBC=Total white blood cell; sEPO=Serum erythropoietin; sTfR=Soluble transferrin receptor; ANOVA=Analysis of variance

Table 2: Causes of chronic kidney disease and other associated clinical conditions in the participants

| Cause of CKD | Male | Female | Total frequency (%) |
|-----------------------------|------|--------|---------------------|
| DM | 3 | 3 | 6 (6.59) |
| HTN | 13 | 33 | 46 (50.54) |
| CGN | 1 | 0 | 1 (1.1) |
| LVH | 1 | 1 | 2 (2.2) |
| Nephrotic syndrome | 2 | 0 | 2 (2.2) |
| Sickle cell nephropathy | 2 | 0 | 2 (2.2) |
| HTN/DM | 10 | 8 | 18 (19.78) |
| HTN/LVH | 3 | 2 | 5 (5.49) |
| HTN/CGN | 2 | 1 | 3 (3.3) |
| HTN/HIV (HIVAN) | 1 | 1 | 2 (2.2) |
| HTN/sickle cell nephropathy | 1 | 3 | 4 (4.4) |
| Total | 39 | 52 | 91 (100) |

HIV=Human Immunodeficiency syndrome; DM=Diabetes mellitus; HTN=Hypertension; CGN=Chronic Glomerulonephritis; LVH=Left Ventricular Hypertrophy; HIVAN=Associated Nephropathy; CKD=Chronic kidney disease

and 41 (45.1%) males with an overall mean age of 47 ± 15 years. The major causes of CKD were hypertension (HTN) (50.54%), diabetes mellitus (DM) (6.59%), and HTN + DM (19.78%) [Table 1]. The mean Hb concentration of the participants was 10.97 ± 2.28 g/dl, the red cell indices were within normal ranges except for Red cell distribution width-Coefficient of variation (RDW-CV) (%) which was elevated (16.29%). Mean serum ferritin, sTfR, and sEpo were 70.58 ± 46.44 ng/ml (interquartile range [IQR] 82.00), 22.9 ± 49.7 ng/ml (IQR 15.00), and 12.49 ± 33.47 IU/L (IQR 6.00), respectively, with a high variance [Table 2]. Serum ferritin and sTfR are consistently low across the stages of CKD (range between 54.54 ng/ml and 88.64 ng/ml), but sEPO for stage 3 and 4 showed a 2-fold increase when compared to normal level at Hb 10.97 g/dl (29.54 IU/L and 38.83 IU/L, respectively) [Table 1]. Correlation

Table 3: Haematologic parameters of the participants

| Haematological variables | Mean±2 SD | IQR |
|--------------------------------------|--------------|--------|
| Hb (g/dl) | 10.97±2.81 | 12.70 |
| TWBC (×10 ⁹ /L) | 6.63±2.79 | 7.70 |
| Platelet count (×10 ⁹ /L) | 256.47±98.71 | 312.00 |
| MCV (fl) | 83.31±8.74 | 88.90 |
| MCH (pg) | 28.34±3.12 | 30.15 |
| MCHC (g/dl) | 34.11±4.93 | 35.20 |
| RDW-CV (%) | 16.29±3.69 | 17.80 |
| Retic count (%), RPI 2.33 | 3.18±4.07 | 2.97 |
| ESR (mm in 1 st h) | 40.11±37.76 | 50.00 |
| Serum ferritin (µg/ml) | 70.58±46.44 | 82.00 |
| sTfR (ng/ml) | 22.90±49.70 | 15.00 |
| sEpo (IU/L) | 12.49±33.47 | 6.00 |

Hb=Haemoglobin; TWBC=Total white blood cell; MCV=Mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MCHC=Mean corpuscular haemoglobin concentration; RDW-CV=Red cell distribution width-coefficient of variation; ESR=Erythrocyte sedimentation rate; sTfR=Soluble transferrin receptor; Sepo=Serum erythropoietin; RPI=Reticulocyte production index; SD=Standard deviation; IQR=Interquartile range

between sTfR and sEpo ($r^2 = 0.96$, $P = 0.001$) [Figure 1], while between sEpo and serum ferritin ($r^2 = 0.02$, $P = 0.185$), and between Hb and stage of CKD undulating ($r^2 = 0.41$, $P = 0.001$) [Figure 2].

DISCUSSION

This study is aimed at improving management of CKD in Nigeria through assessing specifically the EPO activity and erythroblast response as it relates to the hematologic parameters and the type and pattern of anemia in the various stages of this disease. The study revealed female predominance (54.9%) in presenting to the hospital with CKD, this is in contrast to the findings by several researchers; Shittu *et al.* in Ilorin North Central Nigeria, Ijoma *et al.* in South East Nigeria, Abdu *et al.* in Kano North West Nigeria^[10,19,20] and also Iseki in a community survey in Okinawa Japan Asia.^[21] This may be attributed to the major risk factor for developing CKD; DM; and HTN which are noted to be higher among the female participants [Table 2]. In addition, the health seeking behavior of females in the environment is better than in males where females present early with mild symptoms while the males tend to present only when symptoms are severe.^[22]

The mean Hb level observed in this study (10.97 ± 2.81 g/dl), is however, below the cutoff level for females and males as given by NKF-KDOQI (12.0 g/dl females and 13.5 g/dl males) and the European Best Practices Guidelines (11.5 g/dl females and 13.5 g/dl males)^[4,6] yet the Hb level is higher than what was reported by other researchers.^[4,10,20,23] The predominantly normocytic and normochromic anemia [Table 3] consistent with several reports.^[10,20,24,25] This may be explained by the phenomenon referred to as “renal anemia” which is characterized by EPO

resistance and deficiency, leading to a blighted erythropoietic response.^[10,24,26,27] This leads to functional iron deficiency,^[28] and hence, the predominant picture of normocytic and normochromic red blood cells, otherwise known as the anemia of EPO reduction or resistance.^[29,30]

In spite of the normal red cell indices and reticulocyte percentage (Reticulocyte Production Index [RPI] 2.33), this study revealed that the CKD patients have low levels of serum ferritin, sTfR, and an apparently normal sEPO level [Table 3]. Studies have shown that the cause of “renal anemia” is reduction in iron,^[28] relative EPO deficiency and/or EPO resistance,^[2,3,6,27,31] accounting for poor erythropoietic activity with an apparently normal reticulocyte receptor response. Although Shittu *et al.* in Ilorin North Central Nigeria,^[20] and Talwar *et al.* in New Delhi India^[32] reported a much higher reticulocyte count, no reason was given for the findings in both studies. The clinical utility of RPI in management of CKD is still not clear, but as noted by NKF, it is a semi-quantitative measure of erythropoietic activity, and in CKD, there is early release and shortening of the fraction time of reticulocytes production accounting for the normal reticulocyte count which does not increase erythroid production.^[7] It is generally difficult to interpret sEPO level in CKD,^[3] although a “relative erythropoietin” deficiency and resistance are well documented in CKD.^[2,3,5,26,27] In addition, the low set point for EPO production by the kidneys blunts the normal erythroblast receptor response,^[5,27] thus manifesting with impaired EPO response.^[27] Other reasons for the low EPO production and receptor response is the relationship existing between CKD and inflammation, which results in cytokine release and dysregulation blunting the effect of EPO on the marrow.^[6]

Soluble transferrin receptor in this study (22.90 ± 49.70 ng/ml) is low, compared with

the accepted values in CKD.^[13,33-35] This reflects the amount of iron supplied to the blood and the number of precursors, thus, the erythropoietic activity of the erythroblast.^[28] The reason for this low level could be attributed to EPO resistance. Although the study revealed a normal sEPO level (mean sEPO level 12.49 ± 33.47 IU/L is above 25th percentile),^[2] yet the expected commensurate increased soluble transferrin receptors could not occur. This is in keeping with the explanation that “renal anemia” is related to inadequate endogenous EPO response to anemia in CKD.^[2,26,27,31] Serum ferritin levels remain a reliable marker of bone marrow iron stores and one of the most reliable initial diagnostic tests for iron deficiency, with a cutoff for absolute iron deficiency in CKD set at 100 ng/ml.^[34] In this study, serum ferritin level was 70.58 ± 46.44 ng/ml. This could be explained by the fact that ferritin being an acute phase reactant,^[36] its production may have been affected by the inflammatory milieu and the low soluble transferrin receptor response coexisting in the CKD patients studied. For reliable interpretation of serum ferritin, soluble transferrin receptor, which is a negative acute phase reactant, is advised to be assayed.^[13,28,34] It has been shown that in CKD, there is an overall reduction in iron,^[28] and iron deficiency is established when the mean serum ferritin level falls below 100 ng/ml,^[28,34] and red cell distribution width is above 14.50%.^[37,38] The result of this study show mean serum ferritin of 70.57 ± 46.43 ng/ml and RDW-CV of $16.29\% \pm 3.70\%$ supporting the fact of an existing iron deficient state. RDW percentage has been noted to be an early marker of iron deficiency.^[39]

In this study, the relationship between Hb and sEPO is inverse although with a very weak correlation ($r^2 = 0.02$). A negative (inverse) correlation has been observed by many researchers.^[3,5,20,24,40] This is what is seen when there is inadequacy of EPO production by the kidneys in CKD^[26] as well as iron deficiency. The inverse relationship existing between Hb and sEPO is abolished when creatinine clearance gets above 30–40 ml/min.^[3,27]

Associated findings in the study revealed statistically and clinically significant variability of Hb and ESR in the study participants. Variability of such is noted in anemia of CKD,^[41] which cuts across the stages of CKD,^[42,43] signifying differences of mean in each stage of the disease. This positive correlation is consistent with most studies^[3,10,19,24,27] and in agreement with Bathon *et al.*^[40] and Arun *et al.*^[44] However, this does not show any significant impact to the progression of the disease except when inflammation is considered.^[45]

A weak negative correlation was observed in the study between Hb and ESR. The results are in agreement

with other reports,^[5,27,40,41] but in contrast with the Ilorin report^[20] which showed otherwise. The significance of this is the fact that the relationship is an inverse one, whereby as the anemia worsens across the CKD stages the ESR increases, the import of which will give need to monitoring disease progression.

CONCLUSION

Although literature have shown reduction in EPO production as the cause of anemia in CKD, this study revealed that patients with CKD can have apparently adequate EPO and be iron deficient with low erythropoietic activity as noted by the low transferrin receptor response to EPO and poor erythroid activity manifesting as “renal anemia.” The study demonstrated that EPO resistance and iron deficiency contributes to anemia in CKD and serum ferritin can be used to assess iron level of dialysis naïve CKD patients at every stage of the disease. The interaction between EPO with sTfR and Hb is strong to moderate with significant variability in Hb as the disease progresses. Other relationship and associations where they exist are weak.

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Conflicts of interest

There are no conflicts of interest.

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