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Assessment of myeloid response and iron status among Sudanese pediatric ESKD on hemodialysis through reticulocyte parameters and β-globin mRNA expression

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Abstract

Background Sudanese children with End-Stage Kidney Disease (ESKD) often show limited improvement in hemoglobin levels despite treatment with recombinant human erythropoietin (rHuEPO). This study aims to assess the response to rHuEPO therapy by analyzing β-globin mRNA expression and reticulocyte parameters. Additionally, it classifies anemia among Sudanese pediatric patients based on iron status, considering age and gender as biological markers for evaluating treatment response.

Methods A prospective observational cohort study was conducted from January 2019 to February 2020 in Khartoum, Sudan, involving 45 anemic children aged 2 to 15 years diagnosed with ESKD. The treatment protocol included rHuEPO injections and maintenance hemodialysis. Laboratory assessments consisted of complete blood count (CBC), absolute reticulocyte count, ferritin, and transferrin measurements. β-globin mRNA expression was quantified using reverse transcription polymerase chain reaction (RT-PCR), and reticulocyte parameters, including Reticulocyte Hemoglobin Content (CHr), percentage of hypochromic reticulocytes (HYPO%), and Immature Reticulocyte Fraction (IRF), were measured via flow cytometry.

Results Significant variations in hemoglobin levels were observed across different age groups ($p=0.011$). Gender analysis revealed a significant association with IRF, showing a lower IRF in male patients ($p=0.017$). However, there were no significant differences in hemoglobin levels between genders ($p=0.999$). β-globin mRNA expression showed considerable variability, with a strong positive correlation with hemoglobin levels ($r=0.875$, $p<0.0001$).

Conclusion Age and gender significantly influence treatment responses in children with ESKD, highlighting the need to consider growth physiology in anemia management. This study underscores the variability in β-globin mRNA expression and its association with Flow Cytometry parameters, demonstrating their effectiveness in evaluating iron status and guiding rHuEPO dosage.

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Keywords β-globin mRNA expression, End-stage kidney disease (ESKD), Ferritin, Flow cytometry, Hypochromic cells (HYPO%), Immature reticulocyte fraction (IRF), Reticulocyte hemoglobin content (CHr), Sudanese children

Introduction

Chronic Kidney Disease (CKD) in Sudanese pediatric populations presents with varying degrees of anemia, which closely correlates with the progression of kidney damage, up to and including End-Stage Kidney Disease (ESKD). Research by Hussein R et al. identifies glomerulonephritis as the leading cause of ESKD in Sudanese individuals under 18, accounting for 36.8% of cases. Other causes include unknown etiologies (25.3%) and congenital anomalies of the kidney and urinary tract (CAKUT, 14.9%), highlighting a significant gap in epidemiological data regarding pediatric kidney disease in the region [1].

Sudan faces considerable socio-economic challenges that exacerbate these health issues. Despite its abundant natural resources, the country remains below the poverty threshold, struggling with a humanitarian crisis intensified by economic decline and a healthcare system that is unable to meet the growing demands [2]. The ongoing state of emergency, as reported by the United Nations International Children's Emergency Fund (UNICEF), emphasizes the critical need for targeted child survival and development strategies as part of the Country Programme of Cooperation 2018–2021 [3].

Anemia in children with CKD has a multifactorial etiology, primarily resulting from erythropoietin deficiencies and disruptions in iron metabolism, including both absolute iron deficiency (AID) and functional iron deficiency (FID). AID is marked by depleted iron stores, while FID occurs when, despite adequate iron reserves, there is insufficient iron available for erythropoiesis. Additional factors such as inflammation, secondary hyperparathyroidism, and uremic toxins further complicate the condition. The response to Recombinant Human Erythropoietin (rHuEPO) treatment is variable, often reflecting the complexity of anemia in this context, where hypo-responsiveness is a frequent challenge [4].

Evaluating the early myeloid response to treatment is crucial for the effective management of anemia in Sudanese children with ESKD, as it has a direct impact on their growth and quality of life.

The primary objective of this study is to assess the myeloid expression of β-globin mRNA in blood reticulocytes as a marker of response to rHuEPO therapy in Sudanese children with ESKD. The Immature Reticulocyte Fraction (IRF) is utilized as a key indicator of erythropoietic activity, with values ≤ 0.23 indicating an inadequate bone marrow response to anemia, and values > 0.23 prompting further diagnostic investigation into the underlying causes of anemia [5]. Additionally, this study evaluates iron status through the measurement of Reticulocyte Hemoglobin Content (CHr) and the percentage of hypochromic red cells (%Hypo), potentially providing more nuanced insights compared to traditional markers like ferritin and transferrin saturation. By examining the relationships between the response to rHuEPO therapy, reticulocyte β-globin mRNA expression, IRF, CHr, and %Hypo, this study aims to better characterize the type of anemia affecting this population and to refine treatment strategies based on age- and gender-specific physiological responses.

Materials and methodology

Subject population

The study initially enrolled 51 anemic Sudanese children, of whom six withdrew for various reasons. The remaining 45 participants (21 males and 24 females, aged ≤ 15 years) completed six months of treatment and follow-up. All patients received Epoetin beta (Recormon® 4000 IU/ml) and intravenous iron sucrose (100 mg/ml) alongside maintenance hemodialysis. The severity of anemia was classified according to World Health Organization (WHO) hemoglobin concentration norms for age [6]. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend monitoring iron status every three months and evaluating hemoglobin (Hb) levels monthly, targeting an Hb level of 12 g/dl [7].

Causes of withdrawal

Among the six withdrawals, four were males and two females. Reasons for withdrawal included two males being repatriated, one male succumbing to complications from dilated cardiomyopathy, and another being prepared for a kidney transplant. One female achieved stable hemoglobin levels before transplant preparation, while another, suffering from severe hepatitis and requiring recurrent transfusions, passed away.

Ethics

Ethical approval was obtained from the Research Ethics Committee (REC) of Karary University (IRB KU-2018-13), the Ministry of Health-Khartoum State, and Soba University Hospital. Permissions were also secured from the administrations of the participating hospitals, and informed consent was acquired from the guardians of all participants. Strict protocols for confidentiality and anonymity were maintained throughout the study.

Study design

This prospective observational cross-sectional study was conducted from January 2019 to February 2020 in three teaching hospitals in Khartoum State: Soba University Hospital, Gaafar Ibn Ouf Children's Hospital, and Omdurman Children Hospital 'Mohammed Elamin Hamid'. A control group was not included due to the ethical concerns and difficulties associated with blood sampling from healthy children. The absence of a control group is acknowledged, and results are presented as preliminary findings.

Inclusion criteria

Participants included were anemic Sudanese children aged ≤ 15 years with hemoglobin levels ≤ 11.0 g/dl, diagnosed with end-stage kidney disease and undergoing regular hemodialysis. These children were scheduled for routine administration of rHuEPO and intravenous iron.

Exclusion criteria

Children with hemoglobin levels ≥ 12.0 g/dl, those with congenital hemoglobinopathies, CKD associated with cancer, or those receiving treatments that interfere with iron absorption (such as ACE inhibitors and proton pump inhibitors) were excluded.

Sample size justification

A power analysis was conducted to ensure the sample size was adequate. Based on preliminary data, the study aimed for 80% power with a significance level of 0.05, allowing for the detection of statistically significant differences and correlations. The analysis confirmed that the current sample size was sufficient for reliable findings. Although a larger sample would improve generalizability, the rarity of pediatric ESKD in our setting and ethical considerations for recruiting vulnerable populations limited the ability to increase the sample size. Nevertheless, the cohort represents a comprehensive sample for robust analysis and meaningful conclusions.

Patient assessment and treatment protocol

Renal failure was confirmed through Renal Function Tests (RFT) and abdominal ultrasound, with a Glomerular Filtration Rate (GFR) of < 20–30 ml/min. Treatment involved Epoetin beta (Recormon® 4000 IU/ml) and intravenous iron sucrose (100 mg/ml), with dosage adjustments based on FDA guidelines and patient response. The initial dose was 150 units/kg/week, typically administered in one to three doses per week, depending on availability. According to FDA standards, the recommended intravenous iron sucrose dose is 0.5 mg/kg for children ≥ 2 years on hemodialysis and receiving erythropoietin therapy, not exceeding 100 mg per dose every two weeks [8, 9]. Hemodialysis was conducted three times weekly using the GAMBRO system, supervised by pediatric nephrologists, following KDIGO anemia guidelines for CKD [10].

Data collection

A total-coverage sampling strategy was used to recruit 51 children diagnosed with ESKD from several hospitals in Khartoum State. Following the withdrawal of six participants, 45 children completed all study requirements. The sample size was calculated to achieve 90% statistical power at a 5% significance level, with 48 participants required for robust findings.

Statistical analysis

Data analysis was conducted using SPSS version 16, employing Pearson correlation coefficients to identify associations between reticulocyte hemoglobin content (CHr), percentage of hypo chromic red blood cells (%Hypo), and other hematological indices. Regression analysis using R package software (version 4.1.3) was performed to express relationships between quantitative variables, such as manual reticulocyte count, flow cytometry reticulocyte count, and CHr IRF parameters, and β-globin expression results. Receiver Operator Characteristics (ROC) curves assessed the diagnostic performance of CHr and %Hypo for Iron Deficiency Anemia (IDA) and Functional Iron Deficiency (FID). The Marginal Homogeneity Test compared hemoglobin categories at 1st and 6th months, while the Monte Carlo test and Cramer's V statistic elucidated associations between age, gender, and iron status. β-globin mRNA data were analyzed using the Fold Change Expression (FCE) method. Missing data were managed by excluding records with missing values and applying listwise deletion as needed.

Laboratory techniques

Hematology and iron profile analysis

Monthly hemoglobin monitoring was conducted using the Sysmex Automated Hematology Analyzer (KX-21 N). Spectrophotometric techniques were used for iron profile analyses, including serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation [11, 12].

Flow cytometry

Reticulocytes were analyzed using the Beckman Coulter 5-color Cytomics FC500. Thiazole Orange dye was employed to quantify reticulocytes and assess their maturation indicators, such as IRF and CHr [13].

Staining procedure

A stock solution of Thiazole Orange (1 mg/mL) was prepared in methanol and diluted in phosphate-buffered

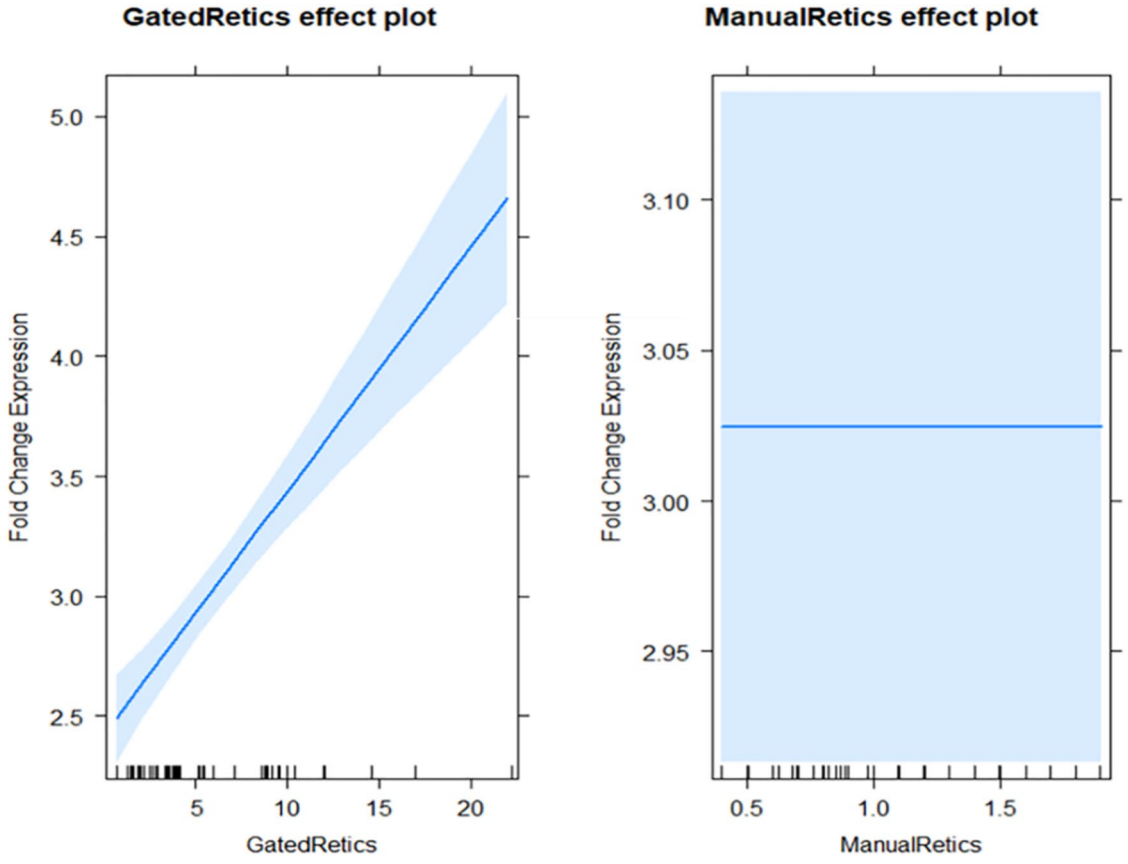

Fig. 1 Comparison between Flow Cytometry and Manual Reticulocyte Counts in β-globin mRNA Expression

Table 1 Diagnosis of anemia among Sudanese children with ESKD by age categories according to KDIGO

Age categories	Hb < 11.0 g/dl	Hb < 11.5 g/dl	Hb < 12.0 g/dl	Total	Monte Carlo Sig. (2-sided)	Cramer's V correlation
0.5–5 years	5 (100%)	0 (0%)	0 (0%)	5	2.594	0.80
5–12 years	12 (100%)	0 (0%)	0 (0%)	12		
12–15 years	24 (85.7%)	1 (3.6%)	3 (10.7%)	28		
Total	41	1	3	45		

saline containing EDTA and sodium azide. EDTA blood from anemic and non-anemic children was stained, incubated, and analyzed using flow cytometry to assess reticulocyte maturation. More details are available in the supplementary files.

Quantification of β-globin mRNA expression

Quantitative Real-Time PCR (RT-PCR) analysis of β-globin mRNA expression was conducted using Maximas SYBR Green/Fluorescein qPCR Master Mix with β-globin-specific primers. Data collection and analysis were performed on the Rotor-Gene Q platform, following the 2-ΔΔCt quantitative method for precise gene expression measurement [14].

Results

Hematological profile and reticulocyte analysis

The complete blood count (CBC) across the study population revealed a normocytic, normochromic red cell morphology. Reticulocyte counts, corrected against 1,000 RBCs and gated via flow cytometry against 50,000 RBCs, showed a significant correlation with β-globin mRNA expression, indicating a robust marrow response ($\beta = 0.10$, $p < 0.001$). This correlation was more pronounced in flow cytometry than in manual reticulocyte counts, which demonstrated a negligible association ($\beta = 4.721 \times 10\text{^-}14$, $p = 1.0$) (Fig. 1) (See Table 1).

Hemoglobin levels over time

The estimated marginal means of hemoglobin (Hb) were analyzed over six months, categorizing the severity of anemia according to WHO age-specific guidelines

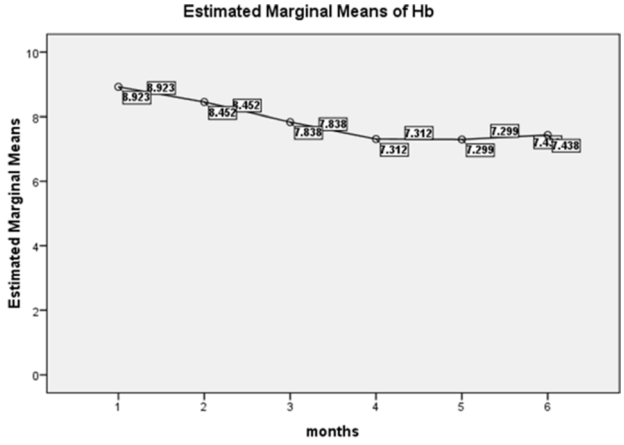

Fig. 2 Estimated Marginal Means of Hemoglobin among Anemic Sudanese Children with ESKD

Table 2 Marginal homogeneity test for hemoglobin categories at the first and sixth month

Hb (1st month)	Hb (6th month)	Total	Marginal Homogeneity Test
	< 11.0 g/dl	< 11.5 g/dl	< 12.0 g/dl
< 11.0 g/dl	30 (93.8%)	1 (3.1%)	1 (3.1%)
< 11.5 g/dl	3 (100%)	0 (0%)	0 (0%)
< 12.0 g/dl	8 (80.0%)	0 (0%)	2 (20.0%)
Total	41	1	3

Table 3 Classification of Anemia at 3rd and 6th Month Follow-Up intervals based on Iron Status

Anemia Classification (3rd month)	Anemia Classification (6th month)	Total	Marginal Homogeneity Test
	Functional Iron Deficiency (FID)	Anemia of other causes	
Absolute Iron Deficiency (AID)	0	1	1
Functional Iron Deficiency (FID)	0	4	4
Anemia of other causes	2	38	40
Total	2	43	45

(Fig. 2). According to KDIGO criteria, hypo-responsiveness to rHuEPO treatment was primarily observed in the 12–15-year age group, with Hb values consistently remaining below 11 g/dl (Table 2).

Longitudinal hemoglobin analysis

A significant shift in hemoglobin categories was observed from the first to the sixth month of treatment ($p=0.01$), suggesting changes in anemia severity over time (Table 2).

Table 4 Impact of gender on Anemia classification based on KDIGO categories

Gender	Anemia Classification	Total	Fisher's Exact Test	Contingency Coefficient
	Functional Iron Deficiency (FID)	Anemia of other causes		
Female	2 (8.0%)	23 (92.0%)	25	0.49
Male	0 (0%)	20 (100%)	20	
Total	2 (4.4%)	43 (95.6%)	45	

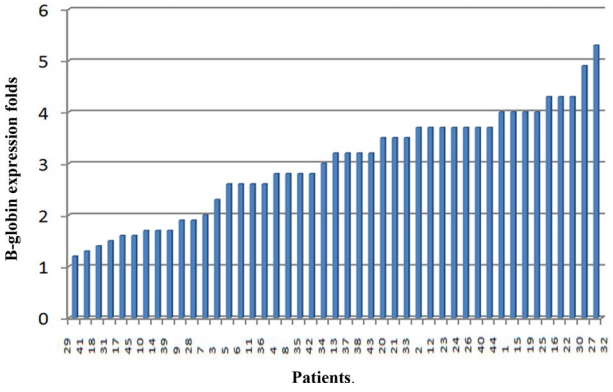

Fig. 3 β-globin expression fold changes among studied patients

Iron status classification

The anemia classification based on iron status was assessed at the third and sixth months. The findings showed a higher prevalence of anemia from other causes compared to functional iron deficiency, with no cases of absolute iron deficiency observed (Table 3).

Impact of demographic factors on anemia

Age was a significant factor influencing iron status over the six-month period ($p=0.49$), while gender did not show a significant impact on the anemia classification based on KDIGO criteria (Table 4).

β-globin gene expression analysis

β-globin gene expression was evaluated in all 45 anemic Sudanese children using RT-PCR. Expression levels varied widely, ranging from 1.2 to 5.3-fold changes, and showed a strong correlation with hemoglobin levels ($r=0.87$, $p<0.001$) (Fig. 3).

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Regression and correlation analyses

Regression analysis demonstrated an association between patient age and CHr (β=0.39, $p=0.04$). Correlation studies revealed that CHr was inversely related to mean corpuscular volume (MCV) and positively correlated with mean corpuscular hemoglobin concentration (MCHC). Figures 4, 5, 6, 7, 8, 9 and 10 depict various ROC curves

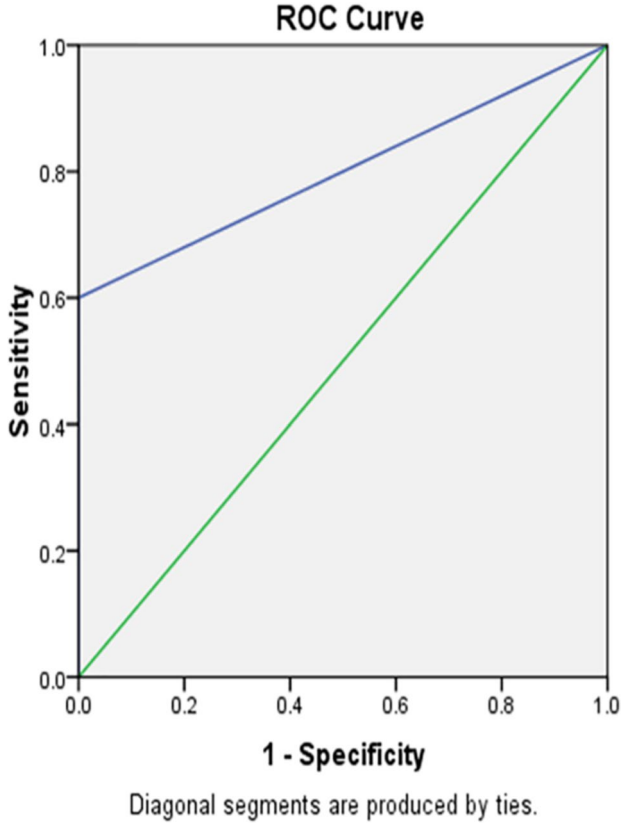

Fig. 4 The area under curve ROC curve expression of anemic Sudanese gender effect on IRF

and correlation graphs highlighting the relationships between hematological indices and anemia.

Receiver operator characteristics (ROC) analysis

ROC curves demonstrated the diagnostic performance of CHr and %Hypo in identifying IDA. Transferrin saturation was a significant predictor of iron status (Figs. 11 and 12).

Sensitivity and specificity of diagnostic tests

CHr showed a sensitivity of 50% and specificity of 4.65% in diagnosing IDA, highlighting the challenges of assessing iron status in pediatric ESKD (Table 5).

Discussion

This study explores the complexities of anemia in Sudanese children with End-Stage Kidney Disease (ESKD) undergoing hemodialysis, focusing on the diagnostic utility of β-globin mRNA expression and reticulocyte parameters. Anemia in pediatric ESKD is often due to insufficient erythropoietin (EPO) production, which is critical for red blood cell synthesis in the bone marrow. Our treatment approach, which combined Epoetin beta (Recormon® 4000 IU/ml) with intravenous iron sucrose (100 mg/ml), underscores the importance of combined therapy for effectively managing anemia in this population.

Influence of iron supplementation and erythropoiesis

Iron supplementation, when administered alongside rHuEPO, enhances erythropoiesis and decreases reliance on blood transfusions. Regular monitoring of iron status

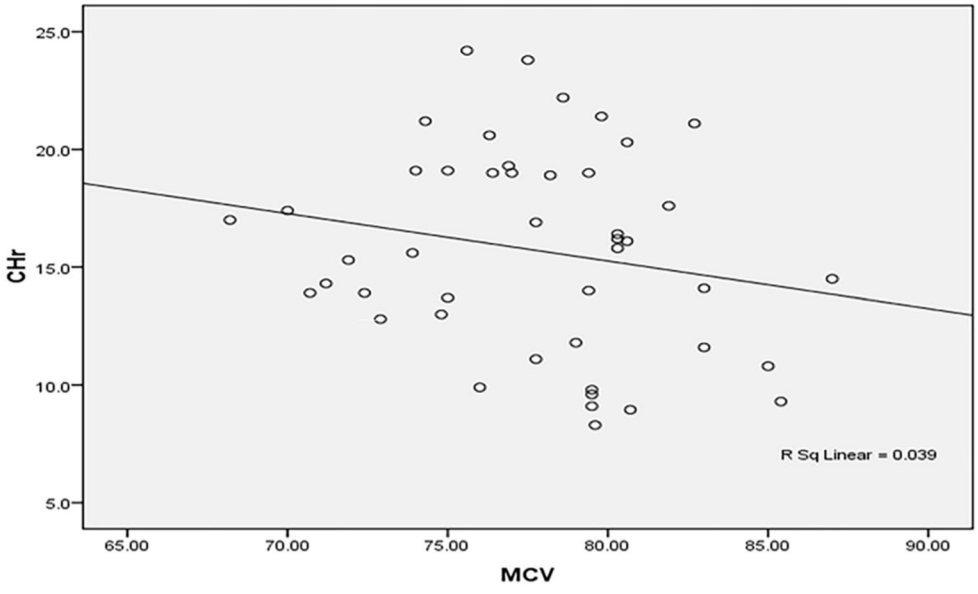

Fig. 5 CHr shows an inverse correlation with MCV among the studied anemic Sudanese children with ESKD

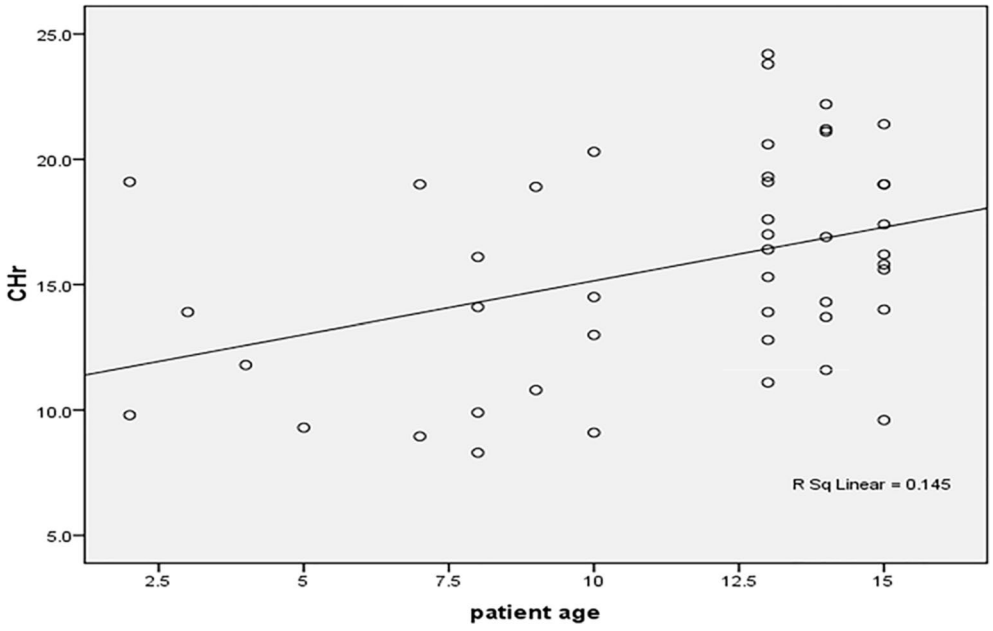

Fig. 6 Significant correlation between patient's age and CHr among the studied anemic Sudanese children with ESKD

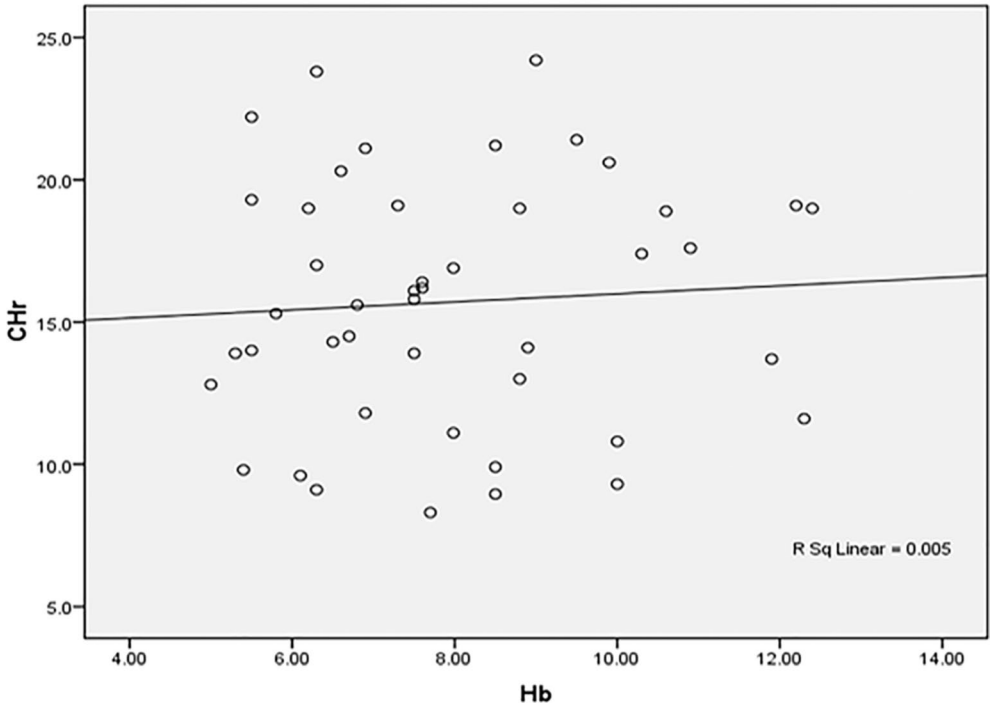

Fig. 7 Positive insignificant correlation between Hb and CHr among the studied anemic Sudanese children with ESKD

and hemoglobin (Hb) levels is crucial for the early detection and management of anemia, ultimately improving clinical outcomes. Our study demonstrates that age significantly impacts iron status, with variations observed in β-globin mRNA expression, CHr, IRF, and mean corpuscular indices. CHr emerged as a sensitive and specific diagnostic tool, particularly for assessing iron deficiency, with transferrin saturation providing more predictive value than ferritin. Interestingly, male patients exhibited a more hypo-responsive pattern to treatment, particularly evident in IRF measures.

Comparative analysis and literature review

Our findings align with Bamgbola et al., who identified age, gender, and other risk factors as determinants of erythropoietin resistance in pediatric dialysis cohorts,

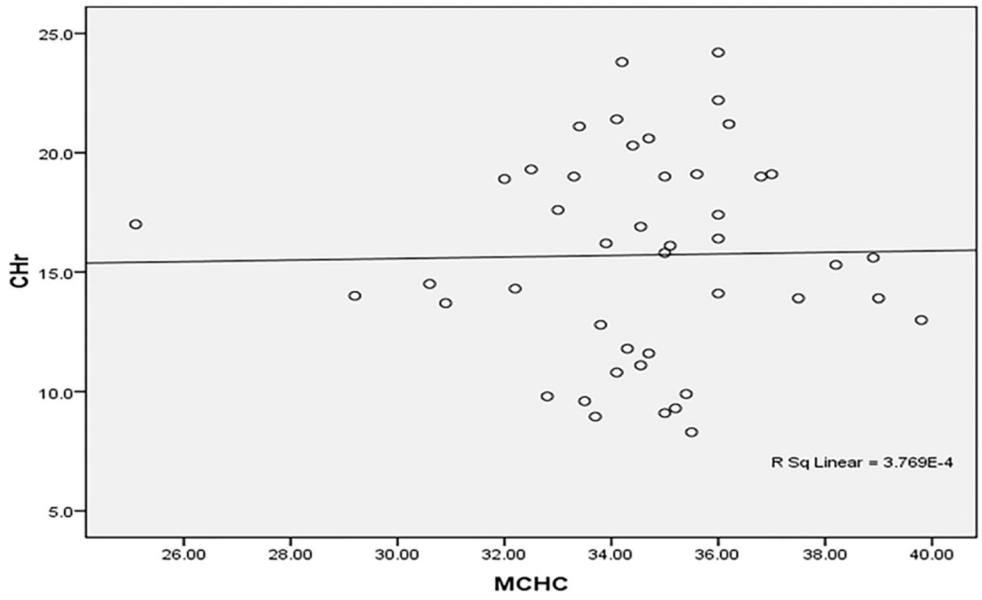

Fig. 8 Positive insignificant correlation between MCHC and CHr among the studied anemic Sudanese children with ESKD

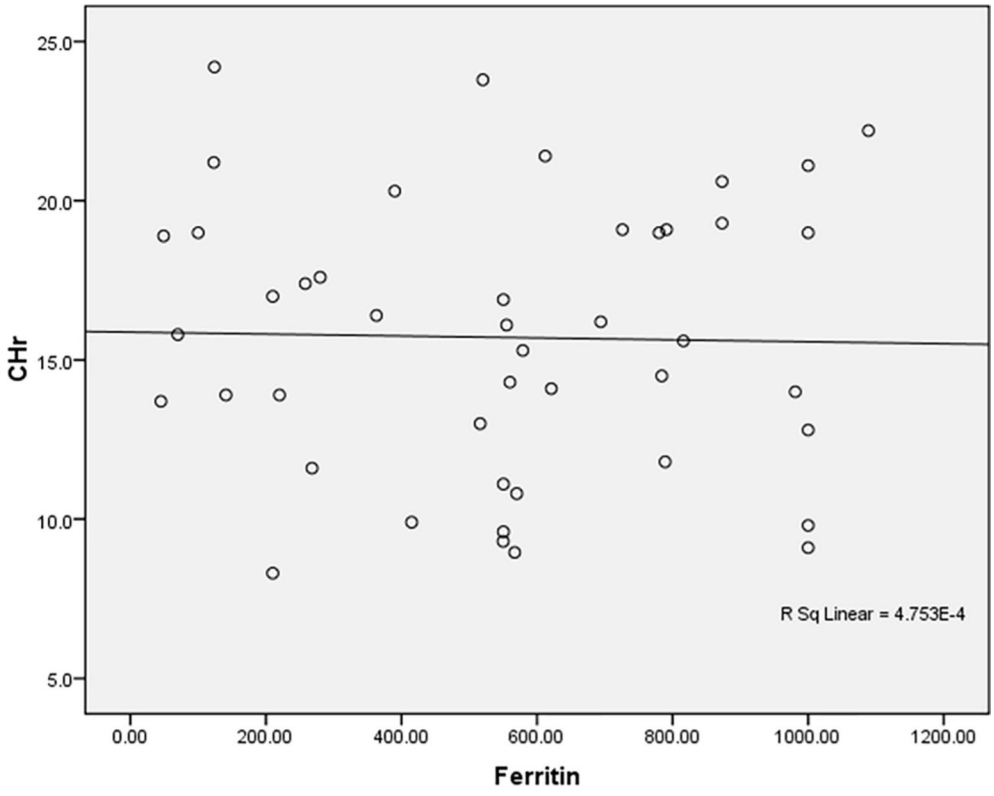

Fig. 9 Positive insignificant correlation between Ferritin and CHr among the studied anemic Sudanese children with ESKD

where factors like chronic inflammation play a significant role in influencing treatment outcomes [15]. Additionally, our results highlight the ongoing challenge of inadequate micronutrient supplementation, a common concern in adult populations with CKD.

Elevated transferrin saturation levels (≥20%) in our cohort suggested a potential risk of iron overload, classifying the anemia as likely driven by non-iron deficiency causes. This finding underscores the utility of CHr and %Hypo as more precise markers for evaluating iron status. Similar conclusions were drawn by Mohsen Saleh et

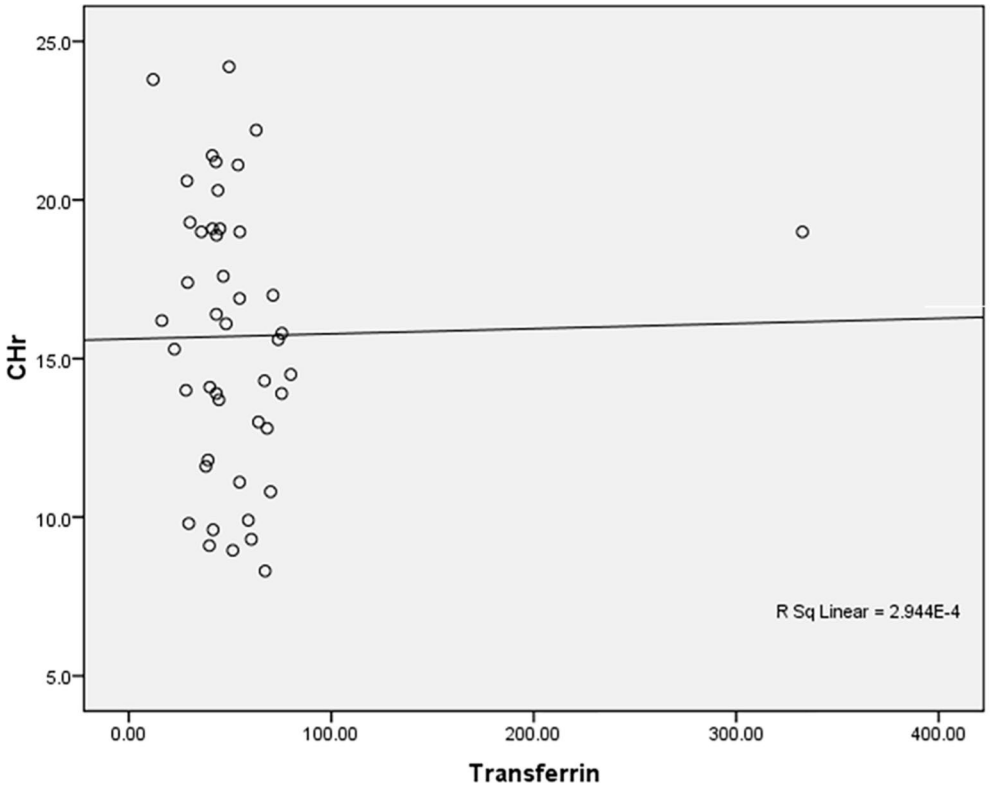

Fig. 10 Negative insignificant correlation between Transferrin and CHr among the studied anemic Sudanese children with ESKD

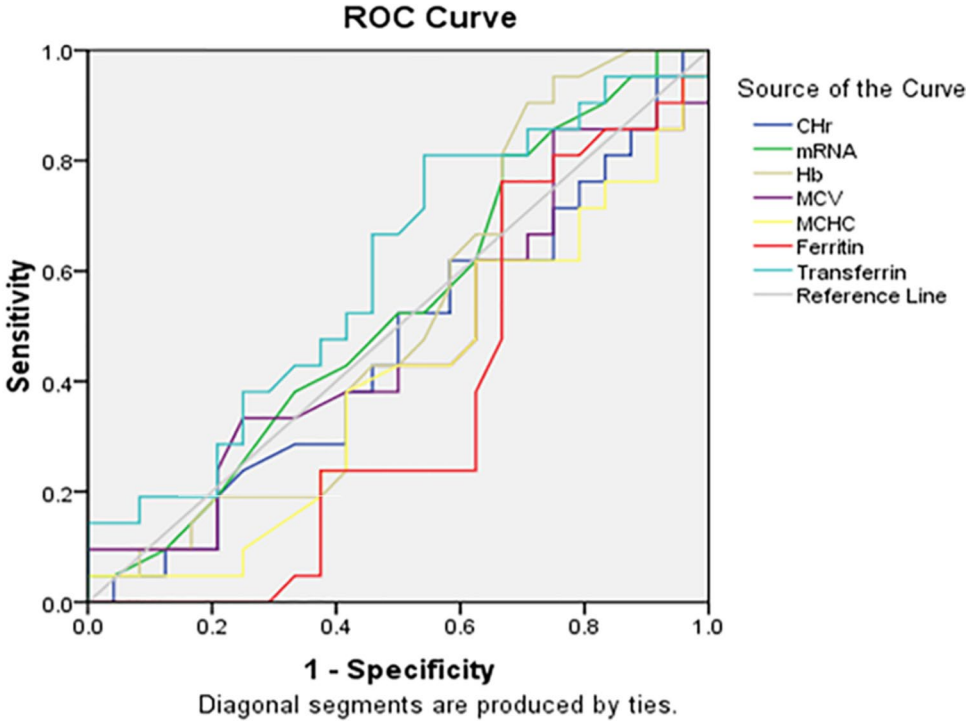

Fig. 11 The ROC curve of CHr, mRNA, and Hb versus gender among the studied anemic Sudanese children with ESKD

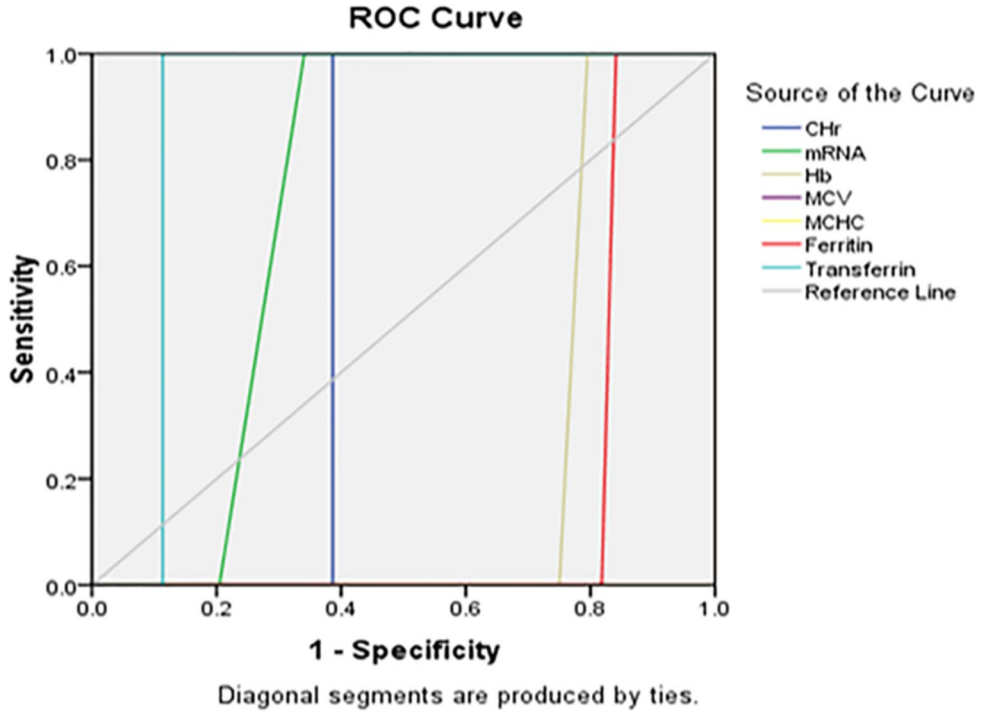

Fig. 12 The ROC curve of CHr, mRNA, Hb versus Hypo% among the studied anemic Sudanese children with ESKD

Table 5 Sensitivity and specificity of CHr in diagnosing IDA among Studied Anemic Sudanese Children with ESKD

Estimated Value	95% Confidence Interval
	Lower Limit
Prevalence	0.04
Sensitivity	0.50
Specificity	0.04

al., who identified iron overload risks in pediatric CKD patients, advocating for cautious iron supplementation [16] .

Limitations of traditional iron markers and role of CHr and HYPO%

Traditional serum markers like ferritin and transferrin saturation are sensitive to inflammatory states and may not reliably indicate iron status [17]. Inflammatory conditions can lead to decreased transferrin levels and, consequently, falsely elevated transferrin saturation. Ferritin is similarly affected by inflammation, malnutrition, and chronic disease, with significant fluctuations in levels (17–70%) [18]. Iron deficiency can manifest before anemia becomes clinically apparent, highlighting the need for more accurate and early indicators like CHr and %Hypo, especially as bone marrow biopsy—the gold standard for assessing iron deficiency—is invasive and not feasible for pediatric patients [19].

CHr provides an early snapshot of iron status and hemoglobin production, reflecting bone marrow activity and response to iron therapy. %Hypo, which measures the percentage of red blood cells with low hemoglobin content (<28 g/dl), serves as a marker of functional iron status, often indicating iron-restricted erythropoiesis before clinical anemia is detectable. Research by Mäkelä et al. demonstrated CHr's superiority over serum ferritin and TSAT in assessing iron deficiency during acute infections, while Dignass et al. found that CHr was a more reliable marker than serum ferritin in anemia associated with chronic disease [20]. Our study corroborates these findings, showing a strong correlation between CHr and age, and confirming its utility in diagnosing iron deficiency anemia (IDA), as supported by Badr et al. [21].

Emerging diagnostic tools

Interestingly, our findings contrast with those of Tessitore et al., who reported that %Hypo was a better predictor of IDA than ferritin, TSAT, and CHr in hemodialysis patients [22]. Such discrepancies highlight the need for population-specific diagnostic strategies, as marker efficacy may vary across different stages of kidney disease and patient demographics. Our results are also in line with Mehta et al., who evaluated CHr and IRF as early indicators of iron therapy response, finding that CHr was the most timely marker for iron supply to developing RBCs [23].

Potential of β-globin mRNA and novel therapeutics

Our analysis further underscores the potential of β-globin mRNA as a biomarker for erythropoiesis and as an early indicator of erythroid response to rHuEPO treatment. This finding is consistent with Ferrer et al., who observed increased β-globin mRNA levels following rHuEPO administration [24]. Furthermore, emerging therapies like hypoxia-inducible factor-prolyl hydroxylase inhibitors (HIF-PHI) offer promising alternatives for managing anemia in CKD, as discussed by Badura et al. [25].

Novelty and incremental value of study findings

This study highlighted the challenges in anemia management among pediatric ESKD patients, notably the poor response to treatment over the follow-up period, necessitating the incorporation of blood transfusion into the treatment regimen. The study identified diverse causes of anemia, including age-related physiological factors, and reported a case of significant menstrual blood loss in a female patient on hemodialysis, prompting the need for coagulation factor assessments.

The study also documented anemia complications, such as fatigue, lethargy, depression, and cardiovascular disorders as the disease advanced.

Importantly, the study introduced novel diagnostic approaches for anemia associated with ESKD, utilizing advanced technologies like flow cytometry and PCR to evaluate bone marrow activity post-treatment. These methods provided an accurate and in-depth assessment, offering an alternative to the painful bone marrow aspiration, which requires supplementary laboratory diagnostics for comprehensive evaluation.

Limitations and future directions

While our study provides a valuable baseline for understanding anemia in pediatric ESKD, certain limitations should be noted. The relatively small sample size and limited follow-up duration may affect the generalizability of our findings. Additionally, the lack of a control group restricts the ability to draw broader comparisons. The variability in clinical characteristics and treatment responses necessitates further research with larger cohorts and longer observation periods to validate these results. Addressing these limitations will enhance our understanding of anemia management in pediatric ESKD and support the development of tailored, effective interventions based on comprehensive physiological assessments.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12882-024-03806-5.

Supplementary Material 1

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Author contributions

1- AA Basic researcher and PhD student 2-AM RT-PCR technical supervisor and training techniques with submitting theoretical Molecular biology courses 3-OA Flowcytomery technical supervision with submitting theoretical Flowcytometry Introduction course 4-EF Flowcytomery technical supervision 5- MO Statistical analysis with multi-regression between Flowctometry and RT-PCR 6- NA Medical clinician supervisor of study cases for Pediatric Kidney Disease and Kidney Transplant 7- NM Professor of Hematology and Molecular Hematology as a Secondary Supervisor of the research 8- NH Professor of Pathology as a main supervisor of the research.

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Data availability

Availability of Data and Materials: Data is available from the corresponding author upon reasonable request.

Declarations

Ethics approval

Ethical approval for this study was obtained from the appropriate ethics committee, and informed consent was secured from all participants' guardians.

Consent to participate

The study objectives and protocols were thoroughly explained to the parents and guardians of the children involved. This included information on laboratory follow-up procedures, contact details of the patients' guardians, and collection of socio-demographic, genetic, and medical histories. It was ensured that all participants' guardians were well-informed and agreed to the study terms, with the promise of transparency in communicating results throughout and after the study period. Participation was voluntary, with no coercion or financial incentives provided. The study benefited participants significantly, offering close clinical follow-up and comprehensive monthly laboratory evaluations, including CBC, RFT, CRP, bleeding profile, and PTH assessments. Blood samples were obtained by nurses from either a peripheral vein or fistula before hemodialysis sessions. Beyond the six-month study period, follow-up was extended to a full year for each patient, depending on their health status. This facilitated enhanced monitoring and informed medical decision-making. The study established a foundation of trust and communication between the patients' parents, the researcher, and the treating physicians. Parents were regularly updated on their child's health and psychological status. In cases where a patient passed away, the family informed the researcher, ensuring transparency about the time, circumstances, and nature of the event.

Ethical considerations for participant withdrawals

For participants who withdrew from the study, psychological support was provided by specialists, and financial assistance came from charitable organizations dedicated to supporting pediatric kidney patients. These considerations were embedded in the treatment protocol, particularly for those returning to their place of origin outside Khartoum State. Risks associated with discontinuation included lack of regular dialysis, inadequate treatment, and insufficient nutritional support. Charitable organizations contributed to patient welfare by offering entertainment and nutritious meals during dialysis, containing energy and protein sources. Medical staff actively advised guardians on proper nutritional practices and emphasized limiting water intake and foods that could exacerbate osmotic imbalances or contribute to high blood pressure. Government agencies provided free access to essential treatments, including calcium supplements, diuretics, and antihypertensive medications, along with the meals during dialysis sessions, reflecting the support available within the local context.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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