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ORIGINAL ARTICLE

Estimation of Anti -erythropoietin Antibody in Children Receiving Erythropiotin Therapy on Hemodialysis

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ABSTRACT:

Background: Anaemia affects many haemodialysis patients for a variety of causes. After treatment, anaemia may still occur due to several causes, such as iron deficiency, elevated urea, poor diet, folate and vitamin B deficiencies, and an ineffective dosage of erythropoietin or an antibody for erythropoietin.

Therefore, our goal was to identify erythropoietin antibody as a contributing factor to resistant anaemia in children receiving frequent haemodialysis. **Methods:** Sixty patients with anaemia, who were getting erythropoietin treatment and haemodialysis, participated in a cross-sectional study. At Zagazig University's Children Hospital Faculty of Medicine, the Paediatric Nephrology Unit evaluated the anti-erythropoietin antibody in every case. **Results:** The neutrophil count, frequency of Epoetin-β administration, and C-reactive protein levels were significant independent predictors of anti-erythropoietin antibody levels. Additionally, there was a significant positive correlation between antibody concentrations and the frequency of Epoetin-β and Epoetin-α administrations. And higher anti-erythropoietin antibody levels were associated with more severe anaemia, with a cut-off of ≥524.3925 predicting severe anaemia with 80% sensitivity and 87.3% specificity. **Conclusion:** In children receiving routine haemodialysis, anti-erythropoietin antibody levels could be a valuable biomarker for assessing the degree of anaemia, providing an efficient tool for clinical decision-making and early intervention.

Keywords: Anti-erythropoietin Antibody; Children; Haemodialysis; Erythropoietin Therapy.

INTRODUCTION

The prevalence of paediatric chronic kidney disease (CKD) ranged from 15 to 74.7 cases per million of the age-related population [1]. Children with chronic conditions exhibit various adverse outcomes due to renal impairment. Anaemia is a common complication in chronic kidney disease [2].

The primary cause of anaemia in patients with chronic renal disease is a relatively low production of erythropoietin (EPO) [3]. It is advised that preventative and control measures be put in place since severe anaemia lowers quality of life and raises the risk of cardiovascular illnesses and death in dialysis patients[4].

In patients with chronic kidney disease, erythropoiesis-stimulating agents (ESAs) are typically used to manage anaemia and lower the requirement for blood transfusions [5]. Epoetin alfa or beta, epoetin alfa biosimilars, and longer-acting medicines such as darbepoetin alfa and methoxy polyethylene glycol-epoetin beta are among the various ESAs that are now on the market [6].

The aetiology of anaemia is multifaceted, and individuals with chronic kidney disease (CKD) have varying response capacities due to competing variables, even though ESAs are proven to be successful in reversing the anaemic state [7]. When a patient uses ESA at higher than typical doses and still does not achieve the correct serum haemoglobin (Hb) concentration, or when higher and higher doses are required to maintain the recommended Hb concentration, this is known as ESA resistance or hypo-responsiveness [8].

While most patients tolerate recombinant human erythropoietin treatment well, a small percentage develop antibodies (anti-erythropoietin antibodies) that can neutralise recombinant proteins or endogenous erythropoietin [9].

Based on our clinical observations of patients who are receiving an optimal dose of erythropoietin-stimulating agents on regular haemodialysis, still experiencing anaemia and requiring blood transfusions, we intended to identify erythropoietin antibody as a contributing factor to resistant anaemia in children undergoing routine haemodialysis, who are receiving erythropoietin-stimulating agents.

METHODS

From January to December 2024, this cross-sectional study was conducted at Zagazig University's Children, Paediatric Nephrology Unit, Hospital Faculty of Medicine. The Institutional Review Board of Zagazig University gave its approval to the study (IRB number 11394-31-12-2023). The eldest children and all parents provided written informed consent.

Inclusion criteria: this study included 60 patients who had been taking recombinant human erythropoietin for more than six months and who were already receiving regular haemodialysis. Both sexes are included, and an erythropoietin dose was required (150 IU/Kg/week).

Patients with haematological malignancies such as leukaemia, aplastic anaemia, bleeding and haemolytic anaemias were excluded from the research.

The World Health Organization's haemoglobin cutoffs for diagnosing anaemia [10], Children aged 5 to 11 had mild anaemia (haemoglobin levels between 11 and 11.4 g/dl), moderate anaemia (between 8 and 10.9 g/dl), and severe anaemia (below 8 g/dl). Children aged 12–14 years and females ≥15 had mild anaemia (haemoglobin 11–11.9 g/dl), moderate anaemia (between 8–10.9 g/dl), and severe anaemia (below <8 g/dl). Males ≥ 15 years had mild anaemia (11–12.9 g/dl), moderate anaemia (8–10.9 g/dl), and severe anaemia (<8 g/dl).

Every patient underwent a thorough history taking, with particular attention paid to the cause, onset, course, and duration of renal disease; history of oedema, hypertension, and urine output; history of dialysis settings, including the onset of dialysis, frequency, duration of each session, duration of dialysis, and size of filter; history of blood transfusions, frequency, and history of drug intake; and clinical examination, which included weight, height, and body mass index. This study comprised sixty children and adolescents receiving haemodialysis. Every patient had previously received routine haemodialysis for a duration varying from nine months to seventeen years.

The age of haemodialysis patients at the time of the study was 6.5 to 23 years. Most patients were hepatitis C virus negative. Most of the studied patients received 3 sessions per week; each session ranged from 3 to 4.5 hours according to clinical, laboratory, and tolerance of the patients for dialysis. Bicarbonate dialysate and either heparin or low molecular weight heparin as an anticoagulant were used in the haemodialysis unit. Frequency of blood transfusion ranged from thrice per month (the least percentage) to once per year (the most percentage). Most of the studied patients were covered by the insurance system, but other patients were covered by ministerial decision and received mainly Epoetin β and/or Epoetin-α.

The following laboratory investigations were done, including complete blood count (CBC) by electrical impedance through the XP device, liver function (total protein, albumin, bilirubin) and kidney function (blood urea and creatinine) by spectrophotometer technique through the COBAS 8000 device, serum calcium and serum phosphorus levels, parathyroid hormone (PTH) levels, C- reactive protein(CRP), procalcitonin, iron profile (serum iron, ferritin, transferrin saturation), and hepatitis C virus. Anti-erythropoietin antibody was measured by using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of anti-erythropoietin antibody in samples.

Statistical analysis:

The software Statistical Package for Social Sciences was used to analyze the data by using version 28. Categorical variables were demonstrated using their absolute frequencies and compared using the chi-square test. Quantitative variables were described using their means and standard deviations or median and interquartile range. Kolmogorov–Smirnov test, Mann-Whitney test, and Kruskal-Wallis test were used. Spearman rank correlation coefficient was used to measure strength and association of correlation. The ROC curve was used to determine the best cutoff of certain quantitative parameters. Linear regression analysis was performed. The level of statistical significance was set at $P<0.05$.A highly significant difference was present if $p\leq0.001$.

RESULTS

This study includes 60 patients on haemodialysis with an age range from 6.5 to 23 years with a mean age of 12.72 years. Females represent 56.7% of them. Body weight has a range from 14 to 63 kg with a mean of 32.51 kg. Mean height is 130.72 cm, and mean BMI is 18.89 (kg/m^2). The unexplained aetiology of CKD represents 21.1% of studied patients, followed by obstructive uropathy at 18.1%. The least percentage of aetiology is structural anomalies (polycystic kidney and single kidney).The largest percentage of haemodialysis patients received blood transfusion once a year (twenty-four patients, 40%); 20% of patients received blood transfusion once a month, but the least percentage of patients received blood three times a month (3.3%). Age at the beginning of dialysis ranges from 3 to 14.5 years, and the median dialysis duration is 3.75 years. The mean duration of the session is 3.87 hours. About 86.7% of patients have three sessions a week. Most of the haemodialysis patients (83.3%) are hepatitis C negative (Table 1).

As regards Table 2, all patients received Epoetin-β (100%), and 39 patients (65%) received it twice a week, and 20% of patients received it three times a week. Thirty-three patients (55%) received Epoetin alpha; among them, 45.4% of patients received it on an infrequent basis.

According to laboratory data for patients, the mean hemoglobin is 9.45 g/dl; all patients (n = 60) who have anemia are classified into mild anemia (15%), moderate anemia (76.7%), and severe anemia (8.3%). Mean corpuscular volume (MCV), mean platelet volume (MPV), white blood cells (WBCs), and platelet counts are 88.6fl, 8.93fl, 7.06 ($10^3/mm^3$), and 260.88 ($10^3/mm^3$), respectively. The median neutrophil and lymphocyte counts are 4.1 and 2.3 ($10^3/mm^3$), respectively. Mean total protein, albumin, calcium, and phosphorus are 6.8 g/dl, 4.08 g/dl, 9.31 mg/dl, and 5.8 mg/dl, respectively. The median C-reactive protein (CRP) and procalcitonin (PCT) are (15 mg/L and 0.19ng/ml). Anti-erythropoietin antibody ranged from 76.199 to 602.23ng/ml. All the patients studied positive for anti-erythropoietin antibody (Table 3).

There was a statistically significant positive correlation between anti-erythropoietin antibody, frequency of blood transfusion, and frequency of epoetin-α. But a statistically significant negative correlation between anti-erythropoietin antibody and frequency of epoetin-β. Also, there was a statistically non-significant correlation between anti-erythropoietin antibody and either age of the patients, age at dialysis, dialysis-related data, or anthropometric data. We found a statistically significant positive correlation between anti-

erythropoietin antibody and CRP, procalcitonin, and neutrophil count. There was a statistically significant negative correlation between anti-erythropoietin antibody and the hemoglobin, mean platelet volume, total calcium, and phosphorus. There was a statistically non-significant correlation between anti-erythropoietin antibody and other laboratory data (Table 4).

The linear regression analysis of factors associated with anti-erythropoietin antibody we found among the factors significantly correlated with anti-erythropoietin antibody: neutrophil count (unstandardized β=11.879, p<0.001), frequency of Epoetin-β (unstandardized β=-117.432, p<0.001), and CRP (unstandardized β=5.87, p<0.001) were significantly independently associated with it (Table 5).

The best cutoff of anti-erythropoietin antibody in prediction of severe anemia is ≥524.3925 with area under curve 0.869, 80% sensitivity and 87.3% specificity, PPV 36.4%, NPV 98% and accuracy 86.7% (p=0.007) (Table 6).

The relation between the use of Epoetin-α and anti-erythropoietin antibody among studied patients showed a statistically significant relation between the level of anti-erythropoietin antibody and the use of Epoetin-α (significantly higher among users) (Table 7).

The level of anti-erythropoietin antibody and the frequency of Epoetin-β use were statistically correlated. A pairwise analysis revealed a substantial difference between the two groups. Additionally, there was a statistically significant correlation between the frequency of Epoetin-α use and the amount of anti-erythropoietin antibody. A paired comparison reveals a significant difference between twice-weekly doses and infrequent users (Table 1 supplementary).

Table (1): Distribution of patients according to baseline data:

Variable	patients N=60	frequency %
Gender		
Female	34	56.7%
Male	26	43.3%
	Mean ± SD	Range
Age (year)	12.72 ± 4.01	6.5 – 23
Weight (kg)	32.51 ± 12.34	14 – 63
Height cm)	130.72 ± 18.17	89 – 167
BMI (kg/m^2)	18.89 ± 3.45	12 – 31.6
Etiology		
Unknown	13	21.1%
Obstructive uropathy	11	18.1%
FSGS	9	13.1%
Neurogenic bladder	7	15%
Atypical HUS	6	10%
RPGN	5	8.1%
Nephrocalcinosis	4	6.5%
SLE	3	5 %
Structural anomalies (Polycystic Kidney-Single kidney)	2	3.1%
Frequency of blood transfusion		
Once/month	12	20%
Twice/month	18	30%
Three/month	2	3.3%
Once/year	24	40%

Variable	patients N=60	frequency %
Twice/year Three/year	3 1	5% 1.7%
Hepatitis C virus Negative Positive	 50 10	 83.3% 16.7%
Number of dialysis (sessions/week) Three times Four times	 52 8	 86.7% 13.3%
	Mean ± SD	Range
Age at beginning of dialysis (year)	8.33 ± 2.56	3 – 14.5
Size of filter	5.17 ± 0.85	3 – 6
Duration of session (hour)	3.87 ± 0.35	3 – 4.5
	Median (IQR)	Range
Duration (period) of dialysis	3.75(1.63 – 5)	0.9 – 17

BMI: Body mass index, FSGS: Focal segmental glomerulosclerosis, HUS: Hemolytic uremic syndrome, RPGN: Rapidly progressive glomerulonephritis, SLE: Systemic lupus erythematosus.

Table (2): Distribution of patients according to treatment-specific data:

Variable	N=60	%
Epoetin-β use	60	100%
Frequency of Epoetin-β Once/week Twice/week Thrice/week	 9 39 12	 15% 65% 20%
Epoetin alpha use No Yes	 27 33	 45% 55%
Frequency of Epoetin-α Once/week Twice/week Infrequent	N=33 9 9 15	 27.3% 27.3% 45.4%

Table (3): Distribution of patients according to laboratory data:

Variable	Mean ± SD	Range
Hemoglobin (g/dl)	9.45 ± 1.09	7.5 – 12
Grades of anaemia	N=60	%
Mild anemia Moderate anemia Severe anemia	9 46 5	15% 76.7% 8.3%
Variable	Mean ± SD	Range
MCV (fl)	88.6 ± 3.84	71 – 97
MPV (fl)	8.93 ± 0.84	7.6 – 11
WBCs (10^3/mm^3)	7.06 ± 2.71	3.1 – 23
Platelet (10^3/mm^3)	260.88 ± 68.21	102 – 385
Total protein (g/dl)	6.8 ± 0.5	6 – 8.1

Variable	Mean ± SD	Range
Albumin (g/dl)	4.08 ± 0.38	3 – 5.3
BUN (mg/dl)	67±13.6	31-45.56
Creatinine(mg/dl)	7.6±2.2	5-15.9
Total calcium (mg/dl)	9.31 ± 1.19	6.6 – 13.7
Phosphorus (mg/dl)	5.8 ± 1.31	3.5 – 8.6
TSAT%	34.78 ± 7.14	22 – 55
	Median (IQR)	Range
Serum parathyroid hormone (PTH)(pg/ml)	141.9(105.75 – 344.45)	7.1 – 1871
Iron (µg/dl)	108.5(102.25 – 115.75)	97 – 125
Ferritin (ng/ml)	328.85(201.25 – 679.5)	102.3 – 5383
CRP (mg/L)	15(13.95 – 16.5)	0.07 – 46
Procalcitonin (PCT)(ng/ml)	0.19(0.1 – 0.24)	0.1 – 0.5
Anti-erythropoietin antibody(ng/ml)	237.22(156.71 – 472.7)	76.199 – 602.23

MCV: Mean corpuscular volume, MPV: Mean platelet volume, WBCs: White blood cells, BUN: Blood urea nitrogen, TSAT%: Transferrin saturation, PTH: Parathyroid hormone, CRP: C-reactive protein, PCT: Procalcitonin.

Table (4): Correlation between anti-erythropoietin level and different data:

Variable	r	P
Age (year)	0.066	0.618
Weight (kg)	0.131	0.32
Height (cm)	0.123	0.348
BMI	0.042	0.751
Age at dialysis (year)	0.066	0.616
Size of filter	0.231	0.076
Number of sessions/weeks	-0.18	0.168
Duration of session (hour)	-0.021	0.873
Duration of dialysis	0.018	0.893
Frequency of blood transfusion	0.523	<0.001**
Frequency of epoetin-β	-0.674	<0.001**
Frequency of epoetin-α	0.887	<0.001**
Hemoglobin (g/dl)	-0.404	0.001**
MCV (fl)	-0.014	0.914
MPV (fl)	-0.263	0.042*
WBCs ($10^3/mm^3$)	0.165	0.208
Neutrophil ($10^3/mm^3$)	0.62	<0.001**
Lymphocyte ($10^3/mm^3$)	-0.05	0.706
Platelet ($10^3/mm^3$)	0.186	0.155
Total protein (g/dl)	-0.059	0.652
Albumin (g/dl)	-0.021	0.875
BUN (mg/dl)	0.312	0.53
Creatinine(mg/dl)	0.06	0.59
Total calcium (mg/dl)	-0.354	0.006*

Variable	r	P
Phosphorus (mg/dl)	-0.436	<0.001**
CRP (mg/l)	0.654	<0.001**
Serum parathyroid hormone(pg/ml)	0.04	0.763
Iron (µg/dl)	-0.182	0.164
Ferritin (ng/ml)	0.187	0.152
TSAT%(ng/ml)	0.142	0.278
Procalcitonin (ng/ml)	0.369	0.004*

BMI: Body mass index, MCV: Mean corpuscular volume, MPV: Mean platelet volume, WBCs: White blood cells, BUN: Blood urea nitrogen, TSAT%: Transferrin saturation, CRP: C-reactive protein.
r Spearman rank correlation coefficient ,**p≤0.001 is statistically highly significant

Table (5): Linear regression analysis of factors associated with anti-erythropoietin antibody:

	Unstandardized Coefficients		Standardized Coefficients	t	p	95.0% Confidence Interval	
	Beta	Std. Error	Beta			Lower	Upper
(Constant)	423.672	59.496		7.121	<0.001**	304.487	542.858
Neutrophile	11.879	3.185	0.347	3.730	<0.001**	5.499	18.260
Frequency of Epoetin β	-117.432	23.190	-0.413	-5.064	<0.001**	-163.887	-70.977
CRP	5.870	1.742	0.288	3.369	<0.001**	2.380	9.360

**p≤0.001 is statistically highly significant, t independent test, std standard deviation
CRP: C-reactive protein.

Table (6): Performance of anti-erythropoietin antibody in prediction of severe anemia

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
≥524.3925	0.869	80%	87.3%	36.4%	98%	86.7%	0.007*

AUC area under curve PPV positive predictive value, NPV negative predictive value *p<0.05 is statistically significant
The best cutoff of anti-erythropoietin antibody in prediction of severe anemia is ≥524.3925 with area under curve 0.869, 80% sensitivity and 87.3% specificity, PPV 36.4%, NPV 98% and accuracy 86.7% (p=0.007)

Table (7): Relation between Epoetin-α and anti-erythropoietin antibody among studied patients

Variable	Epoetin-α		Test	
	Not received	Received	Z	P
	Median (IQR)	Median (IQR)		
Anti-erythropoietin antibody(ng/ml)	155.38(145.97 – 167.4)	395.3(260.49 – 560.18)	-6.652	≤0.001**

Z Mann Whitney test, **p≤0.001 is statistically highly significant , IQR inter quartile range

DISCUSSION

Patients with chronic kidney disease have a higher chance of dying if they are resistant to erythropoietin drugs [11]. While many patients tolerate recombinant human erythropoietin treatment well, a small percentage develop antibodies that can neutralise recombinant proteins or endogenous EPO. The significant number of antibody generation cases has been linked to the subcutaneous administration of epoetin alfa [9].

Transfusion-dependent anaemia and severe pure red cell aplasia can occasionally result from the formation of anti-erythropoietin (anti-EPO) antibodies. According to recent research, pure red cell aplasia caused by anti-EPO antibodies is an uncommon but significant side effect in CKD patients on recombinant human erythropoietin [12].

We sought to determine whether erythropoietin antibody played a role in resistant anaemia in children undergoing frequent haemodialysis.

Sixty haemodialysis patients, ages ranging from 6.5 to 23 years, were enrolled in this study; their mean age was 12.72 years. Of the included patients, 56.7% were female. The predominance of children aged 6.5 to 23 years reinforces the importance of developing age-appropriate dialysis regimens and management strategies, particularly in terms of growth, bone mineral metabolism, and cardiovascular health. Similarly, these findings align with a prior study by Neemat-Allah et al. [13], which reported that the mean age of the included cases was 11.23 ± 4.17 years, and 46% were female. Furthermore, a previous study by Hussein et al. [14] included children on chronic haemodialysis with a median age of 16 (IQR 14-17) years, and 45% were females.

These findings demonstrate a consistent age distribution (mean age of approximately 12-16 years) and a slight female predominance among paediatric haemodialysis patients, suggesting that this age group may be at a critical stage for addressing both physical and psychosocial aspects of chronic kidney disease [15].

In this study, the included cases had body weight ranging from 14 to 63 kg with a mean 32.51 kg. Mean height is 130.72 cm, and mean BMI is 18.89 (kg/m^2). The mean body mass index of 18.89 kg/m^2 in our study suggests a generally healthy weight status, but it is crucial to monitor growth and nutritional status regularly, especially given that paediatric haemodialysis patients may be at risk for growth delays, protein-energy malnutrition, and metabolic disturbances [16].

Similarly, these findings agreed with a prior study by Neemat-Allah et al. [13], which reported that the median weight of cases on haemodialysis was 35.0 kg (range: 25.38–46.5), median height was 135.0 cm (range: 125–150), and median BMI was 18.55 kg/m^2 (range: 14.12–21.5). Similarly, a previous study by McCulloch et al. [17] reported that the weights of patients dialysed ranged from 0.9 to 62.0 kg (median 7.0 kg, IQR 3.0–16.0 kg).

In the current study, unexplained aetiology accounted for 21.1% of chronic kidney disease (CKD) cases, followed by obstructive uropathy at 18.1%. Structural anomalies, such as polycystic kidney and single kidney, represented the lowest percentage of aetiologies.

The higher prevalence of unexplained CKD in this study may reflect regional differences in diagnostic capabilities or delayed presentation, underscoring the need for thorough diagnostic evaluations and early detection strategies. Additionally, the relatively higher rate of obstructive uropathy suggests the importance of preventive measures and early management to reduce the progression to CKD in paediatric populations [18].

Additionally, according to a study by Bello et al. [19], 13% of end-stage renal disease cases had an unexplained origin. Furthermore, a prior study by Sharma et al. [20] revealed that CKD of uncertain aetiology affected around 2.7% of the patients. Of the patients, 6.9% had obstructive uropathy, 3.6% had chronic interstitial nephritis, and 1.5% had autosomal dominant polycystic kidney disease. According to a different study by Salman et al. [21], 9.2%

of CKD causes were attributed to obstructive uropathy.

We found that 40% of haemodialysis patients required blood transfusions once a year, while 20% needed transfusions monthly, and only 3.3% required transfusions three times a month. A study by Neemat-Allah et al. [13] showed that 11% of cases on haemodialysis needed transfusions three times a month. A previous study by Desta et al. [22] showed that 24 (17.3%) of the patients received a blood transfusion while receiving dialysis.

Similarly, a previous study by Chada [23] reported that 18 patients received at least 1 unit of blood, and 26 patients received more than 1 unit of blood. There was a total of 160 units of blood transfused during the 2-year follow-up period with an overall transfusion rate of 3.2%.

The variation in transfusion frequency may reflect differences in anaemia management protocols, access to erythropoiesis-stimulating agents, and the prevalence of underlying conditions such as iron deficiency or chronic inflammation. These findings emphasize the need for optimizing anaemia management strategies, including timely use of iron supplementation and erythropoietin therapy, to minimize reliance on blood transfusions in haemodialysis patients [24].

In this study, the age at the initiation of dialysis ranged from 3 to 14.5 years. The median duration of dialysis was 3.75 years, with an average session lasting 3.87 hours. Most patients (86.7%) underwent dialysis three times per week.

Our results were in accordance with the previous study by Desta et al. [22], showed that the majority of patients (76.2%) had 4 h of dialysis three times each week.

Similarly, a study by Neemat-Allah et al. [13] showed that the median duration of dialysis was 60 months (range: 36–96).

The consistency in session frequency and duration across studies underscores the global standardization of dialysis protocols to optimize treatment outcomes. However, the variability in dialysis duration highlights the need to individualize treatment plans based on patient-specific factors, including age, comorbidities, and response to therapy [25].

Our study reported a significant proportion of patients (83.3%) tested negative for hepatitis C, reflecting substantial progress in infection control and prevention measures in the haemodialysis setting. This rate is higher than those reported in previous studies, such as Neemat-Allah et al. [13], who reported that 35 children tested negative for HCV. In addition, a previous study by Ratiu et al. [26] showed that 67% of patients on haemodialysis tested negative for HCV.

The improvement in our cohort may be attributed to strict adherence to infection control protocols, enhanced screening practices, and the use of antiviral treatments that reduce HCV transmission risk. These findings emphasize the importance of maintaining rigorous infection prevention strategies and early detection to further minimize the prevalence of HCV in dialysis populations [27].

All patients received epoetin-β therapy, with 65% administered the medication twice weekly and 20% three times weekly. Additionally, 55% of the patients received epoetin-α, and among them, 45.4% were treated on an infrequent basis. These findings highlight the widespread use of erythropoiesis-stimulating agents in managing anaemia in haemodialysis patients.

A previous study by Sarhan et al. [28] documented that 58.5% (24 patients) received EPO treatment, indicating variability in practice patterns. The higher erythropoiesis-stimulating agent utilization in our cohort may reflect more aggressive anaemia management strategies, emphasizing the critical role of erythropoiesis-stimulating agents in improving haemoglobin levels and reducing the need for blood transfusions in this population.

Our study showed that the mean haemoglobin level was 9.45 g/dL, mild anaemia (15%), moderate anaemia (76.7%), and severe anaemia (8.3%). The mean values for MCV and MPV were 88.6 fL and 8.93 fL, respectively. Mean WBC and platelet counts were 7.06 ×

$10^3/mm^3$ and 260.88 × $10^3/mm^3$, respectively. Median neutrophil and lymphocyte counts were 4.1 × $10^3/mm^3$ and 2.3 × $10^3/mm^3$.

Similarly, a study by Sarhan et al. [28] reported that the mean haemoglobin, the mean platelet count, the mean corpuscular volume (MCV), and the mean WBC were (9.61 ± 1.64 g/dL ,229.19 ± 47.68 × $10^3/\mu L$,90.62 ± 9.87 fl ,7.32 ± 1.37 × $10^3/\mu L$) respectively. Ahmed et al. [29] revealed the following mean values: total leukocyte count (TLC) at 6.58 ± 2.06 cells/L, haemoglobin at 8.56 ± 1.05 g/dL, and platelet count (PLT) at 191.75 ± 61.17 x10^3/cmm.

Our study reported that the mean total protein, albumin, calcium, and phosphorus levels were(6.8 g/dL, 4.08 g/dL, 9.31 mg/dL, 5.8 mg/dL respectively).

Similarly, in research by Sarhan et al. [27], the mean serum albumin was 3.57 ± 0.39 g/dL, the mean calcium (Ca) was 8.56 ± 0.90 mg/dL, and the mean phosphorus was 4.92 ± 1.49 mg/dL.

A study by Ahmed et al. [29] showed that phosphate was 5.38 ± 1.45 mg/dL, and total calcium was 7.95 ± 0.89 mg/dL.

The variations in the results may reflect differences in nutritional management, supplementation, or dialysis protocols. The higher albumin levels in our cohort could indicate better nutritional status, which is a critical prognostic factor in dialysis patients. These findings emphasize the necessity of individualized care plans, focusing on anaemia correction, bone health, and nutritional optimization to improve clinical outcomes in paediatric haemodialysis populations [30].

All the patients in our research tested positive for anti-erythropoietin antibodies, which ranged from 76.199 to 602.23 ng/ml. Anti-erythropoietin antibodies are present in 30% of included cases, according to a study by Ahmed et al. [29]. According to research by Sarhan et al. [28], 45.6% of patients had anti-erythropoietin antibodies.

The universally positive antibody levels in our study may indicate a higher degree of immunologic sensitization, possibly due to prolonged erythropoiesis-stimulating agent therapy or differing genetic or environmental factors. These results highlight the need for careful monitoring of erythropoiesis-stimulating agent responsiveness and antibody levels, as the presence of anti-erythropoietin antibodies can significantly impair anaemia management and increase the risk of transfusion dependency. Optimizing treatment protocols, including alternative anaemia management strategies, may be critical in improving outcomes for affected children [31].

Our study showed a significant positive correlation between anti-erythropoietin antibody levels and the frequency of blood transfusions, frequency of Epoetin-α administration, CRP, PCT, and neutrophil counts. These findings suggest that higher antibody levels may contribute to increased inflammation, impaired erythropoiesis, and reduced efficacy of erythropoiesis-stimulating agents, necessitating more frequent transfusions and adjustments in erythropoiesis-stimulating agent doses.

Similarly, a study by Sarhan et al. [28] reported a positive correlation between anti-erythropoietin antibody levels and erythropoietin dose per week. In contrast, a study by Ahmed et al. [29] demonstrated no significant correlations between anti-erythropoietin antibody levels and erythropoietin (EPO) therapy duration, dose, and iron dose. This discrepancy might reflect differences in study populations, methodologies, or treatment protocols. Collectively, these findings underscore the importance of routinely monitoring anti-erythropoietin antibody levels in haemodialysis patients to tailor anaemia management strategies, minimize transfusion dependency, and address inflammation-related complications effectively.

Our study revealed a significant negative correlation was observed between anti-erythropoietin antibody levels and the frequency of epoetin-β administration, as well as haemoglobin levels, MPV, calcium, and phosphorus. These findings suggest that higher antibody levels may impair the effectiveness of epoetin-β, leading to reduced haemoglobin

synthesis and disruptions in mineral metabolism. The observed correlations emphasize the potential role of anti-erythropoietin antibodies in exacerbating anaemia and contributing to mineral dysregulation, which are common challenges in managing paediatric haemodialysis patients.

Similarly, a study by Sarhan et al. [28] showed that anti-erythropoietin antibody-positive patients had a strong negative correlation with haemoglobin level. In contrast, a study by Ahmed et al. [29] demonstrated no significant correlations with anti-erythropoietin antibody levels and haemoglobin, mean MCV, mean MCH, creatinine, urea (pre- and post-haemodialysis), serum iron, total iron-binding capacity (TIBC), transferrin saturation, ferritin, phosphate, and total calcium. These contrasting findings highlight the complexity of antibody-mediated ESA resistance and underscore the need for individualized monitoring and treatment adjustments to mitigate the clinical consequences of antibody development in this population.

Our study showed no significant correlation with age, age at dialysis initiation, dialysis-related data, anthropometric data, or other laboratory parameters. This suggests that the development and impact of these antibodies may be influenced more by individual immunological and clinical factors rather than demographic or routine dialysis-related parameters.

In accordance with our results, Ahmed et al. [29] showed no significant correlation between age and dialysis duration. The results support the idea that these variables play a minimal role in the antibody response. These findings emphasize the importance of focusing on immune and inflammatory markers rather than patient demographics or dialysis characteristics when assessing and managing ESA resistance in haemodialysis patients.

Our study identified neutrophil count, frequency of Epoetin-β administration, and CRP levels as independent predictors of anti-erythropoietin antibody levels, with significant p-values (< 0.001). These findings align with previous research indicating that inflammation (as reflected by CRP levels) and neutrophil count are linked to ESA resistance [32].

The negative correlation with epoetin-β administration frequency suggests that higher antibody levels may be associated with more frequent epoetin-β doses, likely due to reduced efficacy of the therapy, which is commonly observed in patients with elevated anti-erythropoietin antibodies. Clinically, these variables emphasize the importance of monitoring inflammatory markers and ESA administration patterns in paediatric haemodialysis patients to better understand and address ESA resistance [33].

Our categorization of anaemia severity revealed that patients with anti-erythropoietin antibody levels ≥524.3925 were more likely to experience severe anaemia, with an AUC of 0.869 showed 80% sensitivity, and 87.3% specificity.

This demonstrates that anti-erythropoietin antibody levels could serve as a useful biomarker for predicting anaemia severity in these patients, providing a valuable tool for clinical decision-making and early intervention. These findings support the clinical utility of assessing antibody levels to better tailor anaemia management strategies in paediatric haemodialysis patients, although further validation in larger cohorts is necessary [34].

In contrast, while our findings are consistent with other studies, some studies, such as those by Ahmed et al. [29], do not have consistently identified similar relationships between inflammation and antibody levels, which may be due to differences in patient populations, treatment regimens, or analytical methods. Therefore, while we advocate for the incorporation of inflammatory markers and ESA usage patterns in managing ESA resistance, additional research is needed to refine and confirm these predictors across diverse clinical settings.

Our study revealed that the anti-erythropoietin antibody levels were significantly higher among patients receiving

Epoetin-α therapy. There was also a significant relationship between antibody levels and the frequency of Epoetin-β administration. Pairwise comparisons revealed significant differences between all frequency groups for Epoetin-β.
Similarly, for Epoetin-α, significant differences were observed between infrequent users and those receiving twice-weekly doses, further supporting the notion that more frequent administration of ESAs could enhance the likelihood of antibody development. This is clinically important, as increased antibody levels may lead to reduced efficacy of ESA therapy and necessitate alternative treatment strategies, such as adjusting ESA doses or switching to other agents. These results underscore the importance of individualized dosing regimens in paediatric haemodialysis patients, considering both the potential for antibody formation and the risk of ESA resistance, to optimize anaemia management and avoid treatment complications.

Similar findings were made by Sarhan et al. [28], who found that 58.5% of patients (24 patients) who received EPO treatment subcutaneously had a significant relationship with the production of anti-EPO antibodies, as opposed to 28.6% of the antibody-negative group (14 patients).

In the other hand, a study by Ahmed et al. [29] revealed that the mean duration of erythropoietin therapy was comparable between antibody-positive and antibody-negative groups, with 6.35 ± 4.35 years in the antibody-positive group and 6.98 ± 4.29 years in the antibody-negative group (p-value = 0.554). Similarly, the weekly erythropoietin dose showed no significant variation, averaging 7739.13 ± 3427.39 IU in the antibody-positive group and 7333.33 ± 3250.54 IU in the antibody-negative group (p-value = 0.630).
The study's strengths:
1) The study effectively highlights the multifactorial nature of anaemia in haemodialysis patients. It underscores the necessity of evaluating anti-erythropoietin antibodies as a potential cause of refractory anaemia, particularly in paediatric patients. This sets a solid foundation for the study, making it clear why this research is significant.
2) The cross-sectional study design is appropriate for identifying the prevalence and predictors of anti-erythropoietin antibodies in this patient population. The sample size of 60 patients appears sufficient for preliminary conclusions, although a larger sample might provide more robust data. Evaluating patients at a single institution could limit the generalisability of the findings.
3) The manuscript reports key predictors of anti-erythropoietin antibody levels, including neutrophil count, Epoetin-β administration frequency, and CRP levels. The significant correlation between antibody levels and the frequency of both Epoetin-β and Epoetin-α administration is noteworthy. This suggests that the more frequent the administration, the higher the antibody levels, potentially exacerbating anaemia.
limitations of the study: The single-centre study design limits the generalisability of the findings.
A larger, multi-centre study would strengthen the evidence base.

CONCLUSION

Anti-erythropoietin antibody levels could serve as a helpful biomarker for estimating the severity of anaemia in paediatric patients undergoing regular haemodialysis, offering a useful tool for early intervention and clinical decision-making.

These findings warrant further studies with a larger population and evaluate the possible potential role for immunosuppressive therapy, which may act against the antibodies to recombinant human erythropoietin.

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Table (1 supplementary): Relation between frequency of use of Epoetin-α, Epoetin-β and anti-erythropoietin antibody among studied patients

Frequency of Epoetin	Anti-erythropoietin antibody	Test		Pairwise comparison
	Median (IQR)	KW	p	
Epoetin-β				
Once/week	590.78(555.77 – 600.74)			P_1<0.001**
Twice/week	237.22(163.54 – 345.97)	28.249	<0.001**	P_2 0.008*
Thrice/week	152.44(147.32 – 173.58)			P_3<0.001**
Epoetin-α				
Infrequent	258.69(237.22 – 301.43)			P_1 0.004
Once/week	505.58(408.05 – 545.91)	28.249	<0.001**	P_2 0.079
Twice/week	590.78(555.77 – 600.74)			P_3<0.001**

KW Kruskal Wallis test **p≤0.001 is statistically highly significant , IQR inter quartile range *p<0.05 is statistically significant p1 difference between first and second groups , p2 difference between second and third groups , p3 difference between first and third groups

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