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Association between anti-erythropoietin antibody levels and treatment response in hemodialysis patients with end-stage renal disease: A cross-sectional study from Iraq

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ABSTRACT

Introduction: The therapeutic efficacy of erythropoietin (EPO) may be diminished by the formation of anti-erythropoietin antibodies (AEAs), potentially resulting in therapy failure.
Objectives: This retrospective study included 84 hemodialysis patients with end-stage renal disease (ESRD) treated with recombinant EPO and aimed to evaluate the association between serum AEA and treatment response.
Patients and Methods: This cross-sectional study included eighty-four Iraqi patients with ESRD who had been on recombinant human EPO for more than 6 months and had hemoglobin levels below 11 g/dL. Serum AEA levels were measured in all patients to evaluate their association with treatment response and to investigate correlations with demographic, clinical, and laboratory data.
Results: The study found that 64.3% of patients displayed increased AEA levels (>20 ng/mL). A poorer hemoglobin response after two months (ΔHb) was associated with increased AEA levels (β = -15.21, P=0.002). Additionally, a higher erythropoietin resistance index (ERI) emerged as a significant positive predictor (β = 1.50, P=0.018), while lower serum albumin demonstrated a protective effect (β = -22.50, P=0.048).
Conclusion: This study indicates a significant prevalence of AEA in patients with ESRD undergoing EPO therapy. Regression analysis revealed that a poorer hemoglobin response (ΔHb) and a higher ERI were independent predictors of elevated AEA titers, while serum albumin was a protective factor.

Implication for health policy/practice/research/medical education:
The significant prevalence of anti-erythropoietin antibodies (AEAs) among Iraqi hemodialysis patients necessitates a policy change to implement targeted screening, focusing on individuals exhibiting a high erythropoietin resistance index (ERI) or inadequate hemoglobin response (ΔHb), which are the primary predictors identified in the study. Monitoring these metrics and optimizing nutritional status, such as serum albumin, is clinically essential. Research should focus on investigating the underlying drivers of AEA in this population. Medical education should incorporate AEA pathophysiology and the analysis of ERI and ΔHb to enhance the identification and management of antibody-mediated treatment resistance.
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Introduction

Anemia is one of the most frequent early complications of chronic kidney disease (CKD) (1). It is associated with a worse quality of life, reduced exercise capacity, decreased mental agility, and an increased prevalence of hospitalization and mortality (2). Anemia is a common consequence in people with CKD in Iraq. A study conducted in Baghdad revealed that all hemodialysis patients experienced anemia, with a notable decrease in red blood cell count detected in 96% of the subjects (3). Erythropoietin (EPO) is a critical hormone in the regulation of erythropoiesis, playing a pivotal role in the

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management of anemia, particularly in patients with CKD undergoing hemodialysis (4). The administration of EPO has become a cornerstone therapy for anemia in this population, significantly improving quality of life and reducing the need for blood transfusions (5).

However, the therapeutic efficacy of EPO can be compromised by the development of anti-erythropoietin antibodies (AEAs), which may lead to a rare but serious condition known as EPO antibody-mediated pure red cell aplasia (6).

The prevalence of AEA in hemodialysis patients receiving EPO therapy remains a subject of concern, as these antibodies can neutralize both endogenous and exogenous EPO, resulting in resistance to treatment and worsening anemia (7,8).

The identification of AEA generally utilizes immunoassays, including radioimmunoprecipitation, or ELISA alongside confirmatory cell-based assays for neutralizing antibodies (9). In instances of AEA-mediated pure red cell aplasia, management strategies involve the prompt cessation of EPO, the use of immunosuppressive therapy (such as cyclosporine), and transfusion support, with certain patients experiencing antibody clearance and a return to erythropoiesis (9). The clinical protocols emphasize the significance of early antibody detection in hemodialysis populations.

Understanding the prevalence of AEA and its impact on the response to EPO therapy is crucial for optimizing treatment strategies and improving patient outcomes (10). Additionally, baseline levels of AEA were identified as independent predictors of increased EPO demand in maintenance dialysis patients (11). To date, no prior studies have been conducted on Iraqi patients with end-stage renal disease (ESRD) to investigate the prevalence of AEA.

Objectives

This study aimed to investigate the relationship between serum AEA levels and treatment response. Ultimately, the findings may inform the development of more effective therapeutic strategies for managing anemia in hemodialysis patients, thereby improving clinical outcomes in this vulnerable population.

Patients and Methods

Study design

This study was a cross-sectional study that included a convenience sample of 84 individuals with ESRD undergoing dialysis. Patients received EPO by the subcutaneous route only. Patients were recruited from the hemodialysis unit at medical city hospital in Baghdad. The data collection continued for six months.

Inclusion criteria

Patients aged 18-80 years old who underwent hemodialysis three times weekly, lasting between 3 and 4 hours. Patients had been receiving EPO for a minimum of 6 months, with hemoglobin levels recorded at less than 11 g/dL at the time of sampling.

Exclusion criteria

Patients exhibiting malnourishment, advanced chronic liver disease, hypothyroidism, active autoimmune disease, Patients with hematological diseases that cause bone marrow failures such as leukemia and aplastic anemia, and those with advanced chronic liver disease child class B and C were excluded from the study, ongoing infections until resolved, undergoing steroid therapy, or experiencing bleeding or hemolysis.

Data collection

Data were collected systematically and categorized into the subsequent domains:

1. Demographic data: Basic demographic information was collected for each patient, encompassing age and sex.
2. Clinical data: A thorough medical history was collected, emphasizing comorbid conditions such as diabetes mellitus (DM), hypertension (HT), chronic hepatitis B, and chronic hepatitis C, along with a detailed drug history.
3. Laboratory data: Venous blood samples were collected from all participants for analysis. Laboratory investigations comprised a complete blood count (white blood cells [WBC]), albumin (mg/dL), glomerular filtration rate (GFR) by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula which utilizes a patient's blood creatinine level, age, and sex to provide a more precise evaluation of kidney function (12), serum creatinine (Scr), serum urea, serum ferritin, parathyroid hormone (PTH), vitamin B12 level, folic acid, aspartate transaminase (AST), and alanine transaminase (ALT). The potential correlation of these parameters with AEA levels was examined. Laboratory technicians were blinded to all patient clinical data during AEA testing to reduce bias.
4. Anthropometric measurements; body mass index (BMI) was assessed utilizing standard methodologies. At the time of study enrollment, the third author measured weight and height using calibrated scales and stadiometers. The calculation of BMI involves dividing weight in kilograms by the square of height in meters (kg/m^2) (13).

Serum AEA measurement

The AEA antibody was measured using an ELISA Kit (YL Biont, Catalog No: YLA0185HU) based on a double-antibody sandwich enzyme-linked immune-sorbent assay technique. An AEA titer below 20 ng/mL will be considered low.

Study outcomes

This study's primary outcome was the prevalence of elevated AEA levels, characterized by a serum titer exceeding 20 ng/mL. Secondary outcomes comprised: 1) the magnitude of AEA titer as a continuous variable; 2) the correlation between AEA titer and demographic and clinical characteristics (e.g., diabetes status, BMI); 3) the correlation between AEA titer and treatment-specific parameters, including the weekly EPO dose (IU/kg/week) and the erythropoietin resistance index (ERI); and 4) the association between AEA titer and hemoglobin response, quantified as the absolute change in hemoglobin levels after two months of therapy (14).

Response evaluation

The treatment response was assessed as a continuous variable, characterized by the absolute change in hemoglobin (ΔHb) from baseline to two months. A secondary binary response outcome was established as an increase in hemoglobin of ≥1.0 g/dL after two months of stable EPO dosing, assuming the absence of confounding factors such as transfusion, iron deficiency, or active infection. Non-response was characterized by the inability to meet this threshold. The ERI was calculated to offer a continuous assessment of erythropoiesis-stimulating agent responsiveness (14). Response evaluation comprises several essential steps to assess the efficacy of EPO therapy. Initially, hemoglobin levels and reticulocyte counts are assessed, followed by an analysis of their correlation with the concentration of AEA. Additionally, the administered dose of EPO is recorded. The ERI is determined by the formula: ERI = (EPO dose per unit body weight [IU/kg]) / (hemoglobin level [g/dL]). The ERI is determined by dividing the average weekly EPO dose (IU/kg/wk) by the mean hemoglobin concentration over the prior two months (g/dL). This index quantitatively measures resistance to EPO therapy (15).

Quality control measures

Stringent quality control protocols were implemented to guarantee the precision and dependability of laboratory measurements. Calibration for equipment (scales, stadiometers) is done before each measurement. In order to mitigate bias during data collection and analysis, blinding protocols were strictly enforced by all personnel.

Statistical analysis

The collected data, along with basic clinical examinations and laboratory investigations, were coded, entered, and analyzed using Microsoft Excel software. The data were imported into the Statistical Package for the Social Sciences (SPSS version 26.0, SPSS, Chicago, IL, USA) for analysis. The qualitative data were reported as numbers and percentages. In contrast, quantitative data were expressed as means with standard deviations (SD) or medians with interquartile ranges (IQR) for nonparametric data, including AEA in blood, ERI, baseline hemoglobin after 2 months, baseline hematocrit, and hematocrit after 2 months. The comparison of two groups with quantitative data was conducted using a two-tailed independent T-test. Univariate and multivariate analyses were employed to evaluate the correlations.

Meanwhile, a P value of less than 0.05 was deemed significant. To meet the assumptions of linear regression, we conducted sensitivity analyses utilizing natural log-transformed AEA titers [ln(AEA)]; the results aligned with those from untransformed data, thereby confirming robustness. Multicollinearity was evaluated through variance inflation factors (VIF), with all values in the final model remaining below 3.0, signifying acceptable levels of collinearity.

Results

The demographic characteristics

Table 1 summarizes the demographic characteristics of the participants. This study included 84 patients. The cohort predominantly included male patients. The median age suggests a participant group that is predominantly middle-aged to elderly. Hypertension was a prevalent comorbidity, with a significant proportion of patients also diagnosed with DM, indicating a notable overlap between the two conditions. Most patients demonstrated elevated AEA titers surpassing the threshold of 20 ng/mL.

The laboratory data

The laboratory data of all participants are demonstrated in Table 2. White blood cell counts were typically within normal limits, averaging 5.44, whereas albumin levels were marginally low at 3.98 mg/dL. Markers of kidney function, such as GFR and serum creatinine, exhibited significant impairment, with average creatinine levels recorded at 9.40 mg/dL, exceeding normal ranges.

The regression analysis of demographics and clinical factors associated with AEA titer

Univariate linear regression revealed multiple factors significantly correlated with elevated AEA titers, such as diabetes, increased BMI, reduced baseline Hb, a smaller ΔHb, elevated weekly EPO dosage, higher ERI, decreased albumin, increased ferritin, fewer dialysis sessions per week, and lower GFR (Table 3). Sensitivity analysis employing ln(AEA) produced consistent outcomes. Variables that were significant in univariate analyses ($P<0.05$) were incorporated into a multivariate linear regression model. The variable "EPO dose (IU/kg/wk)" was omitted because of multicollinearity with ERI, as indicated by a VIF greater than 10. The final multivariate model identified three independent predictors of elevated AEA titer: a reduced (ΔHb) (β = -15.21, $P=0.002$), an increased ERI (β = 1.50, $P=0.018$), and decreased serum albumin levels (β = -22.50, $P=0.048$). The final model accounted for 47.5% of the variance in AEA titers

Table 1. Demographic and clinical characteristics of the study participants (N = 84)

Characteristic	Category/Statistic	Value
Sex	Male	47 (56.0%)
	Female	37 (44.0%)
DM	Yes	35 (41.7%)
	No	49 (58.3%)
HT	Yes	66 (78.6%)
	No	18 (21.4%)
Both DM and HT	Yes	31 (36.9%)
	No	53 (63.1%)
SLE	Yes	1 (1.2%)
	No	83 (98.8%)
Hepatitis C	Yes	27 (32.1%)
	No	57 (67.9%)
Hepatitis B	Yes	14 (16.7%)
	No	70 (83.3%)
Taking ACEi or ARB	Yes	38 (45.2%)
	No	46 (54.8%)
AEA titer	High (>20 ng/mL)	54 (64.3%)
	Low (≤20 ng/mL)	30 (35.7%)
Age (years)	Median (IQR)	57.5 (24.0)
Weight (kg)	Mean ± SD	71.77 ± 17.56
Height (m)	Mean ± SD	1.64 ± 0.10
BMI	Mean ± SD	26.5 ± 5.6
AEA titer (ng/mL)	Median (IQR)	76.79 (78.16)
EPO resistance index (ERI)	Median (IQR)	23.5 (21.0)
Baseline hemoglobin (g/dL)	Mean ± SD	7.21 ± 1.37
Hemoglobin after 2 months (g/dL)	Mean ± SD	8.80 ± 1.90
Baseline hematocrit (%)	Mean ± SD	21.0 ± 3.8
Hematocrit after 2 months (%)	Mean ± SD	26.0 ± 5.0
Duration of dialysis session (h)	Mean ± SD	3.89 ± 0.28
EPO dose per week (IU)	Mean ± SD	13,571 ± 6,170

DM: Diabetes mellitus; HT: Hypertension; ACEi: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin receptor blocker; AEA: Anti-EPO antibody; BMI: Body mass index; EPO: Erythropoietin; IQR: Interquartile range; SD: Standard deviation; SLE: Systemic lupus erythematosus. Categorical data is presented as counts and percentages (n, %), while continuous data is presented using (mean ± standard deviation or median with IQR).

(adjusted R^2 = 0.475). The findings remained consistent when ln(AEA) was utilized as the outcome (Table 4).

Figure 1 and Figure 2 illustrate the key relationships identified in the regression analysis. Figure 1 illustrates a notable inverse correlation between the absolute change in hemoglobin (ΔHb) and AEA titer (r = -0.61, $P<0.001$). Figure 2 illustrates a notable positive correlation between the ERI and AEA titer (r = 0.42, $P<0.001$).

Table 2. Laboratory investigations

Category	Minimum	Maximum	Mean	SD
WBC (×10³ cells/μL)	2	11	5.44	1.66
Albumin (g/dL)	2.3	6.0	3.98	0.59
GFR (mL/min)	3	14	5.8	2.17
Scr (mg/dL)	3.5	19.0	9.40	2.64
Urea (mg/dl)	25	291	122.63	60.19
Ferritin (ng/mL)	20	2000	718.92	348.7
PTH (pg/mL)	13	2476	472.27	457.17
B12 level (pg/mL)	148	250	176.50	16.94
Folic acid (ng/mL)	1.40	2.00	1.71	0.144
AST (U/L)	3	38	14.74	6.1
ALT (U/L)	4	36	14.98	5.78

SD: Standard deviation; WBC: White blood cells; GFR: Glomerular filtration rate; Scr: Serum creatinine; PTH: Parathyroid hormone; AST: Aspartate transaminase; ALT: Alanine transaminase.

Discussion

The study identified a high prevalence (64.3%) of elevated (AEA >20 ng/mL) in Iraqi hemodialysis patients, particularly among those with diabetes, who exhibited significantly higher titers. Clinically significant correlations were observed between AEA levels and elevated BMI, as well as with increased EPO dosing requirements, lower baseline hemoglobin and hematocrit, and diminished Hemoglobin improvement after two months.

The presence of circulating AEA in patients with ESRD who are undergoing regular hemodialysis is a significant concern, as these antibodies can affect the efficacy of EPO therapy. There is a significant prevalence of these antibodies among patients, as evidenced by studies, which is associated with an increased severity of anemia and resistance to treatment (16-18).

In contrast to previous studies that solely assessed the presence or absence of AEA, the present study evaluated the titer of AEA in the patients who participated, which ranged from 0.1 ng/mL to 279 ng/mL in the recruited sample. The study reported that 64.3% of participating patients exhibited elevated levels of AEA antibodies above 20 ng/mL. The current result exceeds findings from prior studies, such as those by Keeppallil et al (16), which indicated a prevalence of AEA antibodies in patients with ESRD undergoing regular hemodialysis at 27.08%.

Another study by Hoppensteadt et al (17) indicated that 8.3% of ESRD patients receiving recombinant EPO developed AEA associated with thrombotic adverse reactions.

Additionally, a study conducted in Egypt evaluated the degree of AEA in hemodialysis patients undergoing EPO therapy. The authors found that 38.9% of patients exhibited AEA in their blood (18). The observed prevalence of AEA (64.3%) in our Iraqi cohort, in comparison to other populations, necessitates further investigation. Multiple

Table 3. Univariate linear regression analysis of factors associated with AEA titer and ln(AEA)

Variable	AEA titer (β, 95% CI)	*P* value	ln(AEA) (β, 95% CI)	*P* value
Diabetes (Yes vs. No)	38.0 (3.1 to 72.9)	0.033	0.97 (0.07 to 1.87)	0.035*
BMI	3.01 (0.10 to 5.92)	0.043*	0.07 (0.002 to 0.14)	0.044*
Baseline Hb (g/dL)	-12.1 (-23.9 to -0.3)	0.045*	-0.32 (-0.63 to -0.01)	0.044*
ΔHb after 2 months	-17.5 (-26.8 to -8.2)	<0.001*	-0.44 (-0.67 to -0.21)	<0.001*
EPO dose (IU/kg/wk)	0.003 (0.001 to 0.005)	0.009*	0.00008 (0.00002 to 0.0001)	0.007*
ERI	1.85 (0.64 to 3.06)	0.003*	0.05 (0.02 to 0.08)	0.003*
Albumin (mg/dL)	-28.9 (-52.6 to -5.2)	0.018*	-0.73 (-1.35 to -0.11)	0.022*
Ferritin (ng/mL)	0.04 (0.001 to 0.08)	0.045*	0.001 (0.0001 to 0.002)	0.030*
Dialysis sessions/wk	-19.6 (-36.6 to -2.5)	0.025*	-0.50 (-0.94 to -0.06)	0.027*
GFR (mL/min)	-6.55 (-12.8 to -0.3)	0.041*	-0.17 (-0.33 to -0.01)	0.041*

BMI: Body mass index; AEA: Anti-EPO antibody; EPO: Erythropoietin; Hb: Hemoglobin; ERI, erythropoietin resistance index; GFR: Glomerular filtration rate; ln(AEA): Natural logarithm of AEA titer.
*Significant association ($P < 0.05$).

factors may contribute to this disparity. Genetic and ethnic variations, including disparities in HLA haplotypes common in Middle Eastern populations, may predispose individuals to AEA development, given that specific HLA alleles are associated with autoimmune responses (19). Secondly, environmental and nutritional factors may be influential; for example, vitamin D deficiency, prevalent in Iraq due to restricted sun exposure and inadequate diet, has been linked to immune dysregulation and heightened autoantibody production (20). Our cohort may exhibit a greater prevalence of unmanaged CKD risk factors, such as HT and diabetes, which are recognized to intensify erythropoiesis-stimulating agent resistance and promote the generation of advanced glycation end products due to sustained inflammatory and oxidative stress. The combined region-specific genetic, environmental, and clinical factors may account for the increased prevalence of AEA observed in our study.

The current study found that the level of AEA was not significantly associated with demographic factors, including age, sex, weight, and height in the patients.

Indeed, age-related factors may affect antibody production variably, depending on the individual's underlying health condition (21).

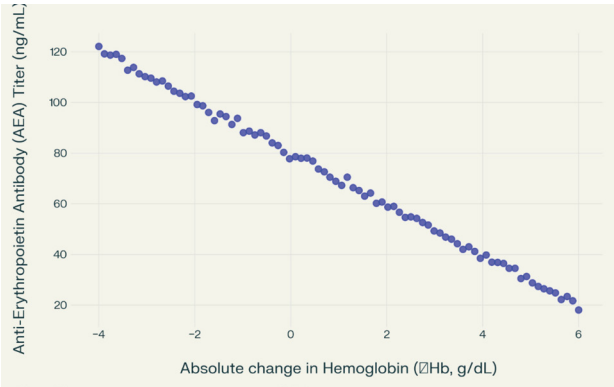

Figure 1. Correlation between hemoglobin response (ΔHb) and AEA titer.

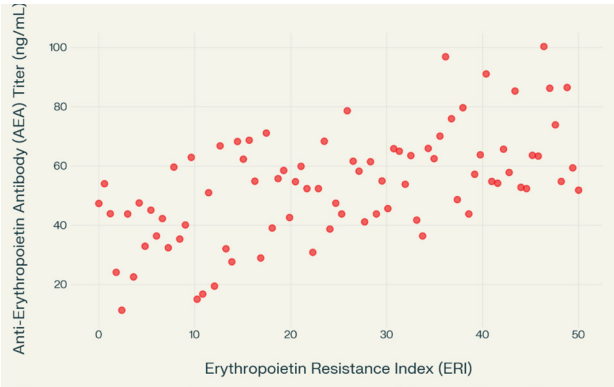

Figure 2. Correlation between the ERI and the AEA.

Table 4. Multivariate linear regression analysis of independent predictors of AEA titer and ln(AEA)

Predictor	AEA titer (β)	*P* value	ln(AEA) (β)	*P* value
ΔHb after 2 months	-15.21	0.002*	-0.39	0.003*
ERI	1.50	0.018*	0.04	0.020*
Albumin (mg/dL)	-22.50	0.048*	-0.57	0.049*
Diabetes (Yes)	25.10	0.112	0.65	0.105
BMI	1.95	0.180	0.05	0.185
Baseline Hb (g/dL)	-8.05	0.150	-0.21	0.152
Ferritin (ng/mL)	0.02	0.210	0.0005	0.215
Dialysis sessions/wk	-12.30	0.105	-0.31	0.110
GFR (mL/min)	-4.20	0.155	-0.11	0.160
Adjusted R^2	0.475		0.469	

BMI: Body mass index; AEA: Anti-EPO antibody; Hb: Hemoglobin; ERI, erythropoietin resistance index; GFR: Glomerular filtration rate; ln(AEA): Natural logarithm of AEA titer.
* Significant association ($P < 0.05$). The final model was adjusted for the variables listed. The variable EPO dose (IU/kg/wk) was excluded from the multivariate model due to multicollinearity with ERI (VIF > 10). The variance inflation factor (VIF) for all included predictors was < 2.5, indicating acceptable multicollinearity.

For instance, the study by Zhang et al (11) indicates that with advancing age, the presence of these antibodies may increase, potentially influencing the requirements for EPO therapy.

In contrast, younger age in pregnant females is associated with a higher prevalence of AEA, as indicated by a study conducted by Nkansah et al (22), which examined the prevalence and relationship of AEA with Plasmodium falciparum malaria and related anemia in pregnant women in northern Ghana.

Consistent with our findings indicating no sex differences in AEA production, a study by Bamgbola et al (23) investigated factors affecting AEA production in ESRD and concluded that gender did not significantly influence EPO resistance in both pediatric and adult patients.

A positive univariate association was identified between BMI and AEA titers, which contrasts with certain studies indicating that obesity may reduce the need for EPO dosage (24). The association may be explained by potential mechanisms, including chronic inflammation and altered ESA pharmacokinetics in obesity (25,26). It is essential to recognize that other factors influenced this relationship, as BMI did not maintain statistical significance in the multivariate regression model ($P=0.180$), indicating that its effect may be mediated or confounded by the primary predictors of ΔHb, ERI, and albumin.

The findings indicated a significant inverse correlation between AEA titer and hemoglobin response (ΔHb), identified as the most potent independent predictor in our multivariate analysis (β = -15.21, $P=0.002$). A strong negative correlation (r = -0.61, $P<0.001$) is visually confirmed in Figure 1. A negative univariate association was observed with baseline hemoglobin; however, this association was not independent in the final model. The consistency observed in multiple studies (16,18,27) highlights a direct mechanistic relationship between AEA presence and impaired erythropoietic response.

The cross-sectional design of this study limits the precise definition of the individual roles of AEA, underlying iron deficiency, and inflammation in ESA hypo-responsiveness, highlighting the need for longitudinal studies to clarify these complex interactions (28).

There was no significant correlation between the number and duration of dialysis sessions and AEA titer. The weekly EPO demonstrated a significant positive association in univariate analysis (Table 3); however, due to severe multicollinearity with the ERI (VIF > 10), it was excluded from the multivariate model. This suggests that the ERI, which integrates both dose and hemoglobin level, serves as a more reliable and independent measure for evaluating AEA-related resistance. Previous studies (11,16,27) indicated a positive correlation between the presence of AEA and the required dose of EPO. Patients possessing these antibodies frequently require higher doses of EPO, which correlates with increased EPO resistance.

The findings of this study indicated no significant correlation between disease conditions, including DM and HT, or the use of medications such as ACE inhibitors or angiotensin receptor blockers (ARBs), and the levels of AEA. This indicates that these comorbidities and medications may not significantly influence the development or modulation of AEA. The findings are consistent with earlier research, which indicates no significant association between AEA levels and prevalent comorbidities (18).

Clinically, these factors may not be regarded as risk factors for the development of acute erythropoietic anemia in patients undergoing EPO therapy. Further research utilizing larger sample sizes and longitudinal designs is necessary to validate these findings and investigate additional potential risk factors for AEA formation.

In contrast to the findings of Sarhan et al (27), which indicated a lower mean ERI, our cohort exhibited a significantly higher ERI (median 23.5, IQR 21.0). Our findings indicate a significant positive correlation between ERI and AEA titer, consistent with the pathophysiology of antibody-mediated resistance. This correlation was evident in univariate analysis (β = 1.85, $P=0.003$) and remained an independent predictor in the multivariate model (β = 1.50, $P=0.018$). The relationship is further substantiated by a significant positive correlation depicted in Figure 2 (r = 0.42, $P<0.001$). The differences observed between our findings and those of Sarhan et al can be attributed to variations in patient demographics, clinical characteristics, or the higher prevalence of AEA in our population, highlighting the impact of population-specific factors.

Multivariate regression analysis identified the independent predictors of elevated AEA titers. The ERI emerged as a significant positive predictor (β = 1.50, $P=0.018$), establishing a quantitative relationship between EPO resistance and antibody levels. Serum albumin levels demonstrated a protective effect (β = -22.50, $P=0.048$), indicating that enhanced nutritional and inflammatory status may lower the risk of AEA development. A diminished (ΔHb) emerged as the most significant negative predictor (β = -15.21, $P=0.002$). Although ferritin and diabetes were significant in univariate analyses, they became insignificant in the multivariate model, suggesting that their effects are not independent but are likely mediated or confounded by the primary predictors of ERI, albumin, and ΔHb.

The high prevalence of AEA (64.3%) is a significant contributor to ESA resistance in Iraqi hemodialysis patients. The independent predictors identified—namely, the inverse correlation between AEA titers and ΔHb, alongside the positive correlation with ERI—offer clinicians a definitive profile for suspicion. A patient necessitating high EPO doses (high ERI) while exhibiting a poor hemoglobin response (low ΔHb) should be

considered as potentially experiencing AEA-mediated resistance. Despite higher AEA levels observed in people with diabetes during univariate analysis, all patients exhibiting this clinical profile should be considered for screening. The protective function of albumin underscores the significance of addressing nutrition and inflammation management. Integrating AEA testing into clinical practice for high-risk patients may facilitate early intervention strategies, including transitioning to non-immunogenic ESA formulations, optimizing iron and nutritional status, and mitigating unnecessary and costly increases in EPO dosage.

The findings corroborate previous research connecting AEA to insufficient ESA response (10,16,18) and broaden the evidence to include underrepresented Middle Eastern populations, where genetic, nutritional, and environmental factors, such as vitamin D deficiency (20) and HLA haplotypes (19), may intensify AEA production. Regular AEA screening in high-risk patients, including those with increasing EPO requirements or decreasing hemoglobin or hematocrit levels, may facilitate the implementation of early intervention strategies. These strategies could involve transitioning to non-immunogenic ESA formulations or incorporating adjunctive therapies such as iron optimization and anti-inflammatory agents. Correspondingly, the inverse correlation between serum albumin and AEA levels highlights the possible influence of malnutrition and chronic inflammation on antibody production. Reduced albumin levels may indicate a pro-inflammatory condition that promotes immune dysregulation and the generation of autoantibodies.

The disconnection between AEA and conventional laboratory markers, such as PTH and ferritin, underscores the need for customized diagnostic strategies. In the future, AEA testing may be integrated into ESA resistance protocols, particularly in regions such as Iraq, where prevalence rates are elevated. Early identification and management of AEA allows clinicians to reduce therapy failure, lower transfusion dependency, and enhance the quality of life for this vulnerable population.

Conclusion

This research demonstrates a notable prevalence of AEA in patients with ESRD receiving EPO therapy. Regression analyses indicated that a lower hemoglobin response (ΔHb) and a higher ERI were independent positive predictors of increased AEA titers, whereas serum albumin served as an independent protective factor. Univariate associations were noted with factors such as BMI and diabetes; however, these factors did not remain independent in the final model. The findings highlight the significance of AEA in treatment resistance and underscore the necessity of incorporating AEA monitoring into the standard management of hemodialysis patients, particularly those with a high ERI and inadequate hemoglobin response despite appropriate dosing.

Limitations and future directions

This study provides valuable insights into the prevalence of AEA among Iraqi hemodialysis patients; however, the use of a single-center convenience sample may restrict the generalizability of the findings, and the exclusion criteria may lead to an underestimation of AEA rates in high-risk populations. The cross-sectional design inhibits the ability to draw causal inferences regarding AEA and EPO resistance. Although ELISA identified AEA, it may overlook neutralizing or low-affinity antibodies that influence EPO efficacy. Additionally, the cross-sectional design limits our ability to ascertain the temporal sequence or causality of the associations identified between AEA titers and factors such as ERI or albumin levels. Unmeasured confounders such as vitamin D, iron status, and inflammation may also affect the results. Future multi-center longitudinal studies are necessary to validate these findings across diverse populations. At the same time, advanced assays, such as cell-based tests, may provide a more detailed characterization of antibody subtypes. Research should investigate biomarkers such as PTH and CRP, genetic predispositions including human leukocyte antigen (HLA) haplotypes, and interventions like alternative erythropoiesis-stimulating agent (ESA) formulations to enhance anemia management in this population.

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Authors' contribution

Conceptualization: Samer Imad Mohammed, Mohammed Yawuz Jamal.
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Conflicts of interest

The authors declare that they have no competing interests.

Ethics issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki. It was approved by the Ethics Committee of the University of Baghdad, College of Pharmacy (Ethical code No. REC-2833-2024). Prior to any intervention, all participants provided verbally informed consent. The authors have fully complied with ethical standards, including adherence to guidelines on plagiarism, data fabrication, and duplicate publication.

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