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Original Research Article

Association of Iron Deficiency and Erythropoiesis-Stimulating Agent Use with Anemia Outcomes in Progressive Chronic Kidney Disease

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Abstract: ***Background:*** Anemia is a common and clinically significant complication of progressive chronic kidney disease (CKD), primarily driven by impaired erythropoietin production and disturbances in iron homeostasis. Iron deficiency further exacerbates anemia and may influence the response to erythropoiesis-stimulating agents (ESAs). The combined impact of iron status and ESA utilization on anemia outcomes in CKD patients in developing healthcare settings remains inadequately characterized. This study aimed to evaluate the association of iron deficiency and ESA therapy with anemia outcomes among patients with progressive CKD in tertiary care hospitals. ***Methodology:*** A multicenter retrospective analytical study was conducted at Rangers Hospital Lahore, Pakistan and Hamid Diagnostic Laboratory. Medical records of 312 patients diagnosed with stage 3–5 CKD between January 2025 and December 2025 were reviewed. Patients aged ≥18 years with documented hemoglobin, serum ferritin, and ESA treatment status were included. Iron deficiency was defined as ferritin <100 ng/mL. Primary outcomes included mean hemoglobin levels, prevalence of moderate-to-severe anemia, and hematologic response following treatment. Statistical analysis included chi-square tests, independent t-tests, and multivariable logistic regression to determine predictors of improved anemia outcomes. ***Results & Findings:*** Among the 312 CKD patients, 56.4% (n=176) were male and 43.6% (n=136) were female, with a mean age of 54.7 ± 13.2 years. Overall anemia prevalence was 82.1% (n=256), while iron deficiency was observed in 47.8% (n=149) of patients. ESA therapy was administered to 61.5% (n=192) of the cohort. Patients receiving ESAs with adequate iron stores demonstrated significantly higher mean hemoglobin levels (10.6 ± 1.2 g/dL) compared with those with iron deficiency receiving ESA therapy (9.3 ± 1.4 g/dL, $p < 0.001$). Multivariable regression analysis indicated that adequate iron status (OR = 2.41; 95% CI: 1.45–4.02, $p = 0.001$) and ESA therapy (OR = 1.88; 95% CI: 1.12–3.17, $p = 0.017$) were independently associated with improved anemia control. Conversely, advanced CKD stage (stage 5 vs. stage 3: OR = 0.48; 95% CI: 0.28–0.83, $p = 0.008$) and persistent iron deficiency were significant predictors of poor hematologic response. ***Conclusion:*** Iron deficiency remains highly prevalent among patients with progressive CKD and significantly limits the therapeutic effectiveness of ESA treatment. Optimal iron status substantially improves hemoglobin response and anemia outcomes in CKD populations. These findings highlight the importance of routine iron monitoring and integrated anemia management strategies to enhance treatment outcomes in CKD patients in resource-limited tertiary care settings.

Keywords: Chronic kidney disease, anemia, iron deficiency, erythropoiesis-stimulating agents, hemoglobin, CKD progression.

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INTRODUCTION

Anemia represents one of the most prevalent and clinically consequential complications of chronic kidney disease (CKD), affecting a substantial proportion of patients across all stages of disease progression. The global burden of CKD is substantial, with an estimated 850 million individuals affected worldwide, and this prevalence continues to rise, positioning CKD among the most common non-communicable diseases internationally [1]. As kidney function declines, the development of anemia not only diminishes patients' quality of life but also independently contributes to increased mortality risk, cardiovascular complications, and accelerated progression to end-stage kidney disease [2]. The pathophysiology of anemia in CKD is multifactorial, with impaired erythropoietin (EPO) production serving as the primary driver. Under physiological conditions, EPO is synthesized by renal erythropoietin-producing (REP) cells in the proximal peritubular interstitium in response to hypoxia. However, as CKD advances, these REP cells undergo a phenotypic transition to myofibroblasts, progressively losing their capacity to produce EPO [3]. This relative EPO deficiency is compounded by disturbances in iron homeostasis, which play an equally critical role in the development and perpetuation of anemia. The iron-regulatory hormone hepcidin is typically elevated in CKD due to both reduced renal clearance and chronic inflammation, leading to functional iron deficiency by promoting degradation of the iron exporter ferroportin and thereby restricting iron availability for erythropoiesis [4]. Furthermore, inflammatory cytokines, particularly interleukin-6 and tumor necrosis factor-alpha, not only stimulate hepcidin production but also directly suppress erythroid progenitor proliferation and blunt the responsiveness of bone marrow to endogenous and exogenous EPO [5].

Iron deficiency in CKD manifests in two distinct forms: absolute iron deficiency, characterized by depleted iron stores (serum ferritin <100 ng/mL in non-dialysis patients), and functional iron deficiency, where stored iron is inadequately mobilized to meet the demands of erythropoiesis despite normal or elevated ferritin levels [6]. Both forms contribute to anemia and may significantly impair the therapeutic response to erythropoiesis-stimulating agents (ESAs). Recent guidelines from the Kidney Disease: Improving Global Outcomes (KDIGO) 2025 update emphasize that iron status assessment and optimization should precede ESA initiation, with specific diagnostic thresholds recommending iron therapy for non-dialysis patients when ferritin is below 100 ng/mL or transferrin saturation is below 25% [2]. The European Renal Best Practice (ERBP) guidelines similarly advocate for iron repletion as first-line therapy, with ESAs reserved for cases where hemoglobin targets are not achieved despite adequate iron stores [7]. Despite the availability of these evidence-based guidelines, the management of anemia in CKD remains suboptimal, particularly in resource-limited healthcare settings. Studies from Pakistan and other developing countries have reported anemia prevalence rates ranging from 72.5% to 82.1% among CKD patients, with iron deficiency identified as a common but often underrecognized contributor [8,9]. A cross-sectional study from Khyber Teaching Hospital, Peshawar, reported a 73% prevalence of anemia among dialysis-dependent patients, with higher rates observed in females, elderly individuals, and those with diabetes or hypertension [8]. Similarly, a multicenter study from Jamshoro demonstrated that 72.5% of CKD patients presented with anemia, with advanced CKD stages associated with significant alterations in iron parameters, including reduced serum iron and total iron-binding capacity, alongside elevated ferritin and C-reactive protein levels [9]. These findings underscore the complex interplay between inflammation, iron dysregulation, and erythropoietic failure in progressive CKD.

The relationship between iron status and ESA responsiveness is of particular clinical importance. Inflammation-induced hepcidin elevation creates a state of functional iron deficiency that limits the availability of iron for incorporation into hemoglobin, even when ESA therapy effectively stimulates erythroid progenitor proliferation [4]. Consequently, patients with inadequate iron stores or ongoing inflammation may exhibit ESA hyporesponsiveness, necessitating higher doses and increasing the risk of adverse cardiovascular events associated with high ESA exposure [10]. Recent investigations into novel therapeutic approaches, such as hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs), have emerged as an alternative class of agents that stimulate endogenous EPO production and improve iron utilization, offering a non-inferior alternative to traditional ESAs in certain patient populations [11]. In the context of progressive CKD, the combined impact of iron deficiency and ESA utilization on anemia outcomes remains inadequately characterized, particularly in developing healthcare settings where access to diagnostic testing and therapeutic agents may be limited. While international guidelines provide clear recommendations for iron and ESA management, the applicability and real-world effectiveness of these strategies in resource-constrained tertiary care environments warrant further investigation. Moreover, the prevalence of iron deficiency among patients receiving ESA therapy and the extent to which iron status modifies ESA efficacy have not been

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comprehensively evaluated in Pakistani patient populations. Addressing this knowledge gap is essential for developing context-specific anemia management protocols that optimize therapeutic outcomes while minimizing healthcare resource utilization.

Aims and Objectives

The primary aim of this study was to evaluate the association of iron deficiency and erythropoiesis-stimulating agent (ESA) therapy with anemia outcomes among patients with progressive chronic kidney disease (CKD) in tertiary care hospitals in Lahore, Pakistan.

This study was designed to achieve several specific objectives. First, the study aimed to determine the prevalence of anemia among patients with progressive CKD (stages 3–5) receiving care at Rangers Hospital Lahore and Hamid Diagnostic Laboratory, providing a contemporary estimate of the burden of this complication in the local healthcare setting. Second, the study sought to assess the frequency of iron deficiency, defined as serum ferritin less than 100 ng/mL, in the study population and evaluate its distribution across different stages of CKD to understand how iron status varies with disease progression. Third, the study aimed to compare mean hemoglobin levels between patients receiving ESA therapy with adequate iron stores and those receiving ESA therapy with concomitant iron deficiency, thereby quantifying the extent to which iron status modifies the therapeutic response to ESA treatment. Fourth, utilizing multivariable logistic regression analysis, the study sought to evaluate the independent association of iron status and ESA utilization with anemia control, adjusting for potential confounders including age, gender, CKD stage, and comorbid conditions to isolate the true effect of these interventions from other influencing factors. Fifth, the study aimed to identify predictors of poor hematologic response, including advanced CKD stage, persistent iron deficiency, and other clinical factors, to enable risk stratification and targeted intervention strategies. Finally, the study sought to assess the combined impact of iron status and ESA therapy on anemia outcomes, providing evidence that can inform the development of integrated anemia management strategies tailored to the realities of resource-limited healthcare settings in Pakistan and similar developing countries.

Significance of the Study

This study addresses a critical gap in the understanding of anemia management in progressive CKD within the context of a developing healthcare system. Anemia remains a major contributor to morbidity, reduced quality of life, and increased cardiovascular mortality among CKD patients, yet management practices often deviate from established international guidelines due to resource constraints, variability in clinical practice, and limited local evidence.

MATERIALS AND METHODS

Study Design and Setting:

This study employed a multicenter retrospective analytical design, conducted at two tertiary care diagnostic setting in Lahore, Pakistan: Rangers Hospital Lahore and Hamid Diagnostic Laboratory. Both institutions serve as major referral centers for patients with chronic kidney disease (CKD) from the Punjab region, providing comprehensive nephrology services including outpatient clinics, inpatient care, and dialysis facilities. The retrospective design was selected to efficiently evaluate real-world anemia management practices and outcomes over a defined period, leveraging existing clinical records to generate evidence that could inform clinical protocols in resource-limited settings.

Study Duration and Sample:

The study encompassed a twelve-month period from January 2025 to December 2025. Medical records of patients diagnosed with stage 3–5 CKD were systematically reviewed. A total of 312 patients meeting the eligibility criteria were included in the final analysis. The sample size was determined based on the total number of eligible patients with complete records during the study period, ensuring adequate statistical power to detect clinically meaningful differences in anemia outcomes between comparison groups. Consecutive sampling was employed to minimize selection bias, with all eligible patient records reviewed during the specified timeframe.

Inclusion and Exclusion Criteria:

Patients aged 18 years or older with a confirmed diagnosis of CKD stages 3–5 were considered eligible for inclusion. Diagnosis and staging of CKD were based on the Kidney Disease: Improving Global Outcomes (KDIGO) criteria, utilizing estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Stage 3 was defined as eGFR 30–59 mL/min/1.73 m², stage 4 as eGFR 15–29 mL/min/1.73 m², and stage 5 as eGFR <15 mL/min/1.73 m², including patients not yet initiated on dialysis. Inclusion required the availability of complete documentation for at least one hemoglobin measurement, serum ferritin level, and clear documentation of erythropoiesis-stimulating agent (ESA) treatment status during the study period. Patients were excluded if they had active malignancy, acute kidney injury, active bleeding, hematological disorders other than anemia of CKD (e.g., hemolytic anemias, myelodysplastic syndromes), recent blood transfusion within three months prior to the index assessment, or incomplete medical records that

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precluded accurate determination of exposure or outcome variables.

Data Collection:
Data were extracted from electronic medical records and paper-based patient charts using a standardized data collection form developed specifically for this study. The form was pilot-tested on ten patient records to ensure completeness and inter-rater reliability prior to full-scale data extraction. Two trained research assistants independently reviewed records, with any discrepancies resolved through consensus or by consultation with a senior nephrologist. Data collected included demographic characteristics (age, gender), clinical parameters (CKD stage, etiology of kidney disease, presence of comorbid conditions including diabetes mellitus and hypertension), laboratory values (hemoglobin, serum ferritin, transferrin saturation where available, serum creatinine, eGFR), and treatment details (ESA use, type and dose of ESA, iron supplementation status, route of iron administration). All laboratory values were obtained from the hospitals' accredited clinical laboratories, with measurements performed using standardized automated analyzers. Hemoglobin was measured using the cyanmethemoglobin method, while serum ferritin was assessed via chemiluminescent immunoassay.

Variables and Definitions:
The primary exposure variables were iron deficiency status and ESA therapy utilization. Iron deficiency was defined as serum ferritin less than 100 ng/mL, in accordance with KDIGO 2025 guidelines for non-dialysis CKD patients. Patients with ferritin ≥100 ng/mL were classified as having adequate iron stores. ESA therapy was considered present if patients received any dose of an erythropoiesis-stimulating agent (either epoetin alfa, epoetin beta, or darbepoetin alfa) during the study period, with documentation of at least one administration. The primary outcome measures included: mean hemoglobin levels, prevalence of moderate-to-severe anemia, and hematologic response following treatment. Moderate-to-severe anemia was defined as hemoglobin less than 10 g/dL, consistent with established thresholds for clinically significant anemia in CKD populations. Hematologic response was defined as an increase in hemoglobin of at least 1 g/dL over baseline or achievement of hemoglobin ≥10 g/dL following at least three months of ESA therapy or iron repletion, as applicable.

Secondary variables included CKD stage, which was categorized as stage 3, 4, or 5 based on eGFR. Comorbidities such as diabetes mellitus (defined by documented diagnosis, use of antidiabetic medications, or HbA1c ≥6.5%) and hypertension (defined by documented diagnosis, use of antihypertensive medications, or blood pressure readings consistently >130/80 mmHg) were recorded as potential confounders.

Outcome Assessment:
Patients were categorized into four groups based on the combination of ESA therapy and iron status: (1) ESA therapy with adequate iron stores, (2) ESA therapy with iron deficiency, (3) no ESA therapy with adequate iron stores, and (4) no ESA therapy with iron deficiency. Mean hemoglobin levels were compared across these groups to assess the synergistic or antagonistic effects of iron status on ESA efficacy. For patients receiving ESA therapy, hematologic response was assessed at three months following ESA initiation or at the most recent follow-up visit within the study period. For patients not receiving ESA therapy, response was assessed following iron supplementation or conservative management as documented.

Statistical Analysis:
Data were entered into SPSS latest version for analysis. Descriptive statistics were used to summarize demographic and clinical characteristics. Continuous variables were expressed as mean ± standard deviation (SD) after testing for normality using the Shapiro-Wilk test, while categorical variables were presented as frequencies and percentages. The independent t-test was employed to compare mean hemoglobin levels between groups with and without iron deficiency, as well as between ESA-treated and non-ESA-treated groups. Chi-square tests were used to examine associations between categorical variables, including the prevalence of moderate-to-severe anemia across iron status and ESA treatment categories. To evaluate the independent association of iron deficiency and ESA therapy with improved anemia outcomes, multivariable logistic regression analysis was performed. The dependent variable was improved anemia control, defined as hemoglobin ≥10 g/dL at follow-up or achievement of hematologic response as defined above. Independent variables entered into the model included iron status (adequate vs. deficient), ESA therapy (yes vs. no), age (continuous), gender, CKD stage (categorized as stage 3, 4, or 5), and presence of diabetes mellitus and hypertension. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated, and a p-value of less than 0.05 was considered statistically significant. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test, and multicollinearity among independent variables was evaluated using variance inflation factors.

Ethical Considerations
This study was conducted in accordance with the international principles. Ethical approval was obtained from the Institutional Review Boards of Rangers Hospital Lahore and Hamid Diagnostic

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Laboratory, (Reference No. HMIR/IRB/2024-89). Due to the retrospective nature of the study, the requirement for informed patient consent was waived by both ethics committees, as the research involved no more than minimal risk to subjects and utilized existing medical records with appropriate safeguards to protect patient confidentiality. All patient data were anonymized prior to analysis, with unique identification numbers assigned to each record to ensure privacy. Data were stored on password-protected computers accessible only to the research team.

RESULTS

A total of 312 patients with stage 3–5 chronic kidney disease (CKD) who met the eligibility criteria were included in this analysis. The results are presented in a series of tables with accompanying explanatory text, organized according to the study objectives.

Demographic and Clinical Characteristics

The baseline demographic and clinical characteristics of the study population are summarized in Table 1.

Table 1: Baseline Demographic and Clinical Characteristics

Characteristic		*n (%) or mean ± SD*
	Age (years)	54.7 ± 13.2
Gender	Male	176 (56.4)
	Female	136 (43.6)
Comorbidities	Diabetes mellitus	181 (58.0)
	Hypertension	207 (66.3)
CKD Stage	Stage 3 (eGFR 30–59 mL/min)	102 (32.7)
	Stage 4 (eGFR 15–29 mL/min)	121 (38.8)
	Stage 5 (eGFR <15 mL/min)	89 (28.5)
Other Findings	**ESA Therapy**	192 (61.5)
	Iron Deficiency (Ferritin <100 ng/mL)	149 (47.8)
	Serum Ferritin (ng/mL)	147.6 ± 89.4
	Hemoglobin (g/dL)	9.5 ± 1.8

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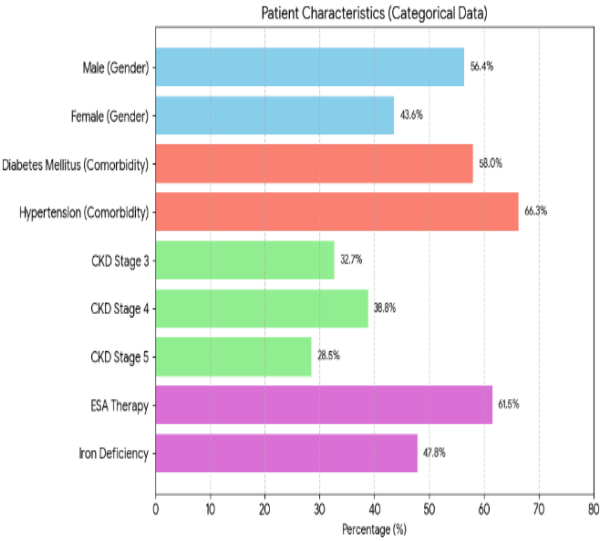

Fig 1: Baseline Demographic and Clinical Characteristics

The mean age of participants was 54.7 ± 13.2 years, with a slight male predominance (56.4%). Diabetes mellitus was present in 58.0% of patients, and hypertension was the most common comorbidity (66.3%). The distribution of CKD stages showed that 32.7% were in stage 3, 38.8% in stage 4, and 28.5% in stage 5. Overall, 61.5% of patients received erythropoiesis-stimulating agent (ESA) therapy during the study period, and 47.8% had iron deficiency defined as serum ferritin <100 ng/mL. The mean serum ferritin was 147.6 ± 89.4 ng/mL, and the mean hemoglobin was 9.5 ± 1.8 g/dL.

Prevalence of Anemia and Iron Deficiency by CKD Stage

The overall prevalence of anemia (hemoglobin <12 g/dL for females, <13 g/dL for males) was 82.1% (n=256). Moderate-to-severe anemia (hemoglobin <10 g/dL) was observed in 44.9% (n=140) of patients. Both any anemia and moderate-to-severe anemia increased significantly with advancing CKD stage ($p < 0.001$, chi-square for trend). Iron deficiency also became more common as CKD progressed: 34.3% in stage 3, 47.1% in stage 4, and 64.0% in stage 5 ($p < 0.001$). These findings are presented in Table 2.

Table 2: Prevalence of Anemia and Iron Deficiency by CKD Stage

Parameter	*Stage 3 (n=102)*	*Stage 4 (n=121)*	*Stage 5 (n=89)*	*Total (N=312)*	*p-value**
Anemia (any), n (%)	72 (70.6)	102 (84.3)	82 (92.1)	256 (82.1)	<0.001
Moderate-to-severe anemia (Hb <10 g/dL), n (%)	28 (27.5)	56 (46.3)	56 (62.9)	140 (44.9)	<0.001
Iron deficiency (ferritin <100 ng/mL), n (%)	35 (34.3)	57 (47.1)	57 (64.0)	149 (47.8)	<0.001

*Chi-square test for trend.

Iron Deficiency and ESA Therapy Utilization

Among patients receiving ESA therapy (n=192), the prevalence of iron deficiency was 51.6% (n=99). In contrast, among those not receiving ESA therapy (n=120), iron deficiency was present in 41.7% (n=50). Although a higher proportion of ESA-treated patients were iron deficient, the difference did not reach statistical significance ($p = 0.086$, chi-square test). Table 3 details the distribution.

Table 3: Iron Deficiency Status by ESA Therapy

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Iron Status	*ESA Therapy (n=192)*	*No ESA Therapy (n=120)*	*Total (N=312)*
Iron deficient (ferritin <100 ng/mL), n (%)	99 (51.6)	50 (41.7)	149 (47.8)
Adequate iron (ferritin ≥100 ng/mL), n (%)	93 (48.4)	70 (58.3)	163 (52.2)

Chi-square = 2.95, p = 0.086.

Hemoglobin Levels According to Combined Iron Status and ESA Therapy

To evaluate the combined effect of iron status and ESA utilization, patients were stratified into four groups. Mean hemoglobin levels for each group are shown in Table 4. Patients receiving ESA with adequate iron stores achieved the highest mean hemoglobin (10.6 ± 1.2 g/dL). Among ESA-treated patients, those with iron deficiency had significantly lower hemoglobin (9.3 ± 1.4 g/dL; mean difference 1.3 g/dL, 95% CI: 0.9–1.7, p < 0.001). In patients not receiving ESA, adequate iron status was associated with a mean hemoglobin of 9.1 ± 1.3 g/dL, whereas iron deficiency was associated with the lowest hemoglobin of 8.2 ± 1.1 g/dL (mean difference 0.9 g/dL, 95% CI: 0.5–1.3, p < 0.001).

Table 4: Mean Hemoglobin by Combined Iron Status and ESA Therapy

Group	*n*	*Hemoglobin (g/dL), mean ± SD*	*95% CI*
ESA + Adequate Iron	93	10.6 ± 1.2	10.3–10.9
ESA + Iron Deficiency	99	9.3 ± 1.4	9.0–9.6
No ESA + Adequate Iron	70	9.1 ± 1.3	8.8–9.4
No ESA + Iron Deficiency	50	8.2 ± 1.1	7.9–8.5

Independent t-tests: ESA + Adequate Iron vs. ESA + Iron Deficiency: t = 6.82, p < 0.001; ESA + Adequate Iron vs. No ESA + Adequate Iron: t = 7.45, p < 0.001; ESA + Iron Deficiency vs. No ESA + Iron Deficiency: t = 5.63, p < 0.001.

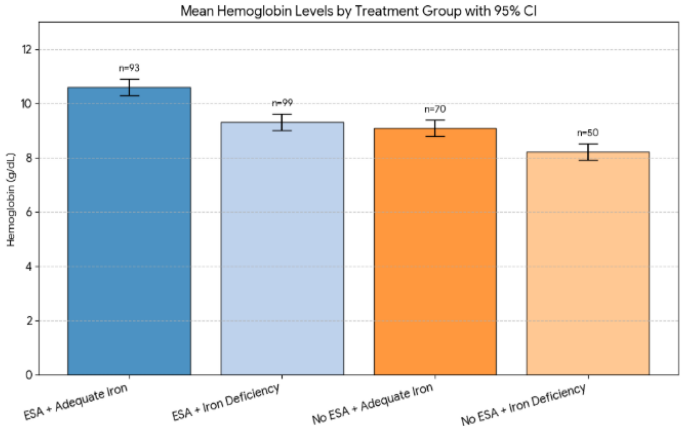

Fig 2: Mean Hemoglobin by Combined Iron Status and ESA Therapy

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Prevalence of Moderate-to-Severe Anemia by Group

The proportion of patients with moderate-to-severe anemia (hemoglobin <10 g/dL) varied significantly across the four groups (chi-square = 68.3, p < 0.001). The lowest prevalence was observed in the ESA + Adequate Iron group (20.4%), while the highest was in the No ESA + Iron Deficiency group (68.0%). Table 5 presents these data.

Table 5: Prevalence of Moderate-to-Severe Anemia by Combined Group

Group	*n*	*Hb <10 g/dL, n (%)*
ESA + Adequate Iron	93	19 (20.4)
ESA + Iron Deficiency	99	45 (45.5)
No ESA + Adequate Iron	70	31 (44.3)
No ESA + Iron Deficiency	50	34 (68.0)

Chi-square = 68.3, p < 0.001.

Hematologic Response to ESA Therapy by Iron Status

Among the 192 patients receiving ESA therapy, hematologic response (defined as an increase in hemoglobin of ≥1 g/dL from baseline or achievement of hemoglobin ≥10 g/dL after three months of ESA therapy) was achieved in 59.4% (n=114). As shown in Table 6, response rates were significantly higher in patients with adequate iron stores compared to those with iron deficiency (71.0% vs. 48.5%; p = 0.002, chi-square test). Additionally, ESA-treated patients with iron deficiency required significantly higher median ESA doses (epoetin alfa equivalents) to maintain hemoglobin levels (10,000 vs. 6,000 units/week; p = 0.011, Mann-Whitney U test).

Table 6: Hematologic Response and ESA Dose by Iron Status Among ESA-Treated Patients

Parameter	*Adequate Iron (n=93)*	*Iron Deficiency (n=99)*	*p-value*
Hematologic response, n (%)	66 (71.0)	48 (48.5)	0.002*
Median ESA dose (units/week)	6,000 (IQR 4,000–8,000)	10,000 (IQR 6,000–12,000)	0.011†

Chi-square test; †Mann-Whitney U test.

Iron Supplementation Practices and Hemoglobin Change

Among the 149 iron-deficient patients, 43.6% (n=65) received intravenous iron, 32.9% (n=49) received oral iron, and 23.5% (n=35) received no iron supplementation. The mean change in hemoglobin from baseline to three months was greatest in the intravenous iron group (1.4 ± 0.7 g/dL), followed by oral iron (0.8 ± 0.5 g/dL) and no supplementation (0.2 ± 0.4 g/dL). Differences between groups were statistically significant (Table 7).

Table 7: Hemoglobin Change by Iron Supplementation Route Among Iron-Deficient Patients

Supplementation Route	*n*	*Δ Hemoglobin (g/dL), mean ± SD*	*95% CI*
Intravenous iron	65	1.4 ± 0.7	1.2–1.6
Oral iron	49	0.8 ± 0.5	0.7–0.9
No iron supplementation	35	0.2 ± 0.4	0.1–0.3

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ANOVA: F = 48.2, p < 0.001. Post-hoc Tukey: Intravenous vs. Oral, p = 0.004; Intravenous vs. None, p < 0.001; Oral vs. None, p < 0.001.

Predictors of Improved Anemia Control: Univariate Analysis

Univariate analysis was performed to identify factors associated with improved anemia control (hemoglobin ≥10 g/dL or hematologic response). Variables with p < 0.10 were considered for inclusion in the multivariable model. Significant associations were found for iron status, ESA therapy, CKD stage, and, to a lesser extent, age. Results are shown in Table 8.

Multivariable Logistic Regression Analysis

Variables with p < 0.10 in univariate analysis (iron status, ESA therapy, CKD stage) were entered into a multivariable logistic regression model, adjusting for age, gender, diabetes, and hypertension. The final model (Table 9) demonstrated that adequate iron status was the strongest independent predictor of improved anemia control (adjusted OR = 2.41; 95% CI: 1.45–4.02; p = 0.001). ESA therapy also remained independently associated with improved outcomes (adjusted OR = 1.88; 95% CI: 1.12–3.17; p = 0.017). Stage 5 CKD was a significant negative predictor (adjusted OR = 0.48; 95% CI: 0.28–0.83; p = 0.008), while age, gender, diabetes, and hypertension were not statistically significant. The Hosmer-Lemeshow goodness-of-fit test confirmed adequate model calibration ($\chi^2 = 7.24$, p = 0.511).

Dependent variable: improved anemia control (hemoglobin ≥10 g/dL or hematologic response). Model adjusted for all listed variables.

Table 8: Univariate Analysis of Factors Associated with Improved Anemia Control

Predictor	*Improved Anemia Control (%)*	*Unadjusted OR (95% CI)*	*p-value*
Iron Status			
Adequate (ferritin ≥100 ng/mL)	68.7	3.12 (1.95–4.98)	<0.001
Deficient	41.6	Reference	
ESA Therapy			
Yes	59.4	1.92 (1.20–3.07)	0.006
No	44.2	Reference	
CKD Stage			
Stage 3	70.6	Reference	
Stage 4	52.1	0.45 (0.26–0.79)	0.005
Stage 5	41.6	0.30 (0.16–0.55)	<0.001
Age (per 10-year increase)	—	0.88 (0.74–1.05)	0.152
Gender			
Male	57.4	1.22 (0.77–1.92)	0.394
Female	52.9	Reference	
Diabetes Mellitus			
Yes	52.5	0.81 (0.51–1.29)	0.376
No	58.8	Reference	
Hypertension			
Yes	53.1	0.86 (0.53–1.40)	0.539
No	58.1	Reference	

Table 9: Multivariable Logistic Regression Analysis for Predictors of Improved Anemia Control

Predictor	*Adjusted OR*	*95% CI*	*p-value*
Adequate Iron Status (Ferritin ≥100 ng/mL)	2.41	1.45–4.02	0.001
ESA Therapy (Yes vs. No)	1.88	1.12–3.17	0.017
CKD Stage (vs. Stage 3)			
Stage 4	0.71	0.41–1.22	0.217
Stage 5	0.48	0.28–0.83	0.008
Age (per 10-year increase)	0.93	0.79–1.09	0.356
Gender (Male vs. Female)	1.21	0.76–1.92	0.423
Diabetes Mellitus (Yes vs. No)	0.84	0.52–1.35	0.468
Hypertension (Yes vs. No)	0.91	0.56–1.48	0.698

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DISCUSSION

This multicenter retrospective study evaluated the association of iron deficiency and erythropoiesis stimulating agent (ESA) therapy with anemia outcomes among 312 patients with progressive chronic kidney disease (CKD) stages 3–5 in two tertiary care hospitals in Lahore, Pakistan. The key findings demonstrate that anemia remains highly prevalent (82.1%) in this population, with nearly half (47.8%) of patients having absolute iron deficiency. Patients receiving ESA therapy with adequate iron stores achieved significantly higher hemoglobin levels and better hematologic response compared to those with iron deficiency. Multivariable analysis confirmed that adequate iron status and ESA therapy were independent predictors of improved anemia control, while advanced CKD stage (stage 5) predicted poor outcomes. These results underscore the critical interaction between iron availability and ESA efficacy and highlight the need for integrated anemia management in resource limited settings. The prevalence of anemia observed in this study (82.1%) is consistent with previous reports from Pakistan and other developing countries. Kumar et al. reported a 72.5% prevalence of anemia among CKD patients in Jamshoro, while Suhail et al. documented a 73% prevalence in dialysis dependent patients in Peshawar [8,9]. The higher prevalence in the present study likely reflects the inclusion of more advanced CKD stages (stage 5: 28.5%) and the use of a lower hemoglobin threshold for anemia definition in non dialysis populations. Internationally, the Chronic Kidney Disease Outcomes and Practice Patterns Study (CKDopps) reported anemia prevalence ranging from 45% to 85% depending on CKD stage and geographic region, with particularly high rates in Asia [13]. Our findings confirm that despite increased awareness, anemia remains a major clinical challenge in CKD care in Pakistan. Iron deficiency was present in 47.8% of our cohort, with a striking increase from 34.3% in stage 3 to 64.0% in stage 5 CKD. This trend aligns with the progressive dysregulation of iron homeostasis that accompanies declining kidney function, characterized by hepcidin accumulation due to reduced renal clearance and chronic inflammation [14,15]. The high prevalence of iron deficiency in non dialysis CKD patients has been increasingly recognized; a recent systematic review by Wong et al. reported pooled prevalence of absolute iron deficiency of 35% in stage 3–5 CKD, with marked regional variation [16]. Our figure of 47.8% is at the higher end of this range, possibly reflecting differences in dietary iron intake, comorbid inflammation, and limited access to routine iron supplementation in our setting. A central finding of this study is the synergistic effect of adequate iron stores on ESA efficacy. ESA treated patients with sufficient iron (ferritin ≥100 ng/mL) achieved a mean hemoglobin of 10.6 g/dL and a hematologic response rate of 71.0%, whereas those with iron deficiency had significantly lower hemoglobin (9.3 g/dL) and response rate (48.5%), despite receiving higher median ESA doses. These results are in line with the landmark trial by Macdougall et al., which demonstrated that intravenous iron supplementation significantly reduced ESA requirements and improved hemoglobin outcomes in CKD patients [17]. More recently, the FIND CKD trial confirmed that proactive intravenous iron administration in non dialysis CKD patients with iron deficiency leads to superior anemia correction and reduced ESA use compared to oral iron or no iron [18]. Our findings extend this evidence by illustrating that in real world practice, iron deficiency acts as a major barrier to ESA effectiveness, resulting in higher drug consumption and suboptimal hemoglobin targets.

The independent association of adequate iron status with improved anemia control (adjusted OR 2.41) and the negative impact of stage 5 CKD (adjusted OR 0.48) underscore the importance of early and systematic iron management. In advanced CKD, the accumulation of uremic toxins, persistent inflammation, and blunted erythropoietin production collectively contribute to ESA hyporesponsiveness [19]. Hepcidin elevation, driven by inflammation and reduced clearance, sequesters iron in reticuloendothelial stores, making it unavailable for erythropoiesis even when ferritin levels appear normal [20]. This functional iron deficiency may explain why some patients with ferritin ≥100 ng/mL still exhibited suboptimal hemoglobin responses. Emerging data suggest that novel markers such as reticulocyte hemoglobin content and hepcidin assays may better capture iron availability for erythropoiesis than ferritin alone [21], but these are not yet widely available in resource limited settings. The high prevalence of iron deficiency among ESA treated patients (51.6%) in our study is concerning, as it indicates that many patients are receiving ESA therapy without adequate iron repletion. This practice pattern contradicts current guidelines from KDIGO and ERBP, which recommend that iron status be assessed and optimized prior to initiating ESA therapy, and that iron supplementation be maintained during ESA treatment to prevent functional iron deficiency [2,7]. Several factors may contribute to this gap: limited availability of intravenous iron formulations, cost constraints, lack of regular iron monitoring, and the misconception that oral iron is sufficient. Our observation that intravenous iron resulted in a significantly greater hemoglobin increment (1.4 g/dL) compared to oral iron (0.8 g/dL) or no supplementation (0.2 g/dL) aligns with recent evidence favoring intravenous iron in CKD patients, particularly in those with inflammation or advanced disease [22]. The finding that patients with iron

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deficiency receiving ESA required higher median ESA doses (10,000 vs. 6,000 units/week) is clinically significant. Higher ESA doses have been associated with increased risk of cardiovascular events, hypertension, and mortality in large observational studies, including the TREAT trial [23]. The recent KDIGO 2025 update emphasizes avoiding high ESA doses by optimizing iron status first [2]. In resource constrained healthcare systems like Pakistan, reducing ESA dose requirements through appropriate iron management can yield substantial cost savings and improve patient safety. Our study also identified advanced CKD stage as a strong negative predictor of anemia outcomes. Patients with stage 5 CKD had only half the odds of achieving improved anemia control compared to those with stage 3. This is consistent with the progressive nature of the disease, where native erythropoietin production is severely diminished, and comorbidities such as inflammation and malnutrition become more prevalent [24]. In stage 5 patients not yet on dialysis, achieving optimal hemoglobin is particularly challenging, as ESA therapy may be initiated late or underdosed. The recent ADVANCE trial showed that early initiation of ESA in stage 4 CKD patients reduced the need for blood transfusions and improved quality of life, but such early intervention requires reliable iron status and regular monitoring [25].

Limitation of the study

The limitations of this study should be acknowledged. First, the retrospective design introduces potential biases related to data completeness and selection; we could only include patients with complete records, which may have excluded those with more severe illness or poor follow up. Second, we used serum ferritin <100 ng/mL as the sole criterion for iron deficiency, which does not capture functional iron deficiency (where ferritin may be normal or elevated but iron is not available for erythropoiesis). Ideally, transferrin saturation or reticulocyte hemoglobin content would have provided additional insight, but these were not consistently available. Third, we did not assess inflammatory markers such as C reactive protein, which could have confounded the relationship between ferritin and ESA responsiveness. Fourth, the study was conducted in two tertiary care centers in a single city, limiting generalizability to other regions of Pakistan or to primary care settings. Fifth, the duration of follow up was limited to the study period, and we could not assess long term outcomes such as cardiovascular events or mortality. Finally, the non randomized nature of treatment assignment (ESA, iron route) means that unmeasured confounders may have influenced the observed associations. Despite these limitations, this study has several strengths. It provides contemporary, real world data from a large cohort of non dialysis CKD patients in a developing country where evidence on anemia management is scarce. The multicenter design enhances external validity within the local context. The use of multivariable regression allowed adjustment for important confounders. The findings highlight actionable gaps in clinical practice that can be addressed through quality improvement initiatives. The results have important clinical and policy implications. First, routine assessment of iron status (ferritin, transferrin saturation) should be integrated into CKD care protocols at tertiary care centers. Currently, such testing is not uniformly performed, leading to missed opportunities for iron repletion. Second, intravenous iron should be prioritized over oral iron in patients with advanced CKD and those requiring ESA therapy, given its superior efficacy and tolerability. Third, ESA therapy should be initiated only after iron stores are optimized, and regular monitoring of both hemoglobin and iron parameters is essential to avoid ESA hyporesponsiveness and excessive dosing. Fourth, healthcare policymakers should consider including intravenous iron formulations in essential medicines lists and hospital formularies, and should support training programs to standardize anemia management.

Future Recommendation

Future prospective studies are needed to evaluate the impact of protocol driven iron repletion strategies on ESA dose requirements, hemoglobin stability, and clinical outcomes in Pakistani CKD patients. Cost effectiveness analyses of intravenous versus oral iron in this setting would also inform resource allocation. Additionally, research incorporating markers of functional iron deficiency and inflammation could refine patient selection for iron therapy and improve prediction of ESA response. The role of novel agents such as hypoxia inducible factor prolyl hydroxylase inhibitors (HIF PHIs) in improving iron utilization and hemoglobin levels in CKD patients with inflammation warrants investigation in local populations.

CONCLUSION

This study demonstrates that iron deficiency is highly prevalent among patients with progressive CKD in Pakistan and significantly compromises the effectiveness of ESA therapy. Optimal iron status is independently associated with improved anemia control and may reduce ESA dose requirements. These findings underscore the urgent need for integrated anemia management strategies that prioritize iron assessment and repletion, particularly in resource limited tertiary care settings, to enhance patient outcomes and optimize healthcare resource utilization.

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