IJMA

International Journal of Medical Arts

VOLUME 6, ISSUE 5, MAY 2024

P- ISSN: 2636-4174
E- ISSN: 2682-3780

Available online at Journal Website
https://ijma.journals.ekb.eg/
Main Subject [Internal Medicine]

Original Article

Serum Erythropoietin Hormone Measurement for Evaluation of Anemia and Red Cell Parameters in Diabetes Mellitus and Diabetic Kidney Disease Patients

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Article information

Received: 08-04-2024

Accepted: 12-05-2024

DOI: 10.21608/IJMA.2024.282217.1954.

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Citation: Alaidy IMM, Hasan FH, Ibrahim EA, Alkhrsawy AMA. Serum Erythropoietin Hormone Measurement for Evaluation of Anemia and Red Cell Parameters in Diabetes Mellitus and Diabetic Kidney Disease Patients. IJMA 2024 May; 6 [5]: 4426-4435. doi: 10.21608/IJMA.2024.282217.1954.

ABSTRACT

Background: Anemia is a common complication of diabetes mellitus and diabetic kidney disease [DKD], leading to significant morbidity and decreased quality of life. Serum erythropoietin [EPO] levels play a crucial role in the regulation of erythropoiesis, and alterations in EPO levels may contribute to the development of anemia in these patients.

Aim of the work: This study aimed to investigate the utility of serum EPO hormone measurement in evaluating anemia and red cell parameters in patients with diabetes mellitus and DKD. The study sought to assess the correlation between serum EPO levels, hemoglobin concentration, hematocrit, and other red cell indices in these populations.

Patients and Methods: A total of 120 patients grouped into 3 groups; 40 patients with diabetes mellitus and no renal abnormality [eGFR>90 ml/min/1.73 m2], 40 patients with DKD [eGFR 15-90 ml/min/1.73 m2], and 40 healthy control. Serum EPO levels were measured and correlated with hemoglobin levels, hematocrit and various red cell parameters. Renal function, HbA1c and iron profile were also collected and analyzed.

Results: The results indicated a significant association between serum EPO levels and anemia severity in patients with diabetes mellitus and DKD. In diabetic patients without renal issues, EPO correlated positively with Hb and ESR but inversely with RBCs, CRP, and serum ferritin. In diabetic patients with CKD, EPO linked positively with Hb and eGFR but negatively with UACR, WBCs, RBCs, and serum ferritin. EPO was a significant predictor of anemia in both groups.

Conclusion: Serum EPO measurement provides valuable insights into the pathogenesis of anemia in diabetes mellitus and DKD patients. Monitoring serum EPO levels may aid in the assessment of anemia severity, guide appropriate treatment strategies, and offer prognostic information regarding kidney function in diabetic individuals.

Keywords: Erythropoietin; Diabetes Mellitus; Chronic Kidney Failure; Anemia; Erythrocyte Indices.

INTRODUCTION

Diabetes mellitus [DM] is a metabolic disorder resulting from either insufficient insulin production or reduced tissue sensitivity to insulin, leading to abnormalities in the metabolism of carbohydrates, fats, and proteins [1]. Diabetes mellitus can be primarily categorized into Type 1 DM [T1DM] and Type 2 DM [T2DM] depending on the need for insulin. T1DM arises from an autoimmune response targeting the pancreas's beta cells responsible for producing insulin, leading to insufficient insulin production. On the other hand, T2DM stems from insulin resistance and is frequently observed in adults [2, 3].

T2DM presents a significant worldwide health challenge due to its linked high rates of illness and death, with its occurrence steadily on the rise. The International Diabetes Federation reported a global diabetes prevalence of 9.3% [463 million individuals] in 2019, with projections indicating an increase to 10.2% by 2030 and 10.9% by 2045 [4].

Endogenous erythropoietin [EPO] is a sugar-protein hormone produced naturally by kidney peritubular cells, primarily responsible for stimulating the production of red blood cells [5]. While the kidneys are the main site of EPO production, small amounts are also synthesized in the spleen, liver, bone marrow, lung, and brain. The level of EPO production is influenced by the oxygen pressure in the environment, with lower oxygen levels leading to higher EPO production [6].

Persistent high blood sugar levels in diabetes mellitus directly contribute to the development of an inflammatory condition [7] through increased expression of proinflammatory cytokines like Interleukin [IL]-1, IL-6, tumor necrosis factor-α [TNF-α], transforming growth factor-β, and interferons [8]. Some of these cytokines play a role in the apoptosis of erythroid progenitor cells [9], have an anti-erythropoietic effect, alter the responsiveness of progenitor cells to erythropoietin [Epo] [10], and facilitate the apoptosis of immature red blood cells, leading to a reduction in the number of circulating red blood cells and eventually causing anemia [11].

Long-term high blood sugar levels in diabetes mellitus can lead to the formation of abnormal red blood cells, increased oxidative stress, and sympathetic nerve damage in the kidneys due to autonomic neuropathy [12]. These conditions create a hypoxic setting in the kidney's interstitium, resulting in reduced production of Erythropoietin by peritubular fibroblasts. Inadequate levels of Erythropoietin serve as a significant factor contributing to anemia in individuals with diabetes mellitus [13].

Diabetic kidney disease, known as chronic kidney disease [CKD] linked to diabetes or diabetic nephropathy, is characterized in both type 1 and type 2 diabetes by the persistent presence of significantly increased albumin levels exceeding >300 mg/24 h [or >200 μg/min], or an elevated albumin-to-creatinine ratio [ACR] surpassing >300 mg/g, validated across a minimum of 2 out of three samples. This definition also requires the simultaneous existence of diabetic retinopathy and the absence of indications of alternative renal conditions [14].

Diabetic kidney disease [DKD] can result in anemia which can lower quality of life and raise risks of illness and death. The reasons for anemia in CKD include reduced production of EPO, deficiencies in iron either absolute or functional, and elevated hepcidin levels due to inflammation [15].

Given the intricate interplay between erythropoiesis, erythropoietin hormone regulation, and the patho-physiology of anemia in diabetes and diabetic kidney disease, investigating serum EPO levels can provide crucial insights into the mechanisms underlying anemia development. Understanding the relationship between serum EPO levels, hemoglobin concentrations, and red cell indices can aid in the early detection, monitoring, and management of anemia in diabetic patients, potentially leading to improved patient care and outcomes.

The aim of this study is to determine anemia and Red Blood Cell abnormalities and their correlation with renal functions in diabetes mellitus and diabetic kidney disease patients by measurement of serum erythropoietin hormone.

PATIENTS AND METHODS

This was a cross-sectional study conducted on diabetes mellitus patients with and without chronic kidney disease at Clinical pathology Department of Al-Azhar University Hospital in Damietta and Damietta Specialized Hospital. All patients were divided into 3 groups according to the MDRD equation [Modification of Diet in Renal Disease].

Group I: Included 40 diabetic patients without renal abnormalities with an eGFR>90 ml/min/1.73 m2.

Group II: Included 40 diabetic patients with variable degrees of DKD with eGFR 15-90 ml/min/1.73 m2

Group III [control group]: Included 40 participants without Diabetes mellitus, CKD or any chronic medical diseases.

Inclusion criteria: Patients with T2DM older than 18 years old. Patients with Diabetic kidney disease on non-hemodialysis conservative therapy.

Exclusion criteria: Critically ill and post-surgery patients, patients with known anemic, hematological or bleeding disorders or any malignancy, patients with history of blood transfusion last 3 months ago and pregnant women.

Data collection

General examination included assessing the patient's overall appearance specially weight, height, Body mass index and vital signs [such as blood pressure, temp., heart rate, respiratory rate] and any signs of distress or discomfort.

For the evaluation of anemia and red cell parameters, Complete Blood Count [CBC] are conducted with RBCs count, Hemoglobin [Hb] level, Mean Corpuscular Volume [MCV], Red Cell Distribution Width [RDW], Mean Corpuscular Hemoglobin [MCH], Mean Corpuscular Hemoglobin Concentration [MCHC], WBC count and differential and Platelet count. In addition, HbA1c, Inflammatory Markers [ESR and CRP], Serum iron and ferritin were determined using an immunonephelometry assay. Urine sample for measuring urine albumin-to-creatinine ratio [ACR] was obtained.

Estimated Glomerular Filtration Rate [eGFR] is calculated using formulas that take into account variables such as creatinine levels, age, sex, and race according to the MDRD equation

$$\mathbf{[GFR\ [mL/min/1.73\ m^2] = 175 \times [S_{cr}]^{-1.154} \times [Age]^{-0.203} \times [0.742\ if\ female]}$$

Serum Erythropoietin Hormone testing was done by Human Erythropoietin, EPO ELISA KIT [ELK Biotechnology cat# ELK1011] and analyzed by Sunrise Absorbance ELISA Reader, Tecom, Austeria, and GmbH. REF: 16039400, SN 607000051.

All participants were examined in the morning after an overnight fast of 10–12 hours. A 3 ml sample of fasting venous blood was taken from all participants. The serum was isolated within 2 hours, frozen at -80° C, and utilized for analysis within 3 months.

Ethical Consideration: Prior to participants' enrollment in the study, the objectives and design of the research, along with the evaluation of risks and benefits, were communicated to them. Their informed consent was obtained. Approval from the Institutional Review Board [IRB] at the Faculty of Medicine, Al-Azhar University, Cairo, Egypt, was secured.

Statistical Analysis: The statistical analysis was performed using SPSS version 28 [IBM Co., Armonk, NY, USA]. Quantitative data were expressed as mean and standard deviation [SD] and analyzed using ANOVA [F] test with post hoc test [Tukey]. Categorical data were presented as frequency and percentage [%] and analyzed using the Chi-square test. Pearson's correlation coefficient was calculated to assess the correlation between two quantitative variables. The diagnostic performance of EPO was evaluated using ROC curve analysis with area under the curve [AUC], where an AUC greater than 50% signifies acceptable performance and an AUC close to 100% indicates excellent test performance. Statistical significance was set at a two-tailed P-value < 0.05.

RESULTS

As demonstrated in Table 1, there was a statistically significant difference among the three groups in terms of age and sex distribution. Patients with chronic kidney disease [CKD] exhibited significantly higher age and male prevalence compared to patients without renal abnormalities and controls. Furthermore, there was a statistically significant variance in BMI among the three groups, with patients with CKD having a notably higher BMI than the control group.

In Table 2, RBCs differed significantly among the studied groups [P=0.002]. WBCs also showed significant variation among the three groups [P=0.01], with a higher count in group I compared to group III [7.62 ± 1.66 vs 6.49 ± 1.49 x 103 cells/µl, P=0.013]. Group I and III had similar WBC counts and both differed insignificantly from group II [p=0.05, p=0.863 respectively].

Hb levels differed significantly among the groups [P<0.001], with a notable decrease in group II compared to groups I and III [11.85 ± 1.22 vs 11.06 ± 1.18, 13.02 ± 1.13 g/dL, P=0.003, <0.001 respectively].

MCV and MCH also exhibited significant differences across the three groups [P<0.001], being lower in groups I and II as opposed to group III [79.31 ± 6.57, 77.49 ± 7.06 vs 93.34 ± 5.6 fL for MCV; 26.26 ± 2.38, 25.61 ± 2.7 vs 28.51 ± 2.62 pg per cell for MCH].

A significant discrepancy was observed in S. iron levels among the groups [P<0.001], with group II having significantly lower levels than group I [P=0.005], and both groups exhibiting lower levels than the control group [31.11 ± 12.59 vs. 42.83 ± 15.20 vs. 57.08 ± 15.96, P< 0.001, <0.001 respectively].

Regarding S. ferritin, a significant difference was found among the three groups [P<0.001], with group II having significantly higher levels than group I [P<0.001], and both groups showing higher levels compared to the control group [201.95 ± 101.18 vs 90.43 ± 31.44 vs 61.75 ± 17.61, P=0.041, <0.001 respectively].

A significant difference in EPO levels was noted among the three groups [P<0.001], as diabetic patients without renal abnormalities and those with CKD had lower EPO levels than the control group [554.14 ± 297.39 vs 338.28 ± 223.79 vs 991.40 ± 521.35 IU/L respectively].

Anemia, defined as Hb < 13 g/dL in men and < 12 g/dL in women, exhibited a statistically significant difference among the three groups [P=0.001]. The incidence of anemia was significantly higher in diabetic patients with CKD compared to those without renal abnormalities or the controls [67.5% vs 95.0%, 20.0% respectively, P=0.001, <0.001]. Furthermore, diabetic patients without renal abnormalities and the controls showed comparable rates of anemia.

A statistically significant difference was observed among the studied groups in terms of HbA1C, with groups I and II being similar while both showing higher HbA1C levels than the control group [P<0.001].

A statistically significant difference was found in terms of inflammatory markers among the three groups [P<0.001]. ESR was higher in group II compared to group I, with both groups showing significantly higher levels than the control group [P<0.001]. Concerning CRP, levels were similar between groups I and II, but significantly higher in both compared to the control group [P= 0.008, <0.001 respectively], as demonstrated in table [3].

In diabetic patients without renal abnormalities, a significant positive correlation was observed between EPO and both Hb [g/dL] [r=0.872, P < 0.001] and ESR [r=0.388, P=0.018]. Conversely, an inverse correlation of significant value was noted between EPO and each of RBCs [r=-0.362, P=0.027], CRP [r=-0.366, P=0.026], and serum ferritin [r=-0.476, P=0.002]. In diabetic patients with varying degrees of CKD, EPO showed significant positive correlations with Hb [g/dL] [r=0.607, P<0.001] and eGFR [r=0.400, P= 0.032]. It was also negatively correlated with UACR [r=-0.423, P=0.003], WBCs [r=-0.56, P<0.001], RBCs [r=-0.565, P<0.001], and serum ferritin [r=-0.490, P=0.001] as shown in table [4].

In diabetic patients without renal abnormalities, EPO was identified as a significant predictor of anemia [AUC=0.714, P value=0.017]. The suggested cut-off value [>405 IU/L] demonstrated 87.7% sensitivity, 66.7% specificity, 72.4% PPV, and 84.2% NPV [figure 3 and table 5].

In diabetic patients with CKD, EPO was found to be a significant predictor of anemia [AUC = 0.829, P value<0.001]. The suggested cut-off value [>430 IU/L] showed 73.3% sensitivity, 78.6% specificity, 81.3% PPV, and 92.1% NPV [figure 4 and table 5].

Table [1]: Baseline characteristics of the studied groups

		Group I [n=40]	Group II [n=40]	Group III [n=40]	P value
Age [years]	Mean ± SD	59.38 ± 10.88	67.13 ± 7.85	34.18 ± 11.19	**<0.001***
	Range	35 – 76	45 - 80	21 – 63	
Pairwise comparison		P1=**0.043**, **P2<0.001***, **P3<0.001***			
Sex	Male	28 [70%]	32 [80%]	13 [32.5%]	**<0.001***
	Female	12 [30%]	8 [20%]	27 [67.5%]	
Pairwise comparison		P1=0.753, **P2=0.046***, P3=0.321*			
BMI [kg/m^2]	Mean ± SD	28.57 ± 3.11	28.29 ± 3.27	26.95 ± 2.81	**0.045***
	Range	24.1 - 37.7	23.7 - 37.5	21.8 - 35.4	
Pairwise comparison		P1=0.753, **P2=0.046***, P3=0.321*			

P1: Comparison between group I and group II, P2: Comparison between group I and group III, P3: Comparison between group II and group III.

Table [2]: Different hematological parameters of the studied groups

		Group I [n=40]	Group II [n=40]	Group III [n=40]	P value
Hb [g/dL]		11.85 ± 1.22	11.06 ± 1.18	13.02 ± 1.13	**<0.001***
		9 – 13.9	9 – 12.8	11.5 – 15.6	
Pairwise comparison		**P1=0.003*, P2<0.001*, P3<0.001***			
WBCs [x10^{3}cells/µl]		7.62 ± 1.66	6.69 ± 2.06	6.49 ± 1.49	**0.01***
		4 - 10.9	3.3 - 10.9	4.5 - 10.3	
Pairwise comparison		P1=0.05, **P2=0.013***, P3=0.863			
PLTs [x10^{3}cells/µl]		294.88 ± 69.19	253.13 ± 41.97	303.35 ± 66.16	**<0.001***
		153 - 455	176 – 367	178 – 425	
Pairwise comparison		**P1=0.007***, P2=0.805, **P3<0.001***			
RBCs [x10^{3}cells/µl]		5.01 ± 0.68	4.69 ± 0.55	5.19 ± 0.65	**0.002***
		3.54 - 6.71	3.41 – 7	3.6 - 5.9	
Pairwise comparison		P1=0.06, P2=0.423, **P3=0.002***			
MCV [fL]		79.31 ± 6.57	77.49 ± 7.06	93.34 ± 5.6	**<0.001***
		64.4 - 91.9	57.4 - 87.7	83 – 104	
Pairwise comparison		P1=0.421, **P2<0.001*, P3<0.001***			
MCHC [g/dL]		33.48 ± 1.3	33.11 ± 1.09	32.74 ± 2.54	0.175
		30.8 - 37	30.1 - 34.8	29.7 – 38	
MCH [pg/cell]		26.26 ± 2.38	25.61 ± 2.7	28.51 ± 2.62	**<0.001***
		21.7 - 31	17.3 - 29.1	20.3 – 34	
Pairwise comparison		P1=0.496, **P2<0.001*, P3<0.001***			
S. iron [mcg/dL]		42.83 ± 15.20	31.11 ± 12.59	57.08 ± 15.96	**<0.001***
		15 – 89	12 – 71	34 – 86	
Pairwise comparison		**P1=0.005*, P2<0.001*, P3<0.001***			
Serum ferritin [ng/mL]		90.43 ± 31.44	201.95 ± 101.18	61.75 ± 17.61	**<0.001***
		47 – 162	96 – 462	28 – 97	
Pairwise comparison		P1=**<0.001***, P2=0.041, **P3<0.001***			
EPO [IU/L]		554.14 ± 297.39	338.28 ± 223.79	991.40 ± 521.35	**<0.001***
		126 - 1127	111 - 991	245 – 1940	
Pairwise comparison		P1=0.065, **P2<0.001*, P3<0.001***			
Frequency of anemia	Non anemic	13 [32.5%]	2 [5.0%]	32 [80.0%]	**< 0.001***
	Anemic	27 [67.5%]	38 [95.0%]	8 [20.0%]	
Pairwise comparison		**P1=0.001*, P2=<0.001*, P3=<0.001***			
HbA1C [%]		7.3 ± 1.47	7.78 ± 1.93	5.2 ± 0.7	**<0.001***
		5.6 - 11	5.2 - 11.5	3 - 6.4	
Pairwise comparison		P1=0.315, **P2<0.001*, P3<0.001***			

P1: Comparison between group I and group II, P2: Comparison between group I and group III, P3: Comparison between group II and group III, Hb: Hemoglobin, WBCs: White blood cells, PLTs: Platelets, RBCs: Red blood cells, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin.

Table [3]: Inflammatory markers of the studied groups

	Group I [n=40]	Group II [n=40]	Group III [n=40]	P value
ESR [mm/hr]	23.03 ± 14.39	32.75 ± 12.35	10.34 ± 4.12	**<0.001***
	3 - 75	18 – 67	3 – 22	
Pairwise comparison	**P1<0.001*, P2<0.001*, P3<0.001***			
CRP [mg/dL]	8.45 ± 9.08	9.85 ± 4.41	4 ± 1.48	**<0.001***
	2 - 55	4 – 22	2 – 7	
Pairwise comparison	P1=0.566, **P2=0.008*, P3<0.001***			

Table [4]: Correlation between EPO and different laboratory investigations in diabetic patients with and without renal abnormalities

	Group I, DM with no renal abnormalities [n=40]		Group II, DKD [n=40]	
	EPO [IU/L]			
	r	P value	r	P value
UACR [mg/g]	-0.1	0.546	-0.423	**0.003***
Hb [g/dL]	**0.872**	**<0.001***	**0.607**	**<0.001***
WBCs [x10^{3}cells/µl]	-0.024	0.886	-0.56	**<0.001***
PLTs [x10^{3}cells/µl]	0.083	0.626	-0.125	0.520
RBCs [x10^{3}cells/µl]	-0.362	**0.027***	-0.565	**<0.001***
MCV [fL]	-0.113	0.504	-0.022	0.909
MCHC [g/dL]	0.067	0.694	0.069	0.721
MCH [pg/cell]	-0.092	0.588	-0.041	0.833
HbA1C [%]	0.247	0.141	-0.163	0.398
ESR [mm/hr]	0.388	**0.018***	-0.220	0.252
CRP [mg/dL]	-0.366	**0.026***	0.045	0.811
Serum creatinine [mg/dL]	-0.084	0.622	0.349	0.063
BUN [mg/dL]	-0.134	0.428	0.114	0.557
Uric acid [mg/dL]	-0.063	0.698	0.092	0.636
eGFR [ml/min/1.73 m^2]	0.096	0.570	0.400	**0.032**
S. iron	-0.121	0.475	-0.269	0.159
S. ferritin	**-0.476**	**0.002***	**-0.490**	**0.001***

r: Pearson's correlation coefficient, *: Statistically significant as P value<0.005

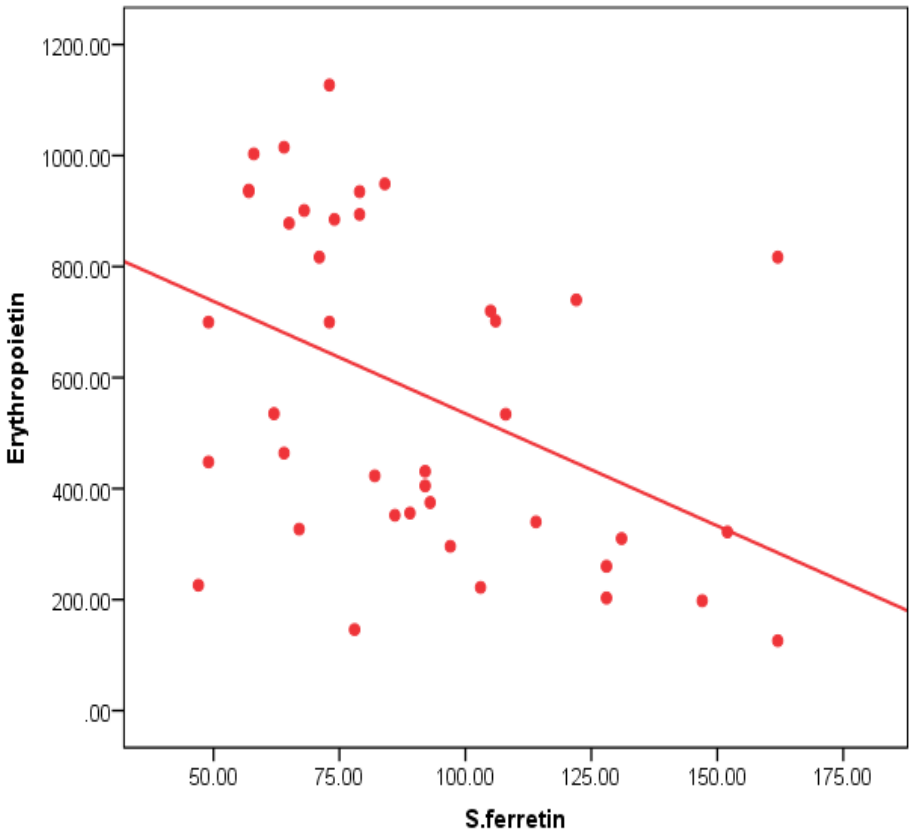

Figure 1: Negative Correlation between Erythropoietin and serum ferritin in diabetic patients with no renal impairment

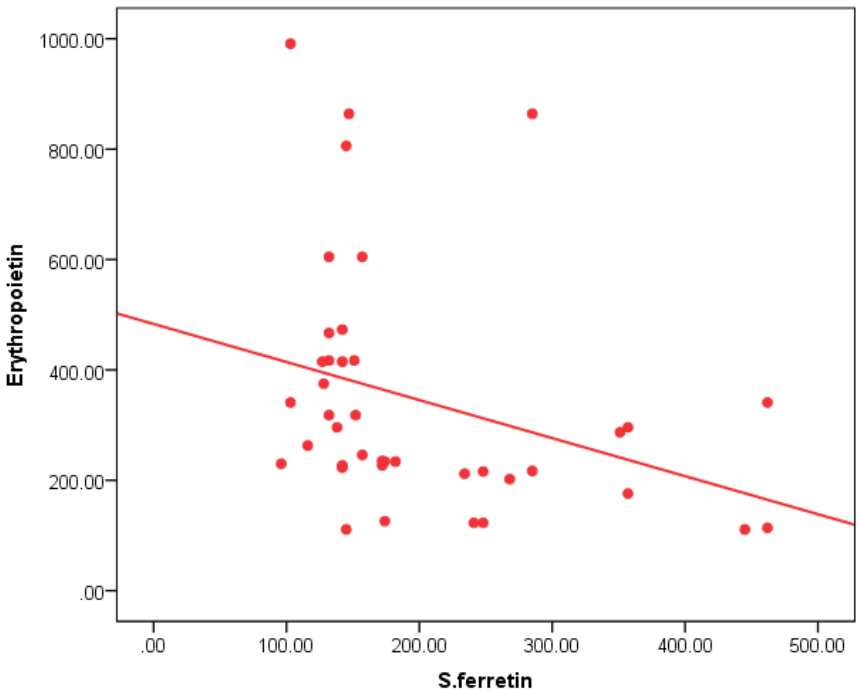

Figure 2: Negative Correlation between Erythropoietin and serum ferritin with variable degrees of CKD

Table 5: Diagnostic performance of EPO in predicting anemia

EPO [IU/L]	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P value
Diabetes with no CKD	>405	87.5	66.7	72.4	84.2	0.714	**0.017***
Diabetes with CKD	>430	93.3	78.6	81.3	92.1	0.829	**<0.001***
Control	>1129	94.1	63.2	71.9	91.5	0.762	**0.002***

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, *: Statistically significant as P value<0.05.

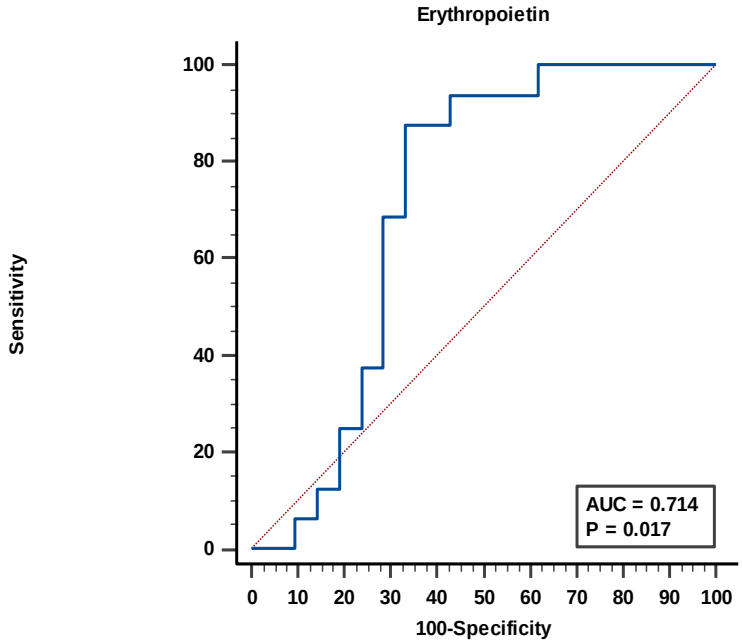

Figure [3]: ROC curve analysis of EPO for predicting anemia in diabetic patients with no renal abnormalities

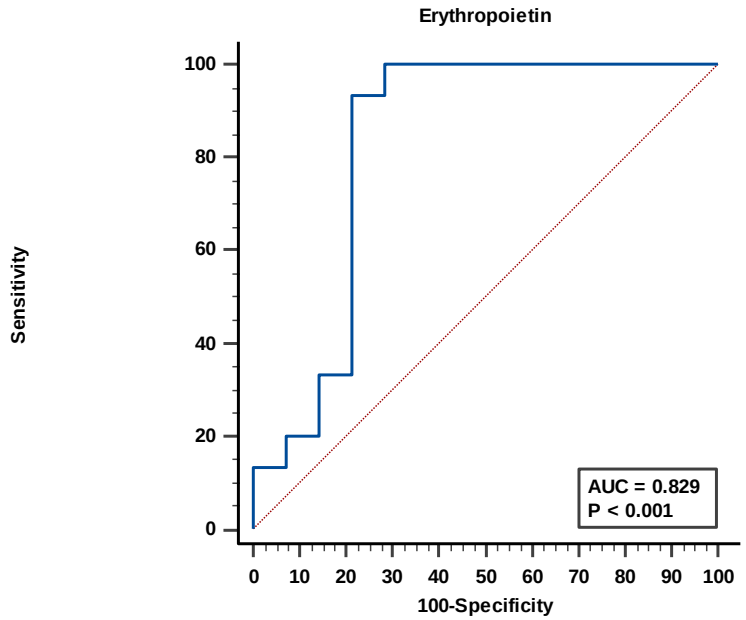

Figure [4]: ROC curve analysis of EPO for predicting anemia in diabetic patients with CKD

DISCUSSION

The results of this study provide important insights into the role of erythropoietin in anemia seen in patients with DM and DKD. Decreased erythropoietin production in the kidneys in response to renal impairment appears to be a key factor influencing anemia in diabetic kidney disease. These results help enhance our understanding of the interplay between diabetes, kidney function, erythropoietin regulation and the development of anemia.

Diabetes mellitus [DM] has been linked to various hematological alterations that impact red blood cells [RBCs]. Elevated blood sugar levels contribute to continuous increases in glycosylated hemoglobin, leading to structural and functional modifications in the hemoglobin molecule, abnormalities in osmotic balance, and changes in cytoplasmic viscosity within each RBC. These modifications can influence several RBC indices such as RBC count, hematocrit [HCT], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and red cell distribution width [RDW] [11, 16, 17].

Diabetic individuals often lack essential nutrients like cyanocobalamin, folate, and iron, leading to various forms of anemia. Metformin can hinder the absorption of cyanocobalamin, causing a deficiency that results in vitamin B12-deficiency anemia [18]. Since anemia and type 2 diabetes exhibit similar symptoms such as pale skin, chest pain, numbness in extremities, coldness, breathlessness, and headaches, anemia often goes unnoticed in diabetic patients. Therefore, detecting anemia in diabetic patients is crucial [19].

The likelihood of anemia occurrence among diabetic patients with kidney disease is notably higher and tends to be more severe at an earlier stage compared to diabetic patients without kidney issues. Individuals with diabetes and anemia who also suffer from heart failure and/or kidney disorders face increased mortality rates. Despite being common, anemia is frequently disregarded in individuals with diabetes, who may face increased susceptibility to the negative impacts of anemia, particularly when coupled with cardiovascular issues and organ damage induced by oxygen deprivation. Anemia can also forecast the advancement of diabetes complications [20-22].

Our results revealed a statistically significant difference among the three groups in terms of EPO levels [P<0.001]. Diabetic patients without renal abnormalities and those with CKD exhibited lower EPO levels compared to the control group [554.14 ± 297.39 vs. 338.28 ± 223.79 vs. 991.4 ± 521.35 IU/L, respectively].

In line with the findings of **Hayder *et al.*** [23] conducted a study involving 172 subjects matched for age and sex, with 130 classified as patients and 42 as healthy individuals, our results demonstrated a notable reduction in EPO levels across all three patient groups compared to the control group [P<0.001]. Furthermore, a substantial

decline in EPO levels was observed in the NIDDM+ESRD group compared to the NIDDM group [P<0.001], with no significant difference noted when compared to the ESRD group. Also, **Sultan *et al.*** [24] who reported that there was a significant difference between the studied groups regarding EPO. In addition, **EL Okel *et al.*** [25] reported that GFR levels showed no significant difference between the studied diabetic and non-diabetic CKD patients.

In patients with DKD, anemia can develop when the glomerular filtration rate [GFR] falls below 45 mL/min/1.73 m2 as a result of reduced erythropoietin [EPO] production in the kidneys, leading to impaired stimulation of red blood cell production in the bone marrow [26].

The study revealed significant positive correlations between EPO and Hb, ESR in diabetic patients without renal issues, and with Hb, eGFR in those with varying CKD degrees. Conversely, EPO showed inverse correlations with RBCs, CRP, UACR, WBCs, and RBCs in the respective groups.

A study by **Panjeta *et al.*** [27] found that the median EPO levels in groups with stage 1 and 2 CKD were significantly higher than the control group [p=0.002 and p=0.018 respectively]. Meanwhile, the median EPO levels in groups with stage 3&4 CKD were significantly lower than the control group [p=0.03 and p=0.011 respectively]. On the other hand, a study by **Sultan *et al.*** [24] demonstrated a strong positive correlation between EPO levels and hemoglobin levels [r=0.56] as well as EPO levels and glomerular filtration rate [GFR] [r=0.54]. They also found a moderate negative correlation between EPO levels and urea levels [r=-0.46] as well as EPO levels and creatinine levels [r=-0.45]. The study measured EPO levels in the blood serum of patients with diabetic kidney disease [DKD].

In diabetic patients without kidney issues, low levels of EPO were a reliable indicator of anemia [AUC=0.714, P=0.017]. Cut-off [>405 IU/L] had 87.7% sensitivity, 66.7% specificity. In diabetic patients with CKD, low EPO predicted anemia [AUC=0.829, P<0.001], cut-off [>430 IU/L] had 73.3% sensitivity, 78.6% specificity.

A study by **She *et al.*** [28] found that decreased EPO may be responsible for worsening kidney structure and function. Their research showed that EPO plays a direct and important role in protecting the kidneys by interacting with and activating erythropoietin receptors on kidney cells, which suppresses apoptosis or cell death.

There are various interrelated factors that lead to declining hemoglobin levels in patients with worsening chronic kidney disease [CKD] over time. However, reduced EPO production by deteriorating kidneys is a key driver, according to another source. The source stated that declining kidney function is a crucial cause of lower hemoglobin levels as CKD progresses [29].

In a study by **EL Okel *et al.*** [25], it was noted that the reduction in HbA1c post-EPO therapy corresponded significantly with the rise in Hb levels in diabetic and non-diabetic CKD populations [r= 879, p < 0.001, and r=−0.879, P < 0.001] respectively. They concluded that changes in Hb percentage should be considered during the evaluation of HbA1c levels when utilizing EPO to treat anemia in patients with diabetes and CKD.

We concluded that EPO is a very specific predictor for diagnosing renal function in diabetic patients with anemia. In those without renal abnormalities, a decreased EPO level was a significant predictor of anemia [AUC=0.714, P value<0.017], with a suggested cutoff value [>405 IU/L] showing 87.7% sensitivity, 66.7% specificity, 72.4% PPV, and 84.2% NPV. In diabetic patients with CKD, a decreased EPO level was also a significant predictor of anemia [AUC=0.828, P value<0.001], with a suggested cutoff value [>430 IU/L] showing 73.3% sensitivity, 78.6% specificity, 81.3% PPV, and 92.1% NPV. Serial EPO measurements can assist in monitoring renal status changes and guiding anemia management in these patients.

Financial and Conflict of Interest: Nil

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IJMA

International Journal of Medical Arts

VOLUME 6, ISSUE 5, MAY 2024

P- ISSN: 2636-4174
E- ISSN: 2682-3780