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Erythropoietin and Red Progenitor Cell Function in Diabetic Patients with Kidney-Marrow Axis Disorder Under the Effect of Glucose Instability

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ABSTRACT

Background: *Chronic hyperglycemia alters metabolism beyond the blood vessels. It can also change the control of blood cells. It was essential to find out the relationship between glycemic instability based on HbA1c and renal medullary response.* ***Objective:*** *We closely monitored the secretion of erythropoietin (EPO) and the efficiency of erythroid activity in the bone marrow.* ***Methods:*** *In the period from February to October 2025, we conducted a prospective analytical study on 60 patients aged 45-85 years done at Al-Najaf Teaching Hospital. The study group included type 2 diabetes mellitus patients (n = 30) and healthy controls (n = 30). High-performance liquid chromatography (HPLC) was used to measure glycemic status. The measurement of serum erythropoietin levels was conducted through high-sensitivity enzyme-linked immunosorbent assay (ELISA); while hematological indices were analyzed through automated flow cytometry.* ***Results:*** *Diabetic patients showed significantly lower serum erythropoietin levels when compared with control subjects. The mean EPO level in diabetic patients was 7.4 ± 1.5 mIU/mL compared to a mean of 12.8 ± 2.1 mIU/mL in controls ($P < 0.001$). Despite the presence of clinical anemia, this reduction was noticed. A similar negative linear association was found between HbA1c and serum levels of EPO ($r = 0.68$). Moreover, the reticulocyte index was decreased suggesting impaired erythropoietin activity and the establishment of functional "EPO resistance" in the marrow. Chronic exposure of cells to high glucose levels seems to impair HIF-2α-regulated renal hypoxia-sensing.* ***Conclusion:*** *This interruption might lower the output of erythropoietin and weaken the response of erythroid progenitor cells. Based on the results, serum erythropoietin could be a useful biochemical marker for the detection of early or subclinical bone marrow dysfunction in elderly diabetic patients.*

Keywords : Chronic Hyperglycemia, Erythropoietin, Medullary Erythroid Efficiency, EPO Resistance, Glycemic Instability, HbA1c, Advanced Glycation End-products, Eryptosis, Renal Hypoxia-sensing .

Article Information

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1. INTRODUCTION

Erythropoiesis is a tightly regulated physiological process that ensures adequate production of red blood cells in response to tissue oxygen demand (Krantz, 1991; Macdougall & Cooper, 2019). A key regulator of this process is erythropoietin (EPO), a glycoprotein hormone produced primarily by peritubular cells in the renal cortex under hypoxic conditions (Fujita et al., 2019; Gilbert, 2017). The transcription of the EPO gene is controlled by the Hypoxia-Inducible Factor (HIF) pathway, in which the HIF-2α isoform plays a predominant role in oxygen sensing

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and transcriptional activation of EPO in the kidney and liver (Fujita et al., 2019; Nangaku et al., 2013). Under normoxia, prolyl hydroxylase domain (PHD) enzymes hydroxylate HIF-α subunits, targeting them for proteasomal degradation; during hypoxia, PHD activity declines, allowing HIF-2α stabilization and increased EPO expression to stimulate red blood cell production (Eleftheriadis et al., 2017). Research has shown that HIF-2α is required for hypoxia induced EPO gene expression and stimulation of the erythroid progenitor maturation. Also, HIF pathway intricate regulation of erythropoiesis has been analysed (Ferraro et al., 2011; Fujita et al., 2019). In T2DM, persistently elevated blood glucose levels lead to metabolic alterations that negatively impact erythropoiesis (Angelousi & Larger, 2015; Alghareeb et al., 2023). A distinctive feature of diabetic metabolic dysfunction is upregulation of reactive oxygen species (ROS) through multiple mechanisms, such as advanced glycation end products (AGEs), activation of pro-oxidative enzymes (Ha et al., 2008; Kim et al., 2021). According to Gilbert and colleagues, oxidative stress caused by hyperglycemia will cause cellular damage along with impairing normal bodily response including response related to adequate production of erythropoietin (Gilbert, 2017; Horta et al., 2022).

The constant oxidative stress present in diabetes impacts the structure and function of erythrocytes. Hyperglycemia stimulates the non-enzymatic glycation of membrane proteins, which decreases membrane fluidity and deformability while also contributing to the premature death of erythrocytes (eryptosis) (Bissinger et al., 2019; Lee & Kim, 2024). Research suggests that reactive metabolites like methylglyoxal enhance phosphatidylserine exposure in the membrane which speeds up erythrocyte disposal thereby worsening oxygen delivery and causing exacerbation of anaemia (Bissinger et al., 2019; Lee & Kim, 2024).

The release of chronic inflammatory cytokines from the adipose tissue in T2DM alters the signalling pathways of erythropoiesis in bone marrow and results in decreased responsiveness of erythroid progenitors for circulating EPO (Sethi et al., 2021; Sharif & Bashir, 2021). Researchers continue to investigate the exact molecular mechanism but clinical evidence suggests that diabetic anemia is mainly due to a decreased production of EPO and defective responsiveness of bone marrow. It is referred to as functional erythropoietin deficiency (Gilbert, 2017; Thomas et al., 2011). Thus, it is critical to comprehend the multi-facetted role of the metabolic dysregulations on the kidney-bone marrow axis for achieving strategies that go beyond glycemic control and address the hematological complications of diabetes (Ferrucci & Fabbri, 2018; Xu et al., 2024).

2. MATERIALS AND METHODS

2.1 Study Period and Setting

Between 1st February 2025 and 1st October 2025, the study was conducted as a prospective analytical study. The clinical part of the study was conducted at Al Najaf Teaching Hospital, Najaf, Iraq. The chosen duration allowed for the observation of potential effects of prolonged fluctuations of glycaemia on metabolic biomarkers throughout different seasonal conditions that may affect glucose homoeostasis in older people (Gikas et al., 2009; Köhlmoos & Dittmar, 2025).

2.2 Ethical Considerations

Ethical approval was obtained from the Najaf Health Directorate Institutional Review Board (IRB) prior to initiating the study. All procedures conformed to ethical principles were adhered to, as outlined in the Declaration of Helsinki, the respect and safety of participants' autonomy and confidentiality. After explaining the purpose of the study, the

procedure of the study and the clinical significance of the measurement of erythropoietin, written informed consent was taken from all subjects. (world medical association, 2013)

2.3 Study Population

The study consisted of sixty subjects who were divided into two groups for comparison. The diabetic group (n = 30) consist of T2DM patients who have persistent impairment in glycemic control. Individuals who are apparently healthy were assigned as controls for age and sex (n = 30) and had no past metabolic disorders. Recruitment of 45–85 years age group was done to avoid confounding of physiological aging on renal endocrine functions and bone marrow responsiveness (Köhlmoos & Dittmar, 2025).

2.3.1 Inclusion and Exclusion Criteria

Individuals in the diabetic group were enrolled if they had T2DM with HbA1c > 7%, indicating poor glycemic control.

- Excluded criteria.
- Kidney disease stage 3 to 5.
- Liver scarring.
- Treatment with recombinant erythropoietin.
- Recent iron supplementation or blood transfusion.

Erythropoietin levels were measured because these levels would not be affected by any pharmacological effect (American Diabetes Association, 2024).

2.4 Blood Sample Collection

In sterile conditions, about 5 mL of fasting venous blood was collected from each subject by sterile venepuncture technique. Blood was collected in gel-activator tubes and allowed to clot at room temperature (~25 °C). After clotting, the tubes were centrifuged at 4000 rpm for 10 minutes. The serum was separated and stored at −80 °C until analysis which ensures biochemical stability (Mosca et al., 2015; Bognetti et al., 1996; Koga et al., 2019).

2.5 Laboratory Analysis

2.5.1 Determination of Glycated Hemoglobin (HbA1c)

HbA1c was estimated by High Perfomance Liquid Chromatography (HPLC) which separates hemoglobin fractions and then measures glycation at the β-chain N-terminal valine. The long-term glycemic control can be assessed using HbA1c (Mosca et al., 2015; Bognetti et al., 1996; Little & Rohlfing, 2013).

2.5.2 Measurement of Serum Erythropoietin

Measurement of serum erythropoietin concentrations was carried out using a high-sensitivity sandwich ELISA, whereby human EPO is captured with monoclonal antibodies and results are read colorimetrically at 450 nm. This method can accurately measure circulating EPO levels (Macdougall & Cooper, 2019).

2.5.3 Assessment of Erythroid Response

The erythroid activities were evaluated indirectly by correlating serum EPO levels with hematologic indices such as red blood cells (RBCs) and reticulocyte (Rx) percentage from an automated hematology analyzer. This evaluation can reveal EPO resistance when progenitor cells show less response to circulating EPO (Macdougall & Cooper, 2019).

2.6 Statistical Analysis

The IBM SPSS Statistics version 26.0 was used to analyze the data. Mean ± SD were used for descriptive statistics. Group comparisons were done by Independent Student's t-test and the relationship between HbA1c and serum erythropoietin by Pearson's correlation coefficient (r). A P-value lower than 0.05 is significant (Field, 2018).

3 . RESULTS

3.1 Demographic and Clinical Characteristics

The study involved a total of sixty samples of which thirty were T2DM and thirty were

age-matched healthy samples between 45 to 85 years. The means age between the two groups was not significantly different (P > 0.05). This shows that alterations in hematology is due to metabolic instability of the animal and not due to natural physiological changes with age (Salman & Abbas, 2023; Robinson et al., 2021).

Table 3.1: Comparison of Demographic and Primary Glycemic Parameters

Parameter	Control Group (n = 30)	Diabetic Group (n = 30)	P-value
Age (Years)	62.4 ± 8.1	64.7 ± 9.5	0.312 (NS)
HbA1c (%)	5.2 ± 0.4	8.9 ± 1.2	< 0.001**
Fasting Glucose (mg/dL)	94.3 ± 10.2	178.6 ± 24.5	< 0.001**

*Data are expressed as mean ± standard deviation, Highly significant at p < 0.001. ***

3.2 Serum Erythropoietin (EPO) Levels

Serum erythropoietin levels in diabetic patients showed a marked decrease compared to the control group, as demonstrated by the ELISA test. Hemoglobin levels were relatively low in the diabetic group, but a marked decrease in renal endocrine response was observed. The renal hypoxia sensing mechanism, mediated by HIF-2α, appears to be impaired as a result of chronic glucose toxicity, as confirmed by this study (Nabil & El-Hefnawy, 2023; Smith & Johnson, 2020).

3.3 Hematological Indices and Medullary Efficiency

Examination of blood parameters indicates a marked reduction of bone marrow erythroid activity in the diabetic group. There was a decrease in the number of red blood cells (RBC) and in reticulocyte index. Thus, it indicates impaired medullary erythropoietic efficiency. The changes may arise due to oxidative stress and glycation of proteins from prolonged excessive blood sugar levels type 2 diabetes that can interfere with normal red blood cell production.

Table 3.2: Bio-Hematological Indices and Hormonal Levels

Parameter	Control Group (n = 30	Diabetic Group (n = 30)	P-value
Serum EPO (mIU/mL)	12.8 ± 2.1	7.4 ± 1.5	< 0.001**
Hemoglobin (g/dL)	14.2 ± 1.1	11.5 ± 1.4	< 0.001**
RBC Count (×10¹²/L)	4.9 ± 0.5	3.8 ± 0.6	< 0.001**
RPI (%)	1.5 ± 0.3	0.8 ± 0.2	< 0.05*

*Data are expressed as mean ± standard deviation, Statistically significant at p < 0.05 *, Highly significant at p < 0.001 ***

3.4 Correlation Analysis (The Biochemical Link)

The Pearson correlation analysis showed strong negative association of HbA1c levels with serum erythropoietin levels (r = −0.68, P < 0.01). Analyzing this biochemical relationship, an increase in glycemic instability progressively suppresses the renal–medullary hormonal axis. Furthermore, we found a moderate positive correlation between serum EPO levels and the reticulocyte index (r = 0.54, P < 0.05), suggesting that optimal erythropoietic activity in the bone marrow is heavily reliant upon adequate stimulation by the hormone (Zhang et al., 2022; Al-Tamimi, 2022).

4. DISCUSSION

4.1 The Molecular Paradox: Renal Hypoxia-Sensing Failure and the "Blunted" HIF-2α Response

The primary finding of this study at Al-Najaf Teaching Hospital is the inappropriately low serum EPO levels (7.4 ± 1.5 mIU/mL) despite the presence of clinical anemia. Under physiological conditions, anemia reduces renal oxygen tension (pO_2), stabilizing Hypoxia-Inducible Factor-2α (HIF-2α) by inhibiting Prolyl Hydroxylase Domain (PHD) enzymes, which in turn induces EPO gene transcription. However, chronic hyperglycemia in type 2 diabetes disrupts this

oxygen-sensing rheostat. The fluctuation of glucose levels activates the polyol pathway and it leads to the conversion of glucose to sorbitol. Due to this biochemical alteration, there is excessive generation of reactive oxygen species (ROS), especially superoxide anion which enhances iron-dependent hydroxylation of HIF-2α and von Hippel-Lindau (VHL) complex through proteasomal degradation. As a result, renal peritubular fibroblasts become "molecularly blinded" to systemic hypoxia, causing EPO mRNA transcription to be suppressed (Heyman & Abassi, 2023; Volpe et al., 2018; Chu et al., 2026).

4.2 Medullary Erythroid Efficiency: The Concept of "EPO Resistance"

A drop in the Reticulocyte Production Index (RPI) to 0.8% confirms unresponsiveness of the bone marrow niche to endogenous EPO therefore functional EPO resistance. When hyperglycemia is present for a long time, it can lead to the formation of advanced glycation end-products (AGEs) that interact with their receptor (RAGE) on the hematopoietic stem cells. Activation of NF-κB signaling and its upregulation of pro-inflammatory cytokines like TNF-α and IL-6.

Cytokines stimulate the production of SOCS3 that binds with JAK2/STAT5 complex. Soc3 inhibits JAK2 phosphorylation so that EPO receptor (EPOR) signalling does not reach the nucleus and promote survival and differentiation of colony-forming unit-erythroid (CFU-E) progenitors. Due to this reason the marrow becomes "chemically deaf" to circulating EPO (Sasaki et al., 2000; Tóthová, 2021).

4.3 Eryptosis: The Accelerated Suicidal Death of Erythrocytes

The production of erythrocytes (red blood cells) is impaired in diabetic patients resulting in anemia. However, anemia is also aggravated by erythrocyte loss through eryptosis (the suicidal death of erythrocytes). High glucose condition activates non-selective cation channels causing Ca influx. Elevated levels of Ca^{2+} in the cytosol can alter the function of calpain, an intracellular protease which degrades cytoskeletal proteins like spectrin and ankyrin and phospholipid scramblase, which facilitates the translocation of phosphatidylserine to the surface of the erythrocyte. Splenic macrophages phagocytose precociously in response to these signals. The shortened lifespan of the RBCs combined with blunted medullary output creates a 'hematological pincer' obnoxious for anemia in aged diabetics (Alghareeb et al., 2023; Lang et al., 2006; Volpe et al., 2018).

4.4 EPO/EPOR Signaling via JAK2/STAT5

Erythropoietin binds to erythropoietin receptor (EPOR), activating the JAK2/STAT5 signaling pathway. Phosphorylation of STAT5 induces survival, development of erythroid progenitors. The binding of SOCS3 to the EPOR-JAK2 complex inhibits signal transduction, leading to functional EPO resistance. The present study follows the pathway for understanding impaired erythropoiesis in diabetes (Sasaki et al, 2000 ; Tóthová, 2021).

4.5 Synergistic Effect of Aging and Glycemic Instability

In the age group of 45-85 years the molecules are affected due to inflammaging. The presence of long-term low-grade inflammation, such as that in rheumatoid arthritis or metabolic syndrome, raises hepcidin levels, which lock away iron inside macrophages, leading to a limit of available iron for hemoglobin and giving rise to functional iron deficiency. The interplay of glucotoxicity, ongoing inflammation, and age-associated immunosenescence gives rise to a special type of pathophysiological phenotype with a severely impaired Kidney-Marrow axis (Heyman & Abassi, 2023; Chu et al., 2026; Drugs activating hypoxia-inducible factors

correct erythropoiesis and hepcidin levels via renal EPO induction, 2023).

5. CONCLUSIONS

The findings of this research indicated that diabetic patients of Najaf have lower hormonal responses Varying levels of blood sugar causes less secretion of erythropoietin. The lowered levels of EPO, despite experiencing anemia, indicates that the chronic high glucose levels are interfering with the normal mechanism of hypoxia in the kidney that regulates EPO production. According to the results of the study a development of bone marrow resistance to erythropoietin stimulation is Housing of advanced glycation end-products (AGEs) phosphorescent hyperglycemia change the bone-marrow microenvironment. As a result, erythroid progenitor cell responsiveness may be impaired, thus causing functional resistance to EPO in the marrow.

The research results furthermore show that glucose control and erythropoiesis are biochemically linked. A reverse association was seen between HbA1cand serum EPO, i.e. greater the HbA1c, lesser was EPO level. In aged diabetic subjects, there is an association between blood glucose level and hemoglobin A1c level. This means effective glycemic control plays an important role in the erythropoietic equilibrium.

6. RECOMMENDATIONS

The clinical monitoring of erythropoietic function would be included in routine clinical care of elderly patients, especially if they have diabetes. Regular measurement of serum erythropoietin (EPO) levels and reticulocyte indices may allow clinicians to detect early or subclinical disturbances in red blood cell production before overt anemia develops.

Kidney-bone-regulation axis will not improve without effective improvements in glycaemic management. Keeping HbA1c below 7 percent helps to maintain erythropoiesis and therefore can help with normal red blood cell production in diabetes.

Further researches are also required to understand the molecular mechanisms responsible for erythropoietic dysfunction in diabetes. The assessment of erythropoietin receptor (EPOR) gene expression may shed light on the mechanisms that impair hormonal response and marrow efficiency.

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