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Original article

# The Association of Glycosylated Hemoglobin with Anemia in non-Diabetic Patients with Chronic Kidney Disease Stage 4 and 5 not on Dialysis

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## Abstract

**Background:** The haemoglobin glycation index (HGI) was created to measure interindividual variation in glycosylated hemoglobin (HbA1c) determined by factors other than blood glucose concentration. The objective of this investigation was to assess the potential role of HbA1c in exacerbating anemia and increasing inflammation in non-diabetic patients with chronic kidney disease (CKD) stages 4-5 not on dialysis and examine anemia–HbA1c correlation. **Methods:** This prospective investigation comprised 100 patients not on dialysis with non-diabetic CKD stages 4-5. The study was conducted at the Internal Medicine and Nephrology Department of Zagazig General Hospital. Patients were randomly selected and divided into two equal categories depending on CKD stage: Group I included individuals with CKD Stage 4, while Group II included individuals with CKD Stage 5. In accordance with HGI value, the following groups were established: Low HGI (n=36), Moderate HGI (n=28), and High HGI (n=36). Group A (n=32) had HbA1c (%) <5.3%, group B (n=38) had HbA1c (%) 5.3–5.7%, and group C (n=30) had HbA1c (%) 5.7–6.5%. **Results:** Fasting plasma glucose, BMI, dyslipidemia, total cholesterol, uric acid, blood urea, ACR, CRP, ESR, NLR, serum transferrin saturation, and serum ferritin level all showed significant positive correlation with HbA1c. There was a significant negative correlation between HbA1c and Hct, platelets, eGFR, Hb, RBCs. **Conclusion:** Correlation analyses confirm that HbA1c negatively correlates with hemoglobin and RBCs, while positively correlating with ferritin, transferrin saturation, and inflammatory indices.

**Keywords:** Anemia; Chronic Kidney Disease; Glycosylated Hemoglobin; Non-Diabetic.

## Introduction

In human population, there are two main categories define the sources of variation in the glycosylated hemoglobin (HbA1c); interindividual variation in blood glucose concentration and idiosyncratic differences in other factors that affect the HbA1c levels. It is supported by the observation that the correlation coefficients between biomarkers inflammation and HbA1c were greater than those for either fasting plasma glucose (FPG) or Hemoglobin Glycation Index (HGI), indicating that variation in HbA1c is a result of the combined influences of variation in both blood glucose concentration and HGI [(1)].

The HGI was developed to quantify interindividual variability in HbA1c levels that cannot be described solely by ambient blood glucose concentrations. In both the Diabetes Control and Complications Trial (DCCT) and its follow-up Epidemiology of Diabetes Interventions and Complications (EDIC) study, the HGI emerged as a strong predictor of microvascular complications in individuals had type 1 diabetes (T1D). This was also documented to be higher in Black children than in white children with T1D. Despite the implementation of standardized care protocols, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial revealed that minoritized groups were less likely to meet their glycemic objectives. This trial also revealed racial disparities in HGI among adults had T2D. Subsequent analyses demonstrated HGI rise was related to elevated cardiovascular risk, particularly among individuals receiving intensive glycemic control. These results indicate that HGI may be indicative of underlying pathophysiological mechanisms, such as chronic inflammation or differential glycation susceptibility, that contribute to cardiovascular disease outside of glycemic exposure [(2)].

Obesity may act as both a confounding variable and a potential mediator in the association between systemic inflammation and HGI, given its role as a chronic source of low-grade inflammation. Findings from multiple regression analyses revealed a significant relationship between obesity and elevated HGI, with obese individuals exhibiting a mean HGI that was 0.029% higher than that of their normal-weight counterparts. Elevated HGI values were significantly related to increase inflammatory biomarkers levels with polymorphonuclear leukocytes, C-reactive protein, and monocyte counts suggesting that HGI may aid surrogate marker for inflammation-related risk in metabolically healthy individuals [(1)].

Anemia is a prevalent comorbidity in chronic kidney disease (CKD) and significantly contributes to the disease burden. It is linked to a decrease in health-related life quality, and rise in mortality and morbidity, an accelerated

decline in renal function, and an increase in healthcare costs. In numerous studies that specifically investigate anemia prevalence in CKD non-dialysis dependent, rates of variable anemia up to 60% have been reported. Anemia becomes more severe and prevalent as the estimated glomerular filtration rate (eGFR) decreases. Anemia in CKD is a multifactorial condition. The classical view has been that endogenous erythropoietin (EPO) the progressive reduction levels is the most significant factor. Nevertheless, anemia in CKD patients is also influenced by other factors [(3)].

CKD patients' anemia management has witnessed a significant transformation. In recent years, intravenous iron supplements have been widely used. Conversely, across various countries and medical units, anemia treatment in CKD cases is inconsistent. The guidelines of KDIGO, ERBP, and NICE are not wholly consistent currently. Regarding the iron targets and optimal Hb, some discrepancies exist. Additionally, these guidelines do not incorporate the most recent research that assesses IV iron safety and efficacy, in addition to alternative strategies for iron repletion. This is expected to lead to a change in clinical practice in the future. IV iron is more effective and safer for patients with PD, renal transplant, and NDCKD, as they have demonstrated. Furthermore, low-frequency administration strategy, the implementation of a high-dose is both safe and beneficial for the health of patients with DD-CKD [(3)].

The study aimed to examine HbA1c and anemia relation, as well as to investigate the potential function of HbA1c in exacerbating anemia and increasing inflammation in non-diabetic patients had CKD stages 4-5 not undergoing dialysis.

## Patients and methods

**Type of study: cross – sectional study**

**Duration of study from October 2024 to April 2025**

This potential study that was showed one hundred patients aged >18 years with non-diabetic CKD stages 4-5 not on dialysis. The study was carried out at Internal Medicine and Nephrology Department in Zagazig General Hospital.

Prior to enrollment, each participant executed a written informed consent form. Each patient was informed of the study's objective and allocated a confidential code number. The investigation was conducted with the approval of the Ethics Committee at the Faculty of Medicine at Benha University.

**Exclusion criteria were** DM (FPG >126 mg/dl or HbA1c> 6.5%), malignant diseases, heart failure, hepatic failure, malnutrition, inflammatory and autoimmune diseases, acute and chronic infection, other causes of anemia, hemorrhage, hematological disorder and children >15 years and adults with CKD diagnosed by anemia when concentration of Hb is lower than 12.0

g/dl in females and lower than 13 g/dl in males [4].

**Grouping:** Patients were selected and separated to both equal groups according to stages of CKD: **Group I (n=50)**: Patients with CKD, Stage 4 (GFR (15-29 mL/min/1.73 m 2) and **group II (n=50):** Patients with CKD, Stage 5 (GFR < 15 mL/min/1.73). Rendering to HGI value: **Group Low HGI (n=36), group Moderate HGI (n=28) and group High HGI (n=36).** According to HbA1c (%): **Group A (n=32):** <5.3%, **group B (n=38):** 5.3–5.7% and **group C (n=30):** 5.7–6.5%.

All patients underwent comprehensive clinical evaluation, which included full history taking **[**Personal history (age, gender, occupation, and demographic details), duration of CKD, known causes of CKD (hypertension, glomerulonephritis), medications: (antihypertensive, antiplatelet agents, statins), symptoms (Fatigue, weakness, shortness of breath, dizziness), dietary habits (Protein intake, fluid intake), smoking/alcohol use and family history of CKD or anemia. **Examination: General examination counting [**measurement of weight, temperature, body mass index (BMI), height, systolic and diastolic blood pressure, vital signs (heart rate, temperature, blood pressure) cardiovascular examination, respiratory examination, abdomen examination and neurological examination].

**Laboratory investigations including** Complete blood count including (red blood cell (RBC) distribution width (RDW), platelet count, white blood cell count, mean corpuscular volume (MCV), hematocrit), renal function tests (Blood urea nitrogen , Serum creatinine, eGFR (ml/min/1.73 using CKD-EPI creatinine),hemoglobin concentration, electrolytes: (Sodium, potassium, chloride, bicarbonate), glucose: (fasting and random), HbA1c%, serum transferrin saturation (TSAT)%,High-sensitivity C-reactive Protein (mg/dl), serum ferritin, ESR, erythropoietin (EPO) levels, urinalysis (Proteinuria, ACR) and lipid profile(LDL),Total cholesterol(mg/dl), cholesterol(mg/dl), HDL cholesterol, and Triglyceride].

**Measurement of HbA1c:**

An immunoturbidimetric method was employed to measure HbA1c levels in whole blood using the COBAS INTEGRA 400 analyzer (Mannheim, Roche Diagnostics GmbH, Germany). This method was chosen for its high accuracy in determining HbA1c levels, which are essential for evaluating long-term blood glucose control. Measuring HbA1c was particularly relevant for investigating its association with anemia in non-diabetic cases had CKD stages 4 and 5 not undergoing dialysis. Since HbA1c provides insight into overall glycemic control over an extended period, it was critical in understanding its potential role in anemia among CKD patients [5].

**Definition of anemia:**

Anemia was classified as anemia if level of hemoglobin was lesser than 13 g/dL for men and lesser than 12 g/dL for women, as per World Health Organization criteria (3).

**Assessment of kidney function (eGFR calculation):**

To determine eGFR; was united equation of Epidemiology Collaboration (EPI) of CKD was employed [5].

$$eGFR = 141 \times \min\left(\frac{\text{Serum Creatinine}}{k}, 1\right)^{\alpha} \times \max\left(\frac{\text{Serum Cre}}{k}\right.$$

Where k was1.0 for males and 0.9 for females, and α is -0.411 for females and -0.302 for males.

**Calculation of hemoglobin glycation index:**

Calculation of HGI involves two sequential steps. First, the predicted HbA1c is estimated using a linear regression model that characterizes the relationship between measured fasting plasma glucose (FPG, mg/dL) and HbA1c. Based on data from treatment-naïve adults in NHANES, the following equation is commonly employed: Predicted HbA1c (%) = 0.024 × FPG (mg/dL) + 3.1[2].

**Calculate HGI:**

Using the formula HGI = observed HbA1c % - predicted HbA1c %, participants are categorized into high (≥0.150) HGI subgroups, moderate (−0.150 to <0.150), and low (<−0.150) [2].

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**Statistical analysis**

The statistical analysis program SPSS v28, developed and maintained by IBM© in Armonk, NY, USA, was utilized. The data was detected for normality using Shapiro-Wilks's test and histograms. The ANOVA (F) test with a Tukey post hoc test was used to evaluate the quantitative parametric data. findings were demonstrated means and standard deviations (SD). To assess the qualitative variables, which were given as percentages and frequencies, the Chi-square test was used. It was determined that there was statistical significance when the two-tailed P value was less than 0.05. Using Pearson correlation looking for two quantitative variables connection [6].

## Results

This figure shows the studied patients distribution. **Figure 1**

Demographic data, lipid profile, and uric acid level were comparable between the two groups. The SBP, DBP, WBCs count, NLR, HbA1c, the absolute reticulocyte count, fasting plasma glucose, CRP, ESR, serum albumin, serum creatinine, blood urea, and ACR were significantly decrease in group I than group II ($p \leq 0.05$). The Hb concentration, RBCs, Hct, and platelet

count were significantly lesser in group II than in group I at the time of laboratory investigations (P <0.05). Nevertheless, HR concerning insignificantly relating both groups. The Hb concentration, RBCs, Hct, and platelet count were significantly lower in group II than in group I at the time of laboratory investigations (P <0.05). Serum ferritin level and eGFR had less significant in group II than group I (P= 0.009). **Table 1**

Based on HGI, the high HGI group had significantly higher age than low and moderate HGI groups (P <0.001, 0.013), and the moderate HGI group had significantly higher age than the low HGI group (P=0.002). high and moderate HGI groups exhibited significantly increased weight, BMI, CRP, serum transferrin saturation, blood urea, and ACR rates and decreased levels of platelets, RBCs, and eGFR than the low HGI group (P <0.05), high and moderate HGI groups had insignificant difference. The comparison between low HGI group, moderate and high HGI groups revealed significantly elevated levels of WBCs, FPG, NLR, serum ferritin level, and ESR (P<0.05), and high HGI group had significantly elevated levels of WBCs, FPG, NLR, serum ferritin level, and ESR than the moderate group (P<0.05). Also, the moderate and high HGI groups revealed significantly lower Hb concentration than the low HGI group (P=0.004, <0.001), and high HGI group also revealed significantly lower Hb concentration than the moderate HGI group (P=0.025). Sex, height, residence, total cholesterol, LDL, serum albumin, triglycerides, and HDL, and serum creatinine had insignificantly among the studied groups. **Table 2**

Based on HbA1c level, the age, WBCs, HbA1c, NLR, CRP, ACR were significantly increase in group C than group B and group A (P <0.001, <0.001) and was significantly higher in group B compared to group A (P <0.001). The weight, BMI, serum transferrin saturation, uric acid and blood urea had higher significant in group B and C compared to group A (P <0.05), with no significant difference between group B and group C. Based on studied groups there was insignificantly different regarding sex, height, residence, the heart rate, the absolute reticulocyte count, serum albumin, LDL and HDL. The vital signs, SBP, FPG, ESR were significantly higher in group C (5.7–6.5%) compared to group A and group B (P=0.001, 0.019), with not significantly different between group B and group A. The DBP, serum creatinine and total cholesterol had higher significant in group C than group A, with no significant difference between group A and group B and between group B and group C. The Hb and Hct were significantly lower in group B and C compared to group A (P <0.05), with no significant difference between group B and group C. The serum ferritin level and triglycerides were significantly higher in group C than group A and group B (P= <0.05), with insignificantly

different about other groups. eGFR was significantly lesser in group C than group A and group B (P <0.001, 0.018) and was significantly lower in group B than group A (P <0.001). **Table 3**

There was a significant negative correlation between HbA1c and the following parameters including Hct, platelets, eGFR, Hb, RBCs. There was a significant positive correlation between HbA1c and age, BMI, HTN, dyslipidemia, SBP, DBP, fasting plasma glucose, total cholesterol, uric acid, and blood urea, ACR, CRP, ESR, NLR, serum transferrin saturation and serum ferritin level. There was an insignificant correlation between HbA1c and sex, CVD, HR, WBCs, serum creatinine, serum albumin, absolute reticulocyte count, HDL, LDL, and triglycerides. **Table 4**

There was negative correlation between HGI, Hb, RBCs, platelets and eGFR significant. There was positive correlation between FPG, BMI, HGI, WBCs, ACR, serum ferritin level and age significant. There was an insignificant correlation between HGI and sex, serum albumin, triglycerides, total cholesterol, serum creatinine, CRP, LDL, HDL, urea and serum transferrin saturation.**Table 5**

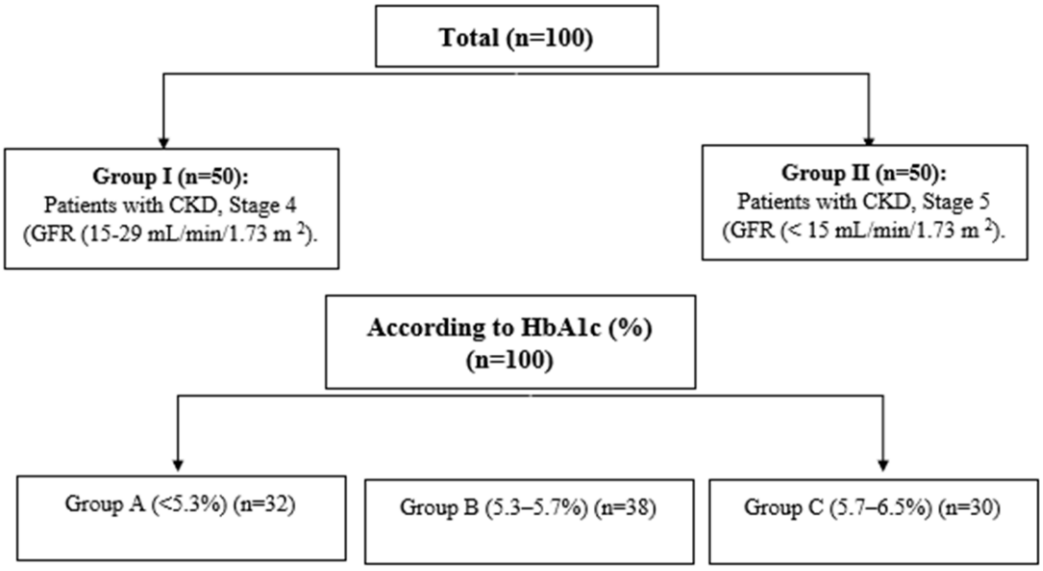


**Figure 1:** Algorithm showing the distribution of the studied patients

**Table 1:** Demographic data, heart rate and blood pressure, laboratory findings, serum ferritin and serum transferrin saturation, lipid profile and renal function tests and serum uric acid level in patients with CKD stage 4 and stage 5

| | | Group I (n=50) | Group II (n=50) | P value |
|---|---|---|---|---|
| **Demographic data** | | | | |
| **Age (years)** | **Mean± SD** | 54.5 ± 11.06 | 57.4 ± 7.84 | 0.134 |
| **Sex** | **Male** | 30 (60%) | 34 (68%) | 0.404 |
| | **Female** | 20 (40%) | 16 (32%) | |
| **Weight (Kg)** | | 84.7 ± 14.02 | 87.9 ± 10.8 | 0.207 |
| **Height (m)** | | 1.67 ± 0.05 | 1.66 ± 0.05 | 0.199 |
| **BMI (Kg/m$^2$)** | | 30.5 ± 5.41 | 32.1 ± 4.43 | 0.105 |
| **Residence** | **Urban** | 27 (54%) | 23 (46%) | 0.423 |
| | **Rural** | 23 (46%) | 27 (54%) | |
| **Heart rate and blood pressure** | | | | |
| **HR (beats/min)** | | 80.2 ± 7.28 | 82.6 ± 7.61 | 0.102 |
| **SBP (mmHg)** | | 134 ± 17.14 | 141.8 ± 15.08 | **0.018*** |
| **DBP (mmHg)** | | 78.0 ± 12.62 | 88.6 ± 15.52 | **<0.001*** |
| **Laboratory findings** | | | | |
| **Hb (g/dL)** | | 10.9 ± 1.81 | 9.2 ± 1.66 | **<0.001*** |
| **RBCs (Million cells/µL)** | | 3.9 ± 0.48 | 3.6 ± 0.59 | **0.001*** |
| **Absolute reticulocyte count (* 10$^9$/L)** | | 43.98 ± 3.57 | 44.5 ± 3.61 | 0.488 |
| **Hct (%)** | | 32.8 ± 5.43 | 27.5 ± 4.97 | **<0.001*** |
| **Platelets (* 10$^9$/L)** | | 221.7 ± 65.09 | 145.7 ± 48.87 | **<0.001*** |
| **WBCs (* 10$^9$/L)** | | 4.7 ± 0.74 | 5.8 ± 0.36 | **<0.001*** |
| **NLR** | | 2.27 ± 0.98 | 2.69 ± 0.95 | **0.034*** |
| **HbA1c (%)** | | 4.9 ± 0.96 | 5.6 ± 0.29 | **<0.001*** |
| **Fasting plasma glucose (mg/dL)** | | 91.7 ± 6.49 | 89.8 ± 6.09 | 0.134 |
| **CRP (mg/dL)** | | 10.4 ± 2.53 | 10.5 ± 2.9 | 0.881 |
| **ESR (mm/h)** | | 12.8 ± 4.11 | 12.3 ± 4.74 | 0.637 |
| **Serum albumin (g/dL)** | | 2.4 ± 0.44 | 2.4 ± 0.52 | 0.677 |
| **Serum ferritin and serum transferrin saturation** | | | | |
| **Serum ferritin level (ng/mL)** | | 104.8 ± 8.32 | 100.5 ± 7.47 | **0.009*** |
| **Serum transferrin saturation (%)** | | 18.2 ± 0.36 | 18.3 ± 0.4 | 0.443 |
| **Lipid profile** | | | | |
| **Total cholesterol (mg/dL)** | | 191 ± 33.34 | 203.6 ± 36.58 | 0.075 |
| **Triglycerides (mg/dL)** | | 167.5 ± 25.56 | 173.1 ± 31.49 | 0.333 |
| **LDL (mg/dL)** | | 169.2 ± 23.75 | 174.4 ± 31.12 | 0.354 |
| **HDL (mg/dL)** | | 48.9 ± 7.5 | 46.9 ± 7.66 | 0.190 |
| **Renal function tests and serum uric acid level** | | | | |
| **Uric acid (mg/dL)** | | 7.6 ± 1.43 | 8.1 ± 1.44 | 0.073 |
| **eGFR (ml/min/1.73 m$^2$)** | | 19.1 ± 5.76 | 13.8 ± 3.69 | **<0.001*** |
| **Serum creatinine (mg/dL)** | | 4.7 ± 1.08 | 5.1 ± 0.86 | **0.029*** |
| **Blood urea (mg/dL)** | | 96.2 ± 24.84 | 119.4 ± 31.03 | **<0.001*** |
| **ACR (mg/g)** | | 288.16 ± 21.14 | 320.2 ± 14.55 | **<0.001*** |

Data was presented as mean ± SD. *: statistically significant P value <0.05. BMI: body mass index. HR: Heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Hb: Haemoglobin, ESR: erythrocyte sedimentation rate, RBCs: Red blood cell count, Hct: hematocrit test, WBCs: white blood cells, neutrophil to lymphocyte ratio, HbA1c: glycated haemoglobin, CRP: c-reactive protein. LDL: low-density lipoprotein, HDL: high-density lipoprotein. eGFR: Estimated glomerular filtration rate, ACR: albumin-to-creatinine ratio.

**Table 2:** Relation between demographic data, laboratory findings, anaemia and the inflammatory markers, lipid profile and renal function tests and haemoglobin glycation index (HGI) value

| | | Low HGI (n=36) | Moderate HGI (n=28) | High HGI (n=36) | P value |
|---|---|---|---|---|---|
| **Demographic data** | | | | | |
| **Age (years)** | | 49.6 ± 8.83 | 56.6 ± 8.61 | 61.9 ± 7.63 | **<0.001*** |
| | | | **P1=0.002*, P2<0.001*, P3=0.013*** | | |
| **Sex** | **Male** | 23 (63.89%) | 17 (60.71%) | 24 (66.67%) | 0.885 |
| | **Female** | 13 (36.11%) | 11 (39.29%) | 12 (33.33%) | |
| **Weight (Kg)** | | 77.7 ± 12.85 | 90.5 ± 8.4 | 89.6 ± 12.83 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*,** P3= 0.762 | | |
| **Height (m)** | | 1.67 ± 0.05 | 1.67 ± 0.05 | 1.65 ± 0.05 | 0.341 |
| **BMI (Kg/m$^2$)** | | 27.9 ± 4.76 | 32.6 ± 3.71 | 32.8 ± 4.88 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*,** P3= 0.856 | | |
| **Residence** | **Urban** | 18 (50%) | 17 (60.71%) | 15 (41.67%) | 0.318 |
| | **Rural** | 18 (50%) | 11 (39.29%) | 21 (58.33%) | |
| **Laboratory findings** | | | | | |
| **Platelets (* 10$^9$/L)** | | 243.5 ± 56.1 | 163.4 ± 51.46 | 139.6 ± 47 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*,** P3=0.059 | | |
| **WBCs (* 10$^9$/L)** | | 4.4 ± 0.66 | 5.5 ± 0.43 | 8.2 ± 1.96 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*, P3<0.001*** | | |
| **Fasting plasma glucose (mg/dL)** | | 83.3 ± 3.44 | 87.8 ± 6.43 | 93.5 ± 6.01 | **<0.001*** |
| | | | **P1= 0.001*, P2 <0.001*, P3<0.001*** | | |
| **Serum albumin (mg/dL)** | | 2.4 ± 0.41 | 2.5 ± 0.53 | 2.4 ± 0.5 | 0.393 |
| **Anemia and the inflammatory markers** | | | | | |
| **Hb (g/dL)** | | 11.3 ± 1.8 | 9.9 ± 1.64 | 9 ± 1.56 | **<0.001*** |
| | | | **P1=0.004*, P2<0.001*, P3= 0.025*** | | |
| **RBCs (Million cells/µL)** | | 4 ± 0.42 | 3.7 ± 0.55 | 3.5 ± 0.6 | **<0.001*** |
| | | | **P1= 0.011*, P2<0.001*,** P3= 0.259 | | |
| **Serum ferritin level (ng/mL)** | | 97.1 ± 8.04 | 101.7 ± 8.19 | 107.1 ± 7.59 | **<0.001*** |
| | | | **P1=0.029*, P2<0.001*, P3=0.009*** | | |
| **Serum transferrin saturation (%)** | | 18.07 ± 0.38 | 18.28 ± 0.4 | 18.39 ± 0.39 | **<0.001*** |
| | | | **P1=0.032*, P2=0.001*,** P3=0.266 | | |
| **NLR** | | 2.13 ± 1.06 | 2.69 ± 1.07 | 3.20 ± 0.9 | **<0.001*** |
| | | | **P1=0.038*, P2=0.001*, P3=0.024*** | | |
| **CRP (mg/dL)** | | 9.2 ± 2.25 | 10.9 ± 2.54 | 12.6 ± 4.05 | **<0.001*** |
| | | | **P1=0.006*, P2 <0.001*,** P3=0.061 | | |
| **ESR (mm/h)** | | 11.2 ± 5.31 | 12.7 ± 5.11 | 15.5 ± 4.05 | **0.002*** |
| | | | P1=0.156**, P2=0.023*, P3=0.034*** | | |
| **Lipid profile** | | | | | |
| **Total Cholesterol (mg/dL)** | | 249.5 ± 21.75 | 242.9 ± 30.46 | 255.7 ± 33.96 | 0.226 |
| **Triglycerides (mg/dL)** | | 162.9 ± 21.57 | 174.7 ± 31.83 | 177.1 ± 35.26 | 0.110 |
| **LDL (mg/dL)** | | 167.1 ± 18.78 | 163.3 ± 25.39 | 169.9 ± 30.03 | 0.580 |
| **HDL (mg/dL)** | | 48.3 ± 7.88 | 50.1 ± 6.75 | 45.7 ± 7.8 | 0.067 |
| **Renal function tests** | | | | | |
| **eGFR (ml/min/1.73 m$^2$)** | | 20.5 ± 5.43 | 15.9 ± 4.51 | 14.3 ± 4.5 | **<0.001*** |
| | | 2 – 30 | 8 – 23 | 6 – 23 | |
| | | | **P1=0.001*, P2 <0.001*,** P3=0.144 | | |
| **Serum creatinine (mg/dL)** | | 4.6 ± 1.16 | 5.05 ± 0.93 | 5.03 ± 0.83 | 0.144 |
| **Blood urea (mg/dL)** | | 88.3 ± 19.71 | 116.9 ± 27.63 | 120.3 ± 31.61 | **<0.001*** |
| | | | **P1<0.001*, P2 <0.001*,** P3=0.656 | | |
| **ACR (mg/g)** | | 286.2 ± 20.04 | 307.6 ± 25.06 | 316.1 ± 15.21 | **<0.001*** |
| | | | **P<0.001*, P2 <0.001*,** P3=0.097 | | |

Data was presented as mean ± SD. BMI: body mass index. *: statistically significant P value <0.05. P1: p value between group low and moderate HGI, P2: p value between low and high HGI, P3: p value between moderate and high HGI. HGI: hemoglobin glycation index. Hb: Hemoglobin, ESR: erythrocyte sedimentation rate, RBCs: Red blood cell count, HCT: hematocrit test, CRP: c-reactive protein. LDL: low-density lipoprotein, HDL: high density lipoprotein. eGFR: Estimated glomerular filtration rate.

**Table 3:** Demographic data, heart rate and blood pressure, laboratory findings, anaemia and inflammatory markers, lipid profile and kidney function and serum Uric acid level according HbA1c categories

| | | Group A (<5.3%) (n=32) | Group B (5.3–5.7%) (n=38) | Group C (5.7–6.5%) (n=30) | P value |
|---|---|---|---|---|---|
| **Demographic data** | | | | | |
| **Age (years)** | | 46.7 ± 6.91 | 57.2 ± 4.65 | 62.5 ± 7.01 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*, P3<0.001*** | | |
| **Sex** | **Male** | 20 (62.5%) | 23 (60.53%) | 21 (70%) | 0.705 |
| | **Female** | 12 (37.5%) | 15 (39.47%) | 9 (30%) | |
| **Weight (Kg)** | | 76.3 ± 12.75 | 90.7 ± 8.79 | 87.7 ± 12.64 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*,** P3= 0.26 | | |
| **Height (m)** | | 1.7 ± 0.05 | 1.7 ± 0.05 | 1.7 ± 0.05 | 0.353 |
| **BMI (Kg/m$^2$)** | | 27.3 ± 4.74 | 32.7 ± 3.32 | 32.1 ± 5.13 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*,** P3= 0.57 | | |
| **Residence** | **Urban** | 17 (53.13%) | 20 (52.63%) | 13 (43.33%) | 0.422 |
| | **Rural** | 15 (46.88%) | 18 (47.37%) | 17 (56.67%) | |
| **Heart rate and blood pressure** | | | | | |
| **Heart rate (beat/min)** | | 80.8 ± 7.84 | 80.9 ± 7.07 | 82.7 ± 7.79 | 0.535 |
| **SBP (mmHg)** | | 131.6 ± 17.43 | 136.3 ± 16.34 | 145.3 ± 13.83 | **<0.001*** |
| | | | P1=0.244, **P2= 0.001*, P3= 0.019*** | | |
| **DBP (mmHg)** | | 77.5 ± 11.64 | 81.3 ± 13.98 | 86 ± 13.03 | **0.040*** |
| | | | P1= 0.224, **P2= 0.009*,** P3= 0.162 | | |
| **Laboratory findings** | | | | | |
| **Absolute reticulocyte count (*10$^9$/L)** | | 44.3 ± 3.93 | 43.8 ± 3.85 | 45 ± 3.4 | **0.435** |
| **Platelets (* 10$^9$/L)** | | 252.3 ± 51.02 | 167.3 ± 51.42 | 131.1 ± 40.08 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*, P3=0.002*** | | |
| **WBCs (* 10$^9$/L)** | | 4.2 ± 0.57 | 5.4 ± 0.12 | 6 ± 0.24 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*, P3<0.001*** | | |
| **HbA1c (%)** | | 4.2 ± 0.57 | 5.4 ± 0.12 | 6 ± 0.24 | **<0.001*** |
| | | | **P1<0.001*, P2 <0.001*, P3<0.001*** | | |
| **Fasting plasma glucose (mg/dL)** | | 88.4 ± 5.17 | 91 ± 6.03 | 94.9 ± 5.47 | **<0.001*** |
| | | | P1= 0.060, **P2 <0.001*, P3=0.008*** | | |
| **Serum albumin (g/dL)** | | 2.3 ± 0.41 | 2.5 ± 0.52 | 2.4 ± 0.48 | 0.374 |
| **Anaemia and inflammatory markers** | | | | | |
| **Hb (g/dL)** | | 11.6 ± 1.52 | 9.5 ± 1.78 | 9.2 ± 1.61 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*,** P3= 0.493 | | |
| **RBCs (Million cells/µL)** | | 4 ± 0.43 | 3.7 ± 0.5 | 3.5 ± 0.63 | **<0.001*** |
| | | | **P1= 0.005*, P2<0.001*,** P3= 0.122 | | |
| **Hct (%)** | | 34.5 ± 4.65 | 28.4 ± 5.33 | 27.6 ± 4.83 | **<0.001*** |
| | | | **P1<0.001*, P2<0.001*,** P3= 0.493 | | |
| **Serum ferritin level (ng/mL)** | | 99.9 ± 7.88 | 101.6 ± 7.98 | 105.8 ± 8.81 | **<0.001*** |
| | | | **P1=**0.407 **P2 =0.007*,** P3=**0.036*** | | |
| **Serum transferrin saturation (%)** | | 18.11 ± 0.31 | 18.33 ± 0.39 | 18.33 ± 0.42 | **<0.001*** |
| | | | **P1=0.031*, P2 =0.020*,** P3=0.737 | | |
| **CRP (mg/dL)** | | 9.2 ± 2.19 | 10.4 ± 2.64 | 13.4 ± 3.87 | **<0.001*** |
| | | | **P1=0.035*, P2 <0.001*, P3<0.001*** | | |
| **ESR (mm/h)** | | 11.3 ± 4.31 | 12.8 ± 4.11 | 15.4 ± 5.05 | **0.002*** |
| | | | P1=0.156**, P2=0.023*, P3=0.034*** | | |
| **NLR** | | 1.70± 0.83 | 2.32 ± 0.98 | 2.88 ± 0.91 | **<0.001*** |
| | | | **P1=0.007*, P2<0.001*, P3=0.019*** | | |
| **Lipid profile** | | | | | |
| **Total Cholesterol (mg/dL)** | | 186.3 ± 30.46 | 197.9 ± 37.1 | 212.6 ± 32.04 | **0.010*** |
| | | | P1= 0.161**, P2=0.002*,** P3= 0.091 | | |
| **Triglycerides (mg/dL)** | | 160.1 ± 20.32 | 173.5 ± 30.25 | 177.2 ± 31.92 | **0.041*** |
| | | | **P1= 0.036*, P2 = 0.014*,** P3=0.627 | | |
| **LDL (mg/dL)** | | 168.5 ± 18.81 | 176.5 ± 31.07 | 169.5 ± 30.92 | 0.419 |
| **HDL (mg/dL)** | | 48.8 ± 7.52 | 49.1 ± 7.62 | 45.3 ± 7.59 | 0.091 |
| **Kidney function and serum Uric acid level** | | | | | |
| **Uric acid (mg/dL)** | | 7.2 ± 1.19 | 8.3 ± 1.43 | 8.2 ± 1.4 | **0.001*** |
| | | | **P1=0.001*, P2 =0.002*,** P3=0.797 | | |

| | | | | |
|---|---|---|---|---|
| **eGFR (ml/min/1.73 m$^2$)** | 21 ± 5.26 | 16.2 ± 4.5 | 13.6 ± 4.34 | **<0.001*** |
| | | **P1<0.001*, P2 <0.001*, P3=0.018*** | | |
| **Serum creatinine (mg/dL)** | 4.5 ± 1.12 | 5 ± 0.93 | 5.2 ± 0.84 | **0.026*** |
| | | P1=0.054, **P2 =0.013*,** P3=0.449 | | |
| **Blood urea (mg/dL)** | 89.7 ± 20.23 | 114.4 ± 29.02 | 118.8 ± 32.76 | **<0.001*** |
| | | **P1<0.001*, P2 <0.001*,** P3=0.558 | | |
| **ACR (mg/g)** | 290.1 ± 19.33 | 307.6 ± 26.58 | 320.9 ± 14.03 | **<0.001*** |
| | | **P1=0.003*, P2 <0.001*, P3=0.016*** | | |

Data was presented as mean ± SD. BMI: body mass index. *: statistically significant P value <0.05. P1: p value between group A and B, P2: p value between group A and C, P3: p value between group B and C. SBP: systolic blood pressure, DBP: diastolic blood pressure. Hb: Haemoglobin, ESR: erythrocyte sedimentation rate, RBCs: Red blood cell count, HCT: hematocrit test, CRP: c-reactive protein. LDL: low-density lipoprotein. eGFR: Estimated glomerular filtration rate.

**Table 4:** Correlation between HbA1c and the clinical, biochemical findings, the inflammatory markers and anemic markers

| | | HbA1c (%) | |
|---|---|---|---|
| | | **R** | **P** |
| **HbA1c and the Clinical, biochemical findings** | **Age (years)** | 0.515 | **<0.001*** |
| | **Sex** | 0.002 | 0.981 |
| | **BMI (Kg/m$^2$)** | 0.278 | **0.005*** |
| | **HTN** | 0.229 | **<0.001*** |
| | **Dyslipidemia** | 0.338 | **0.001*** |
| | **CVD** | 0.059 | 0.559 |
| | **HR (beats/min)** | 0.042 | 0.681 |
| | **SBP (mmHg)** | 0.296 | **0.003*** |
| | **DBP (mmHg)** | 0.209 | **0.037*** |
| | **Absolute reticulocyte count (* 10$^9$/L)** | 0.058 | 0.565 |
| | **Hct (%)** | -0.557 | **<0.001*** |
| | **Platelets (* 10$^9$/L)** | -0.594 | **<0.001*** |
| | **WBCs (* 10$^9$/L)** | 0.068 | 0.510 |
| | **Fasting plasma glucose (mg/dL)** | 0.376 | **<0.001*** |
| | **Serum albumin (g/dL)** | 0.098 | 0.330 |
| | **Total cholesterol (mg/dL)** | 0.369 | **<0.001*** |
| | **Triglycerides (mg/dL)** | 0.155 | 0.124 |
| | **LDL (mg/dL)** | 0.007 | 0.945 |
| | **HDL (mg/dL)** | -0.196 | 0.051 |
| | **Uric acid (mg/dL)** | 0.256 | **0.010*** |
| | **eGFR (ml/min/1.73 m$^2$)** | -0.463 | **<0.001*** |
| | **Serum creatinine (mg/dL)** | 0.122 | 0.226 |
| | **Blood urea (mg/dL)** | 0.339 | **0.001*** |
| | **ACR** | 0.458 | **<0.001*** |
| **Inflammatory markers** | **CRP (mg/dL)** | 0.411 | **0.00١*** |
| | **ESR (mm/h)** | 0.323 | **0.002*** |
| | **NLR** | 0.367 | **0.002*** |
| **Anemic markers** | **Hb (g/dL)** | -0.547 | **<0.001*** |
| | **RBCs (Million cells/µL)** | -0.401 | **<0.001*** |
| | **Serum ferritin level (ng/mL)** | 0.351 | **0.001*** |
| | **Serum transferrin saturation (%)** | 0.260 | **0.009*** |

Data was presented as number. BMI: body mass index, HTN: Hypertension, CVD: Cardiovascular disease, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, Hb: Hemoglobin, RBCs: Red blood cell count, Hct: hematocrit test, WBCs: white blood cells, HbA1c: glycated hemoglobin, LDL: low-density lipoprotein, HDL: high-density lipoprotein, eGFR: Estimated glomerular filtration rate. *: statistically significant P value <0.05, r: correlation coefficient. ESR: erythrocyte sedimentation rate. Hb: Hemoglobin, RBCs: Red blood cell count.

**Table 5: Relation between HGI and the Clinical and Biochemical findings**

| | HGI | |
|---|---|---|
| | **R** | **P** |
| **Age (years)** | 0.279 | **0.028*** |
| **Sex** | -0.063 | 0.626 |
| **BMI (Kg/m$^2$)** | 0.324 | **0.010*** |
| **Hb (g/dL)** | -0.479 | **<0.001*** |
| **RBCs (Million cells/µL)** | -0.319 | **0.011*** |
| **Platelets (* 10$^9$/L)** | -0.439 | **<0.001*** |
| **WBCs (* 10$^9$/L)** | 0.889 | **<0.001*** |
| **Fasting plasma glucose (mg/dL)** | 0.262 | **0.040*** |
| **CRP (mg/dL)** | 0.156 | 0.225 |
| **Serum albumin (g/dL)** | 0.144 | 0.264 |
| **Total cholesterol (mg/dL)** | -0.364 | 0.069 |
| **Triglycerides (mg/dL)** | 0.22 | 0.085 |
| **LDL (mg/dL)** | 0.13 | 0.315 |
| **HDL (mg/dL)** | -0.16 | 0.216 |
| **eGFR (ml/min/1.73 m$^2$)** | -0.370 | **0.003*** |
| **Serum creatinine (mg/dL)** | -0.001 | 0.995 |
| **Blood urea (mg/dL)** | -0.052 | 0.685 |
| **ACR** | 0.442 | **<0.001*** |
| **Serum ferritin level (ng/mL)** | 0.322 | **0.011*** |
| **Serum transferrin saturation (%)** | 0.221 | 0.084 |

Data was presented as number. BMI: body mass index, Hb: Hemoglobin, RBCs: Red blood cell count, WBCs: white blood cells, CRP: c-reactive protein, LDL: low-density lipoprotein, HDL: high-density lipoprotein, eGFR: Estimated glomerular filtration rate. *: statistically significant P value <0.05, r: correlation coefficient. HGI hemoglobin glycation index

## Discussion

Non-diabetic individuals with advanced CKD stages 4–5 face significant health challenges, including an elevated cardiovascular complications risk, progression to ESRD, and increased death rates. Management strategies for these patients often focus on controlling blood pressure, correcting metabolic acidosis, and addressing anemia to improve quality of life and slow disease progression [(7)]. Long-term glycemic control is frequently assessed using HbA1c. Nevertheless, HbA1c levels in non-diabetic cases with CKD, particularly in advanced stages, may be influenced by factors such as the presence of uremic toxins and a reduced RBC lifespan, which could result in inaccurate readings. Recent studies highlight that HbA1c may not reliably reflect glycemic status in non-diabetic individuals with CKD. A nonlinear association has been observed between HbA1c levels and CKD progression, suggesting that both low and high HbA1c values may signal increased risk. These findings emphasize the need for cautious interpretation of HbA1c in this population, as its prognostic value may be influenced by inflammation or metabolic factors unrelated to glucose control [(8)].

Analysis of anemia-related and inflammatory biomarkers across HGI categories revealed significant trends. Hb levels were progressively lower in high and moderate HGI groups than low HGI group, with the high HGI group exhibiting significantly lower Hb than

the moderate group. RBC counts were also reduced in moderate and high HGI groups relative to the low HGI group, though no significant difference was observed between the moderate and high categories. Inflammatory markers including serum ferritin and NLR were significantly raised in moderate and high HGI groups compared to the low HGI group, with further elevation in the high HGI group versus the moderate. Similarly, CRP and serum transferrin saturation were significantly increased in moderate and high HGI groups than in the low HGI group, but did not differ significantly between the moderate and high HGI categories

In the same line with the current results, Fiorentino et al., [9] determined that high and intermediate HGI levels exhibited an increase in inflammatory biomarker levels, counting high sensitivity C reactive protein ,in contrast to low HGI levels. The present findings are contradicted by the fact that there was insignificantly different between the groups with respect to Hb.

In the same context of the current findings, Liu et al., [1] demonstrated that the width of red cell distribution increased gradually in high, moderate, and low HGI levels, while hemoglobin level decreased. HbA1c and HGI were significantly increased in the group with higher CRP, which indicated by results.

In the current study, based on HbA1c level, The Hb and RBCs, Hct and serum ferritin level were significantly decreased in group B (5.3–5.7%) and C (5.7–6.5%) associated to group A (<5.3%), with no difference between group B and group C. NLR was significantly increase in group C than group A and group B and was significantly increase in group B than group A. The Serum transferrin saturation was significantly increase in group C and group B than group A with no difference between group B and group C. Serum albumin and absolute reticulocyte count were insignificantly different between groups.

Also, Amer et al., [10] revealed that in patients with CKD, elevated serum ferritin levels, particularly when combined to high HbA1c, can be indicative of iron overload, inflammation, and potentially worsening kidney function. This situation is complex, as CKD can also cause anemia, which is often treated with iron supplementation, potentially contributing to higher ferritin.

Moreover, Rajagopal et al., [11] study differ from our data as they illustrated that mean in groups with severe anemia the HbA1C% was highest.

The serum ferritin level, RBCs, and HbA1c and Hb, all exhibited significant negative correlations. HbA1c and serum transferrin saturation exhibited a significant correlation.

In agreement with the current findings, Janice et al., [12] shown that HbA1c and ferritin had a positive correlation

However, they are in direct opposition to the present findings, as the HbA1c presented a significant alteration related to MCV, MCH, Hb, and MCH.

There was a significant positive correlation between HbA1c and NLR in present investigation. HbA1c and CRP and ESR exhibited an insignificant correlation.

Kim et al., [13] stated that NLR is generally higher in CKD stage 5 than in CKD stage 4. This elevated NLR in stage 5, which indicates kidney failure, reflects a higher degree of inflammation and immune system activation associated with the progression of kidney disease.

Moreover, Hashmi et al., [14] recommended that anemia is frequently more prevalent and pronounced in CKD Stage 5 than in Stage 4. As renal function declines, the body's capacity to produce erythropoietin, hormone stimulates RBCs production , is reduced, resulting in anemia. Which is further compounded by factors like iron deficiency and chronic inflammation, which are also more pronounced in advanced CKD stages.

In agreement with the current findings, Varma et al., [15] aimed to correlate NLR with glycemic control they reported that the correlation between NLR and HbA1c was significant ($p < 0.001$), signifying elevated NLR values were associated with higher HbA1c percentages. Furthermore, a moderately positive correlation was observed, suggesting that systemic inflammation may play a contributory role in glycemic dysregulation

## Conclusion

Present findings demonstrate both higher HbA1c and HGI levels are strongly associated with worsening anemia, as reflected by lower hemoglobin, RBC counts, and hematocrit values. At the same time, elevated HbA1c and HGI are consistently linked to higher levels of inflammatory markers including CRP, ESR, NLR, and serum ferritin. Correlation analyses further confirm that HbA1c negatively correlates with hemoglobin and RBCs, while positively correlating with ferritin, transferrin saturation, and inflammatory indices.

### Recommendation

We recommend assessing glycosylated hemoglobin and hemoglobin glycation index in non-diabetic patients with chronic kidney disease stages 4-5. Assessing glycosylated hemoglobin and hemoglobin glycation index as predictors of all-cause mortality in chronic kidney disease stages 4-5.

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### Conflicts of interest

Absence of conflicts of interest

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