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Original Article

# Limited effect of coexisting hypothyroidism and iron deficiency on HbA1c in non-diabetic adults: A matched cross-sectional study

Vehbi Sirikci[1,2]

[1]Necip Fazıl City Hospital, Department of Internal Medicine, Kahramanmaraş, Türkiye
[2]Provincial Directorate of Health, Kahramanmaraş, Türkiye

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**Corresponding Author:** Vehbi Sirikci, Necip Fazıl City Hospital, Department of Internal Medicine, Kahramanmaraş, Türkiye, Provincial Directorate of Health, Kahramanmaraş, Türkiye
Email: drvehbisirikci@gmail.com

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**Abstract**

**Aim:** To assess whether coexisting hypothyroidism and iron deficiency are associated with glycated hemoglobin (HbA1c) in adults without diabetes, after accounting for glycemic status, erythrocyte indices, and hematinic markers.
**Materials and Methods:** In this single-center, retrospective, matched cross-sectional study, 143 patients with hypothyroidism who had no diabetes and had received no relevant endocrine or hematinic treatment were matched 1:1 with 143 euthyroid controls on age and sex using propensity scores. Participants were stratified into four combined exposure groups according to thyroid status and iron deficiency. The association with HbA1c was examined using multivariable robust linear regression models with sequential covariate adjustment.
**Results:** Median HbA1c increased gradually from the euthyroid group without iron deficiency to the hypothyroid group with iron deficiency ($p = 0.049$), although the absolute difference between the highest and lowest exposure groups was only about 0.15 percentage points. Combined exposure was associated with HbA1c after adjustment for age and sex ($\beta = 0.142$; 95% CI, 0.039 to 0.245; $p = 0.007$), and after further adjustment for fasting plasma glucose and homeostatic model assessment of insulin resistance (HOMA-IR) ($\beta = 0.131$; 95% CI, 0.029 to 0.233; $p = 0.012$). After adjustment for hemoglobin, MCV, RDW, vitamin B12, and folate, the association was no longer significant ($\beta = 0.067$; 95% CI, -0.041 to 0.175; $p = 0.223$).
**Conclusion:** In adults without diabetes, coexisting hypothyroidism and iron deficiency showed only a limited association with HbA1c. The small observed gradient appears to reflect erythrocyte and hematinic status rather than a synergistic or clinically meaningful metabolic effect.

**Keywords:** Glycated hemoglobin, hypothyroidism, iron deficiency, ferritin, erythrocyte indices, vitamin B12, folate

## INTRODUCTION

Glycated hemoglobin (HbA1c) is a cornerstone biomarker for the diagnosis and monitoring of diabetes because it reflects average glycemic exposure over the preceding two to three months, is relatively unaffected by short-term glycemic fluctuations, and can be measured using standardized methods (1). However, HbA1c is not solely a marker of glycemic exposure; its measured value also depends on the lifespan, turnover, and hemoglobin characteristics of the erythrocyte population exposed to glucose. Conditions that alter erythrocyte survival, erythropoietic activity, or hemoglobin stability may therefore result in falsely high or low HbA1c values independently of mean blood glucose (2). This biological dependence complicates HbA1c interpretation, particularly in common clinical conditions that affect erythrocyte turnover, and underscores the need to consider nonglycemic determinants when interpreting HbA1c. Recent reviews have reaffirmed that erythropoietic factors, including iron, folate, and vitamin B12 deficiency, remain among the principal preanalytical determinants of HbA1c accuracy (3).

Iron deficiency is one of the most common nutritional deficiencies worldwide and remains a major public health problem, particularly among women of reproductive age and individuals living in low- and middle-income countries (4). Iron deficiency has been shown to increase HbA1c independently of glycemia;

proposed mechanisms include prolonged erythrocyte lifespan, increased hemoglobin glycation tendency, and intra-erythrocytic oxidative stress that may facilitate glycation processes (2,5). Hypothyroidism is also a common endocrine disorder that can affect erythrocyte biology. Subclinical hypothyroidism, in particular, has been reported in approximately 4–10% of the adult population, with increasing prevalence with age (6,7). Thyroid hormones are required for normal erythropoiesis, erythrocyte production, and adaptive hematological responses to tissue oxygenation. In hypothyroidism, suppressed erythropoiesis and slowed erythrocyte turnover may contribute to higher HbA1c values without a corresponding change in glycemia; accordingly, HbA1c has been shown to decrease after thyroid hormone replacement in parallel with changes in erythrocyte indices (8).

Iron deficiency and hypothyroidism are not only common conditions individually, but may also coexist in clinical practice through biologically connected pathways. Iron is required for the activity of heme-dependent thyroid peroxidase, a key enzyme in thyroid hormone synthesis; thus, iron deficiency may impair thyroid hormone biosynthesis and contribute to thyroid dysfunction (9). Conversely, hypothyroidism may promote anemia and iron deficiency by suppressing erythropoiesis and affecting gastrointestinal motility and nutrient absorption (10). Despite this biological overlap, the effects of iron deficiency and hypothyroidism on HbA1c have largely been investigated in separate clinical contexts. Although some studies have reported slightly higher HbA1c in individuals with subclinical hypothyroidism without diabetes, most have not comprehensively accounted for iron status, erythrocyte indices, and hematinic markers (11). It therefore remains unclear whether the association with HbA1c when hypothyroidism and iron deficiency coexist reflects a simple additive accumulation, a more pronounced combined effect mediated by shared erythrocyte-related mechanisms, or a limited distributional shift explained mainly by hematological phenotype. In this study, we aimed to evaluate the association between coexisting hypothyroidism and iron deficiency and HbA1c in adults without diabetes who had received no relevant endocrine or hematinic treatment. Participants were stratified into four combined exposure groups according to thyroid and iron status, and the association with HbA1c was examined using multivariable models with sequential adjustment for glycemic parameters, erythrocyte indices, and hematinic markers.

## MATERIALS AND METHODS

### Study design and participants

This single-center, retrospective, matched cross-sectional study was conducted using hospital information system records of adult patients who presented to the Internal Medicine outpatient clinic of Kahramanmaraş Necip Fazıl City Hospital between January 1, 2023, and January 1, 2026. The study protocol was approved by the Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee (TAREK) (Protocol No. 120, Date: 10.01.2026), and the study was carried out in accordance with the principles of the Declaration of Helsinki. Owing to the retrospective design, the requirement for informed consent was waived.

A systematic search of the hospital information system identified individuals aged 18–65 years with complete measurements for thyroid-stimulating hormone (TSH), free thyroxine (T4), HbA1c, fasting plasma glucose, fasting insulin, ferritin, vitamin B12, folate, and complete blood count parameters obtained within the same clinical assessment period. All relevant measurements were required to have been performed at the same visit or within a maximum interval of 15 days. When more than one eligible record existed for the same individual, the earliest visit at which the required laboratory panel was complete and thyroid status could first be classified was selected. Individuals were excluded if they had a known diagnosis of diabetes, used antidiabetic medication, or had HbA1c ≥ 6.5% or fasting plasma glucose ≥ 126 mg/dL; had received thyroid hormone replacement, antithyroid drugs, iron preparations, vitamin B12, or folate supplementation within the preceding three months; had hemoglobinopathy, hemolytic anemia, chronic kidney disease, chronic liver disease, active malignancy, chronic inflammatory disease, or pregnancy; or had a record of blood transfusion, corticosteroid use, immunosuppressive therapy, or chemotherapy within the preceding three months. To reduce the likelihood of active infection or acute inflammation, individuals with a WBC count > 11.000/μL were also excluded.

After eligibility assessment, 143 patients with hypothyroidism constituted the case group. Euthyroid individuals who presented to the same center during the same period and met the same laboratory eligibility and exclusion criteria served as the control pool. From this pool, 143 euthyroid controls were selected through 1:1 matching by age and sex. The final analytical population comprised 286 individuals: 143 hypothyroid cases and 143 euthyroid controls. Balance in age and sex after matching was assessed using standardized mean differences.

### Thyroid and iron status

Participants were classified according to thyroid function tests as euthyroid, subclinical hypothyroid, or overt hypothyroid. Individuals with TSH levels between 0.35 and 4.5 mIU/L and free T4 within the laboratory reference range were considered euthyroid. Subclinical hypothyroidism was defined as TSH > 4.5 mIU/L with free T4 within the reference range, and overt hypothyroidism was defined as TSH > 4.5 mIU/L with low free T4. For the primary analysis, subclinical and overt hypothyroidism were combined into a single hypothyroid group. The distribution of hypothyroidism subtypes was presented descriptively; of the hypothyroid group, 102 had subclinical and 41 had overt hypothyroidism.

Iron deficiency was defined as serum ferritin < 15 ng/mL; individuals with ferritin ≥ 15 ng/mL were classified as having no iron deficiency. Thyroid and iron status were combined to

form four exposure groups: euthyroid without iron deficiency, euthyroid with iron deficiency, hypothyroid without iron deficiency, and hypothyroid with iron deficiency. The euthyroid group without iron deficiency served as the reference group.

### Variables and definitions

Demographic variables were age and sex. Thyroid parameters were TSH and free T4; glycemic and metabolic parameters were HbA1c, fasting plasma glucose, and fasting insulin; and iron and erythrocyte-axis parameters were ferritin, hemoglobin, red blood cell count (RBC), mean corpuscular volume (MCV), red cell distribution width (RDW) and white blood cell count (WBC). Hematinic status was assessed using vitamin B12 and folate levels. HbA1c was treated not as a diagnostic criterion for diabetes but as a continuous biochemical variable that, in individuals without diabetes, may be influenced by nonglycemic factors.

Insulin resistance was calculated using the homeostatic model assessment of insulin resistance (HOMA-IR) formula: HOMA-IR = fasting insulin (μIU/mL) × fasting plasma glucose (mg/dL) / 405. The Mentzer index was calculated as MCV (fL) / RBC ($\times 10^6/\mu L$). Vitamin B12 deficiency was defined as serum vitamin B12 < 200 pg/mL and folate deficiency as serum folate < 4 ng/mL

### Statistical analysis

All analyses were performed in Python version 3.11 (Python Software Foundation, Wilmington, DE, USA) using the pandas, NumPy, SciPy, and statsmodels libraries. A two-sided $p < 0.05$ was considered statistically significant. The distribution of continuous variables was assessed using the Shapiro–Wilk test together with graphical methods. Because the normality assumption was not met for most variables, continuous variables are presented as median (interquartile range, IQR), and categorical variables as n (%). Comparisons between two groups were performed using the Mann–Whitney U test, whereas comparisons across the four combined exposure groups were performed using the Kruskal–Wallis test. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate.

To reduce age and sex imbalance between the case and control groups, propensity score matching was applied. The propensity score was estimated by logistic regression using age and sex. Each hypothyroid case was matched 1:1 to a euthyroid control by nearest-neighbor matching without replacement, using a caliper width of 0.2 times the standard deviation of the propensity score. Balance after matching was assessed using standardized mean differences, with SMD < 0.10 indicating acceptable balance.

The adjusted association of combined thyroid and iron status with HbA1c was evaluated using multivariable linear regression models with sequential covariate adjustment. Heteroscedasticity-consistent HC3 robust standard errors were used in all models. The euthyroid group without iron deficiency was used as the reference category. Regression coefficients (β) were reported as the absolute difference in HbA1c, in percentage points, relative to the reference group. Model 1 was adjusted for age and sex. Model 2 additionally included fasting plasma glucose and HOMA-IR. Model 3, the fully adjusted model, further included hemoglobin, MCV, RDW, vitamin B12, and folate. The sequential models were specified a priori to distinguish glycemic and insulin resistance mechanisms (Model 2) from erythrocyte and hematinic mechanisms (Model 3). Hemoglobin, MCV, and RDW were considered potential mediators of the effect of iron deficiency and hypothyroidism on HbA1c rather than confounders; Model 3 was therefore interpreted as estimating the association that remained after accounting for the erythrocyte pathway.

Model assumptions were evaluated using residual distribution, residual-versus-fitted plots, and influential observations. Multicollinearity among independent variables was examined using the variance inflation factor, with VIF < 5 considered acceptable. Model explanatory power was reported using adjusted $R^2$ values.

## RESULTS

### Baseline characteristics of the study population

Of the records screened through the hospital information system during the study period, 286 adults without diabetes were included in the analysis: 143 patients with hypothyroidism who met the inclusion and exclusion criteria and 143 euthyroid controls matched 1:1 by age and sex using propensity score matching. Within the hypothyroid group, 102 patients (71.3%) were classified as having subclinical hypothyroidism and 41 (28.7%) as having overt hypothyroidism; thus, the study population predominantly consisted of individuals with mild thyroid dysfunction. The groups were similar with respect to the matching variables. Median age was 40 (32–49) years in the hypothyroid group and 39 (31–48) years in the euthyroid group ($p = 0.54$), and female sex accounted for 115 (80.4%) and 112 (78.3%) of the hypothyroid and euthyroid groups, respectively ($p = 0.66$). As expected, thyroid function tests differed markedly between groups: TSH was higher in the hypothyroid group than in the euthyroid group (7.74 [5.38 – 12.60] vs 2.12 [1.34 – 3.06] mIU/L; $p < 0.001$), whereas free T4 was lower (0.96 [0.82 – 1.09] vs 1.17 [1.06 – 1.28] ng/dL; $p < 0.001$).

No statistically significant differences were observed between groups in glycemic or metabolic parameters. Fasting plasma glucose was comparable in the hypothyroid and euthyroid groups (92 [86 – 98] vs 91 [85 – 97] mg/dL; $p = 0.31$). HbA1c was numerically higher in the hypothyroid group, but the difference did not reach statistical significance (5.44% [5.19 – 5.70] vs 5.36% [5.12 – 5.61]; $p = 0.057$). Insulin (9.5 [6.4 – 14.0] vs 8.8 [6.0 – 12.9] μIU/mL; $p = 0.18$) and HOMA-IR (2.20 [1.45 – 3.28] vs 1.98 [1.32 – 2.94]; $p = 0.15$) were likewise similar between groups, indicating comparable baseline glycemic status and insulin resistance profile (Table 1). In contrast, significant differences were observed in iron, erythrocyte, and hematinic

parameters. Ferritin was lower in the hypothyroid group (20.1 [9.6 – 42.5] vs 30.5 [15.2 – 56.8] ng/mL; p = 0.015), and iron deficiency was more frequent (66 [46.2%] vs 48 [33.6%]; p = 0.030). Hemoglobin (12.7 [11.8 – 13.7] vs 13.1 [12.2 – 14.0] g/dL; p = 0.039) and MCV (82.3 [77.7 – 86.6] vs 84.2 [79.8 – 88.3] fL; p = 0.028) were lower, whereas RDW was higher (14.4% [13.5 – 15.7] vs 13.8% [13.1 – 14.8]; p = 0.011). Vitamin B12 was also lower in the hypothyroid group (254 [184 – 362] vs 316 [228 – 430] pg/mL; p = 0.006). No statistically significant differences were found in RBC count, Mentzer index, WBC count, anemia frequency, or folate level (all p > 0.05) (Table 1).

**Table 1. Baseline clinical and laboratory characteristics of non-diabetic adults with hypothyroidism and matched euthyroid controls**

| Variable | Euthyroid controls (n = 143) | Hypothyroidism (n = 143) | p value |
|---|---|---|---|
| **Demographic characteristics** | | | |
| **Age, years** | 39 (31 – 48) | 40 (32 – 49) | 0.54 |
| **Female sex, n (%)** | 112 (78.3) | 115 (80.4) | 0.66 |
| **Male sex, n (%)** | 31 (21.7) | 28 (19.6) | — |
| **Thyroid parameters** | | | |
| **TSH, mIU/L** | 2.12 (1.34 – 3.06) | 7.74 (5.38 – 12.60) | < 0.001 |
| **Free T4, ng/dL** | 1.17 (1.06 – 1.28) | 0.96 (0.82 – 1.09) | < 0.001 |
| **Subclinical hypothyroidism, n (%)** | — | 102 (71.3) | — |
| **Overt hypothyroidism, n (%)** | — | 41 (28.7) | — |
| **Glycemic and metabolic parameters** | | | |
| **Fasting plasma glucose, mg/dL** | 91 (85 – 97) | 92 (86 – 98) | 0.31 |
| **HbA1c, %** | 5.36 (5.12 – 5.61) | 5.44 (5.19 – 5.70) | 0.057 |
| **Insulin, µIU/mL** | 8.8 (6.0 – 12.9) | 9.5 (6.4 – 14.0) | 0.18 |
| **HOMA-IR** | 1.98 (1.32 – 2.94) | 2.20 (1.45 – 3.28) | 0.15 |
| **Iron and hematologic parameters** | | | |
| **Ferritin, ng/mL** | 30.5 (15.2 – 56.8) | 20.1 (9.6 – 42.5) | 0.015 |
| **Iron deficiency, n (%)** | 48 (33.6) | 66 (46.2) | 0.030 |
| **Hemoglobin, g/dL** | 13.1 (12.2 – 14.0) | 12.7 (11.8 – 13.7) | 0.039 |
| **Anemia, n (%)** | 35 (24.5) | 48 (33.6) | 0.089 |
| **RBC, $\times 10^6$/µL** | 4.63 (4.26 – 4.95) | 4.55 (4.18 – 4.88) | 0.23 |
| **MCV, fL** | 84.2 (79.8 – 88.3) | 82.3 (77.7 – 86.6) | 0.028 |
| **RDW, %** | 13.8 (13.1 – 14.8) | 14.4 (13.5 – 15.7) | 0.011 |
| **Mentzer index** | 18.2 (16.5 – 20.1) | 18.5 (16.8 – 20.6) | 0.42 |
| **WBC, $\times 10^3$/µL** | 7.0 (5.9 – 8.1) | 7.1 (6.0 – 8.3) | 0.57 |
| **Hematinic parameters** | | | |
| **Vitamin B12, pg/mL** | 316 (228 – 430) | 254 (184 – 362) | 0.006 |
| **Folate, ng/mL** | 7.2 (5.4 – 9.7) | 6.6 (4.9 – 8.8) | 0.079 |

Data are presented as median (interquartile range) or n (%); Between-group comparisons were performed using the Mann–Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables, as appropriate; Iron deficiency was defined as ferritin < 15 ng/mL; Anemia was defined as hemoglobin < 13 g/dL in men and < 12 g/dL in women; HOMA-IR was calculated as fasting insulin × fasting plasma glucose / 405; Mentzer index was calculated as MCV/RBC; TSH: thyroid-stimulating hormone, free T4: free thyroxine, HbA1c: glycated hemoglobin, HOMA-IR: homeostatic model assessment of insulin resistance, RBC: red blood cell count, MCV: mean corpuscular volume, RDW: red cell distribution width, WBC: white blood cell count

### Combined analysis according to thyroid and iron status

The distribution of glycemic, hematological, and hematinic parameters was examined across the four combined exposure groups defined by thyroid status and iron deficiency. Median HbA1c increased gradually from the euthyroid group without iron deficiency (5.34% [5.10 – 5.58]) to the euthyroid group with iron deficiency (5.39% [5.14 – 5.64]), the hypothyroid group without iron deficiency (5.41% [5.17 – 5.66]), and the hypothyroid group with iron deficiency (5.49% [5.23 – 5.74]) ($p = 0.049$). Although this increase reached statistical significance, the absolute difference in median HbA1c between the highest and lowest groups was limited to 0.15 percentage points. Fasting plasma glucose, insulin, and HOMA-IR did not differ significantly across the four groups (all $p > 0.05$), suggesting that the observed HbA1c gradient was not accompanied by a parallel difference in measured glycemic or insulin resistance markers (Table 2). Iron and erythrocyte parameters differed mainly according to iron deficiency status: ferritin, hemoglobin, and MCV were lower in the iron-deficient groups ($p < 0.001$ for all), whereas RDW was higher ($p < 0.001$). Anemia frequency also differed significantly across the groups, with higher rates observed in the iron-deficient groups ($p = 0.004$). Among hematinic parameters, vitamin B12 level and vitamin B12 deficiency frequency differed across groups ($p = 0.008$ and $p = 0.047$, respectively), with the lowest vitamin B12 values observed in the hypothyroid group with iron deficiency. Folate level, folate deficiency frequency, RBC count, and Mentzer index did not differ significantly across groups (all $p > 0.05$) (Table 2).

**Table 2. Glycemic, iron, erythrocyte, and hematinic parameters according to combined thyroid and iron deficiency status**

| Variable | Euthyroid without iron deficiency (n = 95) | Euthyroid with iron deficiency (n = 48) | Hypothyroidism without iron deficiency (n = 77) | Hypothyroidism with iron deficiency (n = 66) | p value |
|---|---|---|---|---|---|
| **Glycemic and metabolic parameters** | | | | | |
| **Fasting plasma glucose, mg/dL** | 91 (85 – 97) | 91 (85 – 98) | 92 (86 – 98) | 92 (86 – 99) | 0.68 |
| **HbA1c, %** | 5.34 (5.10 – 5.58) | 5.39 (5.14 – 5.64) | 5.41 (5.17 – 5.66) | 5.49 (5.23 – 5.74) | 0.049 |
| **Insulin, µIU/mL** | 8.6 (5.9 – 12.6) | 9.1 (6.2 – 13.3) | 9.2 (6.2 – 13.7) | 9.9 (6.7 – 14.5) | 0.24 |
| **HOMA-IR** | 1.95 (1.28 – 2.88) | 2.05 (1.36 – 3.05) | 2.12 (1.39 – 3.16) | 2.28 (1.52 – 3.39) | 0.21 |
| **Iron and erythrocyte parameters** | | | | | |
| **Ferritin, ng/mL** | 39.8 (24.2 – 69.5) | 9.8 (6.4 – 12.7) | 31.6 (20.1 – 56.2) | 8.9 (5.8 – 12.4) | < 0.001 |
| **Hemoglobin, g/dL** | 13.4 (12.6 – 14.2) | 12.5 (11.7 – 13.4) | 13.0 (12.1 – 13.9) | 12.3 (11.4 – 13.2) | < 0.001 |
| **Anemia, n (%)** | 16 (16.8) | 19 (39.6) | 22 (28.6) | 26 (39.4) | 0.004 |
| **RBC, $\times 10^6$/µL** | 4.67 (4.30 – 4.98) | 4.55 (4.18 – 4.88) | 4.59 (4.22 – 4.91) | 4.50 (4.14 – 4.82) | 0.18 |
| **MCV, fL** | 85.1 (81.3 – 88.9) | 81.5 (76.8 – 85.7) | 83.6 (79.1 – 87.2) | 80.7 (75.9 – 85.1) | < 0.001 |
| **RDW, %** | 13.5 (12.9 – 14.4) | 14.4 (13.5 – 15.8) | 14.0 (13.2 – 15.0) | 14.9 (13.8 – 16.2) | < 0.001 |
| **Mentzer index** | 18.1 (16.3 – 19.8) | 18.3 (16.7 – 20.3) | 18.3 (16.6 – 20.2) | 18.6 (16.9 – 20.8) | 0.37 |
| **Hematinic parameters** | | | | | |
| **Vitamin B12, pg/mL** | 330 (242 – 445) | 292 (205 – 398) | 268 (194 – 376) | 240 (174 – 342) | 0.008 |
| **Vitamin B12 deficiency, n (%)** | 20 (21.1) | 14 (29.2) | 22 (28.6) | 26 (39.4) | 0.047 |
| **Folate, ng/mL** | 7.5 (5.7 – 10.1) | 6.8 (5.0 – 9.2) | 6.8 (5.1 – 9.1) | 6.3 (4.7 – 8.5) | 0.16 |
| **Folate deficiency, n (%)** | 8 (8.4) | 6 (12.5) | 9 (11.7) | 10 (15.2) | 0.48 |

Data are presented as median (interquartile range) or n (%); Between-group comparisons were performed using the Kruskal–Wallis test for continuous variables and the chi-square or Fisher's exact test for categorical variables, as appropriate; Iron deficiency was defined as ferritin < 15 ng/mL; Vitamin B12 deficiency was defined as serum vitamin B12 < 200 pg/mL; Folate deficiency was defined as serum folate < 4 ng/mL; Anemia was defined as hemoglobin < 13 g/dL in men and < 12 g/dL in women; HOMA-IR was calculated as fasting insulin × fasting plasma glucose / 405; Mentzer index was calculated as MCV/RBC; HbA1c: glycated hemoglobin, HOMA-IR: homeostatic model assessment of insulin resistance, RBC: red blood cell count, MCV: mean corpuscular volume, RDW: red cell distribution width

### Multivariable regression analysis for HbA1c

The adjusted association of combined thyroid and iron status with HbA1c was evaluated using three robust linear regression models with sequential covariate adjustment (Table 3, Figure 1). In all models, the euthyroid group without iron deficiency was used as the reference category, and β coefficients represent the absolute difference in HbA1c, in percentage points, relative to the reference group. In Model 1, adjusted for age and sex, only the hypothyroid group with iron deficiency was significantly associated with HbA1c among the four combined categories (β = 0.142; 95% CI, 0.039 to 0.245; p = 0.007). The euthyroid group with iron deficiency (β = 0.052; 95% CI, −0.041 to 0.145; p = 0.271) and the hypothyroid group without iron deficiency (β = 0.071; 95% CI, −0.012 to 0.154; p = 0.093) were not significant. The coefficient for the combined exposure group was close to the sum of the coefficients for iron deficiency alone and hypothyroidism alone. Age was positively associated with HbA1c (β = 0.005; p = 0.018), whereas sex showed no significant association (p = 0.769).

After adding fasting plasma glucose and HOMA-IR in Model 2, the association between combined hypothyroidism and iron deficiency and HbA1c persisted (β = 0.131; 95% CI, 0.029 to 0.233; p = 0.012). Neither fasting plasma glucose (β = 0.003; p = 0.148) nor HOMA-IR (β = 0.013; p = 0.394) was independently associated with HbA1c, and the effect of age was close to the threshold of statistical significance (β = 0.004; p = 0.061). In the fully adjusted Model 3, which additionally included hemoglobin, MCV, RDW, vitamin B12, and folate, the association of the hypothyroid group with iron deficiency with HbA1c lost statistical significance (β = 0.067; 95% CI, −0.041 to 0.175; p = 0.223). The other combined groups also showed no significant association in this model. Hemoglobin, MCV, vitamin B12, and folate were not significant predictors, whereas RDW showed a near-significant positive association (β = 0.021; 95% CI, −0.001 to 0.043; p = 0.063). The variance explained by the models increased progressively with added covariates (adjusted $R^2$: 0.071 for Model 1, 0.101 for Model 2, and 0.184 for Model 3); however, the total variance explained remained low even in the fully adjusted model.

**Table 3. Multivariable robust linear regression models for HbA1c according to combined thyroid and iron deficiency status**

| Predictor | Model 1 β (95% CI) | p | Model 2 β (95% CI) | p | Model 3 β (95% CI) | p |
|---|---|---|---|---|---|---|
| **Combined thyroid and iron status** | | | | | | |
| **Euthyroid without iron deficiency** | Reference | — | Reference | — | Reference | — |
| **Euthyroid with iron deficiency** | 0.052 (-0.041 to 0.145) | 0.271 | 0.049 (-0.043 to 0.141) | 0.297 | 0.022 (-0.075 to 0.119) | 0.654 |
| **Hypothyroidism without iron deficiency** | 0.071 (-0.012 to 0.154) | 0.093 | 0.065 (-0.018 to 0.148) | 0.124 | 0.038 (-0.049 to 0.125) | 0.389 |
| **Hypothyroidism with iron deficiency** | 0.142 (0.039 to 0.245) | 0.007 | 0.131 (0.029 to 0.233) | 0.012 | 0.067 (-0.041 to 0.175) | 0.223 |
| **Age, per year** | 0.005 (0.001 to 0.009) | 0.018 | 0.004 (0.000 to 0.008) | 0.061 | 0.004 (-0.001 to 0.008) | 0.074 |
| **Female sex** | 0.013 (-0.074 to 0.100) | 0.769 | 0.011 (-0.075 to 0.097) | 0.801 | 0.008 (-0.080 to 0.096) | 0.858 |
| **Fasting plasma glucose, per mg/dL** | — | — | 0.003 (-0.001 to 0.007) | 0.148 | 0.003 (-0.001 to 0.007) | 0.181 |
| **HOMA-IR** | — | — | 0.013 (-0.017 to 0.043) | 0.394 | 0.010 (-0.021 to 0.041) | 0.526 |
| **Hemoglobin, per g/dL** | — | — | — | — | -0.021 (-0.056 to 0.014) | 0.240 |
| **MCV, per fL** | — | — | — | — | -0.004 (-0.010 to 0.002) | 0.193 |
| **RDW, per %** | — | — | — | — | 0.021 (-0.001 to 0.043) | 0.063 |
| **Vitamin B12, per 100 pg/mL** | — | — | — | — | -0.012 (-0.030 to 0.006) | 0.188 |
| **Folate, per ng/mL** | — | — | — | — | -0.004 (-0.016 to 0.008) | 0.515 |
| **Adjusted $R^2$** | 0.071 | | 0.101 | | 0.184 | |

β coefficients represent absolute differences in HbA1c percentage points compared with the reference group; Model 1 was adjusted for age and sex; Model 2 was additionally adjusted for fasting plasma glucose and HOMA-IR; Model 3 was additionally adjusted for hemoglobin, MCV, RDW, vitamin B12, and folate; Robust standard errors were used; Iron deficiency was defined as ferritin < 15 ng/mL; β: regression coefficient, CI: confidence interval, HbA1c: glycated hemoglobin, HOMA-IR: homeostatic model assessment of insulin resistance, MCV: mean corpuscular volume, RDW: red cell distribution width, $R^2$: coefficient of determinat

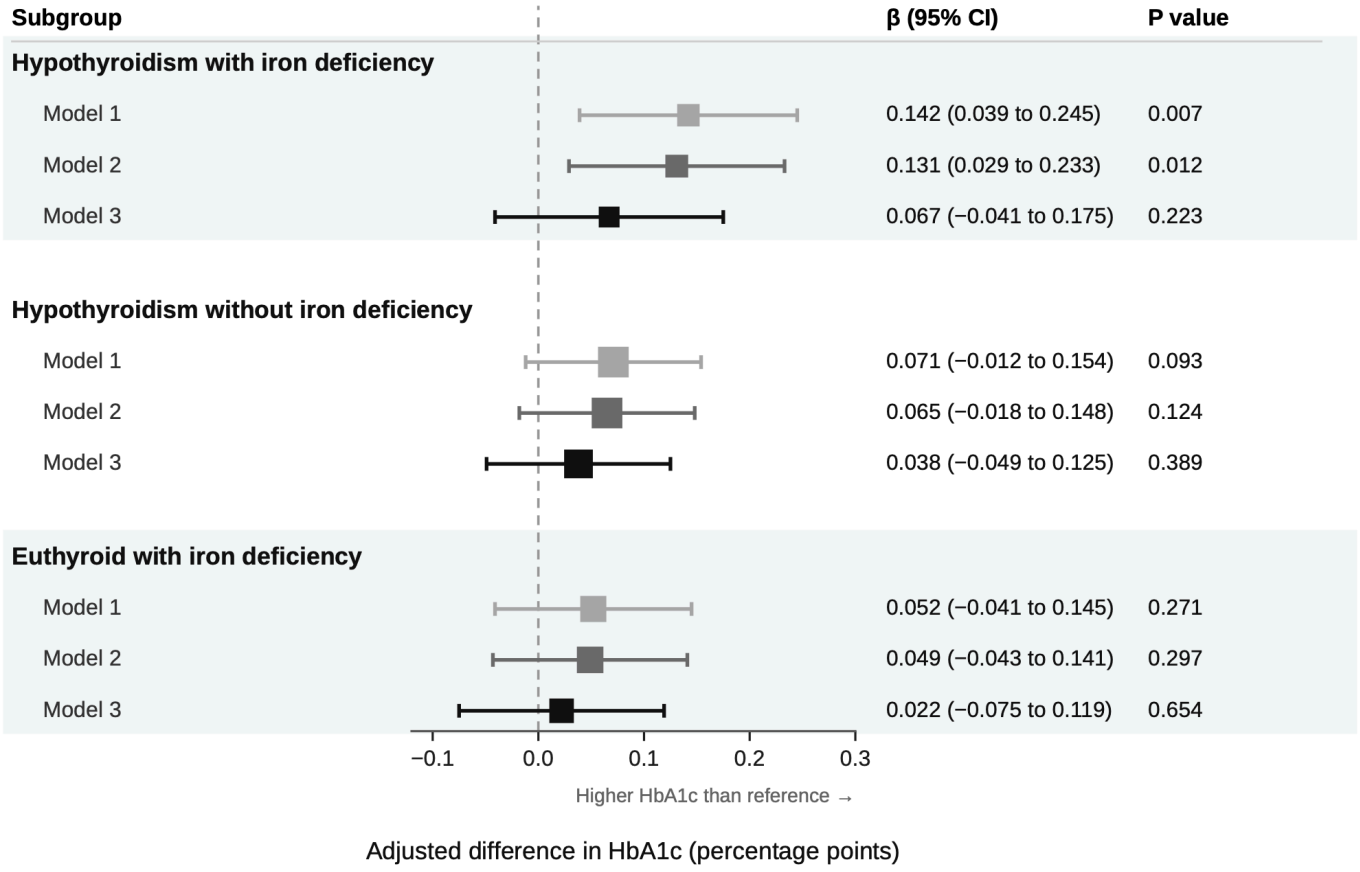


**Figure 1.** Adjusted differences in HbA1c according to combined thyroid and iron status across sequentially adjusted models; Squares are point estimates (β) and lines the 95% CIs relative to the reference group (euthyroid without iron deficiency); the dashed line denotes no difference; Model 1: adjusted for age and sex; Model 2: + fasting plasma glucose and HOMA-IR; Model 3: + hemoglobin, MCV, RDW, vitamin B12, and folate; Robust regression with HC3 standard errors

## DISCUSSION

This matched retrospective study jointly evaluated the association of hypothyroidism and iron deficiency with HbA1c in adults without diabetes who had received no relevant endocrine or hematinic treatment. To our knowledge, this is one of the limited number of studies to examine the separate and combined associations of hypothyroidism and iron deficiency with HbA1c within the same analytical framework. The principal finding was that although HbA1c was higher in the group with combined hypothyroidism and iron deficiency than in the reference group in the age- and sex-adjusted model, this association lost statistical significance after full adjustment for fasting plasma glucose, insulin resistance, erythrocyte indices, and hematinic parameters. The absolute differences in HbA1c between groups remained small; the difference between the highest and lowest exposure groups does not appear large enough to materially affect the diagnostic classification of diabetes. These findings suggest that, in adults without diabetes, the coexistence of hypothyroidism and iron deficiency may be associated with an observable but limited effect on HbA1c, and that this effect may relate more closely to erythrocyte phenotype and hematinic status than to an independent metabolic signal.

The effects of iron deficiency and hypothyroidism on HbA1c have largely been considered separately in the literature. Numerous studies have reported that iron deficiency can increase HbA1c independently of glycemia (2,5,12,13). Proposed mechanisms include prolonged erythrocyte lifespan in iron deficiency, increased glycation tendency due to changes in the quaternary structure of the globin chain, and oxidative stress–mediated glycation related to reduced antioxidant capacity (2,5). This effect has been shown to become more pronounced with increasing anemia severity and to regress after iron therapy (12,13). Nonetheless, the direction of the effect of iron deficiency on HbA1c is not consistent across studies; some have reported lower HbA1c levels or inverse associations in iron deficiency, indicating that this relationship may be sensitive to population characteristics and methodological approach (14-16). Similarly, hypothyroidism has been described as a condition that may contribute to spuriously elevated HbA1c independently of glycemia through suppressed erythropoiesis and slowed erythrocyte turnover. Several studies have shown that the reduction in HbA1c after thyroid hormone replacement parallels changes in reticulocyte count or erythrocyte indices (8,17,18). Notably, the HbA1c difference observed in patients with hypothyroidism has been reported to lose statistical

significance after adjustment for reticulocyte count, suggesting that erythrocyte turnover may be an important mediator of nonglycemic variation in HbA1c (8,18).

The available evidence is particularly inconsistent for subclinical hypothyroidism and mild iron deficiency. Some studies have reported slightly higher HbA1c in individuals with subclinical hypothyroidism without diabetes (11), whereas others have found no consistent association between thyroid dysfunction and glycemic control indicators; subclinical hypothyroidism may increase insulin resistance without being clearly reflected in HbA1c (19), and may even be accompanied by lower HbA1c in individuals with impaired glucose tolerance (20). A recent study in older adults likewise reported an independent association between subclinical hypothyroidism and inadequate glycemic control (21). The direction of the effect of vitamin B12 and folate deficiency on HbA1c is also uncertain. Although megaloblastic changes may theoretically increase HbA1c through larger and longer-lived erythrocytes, other studies have shown that megaloblastic anemia does not significantly alter HbA1c, whereas iron deficiency does (22,23). This heterogeneity suggests that the magnitude of nonglycemic influences on HbA1c may vary according to patient selection, the severity of hypothyroidism and iron deficiency, anemia status, renal function, and accompanying metabolic factors.

In the present study, median HbA1c increased gradually across the four combined exposure groups, from the euthyroid group without iron deficiency to the hypothyroid group with iron deficiency, and this difference reached statistical significance. However, the absolute difference between the highest and lowest groups was only approximately 0.15 percentage points. This magnitude indicates a small distributional shift between subgroups rather than a meaningful HbA1c bias capable of altering clinical decisions. In this respect, the study contributes to the literature by showing how hypothyroidism and iron deficiency relate to HbA1c not only separately, but also when they coexist. The multivariable models reinforce this interpretation. In the age- and sex-adjusted model, combined hypothyroidism and iron deficiency was significantly associated with HbA1c, and this association was largely preserved after adding fasting plasma glucose and HOMA-IR. By contrast, in the fully adjusted model including hemoglobin, MCV, RDW, vitamin B12, and folate, the association lost statistical significance. This pattern suggests that the weak association between combined exposure and HbA1c is more closely related to erythrocyte indices and hematinic status than to a glycemic or insulin resistance–mediated mechanism. Because erythrocyte indices lie on the presumed causal pathway from iron deficiency and hypothyroidism to HbA1c, their inclusion in Model 3 represents adjustment for potential mediators rather than for confounders. The attenuation of the association is therefore best interpreted as evidence that the combined exposure relates to HbA1c largely through the erythrocyte phenotype, consistent with a mediated rather than a direct metabolic effect. This finding is consistent with previous studies reporting that the HbA1c difference in patients with hypothyroidism disappears after adjustment for reticulocyte count (8,18). Although reticulocyte count was not available in our study, the disappearance of the association after adding parameters that reflect erythrocyte phenotype, such as hemoglobin, MCV, and RDW, supports a biological framework similar to that suggested by reticulocyte-adjusted analyses.

Our findings may appear, at first glance, to conflict with the study reporting significantly higher HbA1c in individuals with subclinical hypothyroidism without diabetes (11). However, that study compared groups according to thyroid status alone and did not apply detailed adjustment for erythrocyte indices and hematinic status. In our study, a similar increase was observed in unadjusted and partially adjusted models, but disappeared only after full adjustment. The difference may therefore reflect differences in adjustment strategy and statistical approach rather than a true contradiction. This interpretation is also consistent with studies showing that hypothyroidism-related HbA1c differences are abolished when erythrocyte turnover is taken into account (8,18). The initial hypothesis that hypothyroidism and iron deficiency might exert a combined effect exceeding the sum of their individual effects was based on the premise that both conditions can influence shared biological pathways governing erythrocyte lifespan and turnover. However, the present findings do not provide strong evidence for a clear supra-additive effect. Although the age- and sex-adjusted HbA1c difference in the combined exposure group appeared more pronounced than in the reference group, this difference did not meaningfully exceed the sum of the differences observed in the iron deficiency–alone and hypothyroidism-alone groups. Indeed, the Model 1 coefficient for the combined exposure group ($\beta = 0.142$) was close to the sum of the coefficients for iron deficiency alone ($\beta = 0.052$) and hypothyroidism alone ($\beta = 0.071$) ($\beta = 0.123$). This pattern suggests that the effect of combined hypothyroidism and iron deficiency on HbA1c may reflect a limited and weak additive accumulation rather than a synergistic interaction.

The clinical interpretation of these results should take the characteristics of the study population into account. Because the population consisted predominantly of individuals with subclinical hypothyroidism and mild-to-moderate iron deficiency, the nonglycemic effect on HbA1c may have remained limited. In this relatively homogeneous group—untreated, without diabetes, and with renal dysfunction and major systemic comorbidities excluded—the coexistence of hypothyroidism and iron deficiency was associated with only a small distributional shift in HbA1c that appears clinically limited. More pronounced HbA1c changes reported in the literature have generally been observed in populations with overt thyroid dysfunction, severe anemia, or accompanying renal and metabolic disorders (12,14). In such settings, where the reliability of HbA1c is uncertain, alternative glycemic markers independent of erythrocyte turnover, such as glycated albumin and fructosamine, have been proposed as complementary tools (24,25). Our findings therefore suggest that HbA1c interpretation is not meaningfully distorted in the presence of mild thyroid dysfunction and iron deficiency;

however, this inference should not be generalized to more severe hematological or endocrine phenotypes. From a practical standpoint, HbA1c should be interpreted with caution when hypothyroidism is overt, when iron deficiency is severe, or when marked anemia is present, particularly when the measured value lies close to the diagnostic thresholds for prediabetes or diabetes. In these situations, confirming glycemic status with fasting plasma glucose or an oral glucose tolerance test, reassessing HbA1c after correction of the underlying deficiency, or using erythrocyte-independent markers such as glycated albumin or fructosamine may help avoid diagnostic misclassification (24,25). Recent analyses have similarly examined the extent to which iron deficiency compromises the accuracy of HbA1c in non-anaemic individuals (26).

This study has several limitations. The retrospective, cross-sectional design does not allow assessment of causality or post-treatment changes in HbA1c. Because the population consisted predominantly of subclinical hypothyroidism and mild-to-moderate iron deficiency phenotypes, inferences regarding more extreme clinical states, such as overt hypothyroidism, severe iron deficiency, or marked anemia, are limited. In addition, reticulocyte count was not available; therefore, mechanistic interpretations regarding erythrocyte turnover rely on indirect indicators such as hemoglobin, MCV, and RDW. Furthermore, because these erythrocyte indices are likely mediators of the association rather than independent confounders, the loss of significance in the fully adjusted model should be interpreted as consistent with mediation rather than as evidence of confounding; a formal mediation analysis was not undertaken. Future prospective studies applying formal causal mediation methods, such as structural equation modeling, and incorporating direct markers of erythrocyte turnover would allow this indirect pathway to be quantified rather than inferred. The single-center design and reliance on a single clinical assessment period may also limit generalizability and preclude evaluation of temporal changes.

## CONCLUSION

In conclusion, this matched cross-sectional study shows that, in adults without diabetes who had not received relevant endocrine or hematinic treatment, the coexistence of hypothyroidism and iron deficiency is associated with only a limited difference in HbA1c. The small gradient observed in age- and sex-adjusted analyses lost statistical significance after full adjustment, suggesting that variation in HbA1c may reflect a limited distributional shift related to erythrocyte indices and hematinic status rather than a synergistic or independent metabolic effect. These findings support the view that, in adults without diabetes, mild thyroid dysfunction and iron deficiency do not produce an effect large enough to meaningfully distort HbA1c interpretation. Nonetheless, this association may differ in more extreme phenotypes, such as overt hypothyroidism, severe iron deficiency, or marked anemia. Prospective, multicenter studies incorporating direct markers of erythrocyte turnover, such as reticulocyte count, and tracking post-treatment changes are needed to more clearly delineate the biological and clinical boundaries of this association.

**Conflict of Interests**
*The authors declare that there is no conflict of interest in the study.*

**Financial Disclosure**
*The authors declare that there is no financial support.*

**Ethical Approval**
*This study was approved by the Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee (TAREK) (Protocol No. 120, Date: 10.01.2026).*

**Informed Consent**
*Owing to the retrospective design, the requirement for informed consent was waived.*

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