

## Research Article

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# The independent relationship between hemoglobin A<sub>1c</sub> and homeostasis model assessment of insulin resistance in non-diabetic subjects

## Diyabetik olmayan bireylerde hemoglobin A<sub>1c</sub> ve homeostasis model insülin direnci arasındaki bağımsız ilişki

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**Abstract**

**Introduction:** Determining the factors affecting hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) levels may help better interpretation of HbA<sub>1c</sub> values. In this study, we investigated if insulin resistance is a significant parameter contributing to the variability of HbA<sub>1c</sub> values.

**Methods:** We retrospectively analyzed serum fasting glucose, fasting insulin, 2 h glucose and HbA<sub>1c</sub> records of 18–85 years aged outpatients who underwent a 75 g oral glucose tolerance test (OGTT) in our hospital during the period January 2010–May 2014. Homeostasis model assessment of insulin resistance (HOMA-IR)  $\geq 2.5$  was defined as insulin resistant.

**Results:** Insulin resistant subjects with normal glucose tolerance had significantly higher HbA<sub>1c</sub> and fasting glucose levels compared to insulin sensitive subjects with normal glucose tolerance ( $p=0.002$ ,  $p<0.001$ , respectively). Similarly, insulin resistant subjects with pre-diabetes had significantly higher HbA<sub>1c</sub> and 2-h glucose levels compared to insulin sensitive subjects with pre-diabetes ( $p=0.016$ ,  $p=0.013$ , respectively). Regression analysis showed that HOMA-IR(log) is associated with HbA<sub>1c</sub> levels independent of fasting and 2h glucose concentrations ( $p<0.001$ ). Age

was the variable with highest standardized  $\beta$  coefficient in regression model.

**Conclusion:** Our data showed that HOMA-IR is associated with glycated hemoglobin values independent of glycemic status and the effect of age on HbA<sub>1c</sub> values should not be ignored in non-diabetic subjects.

**Keywords:** HbA<sub>1c</sub>; Insulin resistance; Age; Diabetes mellitus; HOMA-IR.

**Özet**

**Amaç:** Hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) sonuçlarını etkileyen faktörlerin belirlenmesi bu testin daha iyi yorumlanmasını sağlayabilir. Bu çalışmada insülin direncinin HbA<sub>1c</sub> değerlerini etkileyen anlamlı bir parametre olup olmadığı araştırılmıştır.

**Yöntem:** Retrospektif olarak hastanemizde Ocak 2010–Mayıs 2014 tarihleri arasında 75 g oral glukoz tolerans testi (OGTT) yapılan 18–85 yaşlarındaki ayaktan hastaların serum açlık glukozu, açlık insülini, 2.saat glukoz ve HbA<sub>1c</sub> değerlerini analiz ettik. Homeostasis model insülin direnci (HOMA-IR)  $\geq 2.5$  değeri insülin direnci olarak kabul edildi.

**Bulgular:** Normal glukoz toleranslı insülin dirençli bireyler normal glukoz toleranslı insülin duyarlı bireylere göre daha yüksek HbA<sub>1c</sub> ve açlık glukoz seviyelerine sahiptir (sırasıyla  $p=0.002$ ,  $p<0.001$ ). Benzer şekilde prediabetli insülin dirençli bireyler, prediabetli insülin duyarlı bireylere göre daha yüksek HbA<sub>1c</sub> ve 2.saat glukoz seviyelerine sahiptir (sırasıyla  $p=0.016$ ,  $p=0.013$ ). Regresyon analizinde HOMA-IR(log), açlık ve 2.saat glukoz

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konsantrasyonlarından bağımsız olarak HbA<sub>1c</sub> seviyeleri ile ilişkili bulunmuştur ( $p < 0.001$ ). Yaş regresyon modelinde en yüksek standardize beta katsayısına sahip değişken olarak bulunmuştur.

**Sonuç:** Verilerimiz glisemik durumdan bağımsız olarak HOMA-IR'nin glikozile hemoglobin düzeyleri ile ilişkili olduğunu göstermiştir ve diyabetik olmayan bireylerde yaşın HbA<sub>1c</sub> düzeylerine etkisi göz ardı edilmemelidir.

**Anahtar Kelimeler:** HbA<sub>1c</sub>; İnsülin direnci; Yaş; Diabetes mellitus; HOMA-IR.

## Introduction

Insulin resistance (IR) is defined as the impaired ability of target tissues of fat, liver, and muscle to show various metabolic effects of insulin, including glucose uptake [1]. Insulin resistance plays an important patho-physiological role in the development of diabetes, dyslipidemia, hypertension, and cardiovascular disease [2, 3]. Prospective studies have shown that it is a powerful predictor of the likelihood of an individual developing diabetes or cardiovascular disease [4]. Accurate measurement of IR requires complex techniques that are expensive and time-consuming. A number of surrogate indices of IR had been developed. The homeostatic model of assessment-insulin resistance (HOMA-IR), which uses fasting insulin and glucose levels to calculate IR, is the most widely used [5]. However, HOMA-IR results are reasonably correlated with those of clamping studies (the “gold standard”).

Glycated hemoglobin (HbA<sub>1c</sub>) is well recognized and widely used as a measure of glycemic control reflecting the mean blood glucose level over the preceding weeks to months. Until recently, hemoglobin A<sub>1c</sub> has only been used to monitor people already diagnosed with diabetes, serving as the gold-standard measure of glycemic levels over a 3-month period. More recently, as with measures of glucose, HbA<sub>1c</sub> levels have been used to describe a continuum of risk for the development of diabetes and associated conditions. Because it can be measured regardless of food intake, A<sub>1c</sub> is simpler than fasting plasma glucose (FPG) or oral glucose tolerance tests [6].

Recently, the International Expert Committee recommended that HbA<sub>1c</sub> be added to the diagnosis of diabetes mellitus (DM); the 2010 American Diabetes Association (ADA) clinical practice recommendation defining HbA<sub>1c</sub> levels of over 6.5% as DM, and an HbA<sub>1c</sub> between 5.7 and 6.4% as pre-diabetes [7, 8]. It was shown that pre-diabetes is associated with insulin resistance which is a risk factor for development of type 2 diabetes. How well a HbA<sub>1c</sub> level

in the pre-diabetic range (5.7%–6.4%) predicts insulin resistance is not clear. Publications associated with the relationship between HbA<sub>1c</sub> and insulin resistance are limited. Therefore, the aim of the current study was to clarify the relationship between insulin resistance and HbA<sub>1c</sub>.

## Materials and methods

We retrospectively analyzed serum fasting glucose, fasting insulin, 2 h glucose and HbA<sub>1c</sub> records of 18–85 years-old outpatients who underwent a 75 g OGTT in Department of Biochemistry, Tepecik Teaching and Research Hospital during the period January 2010–May 2014. The samples for all parameters were collected in the same day. Patients with a serum creatinine level above reference range and patients with DM according to ADA criteria (fasting glucose  $> 125$  mg/dL or 2 h glucose  $> 199$  mg/dL or HbA<sub>1c</sub>  $> 6.4\%$ ) were excluded. The present study included a total of 365 patients of whom 281 were women and 84 were men. The study was conducted with the approval of the Local Hospital's Ethics Committee.

Glucose tolerance status was assessed with the 75-g OGTT and serum fasting glucose defined according to the 2010 ADA criteria [8]. Our study used the HOMA-IR as the diagnostic criteria for insulin resistance and HOMA-IR  $\geq 2.5$  was identified as an indicator of insulin resistance. HOMA-IR was calculated using the following formula:  $\text{HOMA-IR} = \text{fasting serum glucose (mg/dL)} \times \text{fasting serum insulin value } (\mu\text{U/mL}) / 405$  [5]. Serum glucose levels were measured by the hexokinase method using the Olympus AU 2700 analyzer (Olympus Diagnostics. GmbH, Hamburg, Germany).

Serum insulin levels were measured using direct chemiluminescence technology on Siemens Immulite 2000 XPI analyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA). HbA<sub>1c</sub> analysis was performed by affinity chromatography HPLC on Primus Ultra2 Analyzer (Primus Corporation, Kansas City, Kansas, USA).

## Statistical methods

Statistical analyses were conducted using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA).  $p$ -Value  $< 0.05$  was considered as statistically significant. Data were expressed as mean  $\pm$  SD or with 95% confidence intervals (CI). Independent samples  $t$ -test and  $\chi^2$  test were used for comparison of insulin resistant and insulin

sensitive groups. Multivariate linear regression analysis was used for determining the association between HbA<sub>1c</sub> and independent variables. Variables with non-normal distribution were log transformed.

Results

A total of 365 non-diabetic subjects were included in the study. Mean age of the subjects were 40 ± 11 and 281 (77%) of the subjects were female, while 84 (23%) of the subjects were male. There were 191 individuals with normal glucose tolerance and 174 individuals with pre-diabetes (impaired fasting glucose, impaired glucose tolerance, or both according to ADA criteria). Insulin resistance (HOMA-IR > 2.5) were detected in 147 (40%) of the subjects. Table 1 shows the characteristics of the study population.

The subjects were divided into two groups according to their HOMA-IR values: group 1, insulin sensitive (HOMA-IR ≤ 2.5) and group 2, insulin resistant (HOMA-IR > 2.5). HbA<sub>1c</sub> and other biochemical parameters of insulin resistant and insulin sensitive subjects were compared. Analysis showed that insulin resistant subjects with normal glucose tolerance had significantly higher HbA<sub>1c</sub> and fasting glucose levels compared to insulin sensitive subjects with normal glucose tolerance (p = 0.002, p < 0.001, respectively). Similarly, insulin resistant subjects with pre-diabetes had significantly higher HbA<sub>1c</sub> and 2-h glucose levels compared to insulin sensitive subjects with pre-diabetes (p = 0.016, p = 0.013, respectively) (Table 2).

Table 1: Characteristics of the study population.

n = 365	
Age (mean ± SD)	40 ± 11
Sex	
Male	84 (23)
Female	281 (77)
OGTT	
Normal glucose tolerance	191 (52)
Pre-diabetes	174 (48)
Pre-diabetes	
IFG	112 (64)
IGT	11 (6)
IFG and IGT	51 (30)
HOMA-IR	
> 2.5	147 (40)
≤ 2.5	218 (60)
HbA <sub>1c</sub>	5.5 ± 0.4

Data are expressed as mean ± SD or n (%). IFG, Impaired fasting glucose; IGT, impaired glucose tolerance.

To estimate the independent relationship between HbA<sub>1c</sub> and HOMA-IR, multiple linear regression analysis was used. Age, gender, fasting glucose, 2-h glucose and HOMA-IR were entered in the regression model as independent variables. Since HOMA-IR values had skewed distribution, log transformed HOMA-IR was used in analysis. Regression analysis showed that age, fasting glucose, 2-h glucose, and log transformed HOMA-IR values are significant predictors of HbA<sub>1c</sub>. Higher HOMA-IR values are associated with higher HbA<sub>1c</sub> levels in non-diabetic subjects. Standardized β coefficients demonstrate the relative importance of predictor variables in regression model. In the regression model age showed the highest standardized β coefficient. In non-diabetic subjects age was the most important factor affecting HbA<sub>1c</sub> values (Table 3). HbA<sub>1c</sub> values were also presented in different age groups (Table 4).

Dividing the study population as normal and pre-diabetic subjects and re-performing the regression analysis did not change the results fundamentally. Age was still most effective factor in predicting HbA<sub>1c</sub> values in both normal group and pre-diabetic group. Log HOMA was still a significant variable (data not shown).

To visualize the data a regression plot showing the relation between HbA<sub>1c</sub> and HOMA-IR was generated. The relation between HbA<sub>1c</sub> and HOMA-IR was similar in different sample groups (group 1: fasting glucose < 96 mg/dL, group 2: fasting glucose > 96 mg/dL) (Figure 1).

Discussion

It was clearly reported that lower HbA<sub>1c</sub> values were associated with reduced microvascular and macrovascular complications in diabetic patients [9]. In type 2 diabetes a HbA<sub>1c</sub> target level of < 7% is currently recommended by ADA and HbA<sub>1c</sub> is the basis guiding diabetes therapy. Recently ADA recommended using HbA<sub>1c</sub> also for the diabetes diagnosis and consolidated its importance in diabetes mellitus. The use of HbA<sub>1c</sub> as a diagnostic criterion necessitates a more accurate measurement and careful interpretation. Several factors other than plasma glucose levels were reported to affect glycated hemoglobin levels. Any condition affecting turnover of red blood cells like hemolytic anemia and iron deficiency affect HbA<sub>1c</sub> levels. Previous studies showed that genetic-ethnic factors, sex hormones and age are associated with HbA<sub>1c</sub> values [10–14]. In the present study we report that HOMA-IR is a factor affecting HbA<sub>1c</sub> values independent of fasting and 2-h post-load glucose concentrations. The relationship between glycated hemoglobin

**Table 2:** Demographic and biochemical parameters of insulin resistant and insulin sensitive subjects.

	Normal glucose tolerance			Pre-diabetes		
	HOMA $\geq 2.5$ (n=60)	HOMA $< 2.5$ (n=131)	p-Value	HOMA $\geq 2.5$ (n=100)	HOMA $< 2.5$ (n=74)	p-Value
Age	37 $\pm$ 11	37 $\pm$ 10	0.923	44 $\pm$ 11	44 $\pm$ 11	0.881
Female/male	73%	82%	0.147 <sup>a</sup>	75%	73%	0.763 <sup>a</sup>
HbA <sub>1c</sub> (%)	5.5 $\pm$ 0.4	5.3 $\pm$ 0.4	0.002	5.8 $\pm$ 0.3	5.6 $\pm$ 0.4	0.016
2-h glucose (mg/dL)	96 $\pm$ 22	91 $\pm$ 22	0.229	129 $\pm$ 30	116 $\pm$ 38	0.013
Fasting glucose (mg/dL)	91 $\pm$ 5	87 $\pm$ 7	$< 0.001$	108 $\pm$ 8	105 $\pm$ 9	0.103

Values are presented as mean  $\pm$  SD or %. Independent samples t-test used for comparisons unless otherwise indicated. <sup>a</sup>p-Value determined using  $\chi^2$  test.

**Table 3:** Multivariate linear regression model with HbA<sub>1c</sub> as dependent variable.

Variables	Regression coefficient B	Standardized coefficient $\beta$	p-Value	%95 CI for B
Age	0.010	0.279	<b><math>&lt; 0.001</math></b>	0.007–0.014
HOMA-IR <sub>(log)</sub>	0.263	0.192	<b><math>&lt; 0.001</math></b>	0.133–0.392
Fasting glucose	0.008	0.218	<b><math>&lt; 0.001</math></b>	0.004–0.011
2-h glucose	0.001	0.100	<b>0.040</b>	0.0005–0.0025
Sex	0.056	0.056	0.210	–0.032–0.145

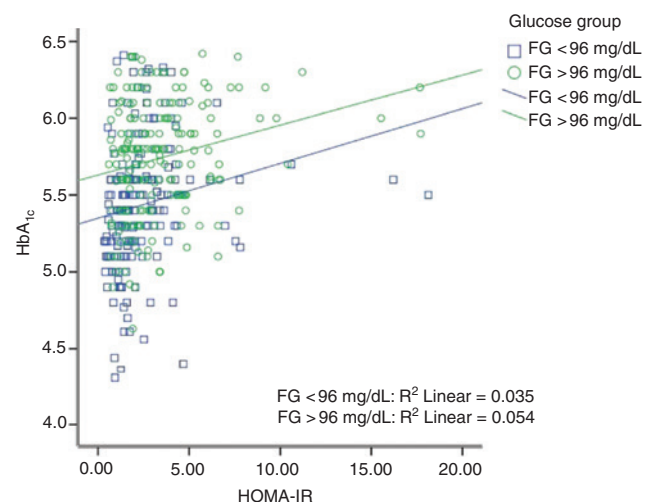
R<sup>2</sup> = 0.30. Significant (p < 0.05) values are presented in bold.

**Table 4:** HbA<sub>1c</sub> values in different age groups.

Age	Normal glucose tolerance (n=191)	Pre-diabetes (n=174)
	HbA <sub>1c</sub>	HbA <sub>1c</sub>
18–30 (n=75)	5.31 $\pm$ 0.38	5.57 $\pm$ 0.31
31–40 (n=107)	5.38 $\pm$ 0.38	5.64 $\pm$ 0.39
41–50 (n=107)	5.55 $\pm$ 0.44	5.74 $\pm$ 0.40
51–60 (n=61)	5.66 $\pm$ 0.34	5.87 $\pm$ 0.31
> 60 (n=15)	5.90 $\pm$ 0.45	6.00 $\pm$ 0.26

levels and glucose levels are higher in diabetic patients than in non-diabetic patients therefore it can be concluded that non-glycemic factors affecting HbA<sub>1c</sub> levels are more important in non-diabetic patients [15].

Previous studies investigated the relationship between insulin resistance and HbA<sub>1c</sub>. Gallwitz et al. showed that with increasing HbA<sub>1c</sub> levels, there was a statistically significant increase in HOMA-IR in patients with type 2 DM [16]. Heianza et al. reported that in subjects without a history of diabetes a HbA<sub>1c</sub> level of  $> 5.9\%$  were significantly associated with higher HOMA-IR values [17]. In another study Borai et al. showed that the correlation between HbA<sub>1c</sub> and insulin resistance were higher in subjects with normal glucose tolerance than in patients with pre-diabetes and diabetes [18]. None of the aforementioned studies reported corrected results independent of

**Figure 1:** Scatterplot showing correlation between HOMA-IR and HbA<sub>1c</sub> in different sample groups. Group 1: fasting glucose  $< 96$  mg/dL. Group 2: fasting glucose  $> 96$  mg/dL.

glycemic status. Venkataraman et al. showed that in a multivariate regression model with HbA<sub>1c</sub> as dependent variable HOMA-IR(log) were significantly associated with HbA<sub>1c</sub> independent of fasting glucose but the effect of post-load glucose on HbA<sub>1c</sub> was lacking [19]. One recent study indicated that HbA<sub>1c</sub> was associated with HOMA-IR independent of 0- and 120-min glucose in pregnant women with gestational diabetes mellitus [20]. The results of our



study reveal that HbA<sub>1c</sub> is associated with HOMA-IR independent of fasting and post-load glucose status also in non-diabetic subjects.

Glycation is the nonenzymatic attachment of free aldehyde groups of carbohydrates to the unprotonated free amino groups of proteins [21]. The binding of glucose molecules to potential glycation sites in hemoglobin molecule leads to formation of HbA<sub>1c</sub>. Via condensation with glucose, hemoglobin A first forms a labile intermediate adduct, which is thereafter rearranged to the more stable ketoamine adduct (HbA<sub>1c</sub>) form [22]. Physiological factors like pH, inorganic phosphate, oxidative stress, deglycation, and Schiff base inhibitors can affect the rate of HbA<sub>1c</sub> formation [23–27]. Another point to be considered is that glycation of hemoglobin occurs in the intracellular compartment. Previously, it was demonstrated that the erythrocyte glucose-to-plasma glucose concentration ratio may affect hemoglobin glycation and contributes to the variation in HbA<sub>1c</sub> levels [28].

Oxidative stress is a process that was proposed to be associated with the multifactorial etiology of insulin resistance. It was shown that plasma markers of oxidative stress were correlated with the degree of insulin resistance [29, 30]. Oxidative stress which is a factor co-existing with insulin resistance may also be responsible for the increased hemoglobin glycation. Oxidative stress biomarkers as lipid peroxides were reported to be associated with hemoglobin glycation [31, 32]. LDL oxidation was also suggested to increase HbA<sub>1c</sub> values [33]. Furthermore there is evidence that antioxidants can partially inhibit the formation of HbA<sub>1c</sub> [31].

Consistent with previous studies, age was a significant factor affecting HbA<sub>1c</sub> levels independent of glycaemia [13, 34]. However the mechanisms involved in the age related HbA<sub>1c</sub> increase remain to be established. Since our model showed that age was the most effective factor contributing to the variation of HbA<sub>1c</sub> levels in non-diabetic subjects it should not be ignored when interpreting an HbA<sub>1c</sub> result. The question whether age-specific diagnostic and treatment criteria would be appropriate was previously mentioned [13].

Due to its retrospective design the current study has some limitations. Firstly the information of some possible confounding variables (e.g. BMI) could not be gathered; therefore confounding factors may exist. Second, HOMA-IR is not the gold standard method for measuring insulin sensitivity. The euglycemic hyperinsulinemic clamp technique is the gold standard for quantifying insulin sensitivity; however, this technique requires insulin infusion and repeated blood sampling. HOMA-IR is a relatively simple method to determine

insulin sensitivity. It can be calculated from a single blood sample and it was reported to have a linear correlation with glucose clamp technique [35].

In conclusion, our data showed that HOMA-IR is associated with glycated hemoglobin values independent of glycaemia and age is a very important factor affecting HbA<sub>1c</sub> values in non-diabetic subjects.

**Conflict of interest statement:** All authors declare that there is no conflict of interest regarding the publication of this article.

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