

## Evaluation of Anemia Effect on HbA1c Level Measurement in Type 2 Diabetic People

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### Abstract

**Objective:** For many years HbA1c used as diagnostic criteria for diabetes, however, measurement of HbA1c has some limitations that cannot accurately assess blood glucose levels in conditions such as changes in red blood cell life. In this study, we evaluated and compared the levels of HbA1c in diabetic, pre-diabetic and non-diabetic individuals to understand the role and relationship of different values of RBC's indices in these conditions.

**Materials and Methods:** This study performed between August to December of year 2021, and the demographic information and hematologic indices of 706 individuals which referred to laboratories in Gorgan city were investigated. According to results of FBS and HbA1c, individuals categorized in three distinct healthy (H), pre-diabetic (PD), and diabetic (D) groups based on latest ADA criteria.

**Results:** Evaluation of HbA1c level based on the presence or absence of anemia showed that in diabetic group with anemia significantly had a lower level  $8.4 (\pm 1.5)$  than people without anemia  $8.6 (\pm 1.5)$  ( $P=0.045$ ).

**Conclusion:** In this study, it was shown that changes in RBC indices in anemia can lead to inaccurate measurement of HbA1c level.


**Keywords:** HbA1c, Diabetes, RBC indices, Hemoglobinopathies, Anemia

QR Code:



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## Introduction

Since the 1990s, by world Health Organization (WHO) and the diabetes associations or societies in many countries Hemoglobin A1c (HbA1c) have recommended as the preferred diagnostic index for monitoring diabetes and later also been introduced as a “gold standard” and diagnostic tool for type 2 diabetes mellitus (T2DM) (1) and American Diabetes Association (ADA) recommended that HbA1c  $>6.5\%$  should be used as diagnostic criteria for diabetes (2). HbA1c is formed by glycosylation of the valine residue NH<sub>2</sub>-terminal of the b-chain of hemoglobin (Hb) (3). However, measurement of HbA1 has some limitations that cannot be accurately assess blood glucose levels in conditions such as changes in red blood cell life (4), Hb metabolic disorders and the use of erythropoietin (5). Anemia is one condition that affects erythrocyte turnover and in anemia such as hemolytic anemia, chronic liver disease, and increased hemolysis from splenomegaly increase reticulocyte and decrease the mean age of erythrocyte, subsequently decreases HbA1c level, furthermore, in hemoglobinopathies such as HbAS, HbAC, HbE, and HbD, can also alter HbA1c level (6), which is argued by physician (7).

The numerous studies have pointed out that any factor causes a decrease the half-life of RBCs, can causes decreased HbA1c, therefore in this study, we evaluated and compared the levels of HbA1c in diabetic, pre-diabetic and non-diabetic individuals to understand the role and relationship of different values of RBC's indices in these conditions.

## Material and methods

In this study 706 individuals base on convenience sampling between august to December 2021, who referred to laboratories in Gorgan city, entered in the study. For this purpose, a checklist was designed and patients' demographic information, laboratory data

were collected. After filling the checklist, patients who were eligible, included in the study.

Exclusion criteria were people less than 40 years, pregnancy or lactation, patients with malignancy, patients with liver, kidney and heart disease, autoimmune diseases.

In fasting condition, 5 cc of blood was taken in appropriate vials for CBC and other requested tests. CBCs were performed by sysmex kx-21-5 cell counter (sysmex, japan) and HbA2 and HbA1C analyzed by Sebia Capillarys 2 Flex Piercing, together (Lisses, France), which this method's accuracy were verified in many studies (8-9).

According to demographic information and laboratory tests (Blood sugar test and HbA1c), individuals categorized in three distinct healthy (H), pre-diabetic (PD), and diabetic (D) groups base on latest ADA criteria (2). Briefly, the patient who had A1c  $\geq 6.5\%$ , OR FPG  $\geq 126$  mg/dL, OR 2-hour plasma glucose  $\geq 200$  mg/dL during an OGTT, OR In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose  $\geq 200$  mg/dL, categorized in diabetic group. The patients who had a lower cut-off value for FPG (100-125 mg/dL) and has additional hemoglobin A1c (HbA1c) based criteria of a level of 5.7% to 6.4% for the definition of prediabetes. Anemia also according to the World Health Organization (WHO) criteria defined as hemoglobin levels less than 12 (g/dl) in premenopausal females and 13 g/dl in postmenopausal females and males of all ages (10).

Data were analyzed using SPSS software version 18. Student t test used for independent samples when normally distributed and Mann Whitney U test when not normally distributed. For comparing categorical data, Chi square test was performed.

## Ethical considerations

The study was approved by the Ethics Committee of the Golestan University of

Medical Sciences (IR.GOUMS.REC.1400.045).

## Results

In this study, 706 people were examined based on age, sex, diabetes status, anemia status, and HbA2 level (Table 1). Patients categorized in diabetes status group according to blood glucose and HbA1c levels according to ADA guideline (2), in anemia status group according to WHO criteria (10), and in HbA2 group in three distinct group, less than 2.2%, between 2.2-3.4%, and more than 3.5% (11).

Evaluation of the level of HbA1c in healthy individuals was  $5.47 (\pm 0.3)$ , in pre-diabetics was  $6.18 (\pm 0.13)$  and in diabetics was  $8.58 (\pm 1.78)$ , which was statistically significant, as we expected ( $P=0.001$ ).

Evaluation of effect of gender on HbA1c level, showed no significant difference

between men and women in group under study separately (Table 2). But the overall mean of HbA1c in women were  $7.32(\pm 2.0)$  and in men were  $6.99 (\pm 1.86)$ , and women statistically had higher levels of HbA1c ( $P=0.004$ ).

In this study the average age in women were  $52.75 \pm 12.48$  and in men were  $53.1 \pm 11.9$ , which was not significantly different ( $P=0.696$ ). Then we analyzed the results according to patients under and above 50 year. Just in healthy group the results were significantly difference, and in patients above 50 year, HbA1c level was 5.64% which was close to pre-diabetic boarder line and had significant different in comparison to patients less than 50 years ( $P=0.001$ ) (Table 3).

Analysis also showed that in healthy individuals the mean age was  $45.7 (\pm 13.4)$ , in pre-diabetic patients it were  $54.26 (\pm 10.08)$ , and in diabetic patients it were  $56.97 (\pm 9.79)$ . ( $P=0.001$ ) (Table 4).

**Table 1. Results of demographic and categorization of patient in the groups under study**

Variable		Frequency	Percent
Gender	female	490	69.4
	male	216	30.6
Age (year)	40- 50	277	39.2
	more than 50	429	60.8
Diabetes Status	non-Diabetic	235	33.3
	pre-Diabetic	94	13.3
Anemia status	Diabetic	377	53.4
	Anemic	256	36.3
HbA2 status (%)	non-Anemic	450	63.7
	<2.2	178	25.2
	2.2-3.5	475	67.3
	>3.5	53	7.5

**Table 2. Evaluation of HbA1c according to gender**

Group	Gender	N	Mean	SD**	P-value*
Healthy	Female	151	5.45	0.35	0.355
	Male	84	5.49	0.32	
Pre-diabetes	Female	68	6.19	0.13	0.494
	Male	26	6.16	0.14	
Diabetes	Female	271	8.65	1.79	0.186
	Male	106	8.38	1.76	

\*based on t-student test, \*\* Standard deviation

**Table 3. Evaluation of HbA1c level based on age in groups under study**

Variable	Age group	N	Mean	SD**	P-value*
Healthy	less than 50	149	5.37	0.34	0.001
	More than 50	86	5.64	0.26	
Pre-diabetic	less than 50	32	6.20	0.15	0.367
	more than 50	62	6.17	0.13	
Diabetic	less than 50	96	8.54	1.73	0.790
	more than 50	281	8.59	1.80	

\*based on t-student test, \*\* Standard deviation

Evaluation of Hb level based on diabetes status showed that its level were in healthy individuals  $12.79 (\pm 1.46)$ , in pre-diabetic individuals  $12.6 (\pm 1.31)$ , and in diabetic individuals  $12.49 (\pm 1.41)$ , that just between healthy and diabetic group showed a significant difference ( $P= 0.011$ ). There was also a significant negative relationship between total hemoglobin and HbA1c ( $P= 0.007$ ).

The overall comparison of the mean level of HbA1c in anemic and non-anemic individuals showed no difference between the two groups ( $P= 0.846$ ). However, in intra group evaluation, HbA1c level based on the presence or absence of anemia showed that in diabetic group with anemia significantly had a lower level than people without anemia ( $P= 0.045$ ) (Table 5).

Another evaluated variant in this study was the amount of HbA2 in patients. The results showed that the level of HbA2 between healthy  $2.5 (\pm 0.7)$  and diabetic groups  $2.25 (\pm 0.65)$  ( $P= 0.001$ ), and also between the pre-diabetic  $2.46 (\pm 0.8)$  and the diabetic group, had a significant difference ( $P= 0.001$ ), but this was not significant between the healthy and pre-diabetic groups.

Also, the study of the difference between HbA1c based on HbA2 groups showed that individuals with  $A2 < 2.2\%$  had higher HbA1c levels  $8.23 (\pm 2.25)$  than those with HbA2 between 2 and 3.5  $6.78 (\pm 1.78)$  ( $P= 0.001$ ), and also in HbA2  $> 5.3$   $6.98 (\pm 1.64)$  ( $P=$

0.001).

The study of the difference of HbA1c based HbA2 levels just in the diabetic group also showed that, people with A2 less than 2.2%  $9.02 (\pm 2.25)$ , had higher HbA1c than the group with HbA2 between 2 and 3.5  $(8.33) (\pm 1.66)$  ( $P= 0.001$ ), and HbA2  $> 3.5$   $(8.35) (\pm 1.39)$  ( $P= 0.001$ ). The results of examining the relationship between HbA1c and HbA2 showed that there is a significant negative relationship between these two factors that decreases the amount of HbA1c with increasing HbA2 ( $P= -0.001$ ).

Comparison of MCV in patients showed that in HbA1c levels, no significant difference was seen between patients with MCV less than 80 fl  $7.6 (\pm 2.1)$  and more  $7.9 (\pm 1.91)$  than 80 fl ( $P= 0.083$ ). Also, according to this classification, there was no significant relationship between HbA1c and MCV ( $P= 0.083$ ).

The results showed that in the control group, CD34 had a significant direct relationship with insulin ( $P= 0.019$ ,  $r= 0.425$ ), and IR ( $P= 0.017$ ,  $r= 0.433$ ). In the DNP group, CD34 had a significant direct relationship with CD133 ( $P= 0.011$ ,  $r= 0.411$ ). In general, CD34 has a significant inverse relationship with FBS ( $P= 0.0001$ ,  $r= -0.425$ ), HbA1c ( $P= 0.001$ ,  $r= -0.384$ ), LDL ( $P= 0.046$ ,  $r= -0.245$ ), and uric acid ( $P= 0.0001$ ,  $r= -0.466$ ). In addition, CD34 has a significant direct relationship with Insulin ( $P= 0.001$ ,  $r= 0.382$ ), IR ( $P= 0.011$ ,  $r= 0.308$ ), and CD133 ( $P= 0.001$ ,  $r= 0.399$ ).

**Table 4. Comparison of Age between three groups of study according to clinical status**

Variable	N	Mean	SD**	P-value*
Pre-diabetic	94	54.26	10.08	0.036
Diabetic	377	56.97	9.79	
Healthy	235	45.71	13.40	0.001
Diabetic	377	56.97	9.79	
Healthy	235	45.71	13.49	0.001
Pre-diabetic	94	54.26	10.08	

\*based on one way ANOVA test, \*\* Standard deviation

**Table 5. HbA1c levels based on patients' anemia status**

Variable	HbA1c level		P-value*
	Anemic	Non-Anemic	
Healthy	5.43 ( $\pm 0.37$ )	5.48 ( $\pm 0.33$ )	0.452
Pre-diabetic	6.17 ( $\pm 0.14$ )	6.19 ( $\pm 0.14$ )	0.215
Diabetic	8.4 ( $\pm 1.5$ )	8.6 ( $\pm 1.42$ )	0.045

\*based on one way ANOVA test

## Discussion

The present study, which aimed to investigate the effect of changing erythrocyte counts on measuring HbA1c levels in healthy and diabetic patients, showed that although the mean age of men and women was not significantly different, but comparing the mean age of patients based on diabetes status showed that the mean age of pre-diabetic individuals was higher than healthy individuals. Also, the mean age of diabetics was higher than pre-diabetics. The relationship between age and diabetes status was also significant.

In the study, Masuch et al. (12) also showed that the percentage of HbA1c will increase with age and linear regression confirmed the positive correlation of HbA1c with age, which was independent of BMI. These results were also consistent with the results of Huang et al. (13).

Another study published in 2017 stated that the current HbA1c threshold (.56.5% or 48mmol/ mol) showed low sensitivity (35.6%) and high specificity (98.9%) in the diagnosis of diabetes (14). The diagnostic efficiency of HbA1c for diabetes decreases with age, and this age effect occurs with decreasing RBCs with age. Therefore, HbA1c may be inappropriate for diagnosing diabetes in the elderly due to the physiological decrease in the number of red blood cells in the elderly (14).

In addition to age, gender is also an important factor affecting HbA1c levels. Our results showed that the mean of HbA1c in women was higher than the mean in men, which was statistically significant.

However, the results of another study showed that HbA1c levels were significantly higher for men than for women among all participants. However, there was no significant positive relationship between HbA1c levels in men for the age group of 50 to 70 years. However, this level was significantly higher in men than women in the age groups of 30-39 and 40-49 years (13). Another study also showed that women had lower HbA1c than men, although this difference was not

significant (12). The inconsistent results in our results were probably due to the greater matching of hemoglobin levels in both genders.

In the study of hemoglobin level based on diabetes status, it was observed that hemoglobin levels were higher in healthy, pre-diabetic and diabetic individuals, respectively, among which, the mean hemoglobin level in healthy and diabetic individuals showed a significant difference ( $P= 0.011$ ). There was also a significant negative relationship between total hemoglobin and HbA1c ( $P= -0.007$ ).

One of the possible mechanisms of increased anemia in patients with diabetes may be because diabetes leads to kidney damage and kidney failure can cause anemia. Healthy kidneys know when the body needs new red blood cells. They secrete a hormone called erythropoietin (EPO), which signals the bone marrow to make more red blood cells. Damaged kidneys do not send enough EPO to meet the body's needs.

On the other hand, people with diabetes are more prone to inflamed blood vessels and micro- and macro-antipathies', which can reduce the lifespan of red blood cells. This can prevent the bone marrow from receiving the signal needed to make more red blood cells. Therefore, keeping diabetes under control and appropriate tests to identify anemia in early-stage diabetic patients can reduce the severity of the complications of anemia in the diabetic population.

Diabetic women and the elderly with diabetes are the most vulnerable group to anemia, so care should be taken in their nutrition and supplements. Physicians should be aware of the risk of anemia in these patients and prescribe vitamin and iron supplements if needed. To maintain glucose levels within the normal range, drug adaptation must be achieved in the diabetic population. Diabetics should be informed of the risk of anemia and other complications of diabetes at the time of diagnosis. Which is significantly increased the risk of cardiovascular disease (15,16).



Negative correlations between HbA1c and MCV, MCH and MCHC were also observed in several studies. Therefore, this study concluded that RBC parameters are a suitable tool parallel to HbA1c and blood glucose for the evaluation of diabetic patients (17-19).

## Conclusions

In this study, it was shown that changes in RBC indices in anemia can lead to inaccurate measurement of HbA1C level.

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## Conflict of Interest

The authors have no Conflict of Interest.

## Authors' contributions

S. K: Collected the data and performed the analysis.

Z. H: Contributed data or analysis tools and wrote the paper.

F. K: Collected the data and performed the analysis.

Kh. K: Conceived and designed the analysis and contributed data or analysis tools.

MT.H: Conceived and designed the analysis and wrote the paper.

All authors have accepted responsibility for the entire content of this manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and approved the version to be published.

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