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Comparıson of the Diagnostıc Value of HbA1c with Fructosamıne in Dıabetes Mellitus

Hesna URAL KAYALIK[1]*, Tuba CANDAR[2], Selda DEMİRTAS[2] and Sema CETIN[3]

[1]Ufuk University, Vocational School of Health Services, Ankara, TURKEY

[2]Ufuk University, Faculty of Medicine, Department of Biochemistry, Ankara, TURKEY

[3]Kirikkale University, Faculty of Sciences and Literature, Department of Biology, Kirikkale, TURKEY

*Corresponding Author E-mail: drhesnaural@gmail.com

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ABSTRACT

HbA1c is the most widely used follow-up test for diabetes mellitus (DM) worldwide. Although the fructosamine test is not widely used, this test can also be useful for the follow-up of DM in the previous 2 weeks.

This study aimed to determine the effect of pathological conditions associated with heme abnormalities by measuring the hemoglobin content in patients with both iron-deficiency anemia and diabetes. To investigate pathological conditions associated with the globin content of hemoglobin, diabetic and prediabetic patients with thalassemia minor were also enrolled. The effects of thalassemia minor on HbA1c levels were evaluated, and serum fructosamine was assayed in both groups to evaluate the accuracy of the results.

Our results demonstrate that while HbA1c levels are altered as a result of hemoglobinopathies related to severe iron deficiency anemia or thalassemia, fructosamine results are not affected by these conditions in either diabetic or prediabetic patients. Furthermore, there was no relationship between HbA1c and fructosamine in either the diabetic or prediabetic groups. These results indicate that the HbA1c test is inadequate for the diagnosis and follow-up of DM patients with heme- or globin-related anomalies. The addition of the fructosamine test is suggested to improve the interpretation of the test results.

Key words: *Diabetes Mellitus, HbA1c, Fructosamine*

INTRODUCTION

Diabetes mellitus (DM) is a disease caused by an insulin deficiency and requires constant medical care. This chronic disease can influence the metabolism of carbohydrates, fats and proteins due to defects in insulin[1]. Chronic diabetic microangiopathy accelerates the development of complications such as disease of the kidney glomeruli, retinal damage, neuropathy, and atherosclerosis[2]. These complications cause thousands of people to die each year worldwide. Therefore, the proper diagnosis and treatment of diabetes is critical.

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HbA1c was known as a follow-up test for diabetes until 2010, when it was reported by the American Diabetes Association (ADA) as a diagnostic test for diabetes. At that time, the reference range for HbA1c was reported to be 4-6%. The primary target HbA1c level suggested by the ADA for the treatment of diabetic patients is below 7%. If the HbA1c level reaches 8%, the treatment regime should be re-evaluated[3,4]. A standardization study of HbA1c conducted by the ADA in 2010 suggested that a HbA1C value of 6.5% may be used as a cut-off value to diagnose diabetes. It was also noted, however, that the HbA1c test result is only valid if it was assayed by a laboratory that is approved by the Diabetes Control and Complications Center (DCCT) and follows the National Glycohemoglobin Standardization Program (NGSP) guidelines[5]. In biochemistry, the addition of glucose molecules to proteins is called glycosylation. In diabetic patients with hyperglycemia, the levels of protein glycosylation are excessive. Increases in blood glucose are correlated with increased levels of non-enzymatic protein glycosylation[6,7]. Hemoglobin (Hb) is the most important glycosylated protein. Once Hb is glycosylated, the glycosylation remains stable throughout the life of an erythrocyte. Hemoglobin glycosylation can be measured by assaying HbA1c, which represents the vast majority of HbA1, and the results are reported as a percentage of total Hb. The amount of glucose bound to hemoglobin within the erythrocytes in the blood is referred to as the HbA1c test. HbA1c is a clinically useful indicator of the mean glucose level during the previous 120- day period (mean erythrocyte life span)[8]. Although the test does not provide information about the daily or short-term fluctuations in blood glucose and does not reflect hypoglycemic episodes, it is the best test of long-term diabetic control. The HgA1c test is an objective measurement technique that does not require the cooperation of the patient. Today, more than 10 glycohemoglobin (GHB) measurements are used in laboratories. These methods fall along a broad spectrum that includes manual minicolumn systems for research purposes as well as automated systems[9,10]. Additional specific proteins can be glycosylated, including lens proteins, erythrocyte membrane proteins, nerve proteins and albumin. For example, serum fructosamine is a serum protein that is subject to non-enzymatic glycosylation. Serum fructosamine usually reflects the degree of hyperglycemia over a period as short as 1-3 weeks in diabetic patients[11]. Fructosamine measurements are not affected by diet, stress, exercise status, cholesterol or triglyceride levels, glucose levels up to 55.5 mmol/L, or bilirubin levels up to 4.0 mg/dl[12-15]. The aim of the study was to determine the diagnostic value of HbA1c in diabetic and prediabetic patients and to compare the use of the HbA1c value with fructosamine. For this purpose, the patients with iron deficiency anemia as an indicator of pathological conditions of heme content were investigated. To study the pathological conditions associated with the globin content of hemoglobin, diabetic and prediabetic patients with thalassemia minor were enrolled in the study. All of the test results were compared with those from a control group.

The effect of iron deficiency on the HbA1c molecule in diabetic patients was investigated separately in patients who were mildly anemic. This common form of anemia is usually ignored because the corresponding Hb levels fall within the range of 11 and 12 g/dl. This type of anemia is sufficiently mild that clinicians may not take it into consideration during routinely measured blood glucose assessments because they are mainly focused on HbA1c results in diabetic patients. Thus, in these patients, HbA1c may fail to accurately reflect the blood glucose level because the structure of heme has been affected by the anemia. Meanwhile, in diabetic patients with Hb values <11 g/dl who exhibit apparent clinical symptoms of iron deficiency anemia, the anemia treatment is applied immediately. Therefore, we specifically focused on different kinds of anemia with respect to the factors mentioned above.

MATERIALS AND METHODS

Our research was performed with the ethics approval of Ufuk University Faculty of Medicine Non-Invasive Research and Evaluation Commission, Ankara, Turkey and followed the guidelines outlined in the Declaration of Helsinki. Our study enrolled a total of 88 diabetic cases with concurrent iron-deficiency anemia (41 women and 47 men). HbA1c levels were evaluated separately for individuals 18-64 years old and those older than 65. Patients with β-thalassemia were also enrolled in the study, and all were less than 65 years old. These patients were subdivided into prediabetic and diabetic subgroups. A total of 30 diabetic patients with β-thalassemia (16 female and 14 male) and 30 prediabetic patients with β-thalassemia (18 women and 12 men) were included.

Diabetic or prediabetic patients were diagnosed according to the 2010 criteria of the ADA. Patients with a fasting blood glucose ≥ 7mmol/L and postprandial blood glucose values or 2 h(two hour) oral glucose tolerance test (OGTT) (75g) ≥11.1 mmol/L were diagnosed as DM; those with fasting blood glucose 5.5-7 mmol/L or postprandial blood glucose values or 2 h OGTT 7.8-11.1 mmol/L were diagnosed as prediabetic[5].

The age, sex, height, weight, history of hypertension and presence of other diseases were documented by administering a questionnaire to the patients. After obtaining informed consent, fasting glucose, OGTT, fructosamine, iron, iron binding capacity and HbA1c were measured. All of the biochemical parameters were assayed by Roche Cobas Integra 800. HbA1c tests were evaluated by HPLC (Chromosystem, Agilent 1100 series). CBC tests were performed automatically (Complete Blood Assay, Beckman Coulter LH 680). TSH and ferritin levels were measured using the Roche Elecsys 2010 (electrochemiluminescent technique). The evaluation of abnormal hemoglobin was performed by HPLC (β-Thalassemia test, Chromosystem, Agilent 1100 series HPLC).

Routine assessments of lipid profiles, liver function, thyroid function and renal function were performed. For this purpose, BUN, creatinine, ALT, AST, GGT, total protein, albumin, direct bilirubin, total bilirubin, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride values were assayed by Roche Cobas Integra 800. Blood samples were taken from healthy volunteers (control group), patients with anemia, and patients with β-thalassemia, and the serum specimens were prepared by centrifugation. Light microscopy imaging was assessed to evaluate erythrocyte deformity.

Data analysis was performed using the SPSS16 Windows program. At the end of the study, the data obtained were analyzed using the independent Student's T-test and ANOVA. Spearman's test was used to assess correlations. For the T-test and ANOVA, different levels of statistical significance were set at $p<0.05$ (significant) and $p<0.01$ (highly significant). For the correlation analysis, a correlation r-value of 0.3 to 0.69 was considered to indicate moderate correlation, and a correlation value of 0.7 to 1 was considered to indicate strong correlation.

RESULTS

We compared the average values of the measured biochemical parameters between patients with diabetes and controls (Table 1). A total of 80 diabetic patients were included. The fasting blood glucose (FBG, n=61), postprandial blood glucose (PBG, n=57), HbA1c (n=80), and fructosamine (n=79) values were 8.4 ± 0.3 mmol/L, 13 ± 0.5 mmol/L, 7.22 ± 0.2% and 286.54 ± 9.9 μmol/L, respectively. The control group comprised 70 people with mean fasting blood glucose (n=67), PBG (n=67), HbA1c (n=70) and fructosamine (n=70) values of 5.3 ± 0.1 mmol/L, 6.8 ± 0.4 mmol/L, 5.26 ± 0.06% and 227.65 ± 2.32 μmol/L, respectively. The mean values for all these parameters were significantly higher in diabetic patients than in the control group ($p<0.01$).

Table 1: The evaluation of differences between the biochemical parameters of the whole patient group and the control group

Diabetic Group	Control Group	
FBG	**8.4 ± 0.3*** (n=61)	5.3 ± 0.1 (n=67)
PBG	13 ± 0.5 * (n=57)	6.8 ± 0.4 (n=67)
HbA1c	7.22 ± 0.2 * (n=80)	5.26 ± 0.06 (n=70)
Fructosamine	286.54 ± 9.9 * (n=79)	227.65 ± 2.32 (n=70)

($p<0.05$ values were considered as significantly different) *

*Difference between Diabetic group and control, $p<0.01$

When the diabetic patients were analyzed as two separate subgroups with HbA1c ≥ 6.5%(n=45) and HbA1c ≤ 6.4% (n=35), we observed no correlation between HbA1c and fructosamine.

The diabetic patients (n=88) with concurrent iron deficiency anemia were divided into two subgroups: Group 1, patients with severe anemia (11 g/dl ≥ Hb, n=50); and Group 2, patientswith mild anemia (12 g/dl ≥ Hb >11, n=38). The results were compared with the control group (n=68).

In Group 1, the average FBG, HbA1c and fructosamine levels were 8.6 ± 0.3 mmol/L, 6.14 ± 1.3% and 283.72 ± 7.2 µmol/L, respectively. Fructosamine and FBG values were significantly higher than in the control group ($p<0.01$) although there was no significant difference with respect to HbA1c values.

In Group 2, the mean FBG, HbA1c and fructosamine levels were 8.6 ± 0.2 mmol/L, 6.33 ± 0.1% 280.29 ± 8.2 µmol/L, respectively and were significantly higher than those of the control group ($p<0.01$) (Table 2).

Table 2: The evaluation of differences between the biochemical parameters of the patient groups (with severe and mild anemia) and the control group

	Group 1	**Group 2**	**Control Group**
FBG	8.6 ± 0.3* (n=50)	8.6 ± 0.2 ** (n=38)	5.3 ± 0.1 (n=68)
HbA1c	6.14 ± 1.3 (n=50)	6.33 ± 0.1 ** (n=38)	5.26 ± 0.1 (n=68)
Fructosamine	283.72 ± 7.2 * (n=50)	280.29 ± 8.2 ** (n=38)	227.65 ± 2.2 (n=68)

($p<0.05$ values were considered as significantly different)

* Difference between group 1 (patients with 11≥ Hb) and control, $p<0.01$

** Difference between group 2 (patients with 12≥ Hb>11) and control, $p<0.01$

Diabetic patients with iron deficiency anemia were divided into two groups with respect to age (18-64 years old (n=48) and ≥ 65 years old (n=40)) to evaluate the effect of age on these tests. In the patients less than 65 years old, the FBG, HbA1c and fructosamine levels were 9 ± 0.2 mmol/L, 6.34 ± 0.1% and 290.86 ± 8.7 μmol/L, respectively, and were significantly higher than the levels in the control group ($p<0.05$). In patients older than 65 years, the FBG, HbA1c and fructosamine levels were 8.4 ± 0.3 mmol/L, 6.11 ± 0.1% and 268.82 ± 8.2 μmol/L, respectively, and only FBG and fructosamine were significantly higher in the patients than in the control group ($p<0.05$) (Table 3).

Table 3: The evaluation of difference between the biochemical parameters of the patient

Groups with Respect to Age	Diabetic Patients with Iron Deficiency 18-64 Age Group (n=48)	Control Group (18-64 Age)	Diabetic Patients with Iron Deficiency older than 65 Age Group (n=40)	Control Group (older than 65 Age)
FBG	9 ± 0.2*	5.3 ± 0.1 (n=32)	8.4 ± 0.3**	5.4 ± 0.2 (n=35)
HbA1c	6.34 ± 0.1*	5.1 ± 0.1 (n=38)	6.11 ± 0.1**	5.36 ± 0.1 (n=32)
Fructosamine	290.86 ± 8.7*	231.65 ± 6.1 (n=36)	268.82 ± 8.2**	220.32 ± 5.8 (n=34)

($p<0.05$ values were considered as significantly different)
* Difference between Diabetic Patients with Iron Deficiency 18-64 age group and control, $p<0.05$
** Difference between Diabetic Patients with Iron Deficiency older than 65 age group and control, $p<0.05$

The effects of thalassemia minor on HbA1c were evaluated, and serum fructosamine was measured to determine the accuracy of the results. β-Thalassemia patients (18-64 years old) were divided in two subgroups: diabetic β-thalassemia (n=30) and prediabetic β-thalassemia (n=30). The means of the measured biochemical parameters were compared with those from the control group (n=32) (Table 4). In diabetic patients with β-thalassemia, the mean FBG, HbA1c, HbA2 and fructosamine values were 9.8 ± 0.3 mmol/L, 5.22 ± 0.2%, 4.18 ± 0.1% and 236.33 ± 4.1 μmol/L, respectively, and were higher than those of the control group. The mean values of RBC and Hb were 4.36 ± 0.1 mil/μL and 9.70 ± 0.2 g/dl, respectively, and were lower than those of the control group. A significant difference was observed with respect to the FBG, fructosamine, Hb and HbA2 tests ($p<0.05$). However, this significance was not observed for the RBC and HbA1c tests (Table 4).

In prediabetic patients with β-thalassemia, the FBG, HbA1c, HbA2 and fructosamine values were 6.4 ± 0.1mmol/L, 4.59 ± 0.1%, 3.81 ± 0.1% and 225.61 ± 2.0 μmol/L, respectively, and were higher than the control group. Similar to diabetic patients with β-thalassemia, the mean values for RBC and Hb (4.62 ± 0.1 mil./μL and 11.26 ± 0.3 g/dl, respectively) were lower than those in the control group. Statistically significant differences were observed for the FBG, fructosamine, Hb and HbA2 tests ($p<0.01$). However, this relationship was not observed for the RBC and HbA1c tests (Table 4).

Table 4: Significant differences in means of biochemical parameters of the patients who were diabetic β-thalassemia, prediabetic β-thalassemia and the control groups

	Diabetic β-Thalassemia Group	Prediabetic β-Thalassemia Group	Control Group
FBG	9.8 ± 0.3 * (n=30)	6.4 ± 0.1 ** (n=30)	5.1 ± 0.1 (n=32)
HbA1c	5.22 ± 0.2 (n=30)	4.59 ± 0.1 (n=30)	4.52 ± 0.1 (n=32)
Fructosamine	236.33 ± 4.1 * (n=30)	225.61 ± 2.0 ** (n=30)	217.07 ± 2.0 (n=32)
RBC	4.36 ± 0.1 (n=30)	4.62 ± 0.1 (n=30)	4.89 ± 0.1 (n=32)
Hb	9.70 ± 0.2 * (n=30)	11.26 ± 0.3 ** (n=30)	12.68 ± 0.2 (n=32)
HbA2	4.18 ± 0.1 * (n=30)	3.81 ± 0.1 ** (n=30)	1.81 ± 0.1 (n=32)

($p<0.05$ values were considered as significantly different)

* The significant difference between the patients of Diabetic β- thalassemia and control group at $p<0.01$

** The significant difference between the patients of Prediabetic β- thalassemia and the control group at $p<0.05$

A statistical evaluation could not be performed for patients with β-thalassemia older than 65 years due to the inadequate number of patients.

The correlation between HbA1c and HbA2 was evaluated in diabetic patients with β-thalassemia (n=30), prediabetic patients with β-thalassemia (n=30) and the control group(n=32). Although HbA1c and HbA2 values were not correlated in the control group (r=0.159), in patients with β-thalassemia, a significant positive correlation was observed between HbA1c and HbA2 (r=0.467 and r=0.359 for diabetic and prediabetic patients, respectively). Thus, HbA1c levels were positively correlated with HbA2 in these patients. Examination of a larger number of cases could strengthen this correlation.

A moderate correlation between Hb and HbA1c was observed in the control group (r=0.451). Similarly, a significant correlation between Hb and HbA1c was detected in diabetic patients with β-thalassemia (r=0.310) and prediabetic patients with β-thalassemia (r=0.307). There was no correlation between HbA1c and fructosamine in diabetic patients with β-thalassemia (r=0.061) or in prediabetic patients with β-thalassemia (r=0.072).

DISCUSSION

Diabetic complications cause death or permanent organ damage in thousands of patients each year worldwide. Therefore, patients must be diagnosed correctly and should be well monitored. Glycohemoglobin (HbA1c), which is among the tests used in the diagnosis and monitoring of diabetic patients, provides information about the amount of glucose in the blood over the last three months. The basic marker for determining long-term glucose levels in diabetic patients is the HbA1c level[1,8,16]. The ADA has proposed the use of the glycohemoglobin test for the follow-up and diagnosis of DM if the test is assayed in specific centers that provides standard and reference methods for the analysis. However, problems surrounding the standardization of this method persist[17]. Both the differences in the methods used and patients' physiological conditions can affect the results. Thus, some healthy people may be diagnosed with diabetes by mistake. Variables that determine the level of HbA1c include the mean serum glucose level within the last 2-3 months, the erythrocyte life span, the erythrocyte plasma membrane permeability to glucose, the tissue oxygen concentration and the

health of the haem and globin structures[18-22].

According to a standardization study reported by the ADA in 2010, a HbA1c ≥ 6.5% should be used for the diagnosis of DM[5]. The ADA declared that the HbA1c test is only acceptable if the test is approved by the Diabetes Control and Complications Center (DCCT) and is assayed by a laboratory that is part of the National Glycohemoglobin Standardization Program (NGSP)[4,5,8]. Although the ADA reported HbA1c ≥ 6.5% as the critical value, inearly 2010, this value was changed to ≥ 6.3% according to suggestions from the National Glycohemoglobin Standardization Program[23].

The confusion about the threshold value of HbA1c encouraged us to clarify this subject. We think that the HbA1c assay is insufficient for the diagnosis of diabetes. Thus, we also measured the serum fructosamine levels in our patients to verify the diagnosis of diabetes. Serum fructosamine, which effectively measures the levels of glycated serum proteins, is an indicator of glycemic control over the prior two weeks, which is equal to the lifespan of albumin[11]. With respect to glycohemoglobin levels, posttranslational glycation of plasma proteins occurs through a slow, non-enzymatic reaction between the amino groups of plasma proteins (mostly albumin) and glucose[24-26]. Human serum albumin turnover (half-life of 14-20 days) is shorter than that of hemoglobin (erythrocyte life span of 120 days), and therefore fructosamine concentration reflects the glycemic index of a shorter time period than does glycohemoglobin concentration. Studies of serum fructosamine demonstrate that serum fructosamine is well correlated with glycohemoglobin[8,27]. It is suggested that the use of serum fructosamine will significantly improve the follow-up and prevention of diabetic complications[11].

In our study, there was no correlation between HbA1c and fructosamine in diabetic patients with β-thalassemia and in prediabetic patients with β-thalassemia. Similar results have been reported by other studies in the literature. One explanation for this phenomenon given by other authors is that HbA1c provides information about the amount of glucose attached tohemoglobin in erythrocytes [6-8], whereas fructosamine provides information about the amount of glucose that binds albumin. This result has been accepted as natural[11,24,25].

In our study, diabetic patients with iron deficiency anemia were separated into two subgroups: Group 1, 11 g/dl ≥ Hb (severe anemia); and Group 2, 12 g/dl ≥ Hb >11 g/dl (mild anemia).

The results of group 2 were similar to predictions from the International Association of Diabetes, which noted that the results of HbA1c are not affected by low Hb levels[5]. However, in patients with diabetes and 11 g/dl ≥ Hb, the FBG, HbA1c and fructosamine tests were significantly higher than in controls, although the HbA1c levels were not significantly increased. This result is inconsistent with the clinical picture. HbA1c levels are valuable only in persons with erythrocytes with a normal life expectancy.

The results of the presented study demonstrate that the HbA1c test is not reliable for the diagnosis of diabetes in patients with severe anemia.

It has been claimed that all GHb methods are insufficient for the long-term follow-up of glycemic control. However, non-hemoglobin-based alternative methods (e.g., serum fructosamine) may be useful in these patients.

In patients with conditions that shorten the lifespan of erythrocytes or reduce the mean erythrocyte life time (e.g., recovery period after acute blood loss or hemolytic anemia), the HbA1c level will be lower and may produce incorrect test results independent of the method used[17]. It has been emphasized that the HbA1c test cannot be used for the metabolic control of diabetes in cases of hemoglobinopathies, anemia, chronic hemolysis, or liver cirrhosis because of the effects of these conditions on erythrocyte lifespan or hemoglobin structure[28].

Several researchers have indicated that false positive values can be noted in patients with uremia, hyperlipidemia, HbF disease, thalassemia, aplastic anemia, myeloproliferativedisease, pregnancy, aspirin intake and alcoholism, whereas the falsely low values can be observed in patients with hemolytic anemia, HbS, HbC or HbD[29]. Minor

increases in HbA2 and HbF in sickle cell anemia, homozygous HbC disease, HbSC disease and β-thalassemia can affect several GHb measurement methods[12]. Our recent case study on a 4-year-old diabetic child revealed that a high HbF level interfered with the HbA1c measurement performed by HPLC (high performance liquid chromatography). However, the fructosamine test was well-correlated with the blood glucose level[30].

In our study, we observed that HbA2 values directly affected HbA1c test results in the group of patients with β-thalassemia. Our results are consistent with the literature. Indeed, the HbA1c test was found to be an inadequate marker with which to diagnose and follow diabetic patients with β-thalassemia. In addition, GHb values have a narrow reference range; in healthy people, these values change very little over time, but the values may vary significantly from person to person[31]. The results of our study also support this finding. Hemoglobin levels are generally below the lower reference values (9-11 g/dl) in patients with β-thalassemia minor. The mean corpuscular volume (MCV) is low, RBC counts are over 5 million/mm3, and the HbA2 level is > 3.5% [32]. Increased inappropriate δ-globin chains in the hemoglobin dimers (HbA2) may cause disruptions in the molecular stability that result in the production of defective β-globulin[33].

A weak positive correlation was observed between Hb and HbA1c in the diabetic patient group with β-thalassemia, the prediabetic group with β-thalassemia and the controls. We believe that this correlation would be strengthened with a greater number of cases.

Our study demonstrates that β-thalassemia pathology affects the stability of the hemoglobin molecule more than does iron deficiency. Blood glucose and fructosamine levels were elevated in both diabetic and prediabetic β-thalassemia patients, whereas HbA1c was notelevated in these patients. This may cause lower HbA1c results than expected (false negative).

Binding of glucose to the NH2 terminus of the globin chain of HbA2 or to the other NH2 molecules on R groups may alter the charge of the Hb molecule, thereby preventing HbA1c glycation. Thus, we believe that the fructosamine assay is superior to HbA1c for following diabetes, especially in patients with β-thalassemia.

Additional evidence that the HbA1c molecule is affected by globin chain abnormalities in patients with β-thalassemia is the good correlation between HbA1c and HbA2 in both diabetic and prediabetic patients. However, this correlation was not observed in the control group. This finding represents additional evidence that HbA1c molecules are affected by globin chain abnormalities in β-thalassemia. This finding demonstrates that the damage to the Hb molecule affects the HbA1c molecule directly.

The correlation of glycohemoglobin with serum fructosamine values is well established in thalassemia[8,27]. Fructosamine is affected by the amount of total protein in the plasma but does not readily change in response to conditions such as hemoglobinopathy or stress; furthermore, it is more stable than HbA1c because it is easy to measure[12-15].

CONCLUSION

We think that it will be more convenient to assess the health of patients by using the HbA1c test together with the fructosamine test during the diagnosis and treatment of diabetic and prediabetic patients. However, additional studies on the glycated albumin test, which has increased in popularity and in routine laboratory measurements, are warranted. Therefore, the glycated albumin test, which reports the percentage of glycosylated serum albumin, can support our arguments in favor of the use of fructosamine and will help further related research.

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