Glycated albumin versus HbA1c as indicators of glycemic control in type I diabetic children with iron deficiency anemia

Mohammed Hashem Mahgoob1, and Mahmoud Mohammed Moussa2
1Department of Pediatrics, Faculty of Medicine, Minia University, Minia, Egypt
2Department of Clinical Pathology, Faculty of Medicine, Minia University, Minia, Egypt

Abstract. We evaluated the clinical usefulness of glycated albumin (GA) and glycated hemoglobin (HbA1c) as indicators of glycemic control in type I diabetic (T1DM) children with and without iron deficiency anemia (IDA). Our prospective cross-sectional study was conducted on 147 T1DM children who were classified into Group I (with IDA) and Group II (without anemia). The participants were classified as controlled and uncontrolled based on mean blood glucose (MBG) in the past 30 days. The 5–12-yr-olds with MBG above 200 and 12–15-yr-olds with levels above 180 mg/dl were considered uncontrolled. HbA1c increased significantly in the participants with IDA compared to those without anemia (p < 0.01). HbA1c in those with IDA showed insignificant difference between the controlled and uncontrolled (p = 0.5), while GA was significantly higher in the uncontrolled than the controlled (p = 0.3). Receiver operating characteristic (ROC) curve analysis showed that GA had 87.2% sensitivity and 75.8% specificity at a cut-off point of 16.9%. HbA1c at a cut-off point of 7.09% showed 80% sensitivity and 57.6% specificity. For prediction of uncontrolled diabetes in children with IDA, we concluded that HbA1c increases significantly in diabetic children with IDA. GA may be a useful alternative biomarker for evaluating the glycemic control in such children.

Key words: type 1 diabetes mellitus, iron deficiency anemia, HbA1c, glycated albumin

Introduction

Uncontrolled type 1 diabetes mellitus (T1DM) in children may result in acute complications, such as hypoglycemia or diabetic ketoacidosis as well as long-term complications, including nephropathy and retinopathy. Therefore, intensive glycemic control is vital for managing such children (1).

Iron deficiency anemia (IDA) is considered the most common cause of anemia worldwide. Anemia is relatively common in children with T1DM and contributes to many clinical aspects of diabetes mellitus (2). Therefore, the possibility of the coexistence of T1DM and iron deficiency is higher than in any other type of anemia (3).

Glycemic biomarkers are used as important tools for assessing whether glycemic control was maintained within the target levels. They are also considered as alternative markers for estimating the risk of chronic complications (4).

Glycated hemoglobin (HbA1c) can be considered as the gold standard for assessing glycemic control (5). However, use of HbA1c has limitations. Conditions that affect the lifespan of red blood cells (RBCs) also affect HbA1c results. RBCs which have a short lifespan secondary to their destruction (as hemolytic anemia, destruction via passage through abnormal heart valves or splenomegaly) will decrease the level of HbA1c, which is irrelevant to the mean serum glucose levels. Hemoglobinopathies, such as sickle cell traits and other abnormal hemoglobin variants, such as hemoglobin C and E may lead to falsely increased or decreased HbA1c levels (6).

Nontraditional markers of hyperglycemia, such as fructosamine, glycated albumin (GA) or 1,5-anhydroglucitol (1,5-AG) may be useful indicators of glycemic control as alternatives to HbA1c in situations where HbA1c has limitations (7).

GA was hypothesized as an alternative marker for predicting glycemic control in diabetic children. It is unaffected by changes in the lifespan of RBCs, as in the case of hemoglobinopathy (8). GA is albumin containing lysine residues bound to glucose (9). It specifically measures the glycation product of albumin; it is not affected by serum albumin levels because its ratio to
total serum albumin is calculated (10). The enzymatic
GA assay is also better standardized and less affected
by pre-analytical variables than fructosamine (9),
which can change due to fluctuations in other serum
protein concentrations. GA can assess glycemic control
in diabetic children during the previous two to three
weeks because of the half-life of albumin (8).

Hence, this study aimed at determining the clinical
usefulness of GA and HbA1c as indicators for glycemic
control in type I diabetic children with and without iron
deficiency anemia.

Patients and Methods

Study design & participants

This was a prospective cross-sectional study. It was
conducted on 147 type I diabetic children [diagnosed
according ADA, 2016 criteria (11)], who had regular
follow ups in the Pediatric Endocrinology Outpatient’s
Clinic, Minia University Children Hospital, Egypt. They
were randomly selected over the period from January
2019 to February 2020. Their ages ranged from 5–15
years. They were classified into 2 groups: Group I
consisted of 72 diabetic children with IDA, and Group
II consisted of 75 diabetic children without anemia.

Anemia was diagnosed according to The World
Health Organization’s (WHO) definition of anemia
(12). Currently, measurement of serum ferritin is
the laboratory test recommended for diagnosing iron
deficiency. The WHO criteria proposed to define depleted
storage iron as 12 μg/L for children under 5 yr and 15
μg/L for those above 5 yr (13).

We excluded T1DM children with history of
other systemic diseases, recent blood loss, including
polymenorrhea in menstruating adolescents; hemoglobinopathies or other types of anemia.

Participants were subjected to a full history check
(including menstrual history in females), thorough
clinical examination, and laboratory investigations,
including complete blood count (CBC), serum ferritin,
serum albumin, mean blood glucose (MBG) of the past
30 days, HbA1c, and GA.

The mean duration of menstrual cycles was obtained
for each female. Menstrual irregularities were defined as
amenorrhea, oligomenorrhea, and polymenorrhea if the
cycle duration was > 90 d, >45 d, or < 25 d, respectively.
A normal cycle was defined as a cycle of 25–45 days’
duration.

Informed written consents were obtained from
the participants’ legal guardians before enrollment in
our study after its approval by the ethical committee
of Faculty of Medicine, Minia University, Egypt (No:
322-11/2018).

Methodology

Complete blood count: Determined by automated
cell counter sysmexKX-21N (TAO Medical Incorporation,
Japan).

Serum ferritin assay: Done by the Eurogenetics
ferritin quantitative ELISA (R&D system).

Mean blood glucose:
– For 30 d, 7-point blood glucose (BG) profiles
were collected (before meals, 90 min after meals, and
at bedtime) by patients at home using a glucometer (14).
– All participants used glucometers (accu-chek
active blood glucose meter manufactured by Roche
Diabetes care-Germany).

– Results were collected and mean blood glucose in
the past 30 d was estimated for each participant.

Classification of the T1DM participants as
controlled and uncontrolled was done according to
Svoren et al., (15) using mean blood glucose in the past
30 days. They were considered controlled when MBG
ranged from 150 to 200 mg/dl for 5–12-yr-olds and 120 to
180 mg/dl for those aged 12–15 yr. They were considered
uncontrolled when MBG was above 200 for 5–12-yr-olds
and 180 mg/dl for 12–15-yr-olds.

Glycosylated Hemoglobin Assay (HbA1c %)
was measured using the Stanbio Glycohemoglobin
procedure for quantitative colorimetric determination
of glycohemoglobin in the blood (Stanbio Laboratory,
Boerne, Texas). GA was determined using an enzymatic
method utilizing albumin-specific proteinase, ketamine oxidase,
albumin assay reagents (LUCICA GA-L; Asahi Kasei
Pharma Co., Tokyo, Japan). First, endogenous glycated
amino acids were eliminated from the sample. This was
done by ketamine oxidase to convert glycated amino acids
into amino acids. Second, GA is hydrolyzed into glycated
amino acids or peptides by the action of albumin-specific
proteinase. Glycated amino acids or peptides were then
oxidized by ketamine oxidase to produce amino acids and
hydrogen peroxide, which was measured quantitatively
by peroxidase enzyme producing a pigment measured
by spectrophotometer. Third, the albumin concentration
was measured using the bromocresol green method. GA
was expressed as a percentage of total serum albumin
[glycated albumin]/ (serum albumin) × 100/1.14 + 2.9
%. The previous formula is as per the manufacturer’s
instructions. The reference range for GA was 11.0% to
16.0%.

Statistical methods

Data were statistically analyzed using SPSS
program (Statistical Package for Social Sciences)
software version 21. Descriptive statistics were expressed
for quantitative data by mean and standard deviation.
They were presented for categorical data as number and
percentage. Analyses were done for quantitative data
using t test. However, for qualitative data, Chi-square
test or Fisher Exact test was employed when appropriate.
The degree of relationship between the variables was
 calculated using the Pearson correlation analysis.
Correlation coefficient (r) ranges from (0–1): weak
(r = 0–0.24), fair (r = 0.25–0.49), moderate (r = 0.5–0.74),

Mahgoob et al.

strong ($r = 0.75–1$). Receiver operating characteristic (ROC) curve analysis was performed using SPSS to determine the optimal cut-off values and diagnostic performance of the variables. The diagnostic sensitivity and specificity were studied using ROC curves. The level of significance was taken at $P$ value $< 0.05$.

**Results**

Our study was conducted on 147 diabetic children who were classified into two groups. Group I included 72 diabetic children with IDA, mean age was $9.9 \pm 3.4$ yr, 35 were males and 37 were females. Group II included 75 diabetic children without anemia, mean age was $10.4 \pm 2.91$ yr, 31 were males and 44 were females.

Our results demonstrated that the T1DM participants with and without IDA showed no significant difference with each other regarding the studied demographic and clinical data (Table 1).

The mean duration of menstrual cycles was obtained for each female patient. Among the participants, 20 (54%) T1DM females with IDA were menstruating and 10 (50%) of them showed oligomenorrhea. While menstruation was reported in 23 (52%) of the females without IDA, 11 (47%) of them showed oligomenorrhea. There was no significant difference between both groups regarding the effect of menstruation on our results.

Table 2 shows that hemoglobin level, mean corpuscular volume, and serum ferritin level were significantly lower in the diabetic participants with IDA than in those without anemia ($p < 0.01$ for all). HbA1c levels were significantly higher in the diabetic participants with IDA than those without anemia ($p < 0.01$). On the other hand, there were insignificant differences between them regarding mean blood glucose levels in the past 30 days and GA.

When we evaluated HbA1c and GA levels in the controlled and uncontrolled diabetic participants without anemia, we found significantly higher levels of both in uncontrolled than controlled participants ($p = 0.01$ and $0.02$, respectively) (Table 3). In those with IDA, there was an insignificant difference between the controlled and uncontrolled regarding HbA1c, while GA was significantly higher in the uncontrolled than the controlled ($p = 0.03$) (Table 4).

Table 4 shows that mean blood glucose levels in the past 30 d showed significant correlations with both HbA1c and GA ($r = 0.73$, 0.47, respectively) ($p < 0.01$), while in those with IDA, the mean blood glucose levels correlated only with GA ($r = 0.52$) ($p < 0.01$).

ROC curve analysis of HbA1c and GA for prediction of uncontrolled diabetes in those with IDA showed that GA had 87.2% sensitivity and 75.8% specificity at a cut-off point $> 16.9\%$, while HbA1c at a cut-off point of $> 7.09\%$ showed an 80% sensitivity and 57.6% specificity (Table 5, Fig. 1).

**Discussion**

In our study, we tried answering two questions. First, “is it accurate to assess glycemic control in T1DM children with IDA using HbA1c?” Second, “what is the usefulness of GA as an indicator of glycemic control in these patients?”

We choose GA as another indicator of glycemic control status as there has been accumulating evidence showing the importance of GA rather than HbA1c in some diseases and pathological conditions (16).

### Table 1. The studied demographic and clinical data between diabetic participants with and without iron deficiency anemia (IDA)

| Variable                          | (Group I) Diabetic children with IDA (n = 72) | (Group II) Diabetic children without anemia (n = 75) | p-value |
|-----------------------------------|---------------------------------------------|-----------------------------------------------------|--------|
| Sex (male/female)                 | 35/37                                       | 31/44                                               | 0.38 NS|
| Age (yr)                          | $9.9 \pm 3.4$                               | $10.4 \pm 2.91$                                     | 0.39 NS|
| Duration of disease (mo)          | $28.5 \pm 16.7$                             | $30.1 \pm 14.5$                                     | 0.54 NS|
| Residence                         | Urban                                       | Rural                                               | 0.42 NS|
|                                  | 27 (37.5%)                                  | 33 (44.0%)                                          |        |
|                                  | 45 (62.5%)                                  | 42 (56.0%)                                          |        |
| Girls with menstruation           | 20 (54%)                                    | 23 (52%)                                            | 0.42 NS|
|                                  | 0.45 $\pm$ 0.76                             | 0.42 $\pm$ 0.67                                     | 0.44 NS|
| Attacks of DKA (in the last year) | 0.88 $\pm$ 1.87                             | 0.74 $\pm$ 2.12                                     | 0.67 NS|
| Attacks of hypoglycemia (in the last year) | 31.8 $\pm$ 14.8                             | 32.7 $\pm$ 12.3                                     | 0.69 NS|
| Weight (kg)                       | 136.1 $\pm$ 16.3                            | 138.2 $\pm$ 13.5                                    | 0.40 NS|
| Height (cm)                       | 17.1 $\pm$ 5.3                              | 16.9 $\pm$ 3.8                                      | 0.80 NS|
| BMI (kg/m²)                       | 103.5 10.4                                  | 101.9 $\pm$ 12.4                                    | 0.39 NS|
| SBP (mmHg)                        | 68.6 $\pm$ 7.6                              | 66.2 $\pm$ 13.1                                     | 0.18 NS|

NS; non-significant. BMI, body mass index; DBP, diastolic blood pressure; DKA, diabetic ketoacidosis; SBP, systolic blood pressure.
Our study results indicate that MBG in the past 30 days showed no significant difference in the diabetic participants with and without IDA. This was in accordance with the studies by Coban (17) and Tarim et al. (18) who found no differences between patients with IDA and healthy subjects regarding mean blood glucose. Hence, we can hypothesize that IDA shows no effect on glycemic control in T1DM children; we can compare between the two groups in our study regarding GA and HbA1c as indicators for glycemic control.
As regards HbA1c, the levels in our study were significantly higher in the diabetic participants with IDA than those without anemia despite comparable MBG in both groups. This result agreed with several studies. For instance, Bhardwaj et al. (19) proved that the level of hemoglobin decreases with increasing severity of iron deficiency in anemic subjects; HbA1c levels increase correspondingly. Higher HbA1c may be explained by deficient RBC production due to the negative iron balance which leads to decrease in iron and hemoglobin. This leads to a slow turnover of RBCs. In this situation, more time for glycosylation of RBCs leads to falsely increased HbA1c values (1).

On the other hand, our results were against those of previous studies, such as Gram-Hansen (20) and Van-Heyningen et al., (21) who reported that there were no differences between the HbA1c levels of anemic patients and controls.

Between the two perspectives, a study by Sinha et al. (22) showed that values of HbA1c decreased with a fall in hemoglobin values and with treatment, these values increased in the following two months.

Glycated albumin in our study showed insignificant difference between the diabetic participants with and without IDA, which may suggest the neutral effect of IDA on GA. As per our knowledge, no other studies were done to evaluate GA in diabetic children with IDA.

Ghassy et al. (23) evaluated the effect of iron deficiency anemia on HbA1c and GA levels in non-diabetic patients and found that HbA1c was significantly higher in IDA before treatment than the normal controls and decreased significantly by iron therapy. However, there was insignificant difference between IDA patients and controls regarding GA. Koga et al. (16) studied usefulness of glycated albumin as an indicator of glycemic control status in type 2 diabetic patients with hemolytic anemia and found that GA is a useful indicator of glycemic control status in patients with hemolytic anemia. Additionally, Moriya et al. (24) evaluated glycemic control in pregnant women with diabetes and IDA and concluded that HbA1c overestimates glycemic control due to iron deficiency in pregnant women with diabetes, whereas GA accurately reflects their glycemic control.

When we evaluated HbA1c and GA levels among the controlled and uncontrolled diabetic participants according to MBG, the diabetic ones without anemia had significantly higher levels of HbA1c and GA among the uncontrolled than the controlled ones. There were also significant positive correlations between MBG and both HbA1c and GA. This agreed with Hempe et al. (25) and Svendson et al., (26) who found a good correlation between HbA1c and mean blood glucose level. Beck et al. (27) proved that GA correlated well with the mean blood glucose values of the preceding 2 wk.

On the other hand, we found significantly higher GA in the uncontrolled than controlled diabetic participants with IDA. There was a significant correlation between MPG and GA in them.

ROC curve analysis showed that GA had higher sensitivity and specificity than HbA1c for prediction of
HbA1c and GA were good parameters for assessing glycemic control in diabetic children without anemia. However, HbA1c increased significantly in diabetic children with IDA. Therefore, a patient’s iron level must be considered while interpreting HbA1c concentrations in T1DM. On the other hand, GA can be a useful alternative marker for evaluating glycemic control during the intermediary time period.

Recommendations

Iron status should be evaluated before any diagnostic or therapeutic decision is made based on HbA1c. GA may be an alternative biomarker for assessing glycemic control in diabetic children with IDA.

References

1. Kang DS, Park J, Kim JK, Yu J. Clinical usefulness of the measurement of serum fructosamine in childhood diabetes mellitus. Ann Pediatr Endocrinol Metab 2015;20:21–6. [Medline] [CrossRef]
2. Kwon E, Ahn C. Low hemoglobin concentration is associated with several diabetic profiles. Korean J Intern Med 2012;27:273-4. [Medline] [CrossRef]
3. World Health Organization. Iron Deficiency Anaemia: Assessment, Prevention and Control: A Guide for Programme Managers. Geneva: World Health Organization; 2001:1-114. WHO reference number: WHO/NHD/01.3 https://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/.
4. Krhač M, Lovrenčič MV. Update on biomarkers of glycemic control. World J Diabetes 2019;10:1–15. [Medline] [CrossRef]
5. Guo Q, Zang P, Xu S, Song W, Zhang Z, Liu C, et al. Time in range, as a novel metric of glycemic control, is reversely associated with presence of diabetic cardiovascular autonomic neuropathy independent of HbA1c in Chinese type 2 diabetes. J Diabetes Res 2020;2020: Article ID 5817074. doi: 10.1155/2020/5817074.
6. Wright LA, Hirsch IB. The challenge of the use of glycemic biomarkers in diabetes: reflecting on hemoglobin A1C, 1,5-anhydroglucitol, and the glycate proteins fructosamine and glycated albumin. Diabetes Spectr 2012;25:141–8. [CrossRef]
7. Parrinello CM, Selvin E. Beyond HbA1c and glucose: the role of nontraditional glycemic markers in diabetes diagnosis, prognosis, and management. Curr Diab Rep 2014;14:548. [Medline] [CrossRef]
8. Ciobanu DM, Bogdan F, Pătruţ CI, Roman G. Glycated albumin is correlated with glycated hemoglobin in type 2 diabetes. Med Pharm Rep 2019;92:134–8. [Medline]
9. Shimizu I, Kohzuma T, Koga M. A proposed glycemic control marker for the future: glycated albumin. J Lab Precis Med 2019;4:23. [CrossRef]
10. Gan T, Li X, Xu G. Glycated albumin versus hba1c in the evaluation of glycemic control in patients with diabetes and ckd. Kidney Int Rep 2017;3:542–54. [Medline] [CrossRef]
11. American Diabetes Association (ADA). Standards of medical care in diabetes. Diabetes Care 2016;39:1–106 https://care.diabetesjournals.org/content/suppl/2015/12/21/39.Supplement_1.DC2/2016-Standards-of-Ca%20tore.pdf.
12. WHO. Hemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MMN/11.1) (http://www.who.int/vmnis/indicators/haemoglobin. pdf, accessed [4/5/2020]).
13. Thuret I. Biological diagnosis of iron deficiency in children. Arch Pediatr 2017;24(5S):S56-S513.
14. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA1c: analysis of glucose profiles and HbA1c in the Diabetes Control and Complications Trial. Diabetes Care 2002;25:275–8. [Medline] [CrossRef]
15. Svoren B, Jospe N. Diabetes mellitus. In: Kliegman RM, Stanton BF, St Germe JW, Behrman RE and Schor NF editors. Nelson textbook of pediatrics. 18th ed. PA: Saunders; 2016. p. 2760-2790.
16. Koga M, Hashimoto K, Murai J, Saito H, Mukai M, Iegame K, et al. Usefulness of glycated albumin as an indicator of glycemic control status in patients with hemolytic anemia. Clin Chim Acta 2011;412:253–7. [Medline] [CrossRef]
17. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol 2004;112:126–8. [Medline] [CrossRef]
18. Tarim O, Kükükerdoğan A, Güney U, Erpal O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. Pediatr Int 1999;41:357–62. [Medline] [CrossRef]
19. Bhargaw K, Sharma SK, Rajpal N, Sachdev A. Effect of iron deficiency anemia on haemoglobinA1c levels. iMedPub j. 2016; 4 (4):123.
20. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. J Intern Med 1990;227:133–6. [Medline] [CrossRef]
21. van Heyningen C, Dalton RG. Glycosylated haemoglobin in iron-deficiency anaemia. Lancet 1985;1: 874. [Medline] [CrossRef]
22. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med 2012;32: 17–22. [Medline] [CrossRef]
23. Ghazy MA, Elbedewy TA, Baiomy N, Hodeib H. Effect of iron deficiency anemia on glycated hemoglobin and glycated albumin levels in non-diabetic patients: role of malondialdehyde. Life Sci J 2016;13: 31–8.
24. Moriya T, Matsubara M, Koga M. Hemoglobin A1C but not glycated albumin overestimates glycemic control due to iron deficiency in pregnant women with diabetes. J Diabetes Metab 2014;5: 445. [CrossRef]
25. Hempe JM, Gomez R, McCarter RJ Jr, Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. J Diabetes Complications 2002;16: 313–20. [Medline] [CrossRef]
26. Svendsen PA, Lauritzen T, Søegaard U, Nerup J. Glycosylated haemoglobin and steady-state mean blood glucose concentration in Type 1 (insulin-dependent) diabetes. Diabetologia 1982;23: 403–5. [Medline]
27. Beck R, Steffes M, Xing D, Ruedy K, Mauras N, Wilson DM, et al. Diabetes Research in Children Network (DirecNet) Study Group. The interrelationships of glycemic control measures: HbA1c, glycated albumin, fructosamine, 1,5-anhydroglucitol, and continuous glucose monitoring. Pediatr Diabetes 2011;12: 690–5. [Medline] [CrossRef]