

# Effect of sickle cell trait and $\beta$ -Thalassemia minor on determinations of HbA1c by an immunoassay method

Suad M. Al-Fadhli, PhD, AbdulHadi A. Ahmad, MSc, Hassan A. Al-Jafer, MD.

## ABSTRACT

**Objective:** Glycated hemoglobin determination is being used worldwide to monitor the efficiency of blood glucose control and to plan treatment in diabetes mellitus patients. Several methods are used for measuring glycated hemoglobin, but a possible interference by hemoglobin variants is a major concern. The use of immunoassay methods with glycated hemoglobin-specific antibodies is supposed to overcome this problem. We are evaluating the effect of the most prevalent hemoglobinopathies in the region (sickle trait hemoglobin and  $\beta$ -Thalassemia) on the immunoassay method used in determining glycated hemoglobin.

**Method:** Eighty-one whole blood sample hemolysates were tested for glycated hemoglobin using Beckman Synchron LX20 system, 37 of these normal adult hemoglobin represented 22 diabetic and 15 non-diabetic samples. Of the remaining 44 samples, 28 were from  $\beta$ -thalassemia minor and 16 from sickle-cell trait sickle trait hemoglobin patients, all non-diabetic. The samples were

collected in ethylenediaminetetraacetic acid anticoagulant and analyzed immediately or stored at 4°C for not more than 2 days.

**Results:** Sickle trait hemoglobin had no effect on glycated hemoglobin measurement by Synchron LX20 while  $\beta$ -thalassemia minor blood elevated the value of glycated hemoglobin to the range of diabetic cases.

**Conclusion:** Synchron LX20 glycated hemoglobin immunoassay method gave falsely high glycated hemoglobin results with  $\beta$ -thalassemia minor patient samples. Therefore, while interpreting the results of Synchron LX20 glycated hemoglobin, the patients history regarding hemoglobinopathies should be checked.

**Keywords:** Diabetes mellitus, glycated hemoglobin, glycohemoglobin, glycosylated hemoglobin, HbA1c, immunoassay.

Saudi Med J 2001; Vol. 22 (8): 686-689

Maintenance of appropriate blood glucose level in diabetes mellitus patients is essential to prevent the development of diabetic complications. The Diabetes Control Complications Trial (DCCT) demonstrated that maintaining near-normal blood glucose level significantly lowered a patients risk of developing complications related to diabetes.<sup>1,2</sup> Measuring blood glucose on a daily basis is not enough. An index of long-term glycemic control is considered valuable in evaluating the diabetic history of the patient, to monitor the efficacy of dietary

control and to plan treatment strategies. The presence of glucose at a high concentration in the diabetic patients blood results in an increased level of glycated hemoglobin (HbA1c) raising the glycation index of hemoglobin. The average life span of erythrocytes is 120 days. The extent of glycation thus represents mean blood glucose levels over the preceding 2-3 months. Measurement of glycated hemoglobin, often as HbA1c, is well established and widely used as an index of diabetic control in most parts of the world.<sup>3</sup> The glycation of hemoglobin is a

From the Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, (Al-Fadhli) Al-Amiri Hospital Medical Laboratory, Ministry of Health (Ahmad and Al-Jafer) State of Kuwait.

Received 30th November 2000. Accepted for publication in final form 19th March 2001.

Address correspondence and reprint request to: Dr. Suad Al-Fadhli, Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, PO Box 31470, Sulaibekhat 90805, State of Kuwait. Tel. +965 983 2214. Fax +965 483 3631. E-mail: Suadq8@yahoo.com

non-enzymatic, non-reversible covalent binding of the beta-chain of hemoglobin with glucose and is a normal biochemical process.<sup>4</sup> The measurement of HbA1c is a simple blood test that can be performed any time of the day without regards to food intake, or blood glucose level at the time of sampling. Although the use of HbA1c measurements to screen for diabetes is not recommended, several reports have explored the feasibility of its use in following up the history of diabetic patients.<sup>5</sup> In Kuwait, diabetes mellitus is reported as a leading chronic disease among the Kuwaiti population and the prevalence is on the increase.<sup>6</sup> The test has assumed great importance to monitor diabetic cases. Several detection modalities are available for measuring HbA1c including high performance liquid chromatography, affinity chromatography, enzyme immunoassay and isoelectric electrophoresis.<sup>7,8</sup> Regardless of which assay is used, certain conditions can interfere with obtaining accurate results such as hemoglobin variants and atypical hemoglobin.<sup>7,8</sup> Hemoglobinopathies are common single gene disorders known in Kuwait. For 2 of the more common disorders, Hb S (hemoglobin structural variant),<sup>9</sup> and  $\beta$ -thalassemia (Hb synthesis variant),<sup>10</sup> the possible analytical interference has not been established with the method used in HbA1c measurement in Kuwait.

In our study, we measured the interference of hemoglobin variants as in beta-thalassemia and sickle cell trait (HbAS), in the analysis of HbA1c using an immunological/colorimetric method (SYNCHRON LX20 system/Beckman Coulter). This method was certified recently (September 2000) by the National Glycohemoglobin Standardization Program (NGSP) for successfully completing precision and bias testing of the method, reagent lots, calibrator lots and instrumentation. For further information please go to website (<http://web.missouri.edu/~diabetes/ngsp/>).

**Methods.** The study was performed in Kuwait where diabetes mellitus and hemoglobinopathies (sickle cell anemia and thalassemia) are considered 2 of the highly prevalent diseases. Eighty-one whole blood sample hemolysates were tested for HbA1c using the SYNCHRON LX20 system. Thirty-seven of these samples had normal adult hemoglobin (HbAA) and represented 22 diabetic patients and 15 normal subjects. Of the remaining 44, all non-diabetic, 28 with  $\beta$ -thalassemia minor and 16 with (HbAS). The samples were collected in ethylenediaminetetraacetic acid (EDTA) anti-coagulant and processed immediately or stored at 4°C for not longer than 2 days. All samples were confirmed for the presence of hemoglobin variants using hemoglobin electrophoresis. Method of HbA1c estimation (SYNCHRON LX20 system): The SYNCHRON LX20 system was used according

to the manufacturer's instructions. Quality control materials obtained from the manufacturer were run with each batch. The system utilizes 2 unique cartridges, total hemoglobin and hemoglobin A1c, to determine HbA1c concentration as a percentage of total hemoglobin. The total hemoglobin concentration is measured by colorimetric method. The SYNCHRON LX20 system automatically proportions the appropriate sample and reagent volumes into a cuvette (1:8.6). The system will then monitor the change in absorbance at  $\lambda$  560. The change in absorbance is directly proportional to the total hemoglobin concentration. Glycated hemoglobin determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. Glycohemoglobin in the sample reacts with specific anti-HbA1c antibody to form a soluble antigen-antibody complex. If there is only one binding site free for the anti-HbA1c antibody in the HbA1c molecule, complex formation does not take place. Then polyhapten are added which react with excess antibodies to form insoluble antibody-polyhapten complex, which can be determined turbidimetrically. The SYNCHRON LX20 system automatically calculates the HbA1c concentration as follows: % HbA1c = HbA1c (g/dl) x 100 / total Hemoglobin (g/dl).

**Table 1** - Glycated hemoglobin (HbA1c) values by Synchron LX20.

NORMAL HEMOGLOBIN (HbAA)		HEMOGLOBINOPATHY	
Diabetic samples n=22 %	Non-diabetic n=15 %	$\beta$ -Thalassemia n=28 %	Sickle cell trait n=16 %
11	5	9	6
6	4	11	4
9	5	7.5	4
6	4	7	5
6	4	7	5
7	5	7	5
6	5	7	6
7	4	6	5
6	6	6	5
6	5	6	5
6	5	7	5
9	6	6.5	5
7	5.5	6	5
8	6	6	5
6	5	7	5
7		7	5
15		7	
7		11	
6		6	
6		6	
6		6	
6		6	
9		6	
		9	
		15	
		10.5	
		10	
		8	
		9	

**Results.** The precision of the assay was assessed using quality control material which in turn runs with the specimens. As shown in Table 1, for all 15 normal subjects tested here, the mean HbA1c value was 5% falling within the reference range, which is based on Kuwaiti Health System (4-6%). All diabetic patients had a HbA1c value higher than the upper limit of the range and were between 6 and 15%. Samples from patients with HbAS had a mean percentage of HbS of 35%. All patients were non-diabetic and the HbA1c concentration tested was within the reference range with a mean value of 5%. In the case of nondiabetic heterozygous  $\beta$ -Thalassemia samples the average percentage of HbF (Fetal hemoglobin) was 4%. All these (n=28) gave HbA1c values of 6-15% comparable to the diabetic samples.

**Discussion.** Glycated hemoglobin (HbA1c) is considered to be representative of the blood glucose level in the past few weeks of the patients medical history. It is being used widely as an index of both long and short-term glucose-control. The National Glycohemoglobin Standardization Program (NGSP) was established to standardize glycohemoglobin test results so that the clinical laboratory results are comparable to those reported in the Diabetes Control and Complications Trial (DCCT) where correlation between mean blood glucose and risk for vascular complications has been established. Factors other than blood glucose concentration have been reported to affect HbA1c value determined by several methods. Variant hemoglobin is one of these. Immunochemical methods, however, behave differently with regards to the specificity of the antibodies. Several laboratories reported that the use of immunoassays (Dako's and Bayer DCA 2000 immunoassays) for the measurement of HbA1c is not affected by Hb variants and derivatives as compared to other HbA1c measurement methods.<sup>8,11,12</sup> However, in cases with HbAS, Dako method gave values for HbA1c lower than the reference range. They reasoned that due to the presence of glycated Hb other than HbA1c, which are not recognized by the antibodies but nevertheless were counted with the total hemoglobin measured. Therefore, they recommended Dako users to establish their own special reference range.<sup>8,11</sup> While in the case of Bayer immunoassay, only one lab was involved in the study using this method, reporting the value of HbA1c in cases with HbAS within the normal range.<sup>8,11</sup> The Beckman Synchron LX20, as mentioned earlier, is based on immunoassay in measuring HbA1c. In cases with HbAS Synchron LX20 measured the HbA1c within the normal range similar to Bayer's immunoassay results. Weykamp et al have studied the interference of hemoglobin variants and

derivatives on glycohemoglobin determination using 16 methods including Dako and Bayer immunoassays.<sup>8</sup> In this study a simulated  $\beta$ -Thalassemia, where one volume of EDTA-blood from a newborn with normal HbF mixed with 30 volumes of an EDTA-blood sample from a healthy adult with HbAA, was tested for HbA1c using immunoassays Dako and Bayer. Both methods gave HbA1c results that were within the reference range. In our study all  $\beta$ -Thalassemia samples (n=28), with an average of 4% HbF, tested on Beckman Synchron LX20 gave an elevated HbA1c result comparable to that of the diabetic samples. The sheep anti-HbA1c antibodies used in Synchron LX20, as claimed by the manufacturer, are very specific with no cross reactivity with any of Hb variants including HbF. It seems that the manufacturer has tested the cross reactivity of the antibodies with pure HbF but not with thalassemic blood. There could be another Hb variant in thalassemic samples that interferes with the HbA1c measurement in the Synchron LX20 method. A comparison between our study and the immunoassays (Dako and Bayer) in the Weykamp study might not be significant because simulated thalassemic samples were used, not real thalassemic blood samples as in our study. We could not exclude the possibility that an Hb variant other than Hb F could be the reason for the high HbA1c value in the non diabetic/thalassemic patients samples. On the other hand, it was reported that in cases where there is high expression of HbF, as in thalassemia cases, the value of detected HbA1c by various methods was falsely elevated. A similar relation was noticed when glycated hemoglobin and HbF levels were compared in red blood cell populations of various ages. Hb F seems to influence glycosylation through its effect on red blood cell survival.<sup>13</sup> This is supported by the finding of a case presented by Egede et al where a patient with hereditary persistence of fetal hemoglobin had a falsely elevated HbA1c, which could be due to the long survival of HbF cells.<sup>14</sup> The patient's general health situation has to be taken into consideration when testing for glycated hemoglobin to avoid false negative and false positive interpretation of HbA1c. An accurate assessment of glycated Hb is important in managing diabetes and preventing long-term complications of the disease. One possible implication of this overestimation is overly rigorous glycemic control with a concomitant increase in hypoglycemia. This is obviously important in patients with both diabetes and thalassemia.

In a country like Kuwait and the neighboring countries in the Arabian Peninsula where both diabetes mellitus and hemoglobinopathy prevalence rates are high, methods used in measuring glycated hemoglobin should be evaluated carefully for interference of hemoglobin variants. Furthermore,

while interpreting the results of HbA1c, the patients history of hemoglobinopathies should be kept in mind.

**Acknowledgment.** We would like express our thanks and gratitude to both Dr. Hafeez and Ms. Doha Alkhrayef for their assistance.

## References

1. The American Diabetes Association: Clinical Practice Recommendations 1998: Standards of medical care for patients with diabetes mellitus. *Diabetes Care* 1998; 21 (Suppl 1): 23-31.
2. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; 329: 977-986.
3. Goldestein D, Wiedmeyer H, England J, Mckenzie E. Glycated hemoglobin: methodologies and clinical applications. *Clin Chem* 1986; 32 (Suppl): 64-70.
4. Bunn HF. Post-translational modifications of hemoglobin. *Hematologia Budapest* 1984; 17: 179-186.
5. The expert committee on the diagnosis and classification of diabetes mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997; 20: 1183-1197.
6. Abdella N, Al Arouj M, Al Nakhi A, Al Assoussi A, Moussa M. Non-insulin-dependent diabetes in Kuwait: prevalence rates and associated risk factors, *Diabetes Res Clin Pract* 1998; 42: 187-196.
7. Roberts WL, Frank EL, Moulton L, Papadea C, Noffsinger JK, Ou CN. Effect of nine hemoglobin variants on five glycohemoglobin methods. *Clin Chem* 2000; 46: 569-572.
8. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Influence of hemoglobin variants and derivatives on glycohemoglobin determinations, as investigated by 102 laboratories using 16 meethods. *Clin Chem* 1993; 39: 1717-1723.
9. Embury SH, Hebbel RP, Mohandas N, Steinberg MH. Sickle cell disease. Basic principles and clinical practice. New York: Raven Press; 1994.
10. Steinberg MH. Review: Thalassemia: Moleuclar pathology and management. *Am J Med Sci* 1988; 296: 296-308.
11. Roberts WL, McCraw M, Cook CB. Effect of sickle cell trait and hemoglobin C trait on determinations of HbA1c by an immunoassay method. *Diabetes Care* 1998; 21: 983-986.
12. Frank EL, Moulton L, Little RR, Wiedmeyer HM, Rohlfing C, Roberts WL. Effect of hemoglobin C and S traits on seven glycohemoglobin methods. *Clin Chem* 2000; 46 (6 Suppl 1): 864-867.
13. Elseweidy MM, Abraham EC. The relationship between fetal hemoglobin level and glycosylation in sickle cell disease. *Am J Hematol* 1984; 16: 375-381.
14. Egede LE, Obah E, Lorch T, Oussova T. Spurious elevation of hemoglobn A1c by hereditary persistence of fetal