

## Effects of $\alpha$ -Thalassemia on HbA<sub>1c</sub> Measurement

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**Background:**  $\alpha$ -Thalassemia is a benign condition that is often present in patients with diabetes mellitus. Here, we evaluated the effects of different genotypes  $\alpha$ -thalassemia on HbA<sub>1c</sub> measurement. **Methods:** A total of 189 samples from nondiabetic patients were analyzed. HbA<sub>1c</sub> analysis was performed by ion-exchange high-performance liquid chromatography, boronate affinity HPLC, immunoassay, and capillary electrophoresis. Fasting glucose, fructosamin, and HbA<sub>2</sub> were also performed. All samples were confirmed by genotyping for thalassemia. **Results:** In patients with two or three functional  $\alpha$ -genes, HbA<sub>1c</sub> values were not significantly different from those of controls ( $P > 0.05$ ); however, in individuals with  $\alpha$ -thalassemia

with one functional  $\alpha$ -gene (i.e., HbH disease), HbA<sub>1c</sub> levels were significantly different from those of controls ( $P < 0.01$ ). HbA<sub>1c</sub> values were significantly lower in individuals with HbH disease than in control individuals and patients in the other two  $\alpha$ -thalassemia groups. For patients with HbH disease, there were no significant differences in the four HbA<sub>1c</sub> measurement systems ( $P > 0.05$ ).

**Conclusions:** In this study, HbA<sub>1c</sub> values in samples from individuals with two or three functional  $\alpha$ -genes basically reflected the normal mean blood glucose level, while those in samples from individuals with one functional  $\alpha$ -gene did not. J. Clin. Lab. Anal. 30:1078–1080, 2016. © 2016 Wiley Periodicals, Inc.

**Key words:** HbA<sub>1c</sub>; HbH disease; mean blood glucose; red blood cell life span;  $\alpha$ -thalassemia

HbA<sub>1c</sub>, a major glycosylated hemoglobin characterized by nonenzymatic binding of glucose to the N-terminal valine residue of the hemoglobin  $\beta$ -chain, is a widely used biomarker in the management of diabetes (1). Hemoglobin variants and thalassemia may affect the reliability of HbA<sub>1c</sub> results due to analytical, biochemical, and biological aspects (2, 3).

Thalassemia is prevalent in the southern region of China. In Guangdong province in China, 12.03%, 3.80%, and 0.63% of individuals are carriers of  $\alpha$ -thalassemia,  $\beta$ -thalassemia, and combined  $\alpha$ -/ $\beta$ -thalassemia, respectively (4). Because of this high prevalence of  $\alpha$ -thalassemia, it is necessary to investigate the applicability of HbA<sub>1c</sub> measurement in patients with  $\alpha$ -thalassemia.

In this study, we investigated the effects of the number of functional  $\alpha$ -genes on HbA<sub>1c</sub> measurement by boronate affinity high-performance liquid chromatography (HPLC; Ultra<sup>2</sup>; Trinity Biotech, Kansas City, MO), capillary electrophoresis (Capillarys 2 Flex Piercing [C2FP]; Sebia, Lisses, France), ion-exchange HPLC (Variant II Turbo 2.0 [VII-T 2.0]; Bio-Rad Laboratories, Hercules, CA) and immunoassay (One HbA<sub>1c</sub> FS [oFS]; Diasys Diagnostics, Holzheim,

Germany). For samples with two or three functional  $\alpha$ -genes, only two methods (Ultra<sup>2</sup> and C2FP) were tested. For samples with one functional  $\alpha$ -gene, including HbH or HbH compound Hb Bart's, all four methods were used. A new calibration was performed for all systems, and the quality controls were within acceptable criteria as defined by the manufacturer. The Hb concentrations of all samples ranged from 67 to 171 g/l. According to the manufacturers' instructions, the above Hb concentrations would not interfere with HbA<sub>1c</sub> measurement.

A total of 189 EDTA whole blood samples from all patients were tested for complete blood counts with red cell indices and HbA<sub>2</sub> using an XN9000 analyzer (Sysmex, Kobe, Japan) and hemoglobin capillary electrophoresis (Sebia). Samples were separated into several aliquots and frozen at  $-80^{\circ}\text{C}$  before HbA<sub>1c</sub>

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analysis. One aliquot was frozen at 2–8°C and was used to confirm the genotyping results. Plasma samples were tested for fasting glucose (FG), albumin, fructosamine, and ferritin immediately after centrifugation. Fifty of the 189 samples were from healthy patients, defined as the control group, had normal α and β genes (no deletions or mutations), Hb, MCV, and HbA<sub>2</sub>. Samples exceeding the FG, fructosamine, and ferritin limit of the reference interval were excluded. One hundred and thirty-nine of the 189 samples from patients with α-thalassemia were divided into three groups: (a) 27 samples from patients with three functional α-genes, including genotypes -α<sup>3.7</sup>/αα and -α<sup>4.2</sup>/αα; (b) 80 samples from patients with two functional α-genes, including genotypes --<sup>SEA</sup>/αα; and (c) 32 samples from patients with one functional α-gene (i.e., HbH disease), including genotypes --<sup>SEA</sup>/-α<sup>3.7</sup> and --<sup>SEA</sup>/-α<sup>4.2</sup>. Samples with one functional α-gene showed elevated HbH fraction range given by C2FP from 2.7% to 12.0%. All identifying information was removed from the samples so that abnormal results could not be linked back to the patient. This study was approved by the Ethics Committee of Peking University Shenzhen Hospital.

All statistical analyses were carried out using SPSS software version 19.0 (SPSS Inc, Chicago, IL). Data are expressed as means ± standard deviations (SDs). Student's *t*-tests were used to compare differences between the three α-thalassemia groups and the control group. Differences with *P*-values of <0.05 were considered statistically significant. The correlation between the HbA<sub>2</sub> values determined by the C2FP HbA<sub>1c</sub> system and the C2FP hemoglobin system was assessed using Pearson's correlation.

Results of Hb, MCV, MCH, and HbA<sub>2</sub> revealed a statistically significant bias between the control and α-thalassemia groups (*P* < 0.01; Table 1). The degree of microcytic (low MCV), hypochromic (low MCH), anemic (low Hb), and HbA<sub>2</sub> abnormalities depended roughly on the number of functional α-genes, with decreases in the above four parameters as the number of functional α-globin chain deficiencies increased (Table 1). However, there were no significant differences in FG or fructosamine between groups (*P* > 0.05). HbA<sub>1c</sub> values were not significantly different between the control and α-thalassemia groups for samples from patients with two or three functional α-genes (*P* > 0.05); however, HbA<sub>1c</sub> values in samples from patients with

TABLE 1. Clinical and Biochemical Demographic Data of the Studied Groups

Items	Group			
	Controls ( <i>n</i> = 50)	Three functional α-genes ( <i>n</i> = 32)	Two functional α-genes ( <i>n</i> = 80)	One functional α-genes (HbH disease) ( <i>n</i> = 27)
Genotype ( <i>n</i> )	αα/αα ( <i>n</i> = 50)	-α <sup>3.7</sup> /αα ( <i>n</i> = 26) -α <sup>4.2</sup> /αα ( <i>n</i> = 6)	-- <sup>SEA</sup> /αα ( <i>n</i> = 80)	-- <sup>SEA</sup> /-α <sup>3.7</sup> ( <i>n</i> = 17) -- <sup>SEA</sup> /-α <sup>4.2</sup> ( <i>n</i> = 10)
Sex				
Male ( <i>n</i> )	<i>n</i> = 15	<i>n</i> = 10	<i>n</i> = 21	<i>n</i> = 7
Female ( <i>n</i> )	<i>n</i> = 35	<i>n</i> = 22	<i>n</i> = 59	<i>n</i> = 20
Age (year)	30.3 ± 7.1	32.7 ± 9.0	29.6 ± 5.4	29.1 ± 11.1
HB (g/l)	140.4 ± 16.1	126.9 ± 18.5*	117.8 ± 13.8*	95.0 ± 13.0*
MCV (fL)	90.0 ± 3.1	82.6 ± 4.1*	71.2 ± 4.3*	65 ± 4.8*
MCH (Pg)	30.2 ± 1.2	27.1 ± 1.5*	21.5 ± 1.2*	18.6 ± 0.9*
HbA <sub>2</sub> (%) <sup>a</sup>	2.9 ± 0.3	2.6 ± 0.3*	2.3 ± 0.2*	1.1 ± 0.3*
FG (mmol/l)	5.03 ± 0.30	5.06 ± 0.37	4.95 ± 0.29	5.16 ± 0.37
Fruc (μmol/l)	209.6 ± 16.7	201.8 ± 19.2	204.2 ± 18.2	212.9 ± 14.8
HbA <sub>1c</sub> (%)				
Ultra <sup>2</sup>	4.90 ± 0.48	4.81 ± 0.38	4.78 ± 0.41	3.84 ± 0.58*
VT 2.0	–	–	–	3.73 ± 0.47
CFP				
HbA <sub>1c</sub>	5.01 ± 0.46	4.93 ± 0.36	4.97 ± 0.33	4.07 ± 0.33 ( <i>n</i> = 18) *
HbA <sub>2</sub> <sup>b</sup>	2.7 ± 0.3	2.4 ± 0.3*	2.1 ± 0.2*	1.0 ± 0.3*
oFS	–	–	–	3.91 ± 0.37

–, HbA<sub>1c</sub> was not analyzed.

\*There was statistically significant bias between the control and test groups (*P* < 0.05).

<sup>a</sup>HbA<sub>2</sub> values from the C2FP hemoglobin system.

<sup>b</sup>HbA<sub>2</sub> values from the C2FP HbA<sub>1c</sub> system.

one functional  $\alpha$ -gene (HbH disease) were much lower than those of controls ( $P < 0.01$ ; Table 1).

C2FP HbA<sub>1c</sub> profiles allowed for easy detection of additional peaks corresponding to HbH and Hb Bart's. Because Hb Bart's peaks were located at the position of HbA<sub>1c</sub> peaks in the C2FP HbA<sub>1c</sub> profiles. Therefore, the C2FP HbA<sub>1c</sub> measurement system could not yield accurate HbA<sub>1c</sub> values in the context of Hb Bart's in nine samples. HbH or/and Hb Bart's peaks were located at the positions of A<sub>1a</sub> and A<sub>1b</sub> peaks in the VII-T 2.0 chromatograms.

For patients with HbH disease, we used Ultra<sup>2</sup> as a comparative system; there were no significant differences in the four assay systems ( $P = 0.08$  for C2FP vs. Ultra<sup>2</sup>,  $P = 0.89$  for VII-T 2.0 vs. Ultra<sup>2</sup>, and  $P = 0.64$  for oFS vs. Ultra<sup>2</sup>).

HbA<sub>2</sub> values from the C2FP HbA<sub>1c</sub> system and CapillaryS2 hemoglobin system were well correlated, but were lower in the C2FP HbA<sub>1c</sub> system (regression equation:  $y = 0.888x + 0.038$ ;  $R = 0.967$ ). Therefore, we concluded that HbA<sub>2</sub> values determined using the C2FP HbA<sub>1c</sub> system were reliable.

HbA<sub>1c</sub> is a measure of hemoglobin glycation, and since normal RBCs have a life span of about 120 days, the HbA<sub>1c</sub> value reflects the patient's mean glycemic level for the previous 3 months before sampling (1, 5). Any conditions altering the RBC lifespan may lead to misinterpretation of the HbA<sub>1c</sub> result (6, 7). Patients with some hemoglobin variants and thalassemia have been reported to have decreased RBC life spans (8). Thus, the HbA<sub>1c</sub> values in patients carrying  $\alpha$ -thalassemia may be misinterpreted if compared with the normal reference cutoff value. For any conditions with abnormal RBC turnover, the ADA recommends the use of glucose criteria exclusively for the diagnosis of diabetes (9). Our study showed that the life span of RBCs is reduced as the number of functional alpha genes is decreased.

In conclusion, we found that HbA<sub>1c</sub> values in samples from individuals with two or three functional

$\alpha$ -genes basically reflected the normal mean blood glucose level, while those in samples from individuals with one functional  $\alpha$ -gene did not. So, for patients with HbH disease, HbA<sub>1c</sub> values cannot be used for the assessment of diabetes because HbA<sub>1c</sub> values do not reflect the mean blood glucose level.

## CONFLICT OF INTEREST

None.

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