

# Causes of Low HbA1c in Malaysian University Hospital

Pavai STHANESHWAR, MD\*; Shireene Ratna VETHAKKAN\*\*; Chia Wei WONG\*\*\*

\*Department of Pathology, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia, \*\*Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, \*\*\*Medical Laboratory Technologist, Division of Laboratory Medicine, University Malaya Medical Centre, 59100 Kuala Lumpur, Malaysia

## SUMMARY

**Introduction:** Glycohemoglobin (HbA1c) most accurately reflects the previous two to three months of glycaemic control. HbA1c should be measured regularly in all patients with diabetes, and values should be maintained below 7% to prevent the risk of chronic complications. Apart from the genetic variants of haemoglobins many other conditions also known to affect HbA1c measurements. In this study we evaluated the conditions that cause low HbA1c results.

**Methods and Materials:** The data was collected retrospectively HbA1c was measured in our laboratory by Biorad Variant II turbo 2.0. The method is based on chromatographic separation of HbA1c on a cation exchange cartridge. This method has been certified by National Glycohemoglobin Standardization Programme (NGSP). 58437 requests were received in a period of one year (January to December 2011). Medical records were reviewed to identify the conditions that might be associated with these low values.

**Results:** Among 58437 samples analysed, 53 patients had HbA1c levels < 4.0%. Fourteen patients had haemoglobinopathy. In 34 patients without Hb variants had conditions such as chronic liver disease, chronic kidney disease, haemolytic anaemia, pregnancy, and anaemia of chronic disease. Five non-pregnant individuals who were screened for diabetes mellitus had HbA1c levels < 4%.

**Conclusion:** Our study underscores the importance of that both laboratories and the physicians should be aware of the factors that can influence the HbA1c results. The haematological status should be taken into consideration for proper interpretation of HbA1c results.

## KEY WORDS:

*Anaemia, haemoglobinopathy*

## INTRODUCTION

Glycated haemoglobin (HbA1c) was initially identified as an "unusual" haemoglobin in patients with diabetes over 40 years ago<sup>1</sup>. HbA1c was introduced into clinical use in the 1980s and subsequently has become an important marker for monitoring diabetes in clinical practice<sup>2</sup>. HbA1c is used routinely in the management of individuals with diabetes mellitus to monitor long-term glycaemic control and assess the risk of developing complications<sup>3,4</sup>. More recently, there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of

diabetes<sup>5</sup>. The 2008 International Expert Committee convened by the American Diabetes Association, European Association for the Study of Diabetes and International Diabetes Federation has recommended that HbA1c is used in the diagnosis of diabetes (HbA1c  $\geq$  6.5%)<sup>5</sup>. This Committee also recommended that persons with an HbA1c level between 6.0 and 6.5% might be considered for diabetes prevention interventions as they were at particularly high risk of progression towards diabetes mellitus.

However, as HbA1c is a measure of non-enzymatic glycation of the  $\beta$ -chain of the haemoglobin molecule, levels may be affected by a variety of genetic, haematologic and illness-related factors<sup>6</sup>. The most common and important factors worldwide affecting HbA1c levels are the presence of concomitant haemoglobinopathies (depending on the assay employed), certain anaemias, and disorders associated with accelerated red cell turnover<sup>6</sup>. In our laboratory, nearly 50,000 specimens are analysed for HbA1c in a year. The reference interval for NGSP certified assay methods is 4-6% (20-42 mmol/mol)<sup>7</sup>. Hb variants and hemoglobinopathies are common in Southeast Asia especially amongst those of Malay and Chinese ethnicity. HbA1c would therefore be of little use in the diagnosis of diabetes in such patients. The present study was undertaken to evaluate the conditions that are associated with HbA1c levels below the reference interval i.e. less than 4%

## MATERIALS AND METHODS

This is a retrospective study. The total number of requests for HbA1c during the period of January 2011 to December 2011 was 58437. Whole blood samples were collected in EDTA tubes for HbA1c analysis. HbA1c is measured in our laboratory by Bio-Rad Variant II turbo 2.0. The method is based on chromatographic separation of HbA1c on a cation exchange cartridge. This method has been certified by National Glycohaemoglobin Standardization Programme (NGSP) and the assay was performed as per the manufacturer's instructions. All HbA1c results were reported after analysing the chromatogram. Other laboratory investigations that were performed were retrieved from the laboratory information system and medical records were reviewed to identify conditions that might be associated with these low values.

## RESULTS

58437 requests were received in a period of one year. 53 patients had HbA1c levels less than 4%. 37% of those with

*This article was accepted: 3 September 2014*

*Corresponding Author: Pavai Sthaneshwar, Department of Pathology, Faculty of Medicine, University of Malaya, Lembah Pantai, 50603, Kuala Lumpur, Malaysia. Email: pavai@ummc.edu.my*

**Table I: Causes for low HbA1c**

N=53

Causes for low HbA1c	
Haemoglobinopathy	14
Haemolytic anaemia	4
Anaemia of chronic disorder	8
Chronic liver disease	9
Chronic kidney disease	8
Pregnancy	5
Non-diabetic patients? Unknown	5

low HbA1c levels were known cases diabetes mellitus. Diabetes mellitus was diagnosed either based on the fasting blood glucose or 2-hour post prandial glucose level. The causes of low HbA1c results are shown in Table I. 14 of these 53 patients (26.4%) had haemoglobinopathy. Hb E homozygous variant was diagnosed in eight, Hb E/β in two and β thalassaemia in the remainder. All the patients who had haemoglobinopathy were Malays except one who was Chinese. Eight of the 53 patients had anaemia of chronic disease with haemoglobin levels < 100 G/L. Liver cirrhosis was the diagnosis in nine subjects and chronic kidney disease in eight. Haemolytic anaemia was the cause of low HbA1c results in four subjects. Five pregnant women, who were screened for gestational diabetes mellitus but found to be negative, had low HbA1c results. Five non-pregnant individuals who were screened for diabetes mellitus had HbA1c levels < 4%. None of these five were on glucose-lowering pharmacological therapy for diabetes mellitus or had any medical problem that could result in low HbA1c results. The fasting glucose levels in these individuals were < 4.5 mmol/L.

**DISCUSSION**

The National Academy of Clinical Biochemistry guidelines<sup>7</sup> recommends that the laboratory should repeat testing for all HbA1c sample results below the lower limit of the reference interval, and if these results are confirmed, the physician should be informed to determine whether the patient has a variant Hb or shows evidence of erythrocyte destruction. Hence this study was undertaken to evaluate prevalence of conditions that can cause low HbA1c values in a multi-ethnic cohort of patients from a tertiary institution in Kuala Lumpur.

We found that less than 1% of samples had glycohemoglobin below the reference interval. The most common cause in almost a quarter of the samples was haemoglobinopathy. HbA1c value was not reported for these patients. Bio-Rad Variant II Turbo 2.0 system will not report HbA1c results if homozygous haemoglobinopathy is present. This can be confirmed by visualising the chromatogram. As per the manufacturer’s information of the method used in the laboratory, HbF levels up to 25% had no significant effect on HbA1c determination<sup>8</sup>. In cases of β-thalassaemia and Hb E/β-thalassaemia the HbF values will be elevated. When the values were above the manufacturer’s recommendation, HbA1c values were not reported by our laboratory.

Chronic diseases not of primary haematological origin accounted for almost half of patients with low A1c in our study population. Of the 53 patients who had HbA1c level <4%, cirrhosis of liver, chronic kidney disease (CKD) and anaemia of chronic disease were diagnosed in nine, eight and eight subjects respectively (Table I). In patients with chronic liver disease (CLD), anaemia, portal hypertension, hypersplenism, and variceal bleeding can be common complications. These factors can alter RBC survival<sup>9</sup>. HbA1C levels in these individuals are frequently falsely low, limiting the utility of A1C measurement as a diagnostic test and monitoring tool. When measuring A1C in patients with CLD, physicians should be fully aware of its limitations. If A1C is measured in these patients, levels of total haemoglobin, reticulocytes, medication use, and liver function tests should be reviewed for better interpretation of the results. HbA1c levels are also unreliable due to altered red blood cell survival, erythropoietin therapy and blood transfusion in patients undergoing haemodialysis. Hence glycosylated haemoglobin may not accurately reflect long-term glycaemic control in patients with diabetes and chronic kidney disease<sup>10</sup>.

In our ACD patients with low HbA1c results, low haemoglobin levels and raised C-reactive protein levels were observed. In patients with anaemia of chronic disease (ACD), there is impaired proliferation and differentiation of erythroid precursors, blunted erythropoietin response and altered life span of RBC<sup>11</sup>. All these factors can contribute to low HbA1c results.

We also found that 5 of our 53 subjects with low HbA1c levels were pregnant mothers in their second and third trimester of pregnancy. O’Connor C et al reported that HbA1c levels were significantly decreased in trimesters 1 and 2 compared to non-pregnant women<sup>12</sup>. During normal pregnancy, a decrease in fasting blood glucose occurs early in pregnancy, mainly between weeks 6 and 10, and is sustained during the remaining part of pregnancy<sup>13</sup>. In addition, erythrocyte lifespan is likely to be decreased in pregnancy. The shorter life span of erythrocytes in late pregnancy may be attributed to higher erythropoietin levels in pregnancy, hence reducing the HbA1c value<sup>14</sup>. This could account for the low HbA1c results observed in pregnant women.

Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, haemolytic anaemia) will falsely lower HbA1c test results regardless of the assay method used<sup>15</sup>. Hence in patients with haemolytic anaemia, HbA1c should not be used for monitoring glycaemic control.

In patients with conditions that predispose to a subnormal glycohemoglobin level, regular home monitoring of capillary blood glucose with a glucometer device or intermittent professional continuous glucose monitoring which evaluates interstitial fluid glucose, can be used as alternative measures of longer-term glycaemic control. In addition to that, measurements of total glycosylated serum proteins or glycosylated serum albumin have been suggested as alternative methods for routine monitoring of glycaemic control in patients with diabetes<sup>16</sup>. It has been suggested as an alternative glycaemic marker in patients with haemoglobinopathies, silent haemoglobin variants or anaemia. However, fructosamine

and glycated albumin are not suitable as a measure of glycaemic control in clinical conditions where protein metabolism is altered<sup>17, 18</sup>.

In conclusion, a low A1c was found in samples processed in a Malaysian lab over a 1 year period. Whilst approximately 26% of subjects in our cohort with a low HbA1c had evidence of a hemoglobin variant, the cause in almost 50% was a condition that was not of primary hematological origin (chronic liver disease, chronic kidney disease or anemia of chronic disease). Our findings emphasize the importance of information concerning clinical conditions affecting glycated haemoglobin evaluation to chemical pathologists and physicians. Physicians must be aware of conditions that may affect HbA1c results and should contact laboratories if discrepancies between clinical impressions and laboratory data are seen. Haematological status should always be considered to ensure the correct interpretation of HbA1c results. Fructosamine or glycated albumin may be used to monitor blood glucose control in patients with conditions that lower A1c, but these parameters have their own limitations.

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