# WHEN PAIRED HBA$_{1C}$ AND FASTING GLUCOSE DON'T MATCH, WHICH IS TELLING THE TRUTH?

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## ABSTRACT

**This article explores the pitfalls in using glycated hemoglobin A (HbA$_{1c}$) as a glycaemic monitoring tool in a patient with alpha-thalassemia intermedia. It includes the methods used for HbA$_{1c}$ measurement, such as charge-based or structure-based, presence of hemoglobin variants, ineffective erythropoiesis, concomitant iron deficiency and peripheral hemolysis. For such cases, the use of blood sugar profiles can be a useful alternative to monitor glycaemic control.**



**Keywords:**
**HbA$_{1c}$, thalassemia, iron deficiency, anemia**

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## PATIENT'S REVELATION: WHAT HAPPENED?

WMW is a 77 year old Chinese gentleman who was seen at the polyclinic regularly for diabetes mellitus after being discharged from the hospital in year 2006. He was diagnosed with alpha-thalassemia intermedia in year 2006 when he first presented with hemolytic anemia. Anemia work-up showed iron saturation of 48% and Vitamin B12 level of 191 pmol/L (reference range 133-675 pmol/L). Folate level was 7nmol/L (reference range 8-30 nmol/L) and he was started on regular folic acid supplement. There was no history of regular blood transfusion and his baseline hemoglobin level ranged from 8.7 to 10.6 g/dL in year 2012.

I saw him during a regular follow up, his baseline dose of oral hypoglycaemic agents was metformin 500mg BD and glipizide 5mg BD. His glycated hemoglobin A (HbA$_{1c}$) was 6.9 % but his paired fasting glucose was 20.2 mmol/L. After ensuring the validity of fasting glucose level by checking the fasting status, overnight heavy meal and hyperglycemia symptoms, I suggested home blood sugar monitoring to monitor glycaemic control. Patient was not competent in using glucometer and depended on his son to do it. However, home blood sugar monitoring was not done as the son worked long hours outside the home. Risk of hypoglycaemia with increased in oral hypoglycaemic agents was discussed with the patient and his son. Careful instructions and patient education on hypoglycemia symptoms were administered. The dose of glipizide and metformin was then increased gradually.

Patient subsequent HbA$_{1c}$ decreased to 4.6% with paired fasting glucose level of 10.5 g/dL after increased of metformin to 850mg TDS and glipizide to 10mg OM and 5mg ON. There was no hypoglycemia symptoms experienced. Glipizide dose was not decreased despite low HbA$_{1c}$ level even though glipizide was halved (glipizide 2.5mg BD) in October 2012 when HbA$_{1c}$ is found to be 5.7%


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## GAINING INSIGHT INTO THE CASE MANAGEMENT: WHAT ARE THE ISSUES?

WMW has a background history of hypochromic microcytic anemia due to alpha thalassemia intermedia with increased hemolysis. A big discrepancy existed between the paired HbA$_{1c}$ and fasting glucose result. In majority of patients, HbA$_{1c}$ is the more reliable marker for glycaemic control while glucose level can fluctuate according to the fasting or prandial state. In this case, reliability of HbA$_{1c}$ results should be questioned in view of low hemoglobin, presence of hemoglobin variant and ongoing increased hemolysis.

The relationship of HbA$_{1c}$ and glucose level was established in the ADAG (A$_{1c}$-derived average glucose) study group[1]. Calculated average glucose levels on linear regression model is equal to [(28.7mg/dl x HbA$_{1c}$) - 46.7mg/dl] or [(1.6mmol/L x HbA$_{1c}$) – 2.6 mmol/L]. This formula can be a rough guide on the expected average glucose level with the reported HbA$_{1c}$ level. The estimated average glucose level for a HbA$_{1c}$ level of 6.9% is 8.4mmol/L. However, the fasting blood sugar for this case was 20.2 mmol/L.

**TABLE 1. ESTIMATED AVERAGE GLUCOSE LEVEL BY LINEAR REGRESSION MODEL: [(28.7mg/dl x HbA$_{1c}$) - 46.7mg/dl] or [(1.6mmol/L x HbA$_{1c}$) - 2.6 mmol/L] [1]**

| HbA1c (%) | Glucose in mg/dl | Glucose in mmol/L |
|---|---|---|
| 4 | 68 | 3.8 |
| 5 | 97 | 5.4 |
| 6 | 126 | 7.0 |
| 7 | 154 | 8.6 |
| 8 | 183 | 10.2 |
| 9 | 212 | 11.8 |
| 10 | 240 | 13.4 |
| 11 | 269 | 14.9 |
| 12 | 298 | 16.5 |
| 13 | 326 | 18.2 |
| 14 | 355 | 19.8 |

The presence of anemia is another confounder leading to incorrect HbA$_{1c}$ measurement for this patient. HbA$_{1c}$ is formed by non-enzymatic addition of glucose to N-terminal valine of the hemoglobin beta-chain[2]. Alpha thalassemia intermedia which is a hemoglobinopathy affecting the alpha globin chain, theoretically will not affect the glycation on N-terminal of beta-chain. However, in a study by Pravatmuang et al, HbA$_{1c}$ levels in HbH disease were found

to be significantly lower by high performance liquid chromatography (HPLC) relative to immunoturbidimetry assays in $HbA_{1c}$ measurement. The observation was postulated due to early elution of beta-4 tetramers and HbH in HPLC chromatogram[3].

Besides that, alpha thalassemia intermedia is associated with ineffective erythropoiesis and peripheral hemolysis. Erythropoiesis is ineffective due to the imbalance in the production of alpha and beta-globin chains. Unstable globin chain tetramers precipitate and oxidise into methemoglobin and hemichromes with eventual separation of heme from globin. The free iron released from heme disintegration catalyzes the formation of reactive oxygen species. It causes oxidation of membrane proteins, structural membrane defects, and exposure of red-cell senescence antigens causing premature cell death within the bone marrow (ineffective erythropoiesis) or peripheral circulation (peripheral hemolysis)[4]. Hence the $HbA_{1c}$ level can be falsely low in this case of HbH disease of alpha thalassemia intermedia.

Reduced life span of the red blood cells due to peripheral hemolysis will affect the $HbA_{1c}$ level as it is a time-weighted measurement of the blood sugar levels. The average life span of red blood cells is 120 days. The $HbA_{1c}$ level at any point in time is contributed by both the oldest and youngest red blood cells. Plasma sugar for the past 30 days contribute to 50% of $HbA_{1c}$; plasma sugar from 30 to 60 days earlier contribute to 25% of HbA1c measurement; and the remaining 25% of $HbA_{1c}$ measurement contributed from plasma sugar of 60 to 120 days earlier. Plasma sugar levels from 90 to 120 days earlier contribute only about 10% of $HbA_{1c}$ measurement[5,6].

## STUDY THE MANAGEMENT: HOW DO WE APPLY IN OUR PRACTICE?

$HbA_{1c}$ measurement is currently a standard measurement for diabetic control. The Diabetes Control and Complications Trial (DCCT) in 1993 found the concentration of $HbA_{1c}$ to be an excellent predictor of diabetes-related long term complications[7]. Any condition that shortens erythrocyte survival or decreases mean erythrocyte age, namely hemolysis and recent blood loss, will falsely lower $HbA_{1c}$ test results regardless of the assay method used [8].

Besides alpha thalassemia, other hemoglobinopathies have different influences on the $HbA_{1c}$ measurement. There are 2 main methods of $HbA_{1c}$ measurement, namely by charge-based or structure-based. Examples of structure-based analysis are immunoassay, which involves antibody recognition of the N-terminal of beta chain of $HbA_{1c}$, and boronate affinity methods. In other common thalassemias that we seen in Singapore, namely beta-thalassemia and Hemoglobin E (HbE) disease, immunoassays and boronate affinity methods may underestimate $HbA_{1c}$ measurement due to the elevated fetal hemoglobin (HbF). Both immunoassay and boronate affinity methods show interference from HbF levels above 10-15%[9,10]. National Healthcare Group Polyclinics use an ion-exchange high performance liquid chromatography (HPLC) method, i.e. charge-based method, for both capillary and venous whole blood sample. Bio-Rad Variant II, one example of HPLC method, only shows interference from Hb F levels of above 25%[9]. However, other studies[11,12] found significantly lower $HbA_{1c}$ values measured by HPLC when compared to the immunoassay, in patients with heterozygous Hb E. The reason postulated is the fact that lysine for glutamic acid substitution at position 26 in HbE was far from the N-terminal of the beta-globin chain where $HbA_{1c}$ glycation and antibody binding took place [11,12].

Other glucose control markers such as fructosamine can be an option in this case as it is not affected by hemoglobinopathies. However, it is not readily available in primary care, more expensive and fluctuates with serum albumin level [13]. Major trials on diabetes do not use fructosamine even though there is generally good correlation between serum fructosamine and $HbA_{1c}$ levels [14].

Another common cause of hypochromic microcytic anemia, other than thalassemia, is iron deficiency anemia. Malondialdehyde increases in patients with iron deficiency anemia and enhances the glycation of hemoglobin[15]. Patients with iron deficiency anemia thus present with higher $HbA_{1c}$. Iron replacement therapy lowers $HbA_{1c}$ in both diabetic and non-diabetic individuals[15-17]. In United States, National Health and Nutrition Examination Survey 1999–2006 reported among women with iron deficiency (at least two abnormalities including free erythrocyte protoporphyrin more than 70 g/dl erythrocytes, transferrin saturation less than 16%, or serum ferritin of less than or equal to 15 g/l) was associated with increased odds of an $HbA_{1c}$ more than or equal to 5.5% before and after adjustment for age and race, waist circumference, parity, and hysterectomy[18]. However, iron status did not significantly affect $HbA_{1c}$ concentrations in a regression study model[19].

In patients with microcytic and hypochromic anemia, pitfalls to $HbA_{1c}$ measurement exist as described above. The common differential diagnoses for hypochromic microcytic anemia are iron deficiency anemia and thalassemias. From the various methods of $HbA_{1c}$ measurements, the $HbA_{1c}$ results can be falsely low in thalassemias and falsely high in iron deficiency anemia. It is reported locally that 4 % of Chinese and Malays possess the gene for alpha thalassaemia, 3% of Chinese, Malays and Indians possess the gene for beta thalassaemia whereas 5% of Malays are heterozygous carriers for HbE compared to < 1% in Chinese and Indians[20]. Iron deficiency anemia however tends to overestimate the $HbA_{1c}$ level. Therefore, a high clinical index of suspicion should exist especially for patients with anemia, when $HbA_{1c}$ level does not correlate with the blood sugar profile despite no recent changes in diet or medication. It will be a good alternative practice to use blood sugar profile as an indicator of diabetes control in patient with thalassemia intermedia and severe iron deficiency anemia.

The use of estimated average glucose level [(28.7mg/dl x $HbA_{1c}$) - 46.7mg/dl] or [(1.6mmol/L x $HbA_{1c}$) – 2.6 mmol/L]

can be considered in routine diabetic care by family physician. It gives a better measurement relates to the numbers that patients get on the glucometer. This gives a better glycemia control reflection during patient education. A wide disparity of estimated average glucose and fasting glucose levels suggests underlying confounders affecting the readings. Besides small chance of picking up "silent" anemia, it does give a clue of a possibility of non-compliance. A much higher estimated average glucose level derived from $HbA_{1c}$ compared to fasting glucose level may signify a bad ambient glucose control for the past 3 months and a very tight control or compliance for the past few days before the laboratory testing, besides attributing it to high post prandial glucose contribution.

## CONCLUSION

This case illustrates an example of $HbA_{1c}$ underestimation in alpha thalassemia intermedia. It reminds us to question the validity of $HbA_{1c}$ results when a discrepancy exists in a paired $HbA_{1c}$ and fasting glucose levels. Home blood sugar profile may provide a more accurate measurement of glycemic control of our diabetic patients with thalassemia intermedia. Estimated average glucose derived from $HbA_{1c}$ can be a useful tool in diabetic care by family physician.

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