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Hereditary Spherocytosis, a Pitfall in the Assessment of Glycaemic Control

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ABSTRACT

The use of glycosylated haemoglobin in the assessment of diabetic control is ubiquitous. Hereditary spherocytosis is a haemolytic anaemia with shortened red blood cell lifespan, which can interfere with the methods of glycosylated haemoglobin measurement. We report a case of hereditary spherocytosis in a young man with type 1 diabetes, and illustrate the discrepancy in the measurements of glycosylated haemoglobin, which were inconsistent with the blood glucose profiles. Fructosamine, an alternative time-averaged indicator of blood glucose level, was advantageous in this particular situation. The awareness of the limitations of glycosylated haemoglobin is essential in the clinical care of patients with diabetes, which is a major health problem in Singapore.

Keywords: glycosylated haemoglobin, hereditary spherocytosis, diabetes, fructosamine, glycaemic control

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INTRODUCTION

Glycosylated haemoglobin (HbA_{1c}) is widely used by physicians in the assessment of glycaemic control in patients with diabetes[1]. It is an indicator of averge blood glucose concentration over two months, and has become one of the cornerstones of the contemporary practice of diabetology. Haemoglobin variants (e.g. sickle cell disease and beta-thalassaemia) and chemically modified derivatives (e.g. carbamylated haemoglobin, which is increased significantly in uraemic patients) are known to interfere with HbA_{1c} measurement methods. To the best of our knowledge, there has not been any previous report on the interference of the measurement of HbA_{1c} in hereditary spherocytosis, which is a haemolytic disorder characterised by anaemia, intermittent jaundice, and splenomegaly. The morphologic hallmark of hereditary spherocytosis is the microspherocyte, which is caused by loss of membrane surface area. It is associated with a shortened lifespan of red blood cells (RBC) due to haemolysis.

Here, we report a case of recently diagnosed diabetes with underlying hereditary spherocytosis, and the subsequent recognition of the discrepancy of the HbA_{1c} measurements.

CASE REPORT

LTC, a 22-year-old man was hospitalised when he presented with three days of fever, mild headache, sore throat, dry cough, and polyuria and polydipsia. He has not experienced any weight loss, and has no past history of diabetes. On further questioning, he revealed that four years earlier, he had been diagnosed with hereditary spherocytosis, for which, he had been followed up by a haematologist annually. His spherocytosis was mild and a splenectomy was deemed unwarranted. He has no family history of hereditary spherocytosis, but his mother had type two diabetes diagnosed in her 30s. He was not on any medication, and denied smoking or drinking.

Physical examination revealed a thin, non-dehydrated, icteric, young gentleman. His body mass index (BMI) was 20 kg/m^2 with weight of 60 kg and height of 1.73 m. His temperature was 37.5°C, and respiratory rate was 20. His pulse rate was 80 beats per minute with a blood pressure of 120/80 mmHg. Heart sounds were normal and chest was clear. The abdomen was soft and non-tender. There was a splenomegaly but no hepatomegaly. The neurological examination was unremarkable.

Laboratory investigations showed that he was anaemic with haemoglobin of 9.1 g/dL (normal range: 13.1 – 17.3); MCV was 82.8 fL (82.0 – 99.0), and white cell and platelet counts were normal. Abundant spherocytes were seen on blood film. Urea and electrolytes were all normal. The capillary blood glucose was raised at 19.0 mmol/L, whereas the plasma glucose was 16.0 mmol/L. Urinalysis was negative for ketones, but revealed 3+ for bilirubin and 1+ for glucose. His HbA_{1c} was 4.2%.

Liver function tests were abnormal with elevated conjugated bilirubin of 104 μmol/L (5 – 30) and aspartate

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transferase of 95 U/L (10 – 50). His serum ferritin and reticulocyte count were raised at 1158.3 µg/L (20.0 – 320.0) and 6.1% (0.5 – 2.5%) respectively. Osmotic fragility of red blood cells was increased at 4.7 g/L NaCl (4.00 – 4.45). CT scan of the abdomen showed a splenomegaly but was otherwise unremarkable.

The diagnosis of type 1 diabetes mellitus was made based on his acute symptoms as well as his young age of onset and low BMI. He was commenced on empirical oral antibiotic for the upper respiratory tract infection, and subcutaneous soluble insulin six units initially and then according to his blood glucose level. Subsequent monitoring of his capillary blood glucose in the following three days showed a persistent hyperglycaemia with a range between 8.9 and 18.5 mmol/L. His insulin requirement was adjusted accordingly, and eventually was fixed at 24 units am and 10 units pm of insulin Mixtard® (30/70) on discharge.

Since discharge, he has been followed up in our diabetes clinic. Further investigations of his diabetes status showed that his fasting C-peptide was 759 pmol/L (364 – 1655) and glutamic acid decarboxylase autoantibodies were negative. His self-monitoring of blood glucose (SMBG) profile at home was reported to be consistently high, which was later found to be the result of his non-compliance with insulin therapy. In spite of the persistent hyperglycaemia on SMBG, ranging from 9.9 to 18.5 mmol/L, his HbA_{1c} result at his first clinic visit was surprisingly low (HbA_{1c} 3.2%). Thus, fructosamine was performed in an attempt to verify the discrepancy, and it was elevated at 378 µmol/L (normal range 0 – 285), confirming the underlying poor glycaemic control. In fact, in the subsequent year of follow-up, this pattern of divergence had been persistent (see Fig. 1), and his glycaemic control was assessed by the measurements of both HbA_{1c} and fructosamine.

Reassessments in the clinic demonstrated that his normochromic, normocytic anaemia had resolved spontaneousy without further relapse in the following year. The ferritin level had normalised; the ongoing haemolysis appeared mild as reflected by a moderately low haptoglobin of 29.0 mg/dL (35.0 – 170.0) and a negative haemosiderinuria. He had no evidence of secondary haemachromatosis. His pituitary hormones and sex hormones were within normal limits. In view of the fact that his hereditary spherocytosis had remained mild, splenectomy was not advised.

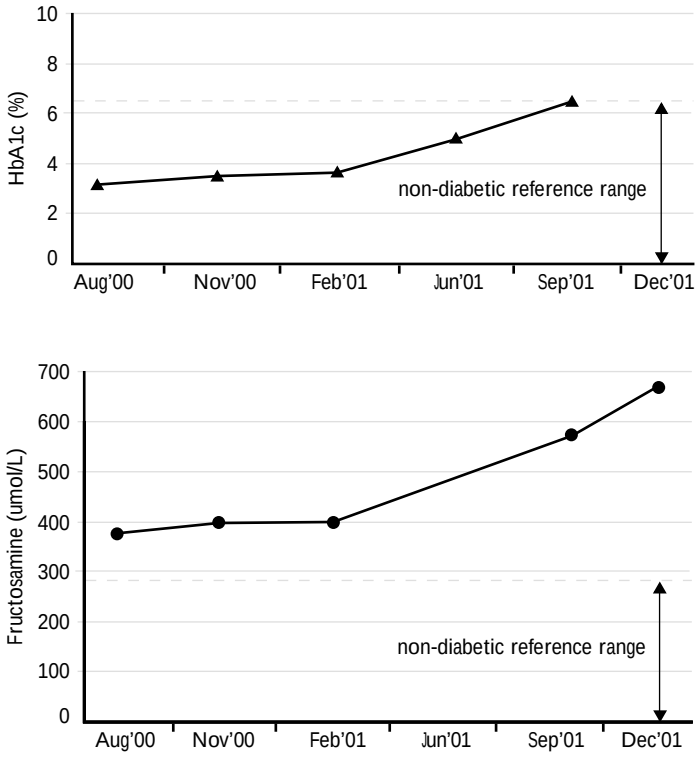

Fig.1 Trends of HbA1c and fructosamine during the period of follow-up. The HbA1c values of this patient were consistently "low" within the non-diabetic range as opposed to the fructosamine levels, which were well above the non-diabetic range, reflecting the observed high blood glucose profiles.

DISCUSSION

Diabetes mellitus is a major health problem worldwide. It has a negative impact on morbidity and mortality from both microvascular and macrovascular complications. The global prevalence of diabetes is predicted to rise from 135 million in 1995 to 300 million by 2025[2]. In Singapore , the prevalence of diabetes has risen from 2% in 1975 to 9% in 1998[3,4]. Given the results of the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabete Study (UKPDS), there is broad consensus on the benefits of good glycaemic control in reducing chronic diabetic complications[5,6]. Measurement of HbA_{1c} is extensively used for monitoring of glycaemic control in clinical practice. Thus, awareness among physicians of the limitations and the possible interference of the HbA_{1c} measurement methods is vital.

HbA_{1c} is formed by the non-enzymatic glycation of the N-terminus of the beta-chain of haemoglobin A. As blood glucose rises, the increase in non-enzymatic glycation of proteins is proportional to both the level of glucose and the lifespan of the protein in the circulation or tissues. Since the average lifespan of RBC is 120 days, HbA_{1c} reflects the mean daily blood glucose concentration over the preceding two to three months.

Hereditary spherocytosis is the most common haemolytic anaemia due to RBC membrane defect with an incidence of approximately one in 5,000 in Europeans[7], but it is thought to be rather less common in South-East Asia[8]. It is an autosomal dominant

inherited haemolytic disorder characterised by anaemia, intermittent jaundice, splenomcgaly, and responsiveness to splenectomy. Nonetheless, up to a quarter of all patients do not demonstrate a dominant inheritance pattern, and the parents of these patients are clinically and hematologically normal. New mutations have been implicated that may explain some of these sporadic cases. There is marked heterogeneity of the clinical features, ranging from an asymptomatic condition to a fulminant haemolytic anaemia. It was therefore unsurprising that our patient did not have any known family history of clinical hereditary spherocytosis, which could be due to spontaneous mutation or the consequence of incomplete penetrance.

The morphologic characteristic of hereditary spherocytosis is the spherocyte, which is coused by loss of membrane surface area, and is characterised by an abnormal osmotic fragility in vitro. Loss of membrane occurs because the spectrin-deficient cytoskeleton is unable to provide adequate support for the lipid bilayer, and it would transform RBCs form from biconcave discs to spherocytic form[9]. This intrinsic red cell defect leads to increased RBCs destruction in the spleen with the consequence of a shortened RBCs lifespan, which results in an artificially low HbA_{1c} level. This effect has been previously documented in a study, in which HbA_{1c} levels correlated with RBCs survival, and patients with haemolytic anaemia had significantly lower HbA_{1c} levels compared to normal controls[10]. Haemoglobinopathies, including beta-thalassaemia, HbSC, HbC and sickle cell disease often show increased amount of minor Hb species, i.e. HbA2 and HbF, which can interfere with HbA_{1c} measurement methods. Additionally, conditions affecting RBC half-life, such as haemolysis, haemorrhage, iron deficiency anaemia, or red cell transfusion can result in similar interference[11]. The other interfering condition is uraemia, whereby chemically modified derivatives, carbamylated Hb, can be as high as 3% of total Hb. Carbamylated Hb has an isoelectric point similar to HbA_{1c} and thus, can interfere with charge-based methods of HbA_{1c} measurement[12].

Evidently, in our case of hereditary spherocytosis with shortened RBC half-life, the HbA_{1c} measurements were falsely low (see Fig. 1), which did not correspond to the poorly controlled SMBG profiles recorded by the patient. In contrast, the elevated fructosamine values were consistent with the underlying SMBG profiles. The use of SMBG as a measure of his glycaemic control was challenging because of his non-compliance. Our attempt in using the two HbA_{1c} measurement methods available in our hospital, i.e. Variant™ II HbA2/HbA_{1c} Dual Program (which utilises the principles of ion exchange high performance liquid chromatography [HPLC]), and DCA 2000® (which is based on a latex immunoagglutination inhibition methodology) produced identical HbA_{1c} readings.

The rationale for using the two different methods was that previous studies have demonstrated that the interfering effect of certain haemoglobinopathies is highly method dependent. For example, it has been shown that patients with HbD had consistently lower HbA_{1c} measured on the HPLC (Bio-Rad Variant) method compared to immunoagglutination (DCA 2000) method[13]. The ion-exchange HPLC method usually indicates the presence of haemoglobin variants but lacks the resolution required for the differentiation of the variants. Thus, the additional peaks due to the haemoglobin variants may result in either falsely low or high HbA_{1c}. In a different way, haemoglobin mutations at the NH_2-terminal of the β-chain can also interfere with the immunoagglutination method by hampering the ability of monoclonal antibody to detect the epitope at the NH_2-terminal of the β-chain. We do recognise the argument for the use of different HbA_{1c} methods based on experience with haemoglobinopathies; but there has not been any previous similar report on hereditary spherocytosis.

In recent years, fructosamine use has declined in favour of the preferred standard HbA_{1c} measurement. Nevertheless, fructosamine remains useful in situations where HbA_{1c} test may not be ideal, such as in haemolytic anaemias or haemoglobinopathies, and where closer period of monitoring is required as in the setting of gestational diabetes. In circumstances where Hb variants are present or RBS turnover rate is increased, fructosamine has been advocated as a suitable alternative[14, 15]. This is because in these specific situations, fructosamine has been observed to correlate better with the underlying glycaemic status than HbA_{1c}[15].

The possible explanation for the advantage of fructosamine over HbA_{1c} in the presence of haemoglobinopathies or haemolytic anaemia is that fructosamine measurement is a reflection of glycated proteins (albumin), which is not affected by haemoglobin variants or increased RBCs turnover rate, whereas HbA_{1c} determination is a measure of the glycated haemoglobin molecules at the NH_2-terminal valines of the β-chains. The shortened RBCs lifespan with the concomitant decreased exposure time of haemoglobin to glucose would lead to a falsely low percentage of HbA_{1c} undergoing glycation.

Fructosamine, like HbA_{1c} is a time-averaged indicator of blood glucose levels. Non-enzymatic condensation of glucose and proteins initially produces an unstable aldimine which converts to a ketoamine. This ketoamine is commonly termed fructosamine owing to its structural similarities to fructose[16]. Its concentration reflects the mean prevailing glucose level during a time interval dependent on the protein lifespan. The turnover of human serum albumin is much shorter (half-life of 14 – 20 days) than that of haemoglobin (RBC life span of 120 days). Consequently, fructosamine determinations provide a means of monitoring blood glucose status over a shorter period (one to three weeks) than HbA_{1c} (approximately 8 weeks)[17]. It has been demonstrated that fructosamine correlated fairly well with HbA_{1c} ($r=0.8$)[18]. Notwithstanding, fructosamine measurement has various limitations, such as interference by hypertriglycaeridaemia and hyperbilirubinaemia. Its values also vary with changes in clearance and synthesis of serum proteins that can accompany liver disease or acute systemic illness, and there is uncertainty whether its values should be corrected for serum protein or albumin concentrations[16].

In the management of patients with diabetes, knowledge of the possibility of various factors, such as haemoglobinopathies and haemolytic anaemia, affecting HbA_{1c} measurement methods is important to avoid misinterpretation of the glycaemic state, especially in view of the relatively high prevalence of haemoglobinopathies in Singapore and South-East Asia. It was estimated that at least 6.7% of the local population were carriers of the genes of alpha and beta thalasaemias, and HbE-thalassaemia[19]. It is therefore vital that haemoglobinopathies or haemolytic anaemia should be taken into consideration when a discrepancy between blood glucose profiles and HbA_{1c} concentrations is observed since haemoglobinopathy is fairly prevalent in our populations. When the clinical impression and HbA_{1c} results do not fit, further investigations are required to elucidate the possible causes as discussed above.

It is recognised that haemoglobin variants and chemically modified derivatives can interfere with HbA_{1c} measurement. However, hereditary spherocytosis interfering with HbA_{1c} methods is less well documented. We hope this case would serve to increase the awareness among physicians of the limitations of HbA_{1c} measurements in conditions where the RBC survival is affected. This has significant clinical relevance in view of the ubiquitous utilisation of HbA_{1c} in the assessment of long-term glycaemic control.

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