

Aberrantly high glycated haemoglobin measurement due to the haemoglobin variant Hb Santa Juana

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ABSTRACT

Various laboratory and patient-related factors can result in falsely high or low glycated haemoglobin (HbA1c) measurements, and haemoglobin (Hb) variants that interfere with laboratory readings is an important cause of this. We report a case of a rare Hb variant, Hb Santa Juana, manifesting as a falsely high HbA1c in a 62-year-old patient with type 2 diabetes mellitus. The patient presented with high HbA1c values that persisted despite the intensification of anti-diabetic treatment. His home blood glucose levels were incongruently low compared to his HbA1c values. Further investigations revealed a family history of the variant Hb Santa Juana. This was confirmed in the patient when his blood was sent for DNA analysis. It is important for clinicians to be aware of the factors that can influence laboratory HbA1c measurements, as clinical decisions on treatment are often based on these measurements.

Keywords: aberrant glycated haemoglobin measurement, haemoglobin variant, HbA1c, Hb Santa Juana

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INTRODUCTION

Glycated haemoglobin (HbA1c) is a validated and widely used measure of glycaemic control. Haemoglobin (Hb) variants can affect laboratory interpretations of HbA1c, thus resulting in discordantly high or low values.⁽¹⁾ Aberrant HbA1c readings secondary to Hb variants is an uncommon occurrence locally, and the exact incidence is unknown. More than 1,000 Hb variants have been reported worldwide.⁽²⁾ We present a case of an Hb variant causing aberrantly high HbA1c values in a patient, and review some of the factors that affect HbA1c measurements.

CASE REPORT

A 62-year-old Chinese man was seen at a second-tier diabetic clinic at the Toa Payoh Polyclinic for poorly

controlled diabetes mellitus. The patient had type 2 diabetes mellitus for 25 years, and his condition was further complicated by peripheral vascular disease, minor coronary artery disease, retinopathy and nephropathy. His HbA1c readings within the last year were noted to be high, ranging from 14% to 15.8%. At presentation at the diabetic clinic, he was on a combination treatment of metformin and twice daily injections of intermediate isophane insulin and short-acting soluble insulin. The patient claimed compliance with his medications and dietary advice, and also monitored his home blood glucose levels regularly. However, it was noted that his blood glucose readings obtained at home and during checkups in the clinic were usually within the range of 4–9 mmol/L and were thus not reflective of his high HbA1c readings. He also reported occasional episodes of hypoglycaemia. The diabetes mellitus nurse educator verified that the readings of his glucometer were accurate and his injection techniques were correct.

An example of the discrepant nature of the patient's blood glucose readings and HbA1c value is shown in the following record. At a clinic visit, the HbA1c reading recorded was 15.8%, while the home glucose monitoring record in the preceding three months was as follows: pre-breakfast 4.6–6.4 mmol/L, two hours post-breakfast 4.5–10.1 mmol/L, pre-lunch 2.4–9.8 mmol/L, two hours post-lunch 5.5–11.1 mmol/L, pre-dinner 3.7–6.6 mmol/L and two hours post-dinner 4.2–6.1 mmol/L.

In view of the consistent pattern of incongruence between the patient's blood glucose readings and his measured HbA1c, his history was reviewed in greater detail. Apart from insulin, he was on captopril, isosorbide dinitrate, nifedipine, simvastatin, vitamin B complex, clopidogrel and pentoxifylline. He denied being on any other drugs. The patient also had no known condition that affects red blood cell turnover, such as asplenia or haemolytic anaemia. He reported no recent episodes of bleeding or blood transfusions. His creatinine level was 80 $\mu\text{mol/L}$, and no uraemia was noted. However, the patient revealed that his son had previously tested positive for haemoglobinopathy. His son's Hb electrophoresis and DNA analysis results revealed an Hb Santa Juana trait.

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Subsequently, the patient underwent a full blood count and Hb electrophoresis. The full blood count revealed a total white blood cell count of $10.4 \times 10^3/\mu\text{L}$, Hb of 12.8 g/L, mean cell volume of 93.3 fL and platelet count of $408 \times 10^3/\mu\text{L}$. The initial thalassaemia screen was inconclusive; HbA2 was quantified at 35.4% (normal range 1.7%–3.2%), HbF < 1.0% (normal range 0.0%–1.0%), and HbH inclusion bodies were not detected. The screen reported a discordant finding of increased HbA2 on high performance liquid chromatography (HPLC) but no corresponding band on alkaline gel, and thus, a repeat thalassaemia screen was suggested.

To confirm the suspicion that the patient's haemoglobinopathy was interfering with the HbA1c measurement of the on-site laboratory, a blood sample was drawn and sent to two laboratories for testing on the same day. The HbA1c value measured at the on-site laboratory using HPLC was 17.5%, while that measured at the off-site laboratory at Alexandra Hospital using turbidimetric inhibition immunoassay was 6.3%. The results showed that the on-site laboratory generated a falsely elevated HbA1c value, while the off-site laboratory generated a value more concordant with the patient's home blood glucose levels. The patient's insulin was stopped, and he was switched to the oral agent tolbutamide. He did not experience any further episodes of hypoglycaemia.

Further analysis of the patient's blood was performed at the National Thalassaemia Registry laboratory. Hb electrophoresis quantified HbA2 at 1.4% and HbF at 0.3%. It also detected the presence of the Hb variant, Hb Santa Juana, at 14.9%. DNA analysis revealed a mutation at codon 108 (ACC/AGC) at the beta gene, which is consistent with the Hb Santa Juana (Hb Serres) trait. This was also consistent with the Hb analysis results of the patient's son. Hb Santa Juana in this patient merged with HbA2 in the chromatogram analysis of the initial thalassaemia screen, thus leading to a falsely elevated HbA2 measurement of 35.4% and an inconclusive result. It also accounted for the falsely high HbA1c value measured using the on-site HPLC laboratory method.

DISCUSSION

This is an interesting case of an otherwise clinically silent Hb variant, Hb Santa Juana, which manifested as a discordantly high HbA1c level using the HPLC laboratory method of measurement. Glucose binds to many proteins in blood by a non-enzymatic two-step process; a reversible step resulting in the formation of a Schiff base followed by an irreversible Amadori rearrangement. HbA1c is formed when glucose attaches to one or both N-terminal valines of the beta chains within

the Hb tetramer and is the main fraction of Hb bound to glucose.⁽³⁾ It reflects the mean blood glucose level over the last 120 days, which is the average life span of red blood cells. Interestingly, a non-linear relationship of glycosylation has been observed, with 50% of HbA1c correlating with the mean glucose level within the last 30 days.⁽⁴⁾ Hence, a relatively recent change in mean plasma glucose can result in a clinically significant change in HbA1c.

The Diabetes Control and Complications Trial and United Kingdom Prospective Diabetes Study Group were large trials that have demonstrated that HbA1c levels are directly related to the risk of suffering from the complications of diabetes mellitus such as stroke and ischaemic heart disease.^(5,6) HbA1c is the most widely used test to assess and monitor glycaemic control, and it strongly correlates with blood glucose levels.⁽⁷⁾ It is also increasingly used as an outcome measure of the performance of clinical practice in managing diabetes mellitus, e.g. in the United Kingdom Quality and Outcomes Framework. In view of the evidence that HbA1c is a strong predictor of new-onset diabetes mellitus,⁽⁸⁾ the American Diabetes Association promulgated the use of the HbA1c test in the diagnosis of diabetes mellitus and for identifying pre-diabetes mellitus in its new Clinical Practice Recommendations.⁽⁹⁾ Owing to the importance of HbA1c as a monitoring and diagnostic tool in diabetic patients, it is essential to be aware of conditions that can affect laboratory HbA1c values apart from plasma glucose levels.

Both patient and laboratory factors can result in misleading HbA1c values. Firstly, conditions that cause increased turnover and reduced average life span of the red blood cells can lead to lower HbA1c values. These conditions include active bleeding, haemolytic disease, haemoglobinopathies and myelodysplastic disease.⁽¹⁰⁾ Secondly, a patient with renal failure and uraemia can have high concentrations of carbamylated haemoglobin, resulting in aberrantly high HbA1c.⁽⁴⁾ To complicate the picture, treatments such as dialysis may shorten the life span of red blood cells. Falsely elevated HbA1c measurements may also be obtained when red blood cell turnover is low, resulting in higher proportions of older red blood cells, such as in iron, B₁₂ or folate deficiencies.⁽¹¹⁾ Haemoglobinopathies can affect HbA1c values in three ways,⁽¹⁾ namely by influencing the binding of glucose to haemoglobin, by affecting peak measurements on chromatography and by increasing the risk of haemolysis and hence decreasing the lifespan of red blood cells.

There are various laboratory methods for measuring HbA1c. The most common methods used

are boronate affinity chromatography, cation-exchange chromatography, electrophoresis and immunoassay.⁽¹⁾ With a single Hb variant such as sickle cell Hb or HbS, the HbA1c value may be falsely high or low depending on the method used.⁽³⁾ In this case report, the patient's variation in beta Hb chain resulted in a falsely high HbA1c of 17.5% when measured by ion-exchange HPLC. During ion exchange chromatography, different Hb species elute separately due to their different charges. The concentration of each Hb species is measured by a spectrophotometer and quantified by calculating the area under each peak.⁽³⁾

The on-site HbA1c measurement machine used in our clinic, the Bio-Rad Haemoglobin A1C Programme, is based on the principles of HPLC. In the patient's sample, an extra peak eluted at 1.61 seconds. Due to the abnormal peak detected on HPLC, the on-site laboratory reported a falsely high HbA1c result. On the other hand, the turbidimetric method relies on anti-HbA1c antibodies that react with glycohaemoglobin, and is thus not affected by a patient's beta Hb variant. This method gave a value of 6.3% in our patient, which was concordant with his blood glucose levels.

Throughout the world, other Hb variants have reportedly surfaced when aberrant HbA1c measurements were observed. Some examples are Hb Takamatsu (Beta 120 Lys → Gln) and Hb G-Szuhu (Beta 80 Asn → Lys)⁽¹²⁾ and Hb Camperdown (Beta 104 Arg → Ser).⁽¹³⁾ These Hb variants are otherwise clinically asymptomatic. To date, 1,040 Hb variants have been identified.⁽²⁾ Internationally, HbS and HbC are the commonest haemoglobinopathies, and the prevalence of Hb variants vary in different parts of the world.⁽³⁾ Hb Santa Juana, also known as Hb Serres, has been more widely reported in Greek and Mexican populations.⁽²⁾ It is a rare Hb variant in this region; the National Thalassaemia Registry of Singapore has confirmed that our patient and his son were the only cases of Hb Santa Juana recorded at the registry when this case report was being written. This Hb variant has normal stability and decreased affinity for oxygen, and presents clinically as mild anaemia.⁽²⁾ At the time of writing, a literature search did not yield any similarly reported cases of Hb Santa Juana interfering with HbA1c measurement.

Attempts have been made to characterise the effects of Hb variants on HbA1c measurement using different laboratory methods.⁽³⁾ However, these are far from comprehensive due to the number of Hb variants and the possible laboratory methods. In Singapore, the exact prevalence of Hb variants among patients with diabetes mellitus is unknown. They can also affect

HbA1c measurement in subtle ways, leading to clinically significant differences in results. Researchers have suggested that this problem may be more prevalent in clinical practice than previously known.⁽¹⁴⁾ Studies have also attempted to characterise the relationship between HbA1c and average glucose levels. A useful formula is: estimated average glucose (mg/dL) = $28.7 \times \text{HbA1c} - 46.7$.⁽¹⁵⁾ Using this formula, an HbA1c value of 8% yields an estimated average glucose level of 183 mg/dL or 10.2 mmol/L.

When there are inconsistencies between a patient's home blood glucose monitoring and laboratory-measured HbA1c, one should suspect a falsely elevated or lowered HbA1c result. Suspicion should also be raised when HbA1c is more than 15%, or when there is a significant change in a patient's HbA1c together with a change in laboratory HbA1c assay method.⁽¹⁴⁾ A suggested algorithm for investigation is to check the accuracy of the patient's glucometer by comparing the laboratory plasma glucose measurements with the home monitoring measurements.⁽⁴⁾ If these values tally but the HbA1c values are discordant, one should clinically assess for conditions that can affect HbA1c measurements, such as anaemia, a shortened red blood cell life span or haemoglobinopathies. One can repeat the HbA1c measurement with a different assay method to observe for discrepancies. Further investigations that could be helpful include a full blood count, reticulocyte count, serum haptoglobin and Hb electrophoresis, where relevant. In some cases, one may have to perform sequencing of the globin genes to characterise the variant type. Fructosamine, a product of serum protein glycation, has been suggested as an alternate measure of glycaemic control when HbA1c measurement is unreliable.⁽¹⁾ It is independent of Hb variants and positively correlated with HbA1c.⁽¹⁾ However, fructosamine only reflects blood glucose concentration in the previous 2–3 weeks. Unlike HbA1c, the correlation between fructosamine and complications of diabetes mellitus has not been robustly evaluated in large randomised trials.

In conclusion, we reported a case of aberrant HbA1c measurement due to the Hb variant, Hb Santa Juana. The association between haemoglobinopathies and discrepant HbA1c measurements is well documented; however, this is the first reported case involving Hb Santa Juana. In the local context, this Hb variant is rare and would otherwise be clinically silent in this patient. The HbA1c test remains an essential tool in monitoring glucose control in patients with diabetes mellitus, and it has recently been recommended as a diagnostic test for diabetes mellitus. This case report serves as a reminder that Hb variants

can result in discordant and misleading HbA1c values. A high index of suspicion is required, especially in cases with a family history of haemoglobinopathy, and where the reported HbA1c value appears incongruent with the blood glucose profile of the patient. From a wider perspective, this case reminds us of the importance of interpreting laboratory measurements in the clinical context of the patient, i.e. to treat the patient and not a laboratory value.

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