



Spurious HbA1c results in patients with diabetes treated with dapsons

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Summary

Measurement of glycated haemoglobin (HbA1c) has been utilised in assessing long-term control of blood glucose in patients with diabetes, as well as diagnosing diabetes and identifying patients at increased risk of developing diabetes in the future. HbA1c reflects the level of blood glucose to which the erythrocyte has been exposed during its lifespan, and there are a number of clinical situations affecting the erythrocyte life span in which HbA1c values may be spuriously high or low and therefore not reflective of the true level of glucose control. In the present case series, we describe the particulars of three patients with diabetes who had spuriously low HbA1c levels as a result of dapsons usage. Furthermore, we discuss the limitations of HbA1c testing and the mechanisms by which it may be affected by dapsons in particular.

Learning points:

- Various conditions and medications can result in falsely low HbA1c.
- Dapsons can lead to falsely low HbA1c by inducing haemolysis and by forming methaemoglobin.
- Capillary glucose measurement, urine glucose measurements and fructosamine levels should be used as alternatives to HbA1c for monitoring glycaemic control if it was falsely low or high.

Background

HbA1c reflects the level of blood glucose to which the erythrocyte has been exposed during its lifespan, and there are a number of clinical situations affecting the erythrocyte life span in which HbA1c values may be spuriously high or low and therefore not reflective of the true level of glucose control. In the present case series, we describe the particulars of three patients with diabetes who had spuriously low HbA1c levels as a result of dapsons usage. Furthermore, we discuss the limitations of HbA1c testing and the mechanisms by which it may be affected by dapsons in particular.

Case presentation

Patient 1

A 34-year-old male was referred to the diabetes clinic with a presumptive diagnosis of type 2 diabetes mellitus (T2DM) made 2 years previously. His diabetes regimen included metformin, gliclazide and dapagliflozin. His daughter had a history of type 1 diabetes mellitus (T1DM), and he himself had coeliac disease with associated dermatitis herpetiformis, for which he received 100 mg of dapsons daily. In clinic it was noted that he had experienced significant osmotic symptoms (polyuria and polydipsia) in the preceding 18 months along with a 9 kg weight loss.



With regard to his self-monitored capillary glucose levels the patient reported elevated readings ranging from 8 to 22 mmol/L with no hypoglycaemic events. A random blood glucose level checked in the diabetes clinic was elevated at 18 mmol/L. Despite all of the above, however, his HbA1c was 40 mmol/mol, consistent with excellent glycaemic control.

Given this patient's autoimmune history, osmotic symptoms and weight loss, a diagnosis of T1DM was considered in clinic and a basal bolus insulin regimen was commenced. On subsequent follow-up visits his glucose readings improved significantly (although remaining above target) and his HbA1c (IFCC) decreased to 31 mmol/mol in the absence of significant hypoglycaemia. A blood sample for fructosamine level was taken with the result returning at 401 µmol/L and a normal range (NR) of 205–285 µmol/L. This provided further confirmation of suboptimal glucose control and provided further support to the suspicion of a spuriously low HbA1c result likely secondary to dapsone treatment. Additional investigations to determine the mechanism by which dapsone may have affected HbA1c in this case were performed including HbA1c assay on different laboratory platforms (the results of which are shown in Table 1) and were suggestive of methaemoglobinaemia and chronic low level haemolysis.

Patient 2

A 38-year-old female was diagnosed with type 1 diabetes following presentation with osmotic symptoms of hyperglycaemia and a random glucose of 17 mmol/L. Her HbA1c was 75 mmol/mol at the time of diagnosis. She had a history of hypothyroidism and testing revealed

positive antibodies against glutamic acid decarboxylase (GAD). She was commenced on a basal bolus insulin regime. She represented 4 months later with a severe chronic urticarial rash and her dermatologist commenced her on dapsone, titrating her dose up to 200 mg daily. Two months later, at a routine diabetes follow-up clinic, it was noted that her HbA1c had fallen to 22 mmol/mol despite persistent hyperglycaemia on capillary glucose readings. Fructosamine levels were elevated at 356 µmol/L (NR 205–285 µmol/L), confirming suboptimal glucose control. The results of additional investigations are shown in Table 1 and were suggestive of increased red cell turnover.

Patient 3

A 67-year-old woman with type 1 diabetes treated with basal bolus insulin had achieved good glucose control, as evidenced by HbA1c readings ranging between 44 and 52 mmol/mol during 7 years of follow-up. She was commenced on dapsone for the treatment of granuloma annulare. Four months later her HbA1c had fallen to 25 mmol/mol, but review of her capillary glucose readings demonstrated that the majority were within or above the recommended target range. She had normal hypoglycaemia awareness and reported only three episodes of symptomatic hypoglycaemia in the preceding 4 months. Her fructosamine level was 320 µmol/L (NR 205–285 µmol/L), confirming hyperglycaemia and a spuriously low HbA1c. The results of additional investigations are shown in Table 1 and were suggestive of methaemoglobinaemia and chronic low level haemolysis.

Table 1 Laboratory investigations.

Tests	Patient 1	Patient 2	Patient 3	Reference range
HbA1c (off dapsone)	Patient remains on dapsone	75	44–52 (pre-dapsone)	20–42 mmol/mol
HbA1c (on dapsone)	31	22	25	20–42 mmol/mol
HbA1c (on dapsone) immunoassay*	30.3	N/A	N/A	20–42 mmol/mol
HbA1c (on dapsone) capillary electrophoresis	31	N/A	N/A	20–42 mmol/mol
Fructosamine	401	356	320	205–285 µmol/L
Haemoglobin	14.1	10.8	12	g/dL
MCV	88.7	93	108	83–98 fl
Methemoglobin	6	N/A	4.7	0–3%
Haptoglobin	<0.24	<0.1	<0.24	0.45–2.05 g/L
Reticulocytes	94 × 10 ⁹	105.9 × 10 ⁹	4.3	0.4–1.8% (20–80, patients 1,2)
Bilirubin	10	24	12	0–21 µmol/L
LDH	260	319	245	135–250 IU/L

*Abbott Architect Assay.

Treatment

In this case series we showed a lab error only with no treatment to correct this error.

Outcome and follow-up

Other tests, such as capillary glucose measurement, and fructosamine levels were used as alternatives to HbA1c for monitoring glycaemic control in this cohort.

Discussion

In the present case series, we present the details of three patients who had very significant and spurious reductions in their HbA1c values occurring secondary to treatment with dapsonsone. In all three, prompt recognition of the dapsonsone effect avoided inappropriate modifications to their diabetes treatment, although it is possible that in the first case the spurious HbA1c results delayed the recognition of T1DM prior to his presentation in our clinic. Thus, this case series illustrates the importance of education regarding this specific effect of dapsonsone, along with an awareness of the limitations of HbA1c testing in general. It should be emphasised that the accurate monitoring of glycaemic control via HbA1c is an essential part of diabetes management. Chronic hyperglycaemia directly results in increased rates of retinopathy, nephropathy and neuropathy, and both the DCCT and UKPDS trials demonstrated that the variable that correlated to the greatest degree with the risk of these microvascular complications was HbA1c. With the relevance of this test in mind, therefore, the present discussion shall focus on the mechanisms by which HbA1c is calculated and the mechanisms by which this reading may become inaccurate.

Adult haemoglobin (Hb) consists of 97% HbA, which contains two alpha and two beta chains. Circulating blood

glucose binds to the N-terminal valine of the β -chain of haemoglobin A to form HbA1c (1) (Fig. 1). In individuals with poorly controlled diabetes, and with higher than normal levels of circulating glucose, the percentage of HbA which becomes glycated is greater than that of the healthy population. The increase in glycation and the consequent HbA1c value depends on the degree of hyperglycaemia to which the red blood cells (RBCs) are exposed, and the duration of this exposure. HbA1c, therefore, ultimately reflects the mean blood glucose concentration over the lifespan of the RBC, which is 120 days on average. It should be noted that the HbA1c is a weighted mean, however, and correlates best with blood glucose readings from the most recent 4 weeks (1).

The laboratory measurement of HbA1c is based on our knowledge of the changes that occur following glycation of the haemoglobin molecule. Glycation of the N-terminal residue changes its structure and decreases the positive charge of HbA. As a result, a slight difference in isoelectric point between HbA1c and HbA0 allows them to be separated by charge, while the presence of the glucose adduct on HbA1c allows for separation by structural difference (2). Thus, methods of HbA1c analysis can be divided into two categories: those based on the charge differences (such as in cation-exchange chromatography, electrophoresis, and isoelectric focusing) and those based on the structural differences (such as in boronate affinity chromatography and immunoassay) (2).

With the mechanisms of Hb glycation in mind, and the methods by which it may be measured, it is unsurprising that a significant number of factors can interfere with the accuracy of HbA1c as a measure of glycaemic control in clinical practice. Whether these factors interfere with the methods of HbA1c measurement in the laboratory or whether they directly affect HbA1c levels *in vivo*, the clinical concerns is that of 'falsely' elevated or 'falsely' lowered values; values which do not accurately reflect the ambient glucose concentration and which give the

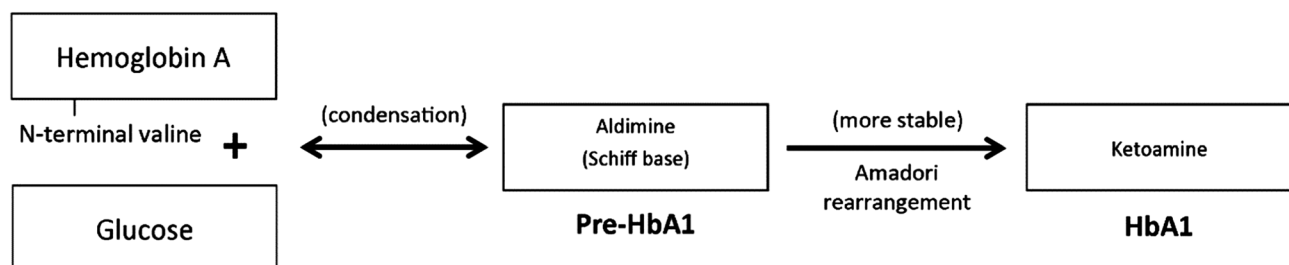


Figure 1
Formation of glycated haemoglobin (HbA1).



false impression that glycemic control is better or worse than actually is the case. It is important that clinicians are aware of these factors and that important therapeutic decisions are not taken on the basis of misleading and inaccurate HbA1c results.

The most well-described co-morbid conditions that can interfere with HbA1c measurement and interpretation are listed in Table 2. Of particular note are factors that alter RBC survival and variants of Hb. Low RBC turnover leads to a greater proportion of older red cells in the circulation – these cells are exposed to circulating glucose for longer, leading to a ‘falsely’ high HbA1c. High RBC turnover, conversely, leads to a greater proportion of younger red cells, and a ‘falsely’ low HbA1c is the result. Abnormal Hb variants can also interfere with measurement of HbA1c by some of the older laboratory methods, for example, falsely elevated HbA1c values in patients with haemoglobin F (HbF) or falsely low values in patients with haemoglobin S (HbS). Chronic kidney disease (9) and severe hyperbilirubinaemia can also falsely increase A1c by interference with the laboratory assay (3).

As illustrated in Table 3, there are also a number of drugs that can interfere with HbA1c measurement and interpretation. All the patients we describe were exposed to the medication dapsonsone. Dapsonsone is an antibiotic used for certain inflammatory, infective and dermatological conditions, for example, dermatitis herpetiformis, leprosy, and pemphigus vulgaris. More than 10% of patients

treated with dapsonsone develop haematologic side effects including haemolysis, anaemia, methaemoglobinaemia, and agranulocytosis, and it is via its haematological side effects that dapsonsone can interfere with accurate measurement of HbA1c (15).

The first mechanism whereby dapsonsone can interfere with accurate HbA1c measurement is by induction of haemolysis, which is effected via a direct toxic effect of its N-hydroxy metabolites on RBCs leading to red cell sequestration in the spleen. This process of RBC destruction by haemolysis reduces erythrocyte lifespan and therefore lowers HbA1c. Although the mean fall in haemoglobin in patients developing dapsonsone-induced haemolysis is reported to be in the order of 2g/dL (16), the presence of haemolysis may not always be readily detectable as, in some patients, the bone marrow may be able to compensate for erythrocyte loss by manufacturing and releasing more erythrocytes. Our first patient had normal haemoglobin, normal MCV, and normal bilirubin, so it would not have been immediately clear that his dapsonsone therapy may have been causing a low HbA1c via haemolysis – but further more detailed testing revealed an elevated reticulocyte count and a low haptoglobin suggesting haemolysis. Patients 2 and 3, in contrast, had a more recognisable anaemia process present.

The second means by which dapsonsone can interfere with HbA1c measurement is via the formation of

Table 2 Conditions that may interfere with HbA1c measurement and interpretation.

Conditions	Effect on HbA1c values	Mechanism
Factors interfering with HbA1c values		
Increased RBC turnover	Decrease	Shortened exposure of red cells to glucose
Haemolytic anaemia (4)		
Acute blood loss (5)		
Recent blood transfusion (6)		
Treated iron, vitamin B12 or folate deficiency (3)		
Splenomegaly (3)		
Decreased RBC turnover	Increase	Prolonged exposure of red cells to glucose
Reticulocytosis (3)		
Iron deficiency (3)		
Vitamin B12 deficiency (3)		
Haemoglobinopathy	False decrease	Altered Hb
*Fetal Hb (8)	False decrease	
*Hb variants (e.g. HbS, HbC) (8)	False decrease	
Interference with assays	False increase	β chain mutation
Uraemia (9)	False increase	Formation of carbamyl-haemoglobin
Severe hyperbilirubinemia (3)	False increase	Unknown
Other	False increase	Formation of haemoglobin-acetaldehyde (HbA1-AcH) compound
Chronic alcohol consumption (10)	False increase	
Chronic liver disease (10)	Variable	Multiple factors (e.g. anaemias, splenomegaly)

*Only interfere with some of the methods used to measure HbA1c.



Table 3 Drugs that may interfere with HbA1c measurement and interpretation.

Drugs affecting HbA1c	Drugs	Effect on HbA1c	Mechanism
Erythrocyte destruction	Dapsone (11) Ribavirin (11) Phenazopyridine (11) Rifampin (11) Trimethoprim-sulfamethoxale (11)	Decrease	Hemolytic anaemia
Altered haemoglobin	Hydroxyurea (11) Dapsone (7)	Decrease Decrease	Increase haemoglobin F proportion Formation of methaemoglobin
Altered glycation	High doses of vitamin C (12)	Decrease	Competitive inhibition of glycosylation
Other	Opiates (13) Aspirin (11) Erythropoietin therapy (14)	Increase Increase Decrease	Unknown Altered glycation of Hb Formation of new RBCs

methaemoglobin (7, 11), which interferes with the high-performance liquid chromatography assay often used to measure HbA1c, resulting in a falsely low HbA1c value. Methaemoglobin refers to the transformation of an iron atom in Hb from the ferrous (Fe²⁺) state into the ferric (Fe³⁺) state as a result of oxidation. The presence of ferric heme molecules causes a structural change in the Hb molecule resulting in reduced oxygen-carrying capacity and impaired unloading of oxygen at the tissue level – a ‘left shift’ in the oxygen saturation curve. This can result in a functional anaemia, where Hb levels appear normal, but the ability of Hb to carry and deliver oxygen to the tissues is impaired. When RBCs containing methaemoglobin at levels greater than 1% of the red blood cell population are present, methaemoglobinaemia is said to be present, symptoms of which include shortness of breath, headache, fatigue, with coma, seizures and death in severe cases. Patients may be asymptomatic, however, if the level of methaemoglobin is <15%. Acquired methaemoglobinaemia occurs most commonly in association with use of various drugs and toxins, and along with topical anaesthetic agents, and dapsons is the commonest cause. In addition to haemolysis, patients 1 and 3 in this case series were found to have elevated methaemoglobin levels, and therefore, we conclude that these patients had two factors, both caused by dapsons treatment, which resulted in a low HbA1c (7).

As with many processes that can adversely affect HbA1c measurement, the response to misleading HbA1c values in the context of dapsons is to rely instead on alternative markers of glycaemic control. In addition to self-monitored capillary glucose readings, fructosamine levels, which measure glycation of serum proteins (mainly albumin), can serve as an alternative measure of glycaemic control, but reflect a shorter time period, approximately 2–3 weeks (17). We note that in all of the patients in this case series the fructosamine levels were

high and helped to confirm our clinical suspicion of poor glycaemic control.

In conclusion, HbA1c is a crucial measure of long-term glycaemic control, and correct interpretation of HbA1c readings in clinical practice is vital to facilitate the optimal treatment of patients with diabetes. With modern methods of HbA1c measurement, haemoglobinopathies do not interfere with accurate HbA1c estimation, and other common factors such as iron deficiency can usually be detected via analysis of the full blood count indices. Dapsone treatment, on the other hand, may significantly interfere with HbA1c without any obviously detectable abnormality on a full blood count and with minimal or no symptoms. Clinicians practising in the area of diabetes care should be aware that HbA1c levels can be misleadingly low in patients treated with dapsons. A thorough medication history should be taken in patients with apparently contradictory HbA1c results; and other tests, such as capillary glucose measurement, urine glucose measurements and fructosamine levels should be used as alternatives to HbA1c for monitoring glycaemic control in this cohort.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent

Verbal and written consents were given.

Author contribution statement

All the authors are the treating physicians of the three patients.



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