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CASE REPORT

A Case of Pseudoelevation of Glycosylated Hemoglobin

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SUMMARY

Background: **Glycosylated hemoglobin (HbA1c) is a stable compound in human blood that covalently binds the N-terminal valine residue of the β-chain in hemoglobin A to the free aldehyde group of glucose. It can reflect the average blood glucose level of patients in the past 2 - 3 months. Therefore, the accuracy of HbA1c detection results is of great significance for the diagnosis and differential diagnosis of diabetes.**
Methods: **We report a case of false elevation of HbA1c measured by a high-performance liquid chromatography (HPLC) system. Using different detection platforms and capillary electrophoresis analysis to identify the causes of abnormally elevated HbA1c levels.**
Results: **HbA1c levels decreased to the normal reference range on a different testing platform. Meanwhile, capillary electrophoresis analysis showed the presence of hemoglobin variants. Therefore, the patient's HbA1c result is abnormally elevated, which is considered a pseudo elevation caused by hemoglobin variants.**
Conclusions: **When HbA1c is abnormally high but there is no clinical symptom of diabetes, the staff should consider the possibility of hemoglobin variants interfering with HbA1c detection. They should simultaneously use different methods to detect HbA1c and conduct hemoglobin component analysis if necessary.**
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KEYWORDS

glycosylated hemoglobin, interference, hemoglobin variant

INTRODUCTION

HbA1c is an important indicator for diagnosing diabetes, evaluating the blood sugar control of diabetes patients, and guiding the development of treatment plans [1]. The result of HbA1c is not only related to the average blood glucose in the body, but also influenced by various factors such as red blood cell lifespan, hemoglobin variants, and medication [2]. We found one case of pseudoelevation of HbA1c due to interference from hemoglobin variants. The specific situation is as follows:

CASE PRESENTATION

The patient is a 46-year-old female. On July 12, 2024, she was hospitalized due to a car accident. Admission

Table 1. The results of serum biochemistry and HbA1c.

Test items	Results	Reference value
C-reactive protein (CRP)	108.95	0 - 6 mg/L
Amylase (AMY)	91.6	40 - 132 U/L
Total protein (TP)	51.3	65 - 85.00 g/L
Albumin (ALB)	34.4	40 - 55 g/L
Total cholesterol (TC)	2.67	2.84 - 5.69 mmol/L
Triglyceride (TG)	2.0	0.56 - 1.70 mmol/L
Fasting blood glucose	6.00	3.9 - 6.10 mmol/L
HbA1c	38.2	4 - 6.3%

auxiliary examination: Abdominal enhanced CT showed left kidney and spleen contusion and laceration and a renal subcapsular hematoma. Serum biochemical indicators are shown in Table 1: CRP (108.95 mg/L) significantly increased, triglycerides slightly increased (2.0 mmol/L), and fasting blood glucose (6.0 mmol/L, within the normal reference range). At the same time, the patient's HbA1c was detected by high-performance liquid chromatography (HPLC), and the result was 38.2%, far exceeding the normal range. The next day, we drew blood again to monitor blood sugar levels, which were 5.29 mmol/L.

Since the patient's blood sugar level is normal and there is no history of diabetes, laboratory personnel have doubts about such high HbA1c. The staff first checked the status of the specimen and did not find any abnormal conditions such as agglutination or lipid turbidity. Simultaneously checking the instrument quality control, it was found that it was completely under control, ruling out the possibility of detection errors caused by specimen status and instrument reasons. The staff immediately collected a new blood sample from the patient and analyzed it. The results were consistent with the previous ones. Analyzing the patient's case, it was found that the patient was involved in a car accident and had bleeding. On the same day, 200 mL of red blood cells were infused. Because the production of glycated hemoglobin is a slow process, theoretically infusing fresh red blood cells can lead to low glycated hemoglobin. Why does the patient's HbA1c not decrease but instead increase? Considering the possibility of interference in HbA1c detection. We sent the specimens for boronate affinity binding and HPLC platform testing and found that the results were within the normal range, consistent with the patient's clinical presentation (Table 2). In order to further identify specific interferents, we analyzed the patient samples using a capillary electrophoresis method and found that the Z11 hemoglobin variant was as high as 35.2% (Table 3). After discussing with clinical doctors, we believe that the abnormal increase in HbA1c in the patient is due to pseudo elevation caused by interference from hemoglobin variants, and suggest further genetic testing.

DISCUSSION

Glycosylated hemoglobin (HbA1c) plays an extremely important role in the screening, diagnosis, and treatment of diabetes [1]. At present, the main methods for detecting HbA1c include capillary electrophoresis and high-performance liquid chromatography (HPLC), boronate affinity binding, and HPLC. Factors such as hemoglobin variants, carbamoylated hemoglobin, and hematological disorders can interfere with different detection methods to varying degrees [2].

Hemoglobin is an important carrier of HbA1c, so any abnormal changes in hemoglobin structure can directly affect the detection of HbA1c [3]. In this case, the patient had normal fasting blood glucose and blood lipids and had received fresh red blood cell transfusions. The clinical manifestations are inconsistent with extremely elevated glycated hemoglobin. Studies have shown that HPLC can cause false positives in detection results due to interference from hemoglobin variants, while boronate affinity binding and HPLC can effectively avoid interference from variant hemoglobin [4,5]. When using HPLC method, due to the co elution of hemoglobin variants and HbA1c, the two cannot be effectively separated, resulting in a false increase in HbA1c. When using boronate affinity binding and HPLC methods, HbA1c combines with borate through its diol structure and remains on the chromatography column. Non glycosylated components such as hemoglobin variants are eluted from the chromatography column, and then HbA1c are replaced from borate by a reagent, thus successfully avoiding interference from hemoglobin variants. We sent the specimens to an external platform using boronate affinity binding and HPLC to remove interference and obtain results consistent with clinical symptoms. Capillary electrophoresis is a technique that utilizes the difference in migration speed of different components in an electrolyte solution within a capillary tube under the action of an electric field to achieve separation.

When measuring hemoglobin, different hemoglobin variants have different migration speeds in an electric field due to their varying molecular sizes, shapes, and charges. This technique can effectively distinguish between different hemoglobin variants [6]. We used a capillary electrophoresis method to analyze the hemoglobin components of patient samples and found that the Z11 hemoglobin variant in patients was as high as 35.2%. We speculate that the presence of this variant leads to a false increase in HbA1c.

In summary, this case emphasizes that if laboratory personnel discover abnormal HbA1c results or differences from clinical diagnosis, further confirmation of the pres-

Table 2. The results of HbA1c in different detection platforms.

	HPLC	Boronate affinity binding and HPLC
HbA1c	**38.2%**	**5.67%**
Reference ranges	**4 - 6.3%**	**4 - 6%**

Table 3. Capillary electrophoresis results of hemoglobin component.

Hemoglobin component	Results	Reference value
HbA1	**61.5%**	**95 - 97.9%**
HbF	**0%**	**0 - 2.1%**
HbA2	**3.3%**	**2 - 3.5%**
HbC	**0%**	**0%**
HbD	**0%**	**0%**
HbE	**0%**	**0%**
HbS	**0%**	**0%**
Z10	**0%**	**0%**
Z11	**35.2%**	**0%**

ence of hemoglobin variants is necessary, and other appropriate testing methods should be used to avoid misdiagnosis and unnecessary treatment.

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Declaration of Interest:
All authors declare that they have no competing interests.

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