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CASE REPORT

Abnormal High HbA_{1c} Caused by Hb Takasago Variant Firstly in a Chinese Pedigree

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SUMMARY

Background: **Over a thousand types of hemoglobin variants have been reported. It is necessary to understand the impact of variants on the accuracy of various methods. The effect of hemoglobin variants on HbA1c determination depends on different detection methods.**
Methods: **A 53-year-old female presented with abnormally elevated glycated hemoglobin (HbA1c) in a routine medical check-up. Capillary electrophoresis (CE) indicated the presence of hemoglobin variant. In the family screening, we found her mother, sister, and daughter all had the same heterozygous mutation (c.397A > G, K133E) in the HBB gene.**
Results: **This is the first known familial case of Hb Takasago in China. Improving the understanding of hemoglobin variants has important medical significance. In the case of abnormal HPLC chromatograms, it is crucial to apply appropriate detection methods, find accurate causes, and communicate with clinicians in a timely manner.**
Conclusions: **The use of appropriate HbA_{1c} detection methods that are not affected by related variants can ensure the accuracy of the results. In the existence of abnormal HPLC chromatograms, it is necessary to communicate with clinical clinicians. Given its heredity and prevalence, effective screening for abnormal hemoglobinopathies is of great significance for improving the quality of the population.**
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KEYWORDS

$HbA_{1c,}$ hemoglobin variants, genetic, pedigree

INTRODUCTION

Glycated hemoglobin (HbA_{1c}) is an important indicator of long-term glycemic control and diabetic complication risk recommended by the American Diabetes Association and World Health Organization (WHO), which has been considered the gold standard for monitoring and evaluating the treatment of diabetes [1]. In the treatment of diabetes, HbA1c should be controlled at < 7% (53 mmol/mol) much as possible [2]. The significance of HbA_{1c} measurement in diabetes management has been widely recognized.
The most common method for HbA_{1c} measurement is high-performance liquid chromatography (HPLC) [3] which separates hemoglobin species by charge differ-

ence to calculate HbA_{1c}. Capillary electrophoresis (CE) is a modern self-resolving technology that performs self-separation at different migration speeds under the action of electric field force and the guidance of solute early electrophoresis control separation technology. The accuracy of HbA1c with hemoglobin variants is an analytical challenge in clinical laboratories. At present, over a thousand types of hemoglobin variants have been reported [4]. It is necessary to understand the impact of variants on the accuracy of various methods. The effect of hemoglobin variants on HbA_{1c} determination depends on different detection methods.

Here, we describe a unique hemoglobin variant (c.397A > G, K133E) and its heredity characteristics in a female patient with abnormally elevated HbA_{1c}. The two methods, HPLC and CE, differ in the detection of HbA_{1c} in such patients.

CASE REPORT

Clinical features

A 53-year-old female presented with an abnormal elevation of HbA_{1c} in a routine medical check-up. The preliminary evaluation by high-performance liquid chromatography (HPLC) with the Tosoh G8 analyzer showed an HbA_{1c} value of 44.6%, with a reference interval of 4.5% (26 mmol/mol) - 6.1% (43 mmol/mol). The value was too high to be standardized to the International Federation of Clinical Chemistry (IFCC) reference measurement procedure, because the National Glycohemoglobin Standardization Program (NGSP) standard requires that the HbA_{1c} value in NGSP units must be between 3% and 20% (http://www.ngsp.org/). The patient had no family history of diabetes but a medical history of thyroid cancer surgery.

Hematologic examination

At the same check, a mild increase of serum thyroid-stimulating hormone (TSH) to 4.410 mU/L (reference interval 0.27 - 4.2), fasting plasma glucose (FPG) of 5.39 mmol/L (reference interval 3.90 - 5.90 mmol/L), and glycated albumin (GA) of 12.55% (reference interval 9 - 14%) was observed. The synchronous biochemistry and hematology results were all within the reference interval (Table 1).

Chromatograms analysis

The HPLC chromatogram of the proband indicated a large abnormal peak, leading to an inaccurate HbA_{1c} value of 44.6% (Figure 1A). On retesting, the capillary electrophoresis (CE) result of HbA_{1c} was 5.2% (33 mmol/mol), indicating the presence of a hemoglobin variant (assayed by Capillary3 TERA analyzer) (Figure 1B). The analysis revealed an abnormal peak eluted between HbA_{1c} and HbA_0. Therefore, the patient was suspected of hemoglobinopathy and underwent further examination.

Gene sequencing and pedigree analysis

Gene sequencing identified a heterozygous mutation (c.397A > G, K133E) in the HBB gene. According to the HbVar (http://globin.bx.psu.edu/hbvar/hbvar.html), a database of human hemoglobin variants and thalassemia mutations, the hemoglobin genotype was named Hb Takasago, an extremely rare variant and no related cases had been reported. It has never been found in the Human Genetic Variation Database (HGVD) (http://www.Genome.med.kyoto-u.ac.Jp/SnpD B/index.html). During the family screening, we found her mother (I - 2), sister (II - 5), and daughter (III - 2) all had the same mutation (Figure 1C). Apart from HbA_{1c}, other laboratory investigations of the family were normal (Tabel 1). The abnormal HbA_{1c} results of the patient's family members were as follows: 44.9% for her mother, 43.3% for her sister, and 44.4% for her daughter. This is the first case of pedigree genetics caused by the Hb Takasago.

DISCUSSION

Unexpectedly, despite the abnormal HbA_{1c} results, none of the patients had obvious hemolysis, anemia, or hyperglycemia, and the results of biochemical and blood routine results were all in the normal range, also mentioned in Cohen RM et al.'s study [5]. It reminds us to distinguish and address disease conditions caused by inconsistent FPG and HbA_{1c}. In this case report, gene sequencing identified the presence of hemoglobin variants.

The presence of hemoglobin variants can impact HPLC assay at the analytic level [6]. The HPLC method calculates the percentage of HbA_{1c} area relative to the chromatograms of total area, but due to the presence of variants, the value of the HbA_{1c} increases, resulting in higher calculated values [7]. Some researchers have pointed out the chromatogram of samples with abnormal hemoglobin is different from that of normal samples [7], making it difficult to determine the accurate HbA_{1c} value. CE can sensitively reflect mutation peaks and issue warnings, and HbA_{1c} measurement is not compromised by common hemoglobinopathies [8]. Screening only through blood cell analysis may lead to missed diagnosis, while CE can effectively identify abnormal hemoglobinopathies [8]. For patients with the Hb Takasago trait, CE may be a supplemental assay to HPLC.

We observed the characteristics of this hemoglobin variant were reflected on the HPLC chromatogram. Also, it enriched the CE chromatogram with the hemoglobin variant. Chromatograms, as laboratory evidence, are especially important for HbA_{1c} detection and deserve more attention. The laboratory staff needs to be serious about various factors that may interfere with the results and communicate with clinicians in good time to explain the situation and provide suggestions.

Therefore, we recommend the use of alternative indicators such as GA to monitor and evaluate blood glucose

Table 1. Patient biochemistry and hematology results at presentation.

Analyte	I - 2	II - 1	II - 4	II - 5	III - 1	III - 2	III - 3	Reference interval
HbA_{1c}, %	44.90	5.7	44.6	43.3	5.1	44.4	5.3	4.5 - 6.1
RBC, x 10^{12}/L	4.63	4.43	4.05	3.98	4.11	4.09	4.38	male (4.3 - 5.8)
								female (3.8 - 5.1)
HB, g/L	146	132	120	121	124	122	134	male (130 - 175)
								female (115 - 150)
MCV, fL	97.2	89.2	94.1	95	89.3	92.1	91.1	82 - 100
MCH, pg	31.5	29.8	29.6	30.4	30.2	30.3	30.6	27 - 34
MCHC, g/L	324	334	315	320	338	316	336	316 - 354
FPG, mmol/L	6.47	5.69	5.39	4.73	4.44	4.9	5.26	3.90 - 5.90
GA, %	12.34	12.63	12.55	12.14	12.24	12.25	12.52	9 - 14
TBIL, μmol/L	12	9.8	9.6	9.8	8.4	9.1	5.7	5.0 - 28.0
IBIL, μmol/L	9.1	7.4	6.5	6.8	5	5.9	3.3	< 20
DBIL, μmol/L	2.9	2.4	3.1	3.0	3.4	3.2	2.4	< 8.8
TP, g/L	75.6	79.5	78.3	72.7	85.6	75.1	71.4	65.0 - 85.0
ALB, g/L	45.7	49.4	49.8	47.1	45.9	46.2	48.6	40.0 - 55.0
GLB, g/L	29.9	30.1	28.5	25.6	39.7	28.9	22.8	20.0 - 40.0

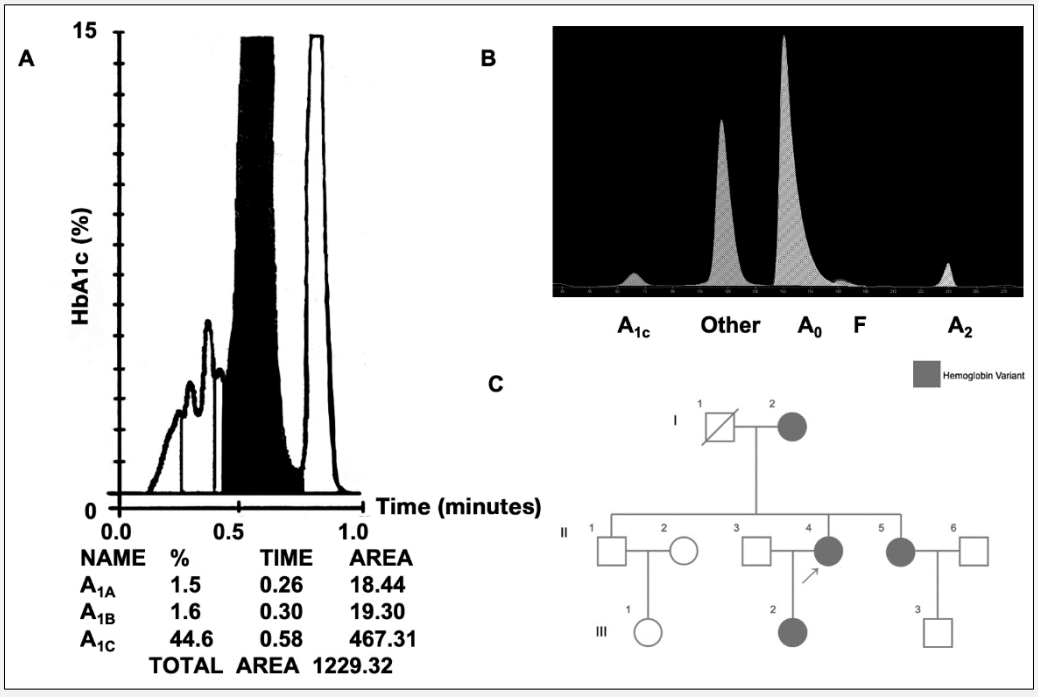

Figure 1. A. HPLC chromatogram of the proband. Dark indicates a big abnormal peak. The HbA_{1c} measured was 44.6%, A_{1A} was 1.5%, and A_{1B} was 1.6%. B. Capillary electrophoresis of the proband showed a normal result. Another peak (38.3%) between HbA_{1c} and HbA_0 was identified. The HbA_{1c} measured was 5.2%. C. Pedigree analysis for hemoglobin variant. I - 2, II - 4, II - 5, and III - 2 all have the same hemoglobin variant.

levels for patients with abnormal hemoglobin. GA is a biomarker for short-term (2 - 3 weeks) glycemic control, which can reflect glycemic control more quickly and clearly than HbA_{1c}, thereby evaluating the effect of diabetes treatment in a more timely manner [9].
Thalassemia is a monogenic genetic disorder with a high prevalence in the southern region of China [10]. Further investigation into the patient's family revealed that her mother, sister, and daughter had the same mutation (c.397A > G, K133E) on the HBB gene. This mutation has never been reported in China. Although patients with this gene mutation do not develop obvious clinical signs and symptoms, the potential risk is unclear which is different from the research results of Ruetsch C et al. [11]. This rare mutation in the Chinese pedigree can enrich the spectrum of thalassemia.

CONCLUSION

We report a family case of high HbA_{1c} with no clinical symptoms and demonstrate that the high HbA_{1c} results were caused by a rare variant on the Tosoh G8 analyzer. The causes of Hb Takasago variant and abnormal HbA_{1c} value are still not fully understood. The use of appropriate HbA_{1c} detection methods that are not affected by related variants can ensure the accuracy of the results. In the existence of abnormal HPLC chromatograms, it is necessary to communicate with clinical clinicians.

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Data Availability Statement:
All data generated in this study are included in the main text.

Ethical Statement:
The present study protocol was reviewed and approved by the West China Hospital's Ethical Review Committee (approval No. 228-2015), and conducted in accordance with the Declaration of Helsinki.

Consent for Participation:
Informed consent was obtained from all participants involved in the study.

Consent for Publication:
Informed consent was obtained for the publication of all patient images/data.

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Declaration of Interest:
The authors declare that they have no conflicts of interest.

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