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CASE REPORT

False HbA_{1c} Value due to a Rare Variant of Hemoglobin J-Cubujuqui

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SUMMARY

Background: **Hemoglobin (Hb) J-Cubujuqui is a rare Hb variant, and reports about it are very limited. There are no descriptions that it affects the results of glycated Hb.**
Methods: **In this study, we describe a rare variant discovered during newborn screening. Both high-performance liquid chromatography (HPLC) and capillary electrophoresis for hemoglobin analysis displayed abnormal peaks. The Hb variant was confirmed by Sanger sequencing.**
Results: **The pedigree study shows the variant was inherited from the newborn's father. His fasting blood glucose (FBG) level was 5.5 mmol/L. HbA_{1c} measured by HPLC was falsely low in her father (2.41%), whereas that measured by immunoassay was normal (5.11%). Sanger sequencing revealed a heterozygous mutation (CGT>AGT) at amino acid position 141 of the α1 gene, corresponding to Hb J-Cubujuqui [α1 141(HC3) Arg→Ser (CGT>AGT); *HBA1*:c.424C>A (or *HBA2*)].**
Conclusions: **This is the first report that Hb J-Cubujuqui interferes with the measurement of HbA_{1c} and prompts clinicians to pay attention to the accuracy of glycated Hb results.**
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KEYWORDS

HbA_{1c}, glycated Hb, Hb J-Cubujuqui, variant

INTRODUCTION

Glycated hemoglobin (HbA_{1c}) is widely considered a marker for diagnosing and monitoring diabetes [1]. Some hemoglobin (Hb) variants may interfere with HbA_{1c} measurement and produce false results [2,3]. Hb variant is often detected during thalassemia screening, HbA_{1c} measurement, and newborn screening [4-6]. HbA_{1c} can be measured by immunoassay, affinity assays, capillary electrophoresis (CE), and high-performance liquid chromatography (HPLC). Reports show that cases with an Hb variant are more likely to present falsely low or high HbA_{1c} values using the HPLC method [7,8]. Here, we describe a rare variant of Hb J-Cubujuqui in a patient with no diabetes that showed a false low HbA_{1c} value measured by HPLC but a normal value

Table 1. Hematological and molecular results of the family with Hb J-Cubujuqui.

Parameters	Newborn	Her mother	Her father
Gender/age (years)	F/0	F/35	M/35
Hb (g/dL)	18.3	11.7	16.3
MCV (fL)	111.0	81.7	87.8
MCH (pg) CE	36.6	27.4	30.6
Hb A (%)	24.2	97.2	76.2
Hb J-Cubujuqui (%)	2.0	0	21.7
Hb A2 (%)	0.5	2.8	2.1
Hb J-Cubujuqui -F (%)	11.4	0	0
Hb F (%)	61.9	0	0
HPLC			
Hb A0 (%)	N	N	71.3
Hb J-Cubujuqui (%)	N	N	0
Hb A2 (%)	N	N	2.3
Hb J-Cubujuqui -A2 (%)	N	N	0
α-globin Genotype	$\alpha\alpha^{\text{J-Cubujuqui}}/\alpha\alpha$	αα/αα	$\alpha\alpha^{\text{J-Cubujuqui}}/\alpha\alpha$

N - No detection.

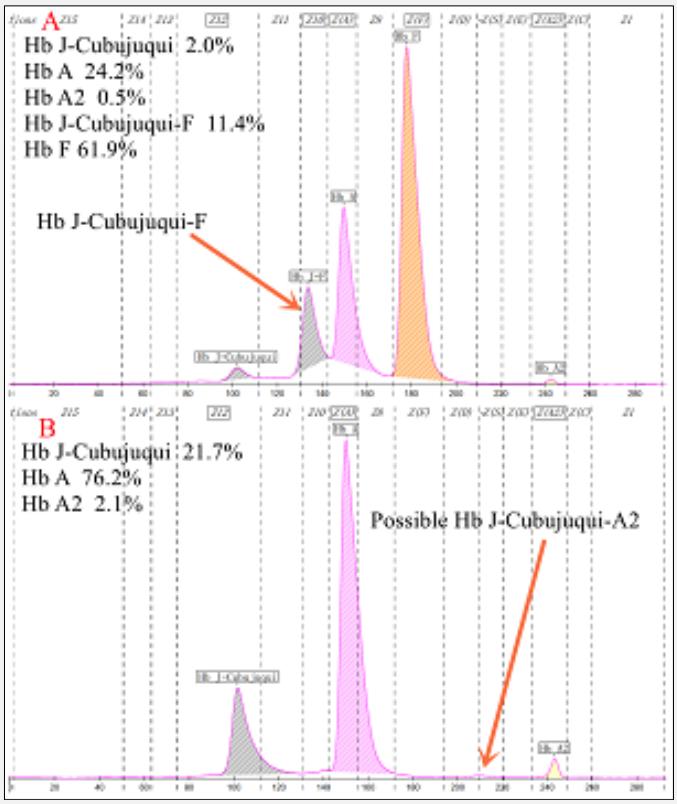
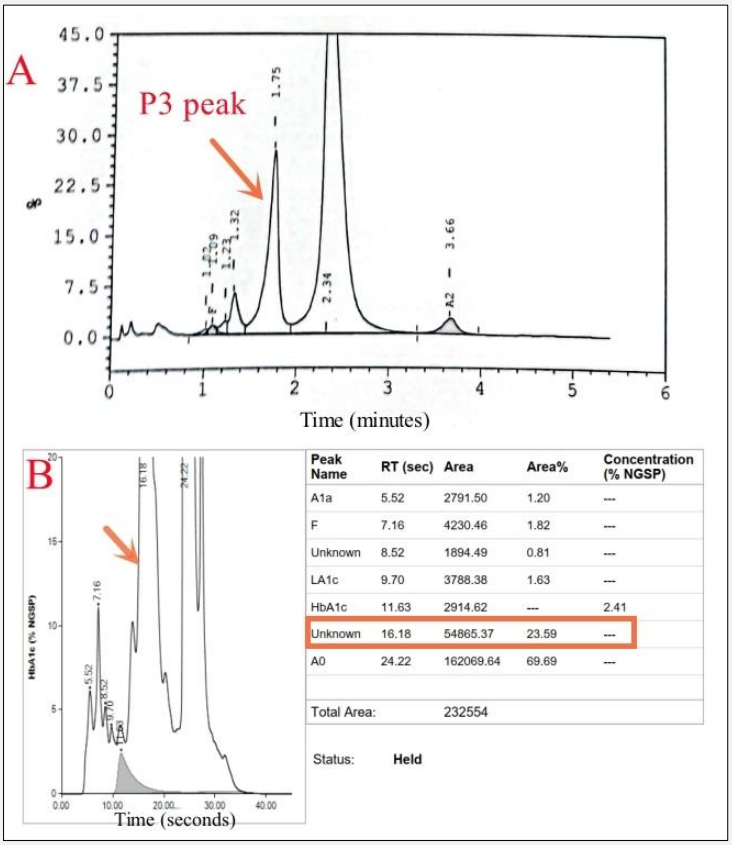


Figure 1. The electrophoresis results of the newborn (A) and her father (B) by CE.



Peak Name	RT (sec)	Area	Area%	Concentration (% NGSP)
A1a	5.52	2791.50	1.20	---
F	7.16	4230.46	1.82	---
Unknown	8.52	1894.49	0.81	---
LA1c	9.70	3788.38	1.63	---
HbA1c	11.63	2914.62	---	2.41
Unknown	16.18	54865.37	23.59	---
A0	24.22	162069.64	69.69	---
Total Area:		232554		

Status: **Held**

Figure 2. Hb A_{1c} (A) measurement and Hb analysis (B) for the newborn's father by HPLC.

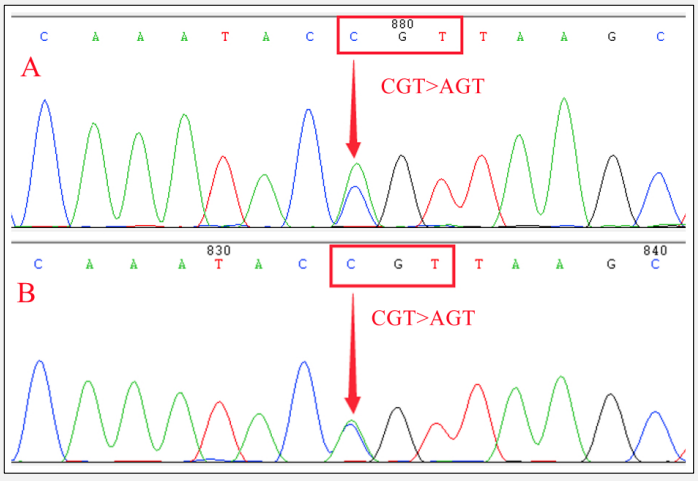


Figure 3. Sanger sequencing of the α-globin gene revealed heterozygosity for the CGT>AGT mutation at codon 141, corresponding to Hb J-Cubujuqui.

by immunoassay.

CASE REPORT

The Guangxi Zhuang Autonomous Region is a province in southern China where thalassemia is prevalent. Newborn screening for α-thalassemia has been conducted at our hospital since 2013. In this study, the infant with the Hb variant was detected during a routine newborn screening. Subsequently, the newborn's parents were recalled and recommended for thalassemia screening. Their blood samples were collected after written informed consent was obtained. The hematological parameters of the newborn's father showed the following: Hb level 16.3 g/dL, red cell count (RBC) 5.32 x 10^{12}/L, mean corpuscular volume (MCV) 87.8 fL, and mean corpuscular Hb (MCH) 30.6 pg (Sysmex XN 1000; Sysmex Corporation, Kobe, Japan). The results of the newborn and her mother from the complete blood count are shown in Table 1.

Hb analysis was performed by CE (Capillary S2 Flex Piercing; Sebia, Lisses, Paris, France). The electropherogram of newborns revealed abnormal peaks in zone 10 and zone 12, with values of 11.4% and 2.0%, respectively (Figure 1A). The CE results of the newborn's father showed an abnormal peak in zone 12 (21.7%) and a possible shadow peak in zone 5 (Figure 1B). Compared with CE, HPLC showed no abnormal peaks, but there was a high-value P3 peak (20.8%), suggesting the presence of the Hb variant (Figure 2A) (VARIANT II^{TM}; Bio-Rad, Hercules, CA, USA). To investigate whether this variant interfered with HbA_{1c} measurement, we performed the glycated hemoglobin test by HPLC (D100; Bio-Rad, Hercules, CA, USA). The chromatogram presented HbA_{1c} results out of range (2.41%), possible variant interference, and HbA_{1c} peak shape tailing (Figure 2B). However, his fasting blood glucose (FBG) (5.5 mmol/L) (AU680; Beckman Coulter, Kraemer Boulevard Brea, CA, USA) and HbA_{1c} measurement (5.11%) by immunoassay were normal. The Gap-PCR was used to identify four common Chinesea-thalassemia deletion mutations (-$\alpha^{4.2}$, -$\alpha^{3.7}$, --^{SEA}, and –^{THAI}) (Yishengtang Biotech, Shenzhen, China). PCR and reverse dot-blot hybridization were used to detect three common non-deletional mutations of the α-globin gene, including Hb Quong Sze (Hb QS, *HBA2*:c.377T>C), Hb Constant Spring (Hb CS, *HBA2*:c.427T>C), and Hb Westmead (Hb WS, *HBA2*:c.369C>G) (Yaneng Biotech, Shenzhen, China). Neither test discovered any mutations, despite the father's decreased HbA_2 level. Sanger sequencing of the α-globin gene was performed to identify the Hb variant with a 3500 XL sequencer (Applied Biosystems, Foster City, CA, USA). We found a substitution of C>A at codon 141 in the α-globin gene, corresponding to Hb J-Cubujuqui [α1 141(HC3) Arg→Ser (CGT>AGT); *HBA1*:c.424C>A (or *HBA2*)] (Figure 3).

DISCUSSION

Hb J-Cubujuqui was first reported by Saenz GF et al. in Costa Rica as a silent clinical variant [9]. However, the variant exhibits a high affinity for oxygen in another report. To date, five other variants caused by the mutation at codon 141 in the *HBA* gene were described in the HbVar database, including Hb Nunobiki [α1 141(HC3) Arg→Cys (CGT>TGT); *HBA1*:c.424C>T (or *HBA2*)], Hb J-Camagüey [α1 141(HC3) Arg→Gly (CGT>GGT); *HBA1*:c.424C>G (or *HBA2*)], Hb Suresnes [α1 141 (HC3) Arg→His (CGT>AAT); *HBA1*:c.425G>A (or *HBA2*)], Hb Legnano [α1 141(HC3) Arg→Leu (CGT> ATT); *HBA1*:c.425G>T (or *HBA2*)], and Hb Singapore [α1 141(HC3) Arg→Pro (CGT>ACT); *HBA1*:c.425G >C (or *HBA2*)]. There are no reports on the effect of these five variants on HbA_{1c} measurement.

Hb J-Cubujuqui is the first variant at position 141 of the HBA gene described to interfere with glycation measurement. In this study, the chromatogram showed an unknown peak directly behind the HbA_{1c} peak, which likely corresponds to Hb J-Cubujuqui. It is well known that chromatographic methods calculate the % HbA_{1c} as follows: % HbA_{1c} = 100* HbA_{1c}/(HbA_{1c} + HbA_0). According to the equation, the denominator is overestimated, yielding falsely low HbA_{1c} results. Therefore, when Hb J-Cubujuqui is present, HPLC is not the most appropriate for quantifying HbA_{1c} and monitoring long-term glycemic control. An alternative method such as immunoassay should be chosen. In addition, the chromatogram should also be carefully checked to detect the possible presence of Hb variants when HPLC is used to screen for hemoglobinopathies.

The mechanism by which Hb variants interfere with HbA_{1c} measurement depends on the method and can be divided into physiological or analytical factors. Physiologically, Hb variants may impair the process of HbA_{1c} formation, leading to an undervaluation of HbA_{1c}. Analytically, if the Hb variant carries a similar charge to HbA_{1c}, it may be disturbed by co-eluting with the HbA_{1c} peak during measurement. This suggests an important flaw in the use of HbA_{1c} as a marker for diabetes and should alert clinicians to consider Hb variants when HbA_{1c} is outside the normal range and not consistent with the clinical situation. In this study, the limitation was that CE was not used to detect the value of HbA_{1c} due to the lack of reagents.

CONCLUSION

This case emphasizes that Hb variants may cause misleading HbA_{1c} values and affect the interpretation of the results. When using HbA_{1c} to diagnose and monitor diabetes, clinicians should suspect Hb variants if the HbA_{1c} value is inconsistent with the clinical situation. Where indicated, glycated albumin, fasting glucose, or oral glucose tolerance tests can be used as alternative measuring indicators and assessments of its control.

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Declaration of Interest:
The authors report no conflicts of interest relevant to this article.

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