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CASE REPORT

Interference of Hb J-Sardegna on HbA1c Quantification in a Diabetic Patient

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SUMMARY

Background: **Hb J-Sardegna represents a rare hemoglobin (Hb) variant with no previously documented interference in HbA_{1c} quantification. In this study, we report the first case of Hb J-Sardegna incidentally identified in a diabetic patient, demonstrating significant discordance between measured blood glucose levels and disproportionately elevated HbA_{1c} values.**
Methods: **The patient presented to our institution for follow-up evaluation of thyroid nodules, during which diabetes screening was clinically indicated. Plasma glucose levels were measured using biochemical analyzers, while HbA_{1c} quantification was performed by high-performance liquid chromatography (HPLC). Hb fractions analysis was conducted by capillary electrophoresis (CE), with subsequent confirmation of the suspected variant through Sanger sequencing.**
Results: **Laboratory investigations revealed markedly elevated glucose levels (fasting: 8.79 mmol/L; 2-hour postprandial: 18.09 mmol/L), while HPLC analysis demonstrated a discordantly elevated HbA_{1c} of 32.23%. CE identified an abnormal Hb fraction (Hb J peak: 31.8%), with subsequent Sanger sequencing confirming the presence of Hb J-Sardegna (*HBA2*:c.151C>G) and the intronic variant IVS-II-89T>C (*HBA1*:c.301-61T>C). Notably, all hematological parameters remained within normal ranges.**
Conclusions: **This study reports for the first time that Hb J-Sardegna can interfere with HbA_{1c} quantification, highlighting the importance for laboratory technicians to carefully interpret chromatographic profiles when analyzing HbA_{1c} results.**
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KEYWORDS

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INTRODUCTION

Glycated hemoglobin (HbA_{1c}) serves as a pivotal biomarker in diabetes management [1]. First identified in 1968 as an abnormal Hb component in diabetic patients, HbA_{1c} was subsequently characterized as the non-enzymatic glycation product formed between glucose and the N-terminal valine of the Hb β-chain [2]. After decades of investigation, HbA_{1c} has been established as the gold standard for evaluating glycemic control, uniquely reflecting average blood glucose levels over

the preceding 2 - 3 months. Following WHO's 2011 diagnostic incorporation (≥ 6.5%), China implemented HbA1c as a definitive criterion in 2019 [3].

Notably, despite advantages including fasting-independent nature and low biological variability, HbA_{1c} measurement carries clinical limitations. Biological confounders encompass erythrocyte lifespan alterations (e.g., shortened cycle in hemolytic anemia) and bone marrow dysfunction (e.g., impaired erythropoiesis in iron-deficiency anemia) [4]. Methodological interference arises from Hb variants (e.g., HbS, HbE), while metabolic disorders like uremia may alter erythrocyte microenvironment and glycation kinetics [5,6]. These complexities necessitate multidimensional assessment using serum fructosamine and continuous glucose monitoring, particularly in pregnancy or hemoglobinopathies.

Here, we present a rare variant Hb J-Sardegna incidentally diagnosed in a diabetic patient. Discrepancies between HPLC-measured HbA_{1c} and blood glucose levels prompted suspicion of hemoglobinopathy, confirmed through Hb electrophoresis. To our knowledge, this represents the first documented case of Hb J-Sardegna interfering with HbA_{1c} quantification.

CASE REPORT

A 53-year-old Chinese male with a history of thyroid nodules presented to our institution for routine follow-up. The patient denied a personal or familial history of diabetes mellitus or hemoglobinopathies. As part of age-appropriate metabolic screening, clinicians ordered fasting plasma glucose (FPG) and HbA1c measurements alongside complete blood count (CBC), hepatic/renal function panels, and lipid profiling.

Biochemical analysis revealed elevated glucose levels: FPG 8.79 mmol/L (reference: 3.9 - 6.1 mmol/L) and 2-hour postprandial glucose 18.09 mmol/L (reference < 7.8 mmol/L) (AU680; Beckman Coulter, Kraemer Boulevard, Brea, CA, USA). However, HPLC-based HbA_{1c} quantification demonstrated a discordantly extreme value of > 32.23% (reference ≤ 6.5%), suggesting potential interference from Hb variants (D100; Bio-Rad, Hercules, CA, USA). To investigate this possibility, Hb fractions were performed by capillary electrophoresis (CE) (CapillaryS2 Flex Piercing; Sebia, Lisses, Paris, France). Analysis of Hb fractions identified abnormal migration patterns: HbA (65.8%), HbF (0.7%), HbA2 (1.7%), with two distinct aberrant peaks corresponding to HbJ (31.8%) and HbD (0.7%). Based on the electrophoretic characteristics of α-globin chain variants, which produce distinct migration bands corresponding to their α-chain composition, these bands were attributed to α-globin chain variants.

To molecularly confirm the suspected Hb variants, bidirectional Sanger sequencing of the *HBA1* and *HBA2* genes was performed [7]. Sequencing analysis identified a heterozygous missense mutation (CAC>GAC) at codon 50 of the *HBA2* gene (*HBA2*:c.151C>G, p.His50Asp), corresponding to the known Hb J-Sardegna variant. Furthermore, a previously unreported splice region variant was detected in intron 2 of the *HBA1* gene (*HBA1*:c.301-61T>C, IVS-II-89T>C). Database interrogation (ClinVar, HbVar) and literature review confirmed this intronic mutation represents a novel mutation with no prior clinical reports.

Additional laboratory investigations revealed normal hepatic and renal function profiles. Lipid panel analysis demonstrated isolated hypertriglyceridemia (2.94 mmol/L; reference range: < 1.7 mmol/L). CBC parameters showed Hb at the upper reference limit (175 g/L; normal range: 130 - 175 g/L), with normocytic and normochromic erythrocyte indices: mean corpuscular volume (MCV) 89.0 fL (reference: 82 - 100 fL) and mean corpuscular Hb (MCH) 30.1 pg (reference: 27 - 34 pg).

DISCUSSION

Hb J-Sardegna is a Hb variant caused by a missense mutation in the *HBA* gene (α50[CE8]His→Asp), resulting in the substitution of histidine by aspartic acid at position 50 of the α-globin chain [8]. This variant, first found in 1969, has different geographical distribution patterns, with a prevalence of 0.25% in northern Sardinia but an exceedingly low prevalence in the Chinese population [9,10]. This clinically benign hemoglobinopathy exhibits unique oxygen-binding characteristics. Unlike normal adult Hb (HbA), Hb J-Sardegna demonstrates elevated oxygen affinity specifically under conditions of physiological 2,3-diphosphoglycerate (2,3-DPG) concentration, a critical allosteric regulator of Hb function [11].

This case represents the first documented instance of Hb J-Sardegna interfering with HPLC-based HbA_{1c} quantification, resulting in discordance between glucose levels and HbA_{1c} values. While previous reports have described Hb J-Sardegna in non-diabetic populations, its coexistence with diabetes mellitus and subsequent impact on glycemic monitoring remained unexplored. Mechanistically, hemoglobinopathies influence HbA_{1c} measurement through three principal pathways: 1) altered Hb glycosylation kinetics, 2) reduced erythrocyte lifespan, and 3) chromatographic co-elution artifacts [12]. In this case, the co-elution of Hb J-Sardegna with HbA_{1c} derivatives led to significant overestimation by HPLC, thereby exposing critical limitations in HbA_{1c}'s reliability as a diabetes biomarker under such pathophysiological conditions. Regrettably, the lack of immunoassay-specific reagents in our laboratory precluded evaluation of potential interference by Hb J-Sardegna with immunoassay-based HbA_{1c} measurements.

Hb J-Sardegna, a benign genetic variant that does not cause clinical disease, may lead to measurement errors in HbA_{1c} values. Similar Hb variants interfering with HbA_{1c} quantification are occasionally encountered in clinical practice, particularly in regions with a high

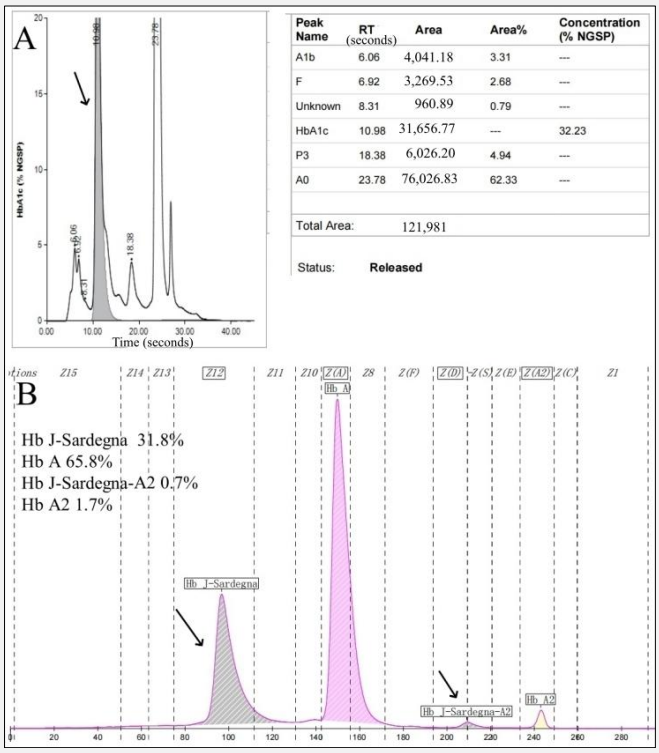

Figure 1. HbA_{1C} measurement with HPLC (A) and Hb analysis by CE (B) in this patient.

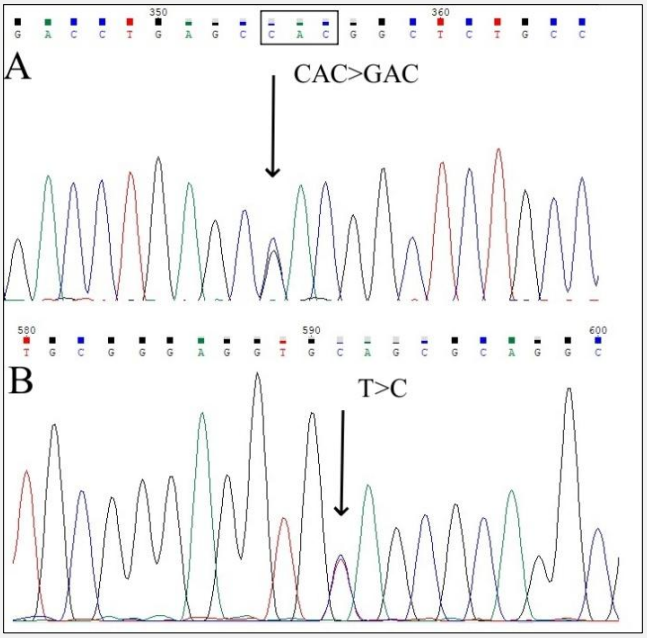

Figure 2. Sanger sequencing of this patient identified two genetic alterations: a missense mutation at codon 50 of the *HBA1* gene (CAC>GAC, p.His50Asp) (A) and a splice-region variant in intron 2 of the *HBA2* gene (*HBA2*:c.301-61T>C) (B).

prevalence of hemoglobinopathies [6,7]. Clinicians should be aware of the possibility of Hb variants when encountering abnormal HbA_{1c} values (e.g., below the normal range or inconsistent with clinical observations). Additionally, the selection of inappropriate detection methods may result in falsely elevated or reduced HbA_{1c} values due to Hb variants, leading to misdiagnosis or a false impression of well-controlled diabetes. Notably, a novel mutation was identified in intron 2 of the *HBA1* gene (*HBA1*:c.301-61T>C, IVS-II-89T>C). To our knowledge, this variant represents a previously unreported mutation with no documented phenotypic associations. Although localized to a splice region where sequence variations may alter RNA processing, the absence of hematological abnormalities - evidenced by normocytic erythrocyte indices (MCV 89.0 fL, MCH 30.1 pg) - suggests this likely represents a benign polymorphism.

This study highlights the necessity for clinical laboratories in hemoglobinopathy-endemic regions to recognize the inherent limitations of HbA_{1c} testing. Technologists should systematically interrogate chromatographic profiles to verify analytical validity, particularly when aberrant patterns emerge. Furthermore, in alignment with the Chinese expert consensus on HbA_{1c} testing, we advocate implementing routine Hb fractionation analysis prior to HbA_{1c} quantification in high-prevalence populations to preempt Hb variant-related interference. For patients with confirmed Hb variants, glycemic monitoring should be supplemented with non-Hb-dependent biomarkers - such as glycated albumin, fructosamine, or continuous glucose monitoring (CGM) - to ensure accurate longitudinal assessment of glycemic control.

CONCLUSION

This case underscores the potential for Hb variants to confound HbA_{1c} measurements, thereby compromising their diagnostic reliability in diabetes management. Clinicians should maintain a high index of suspicion for hemoglobinopathies when encountering discordance between HbA_{1c} values and clinical presentation. In such scenarios, alternative glycemic monitoring strategies - including glycated albumin, fasting plasma glucose, or oral glucose tolerance testing - may provide more accurate metabolic assessment and therapeutic guidance.

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

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