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CASE REPORT

First Family Case of Hemoglobinopathy Titusville in China with Falsely Low SpO2 and Unmeasurable SaO2

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SUMMARY

Background: **Normal hemoglobin is a tetrameric structure, consisting of two alpha-globin chains and two nonalpha (beta, gamma, delta) chains. Hemoglobinopathies occur when the presence of gene mutations affect the molecular structure or expression of the globin chains.**
Methods: **We reported the case of a 9-year-old Chinese girl who presented with abnormal low oxygen saturation values on pulse oximetry and no oximetry results were obtained during blood gas analysis (BGA).**
Results: **High-performance liquid chromatography (HPLC) and capillary electrophoresis demonstrated that the presence of a low oxygen affinity hemoglobin variant, characterized as hemoglobin Titusville, was proven by gene sequencing. The patient's mother and aunt also carry the hemoglobin variant, representing the first Chinese family case reported.**
Conclusions: **Hemoglobin Titusville is a rare genetic hemoglobin structural defect. early diagnosis can help patients and clinicians avoid unnecessary anxiety and costly or excessive clinical investigations.**
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KEYWORDS

hemoglobinopathy, pulse oximetry, hemoglobin Titusville, low oxygen affinity hemoglobin variant

INTRODUCTION

Hemoglobin is composed of two alpha-globin chains and two non-alpha (beta, gamma, delta) chains, resulting in a tetrameric structure that contains four heme groups capable of binding to oxygen. Hemoglobinopathies are caused by gene mutations that affect the molecular structure or expression of the globin chains [1]. Approximately 269 million people are estimated to be carriers of mutations of the globin chains, most of whom do not have any known clinical manifestations [2].

Arterial oxygen saturation (SaO2) is measured by co-oximetry with arterial blood gas analysis, which is the gold standard for assessing the body's oxygen supply. However, it is invasive, costly, and time-consuming. Therefore, pulse oximetry is commonly used to evaluate

Table 1. Clinical characteristics of the patient at hospital admission.

Clinical characteristics	
Age (years)	9
Gender	female
WBC (x 10^9/L)	7.1 (N)
HGB (g/L)	115 (N)
PLT (x 10^9/L)	195 (N)
MCV (fL)	89.1 (N)
CRP (mg/L)	< 0.5 (N)

WBC - white blood cells, HGB - hemoglobin, PLT - platelets, MCV - mean corpuscular volume, N - values within the normal range.

Table 2. Blood parameters measured on different days. Gas analysis of the blood of the patient was performed using a GEM5000 gas analyzer on different days.

Day	Before admission	After admission
Parameters		
pH	7.460	7.410
PaO2 (KPa)	34.0	13.4
PaCO2 (KPa)	3.1	4.4
SaO2 (%)	unmeasured	unmeasured
HbCO (%)	error in measurement	
HHb (%)		
MetHb (%)		
HbO2 (%)		

pH - potential of hydrogen, PaO2 - partial pressure of oxygen in arterial blood, PaCO2 - partial pressure of carbon dioxide in the arterial blood, SaO2 - arterial oxygen saturation, HbCO - carboxyhemoglobin, HHb - deoxyhemoglobin, MetHb - methemoglobin, HbO2 - oxyhemoglobin.

arterial oxygen content noninvasively at the fingertip or the ear. There are many factors that can lead to low peripheral oxygen saturation (SpO2), with the most common being inadequate peripheral blood perfusion. However, factors such as carboxyhemoglobin, methemoglobin, skin pigmentation, hyperbilirubinemia, and anemia may also cause low SpO2.

We report a case of hemoglobin (Hb) Titusville, a low-oxygen-affinity hemoglobin variant causing low oxygen saturation in a healthy Chinese child. This case represents the first reported case of hemoglobin Titusville in a family of Chinese descent. Identifying variant hemoglobins plays a crucial role in minimizing the need for extensive investigations on certain individuals, as highlighted by this case report.

CASE REPORT

The patient, a 9-year-old girl, was admitted to the hospital initially due to low oxygen saturation for more than 3 months and fever for more than 3 days. In blood samples, C-reactive protein (CRP) was normal (< 0.5 mg/L); cardiac troponin I (cTnI) and B-type natriuretic peptide (BNP) were negative; and white blood cell count (7.1 E9/L), hemoglobin (115 g/L), and platelet count (195 E9/L) were all normal (Table 1). The result of the influenza A (H1N1) test was positive. Bedside echocardiography and electrocardiogram (ECG) indicated normal brain function. Small nodules in the outer basal segment of the right lobe of the lung were observed by plain chest scan and lung high-resolution thin layer scan, indicating the possibility of inflammatory nodules. The peripheral oxygen saturation (SpO2) was 88% by pulse oximetry. Two BGAs were performed at different times, and the following parameters were not measured by the blood gas analyzer: oxyhb (HbO2), deoxyHb (HHb), carboxyHb (COHb), and metHb (MetHb). In addition, because it is calculated from HHb and HbO2 values, SaO2 was also not measurable by the device (Table 2). Due to the failure to detect these parameters by BGA, it was necessary to determine whether the patient had hemoglobinopathy. Interestingly, the patient's mother, aunt, and grandfather were all found to have low oxygen saturation, and the percutaneous oxygen saturation was mostly maintained between 85% and 89% for more than 3 months. Hence, additional analyses were conducted to define the biological characteristics more clearly.

High-performance liquid chromatography (HPLC) was conducted to determine whether there was a hemoglobin variant. An unknown Hb fraction was found with a retention time of 28 minutes at a proportion of 29.63%, contrasting with a normal Hb pattern (Figure 1). These data were confirmed by capillary electrophoresis, showing a slow elution of the Hb variant at a concentration of 20% with 76.5% HbA and 2.4% HbA2, in contrast with a patient with normal Hb analyzed by capillary electrophoresis showing the relative levels of HbA (76.5%) and HbA2 (2.4%) (Figure 1). The diagnosis of hemoglobin Titusville was confirmed through gene sequencing studies, which revealed that the patient was heterozygous for the alpha 1 globin gene mutation Cd 94 GAC>AAC; c.283G>A. We also screened other members of the family and found that her mother and aunt were carrying this Hb Titusville variant (Figure 2). The patient has provided informed consent for publication of the case. This is the first Chinese family case with this hemoglobinopathy to be reported.

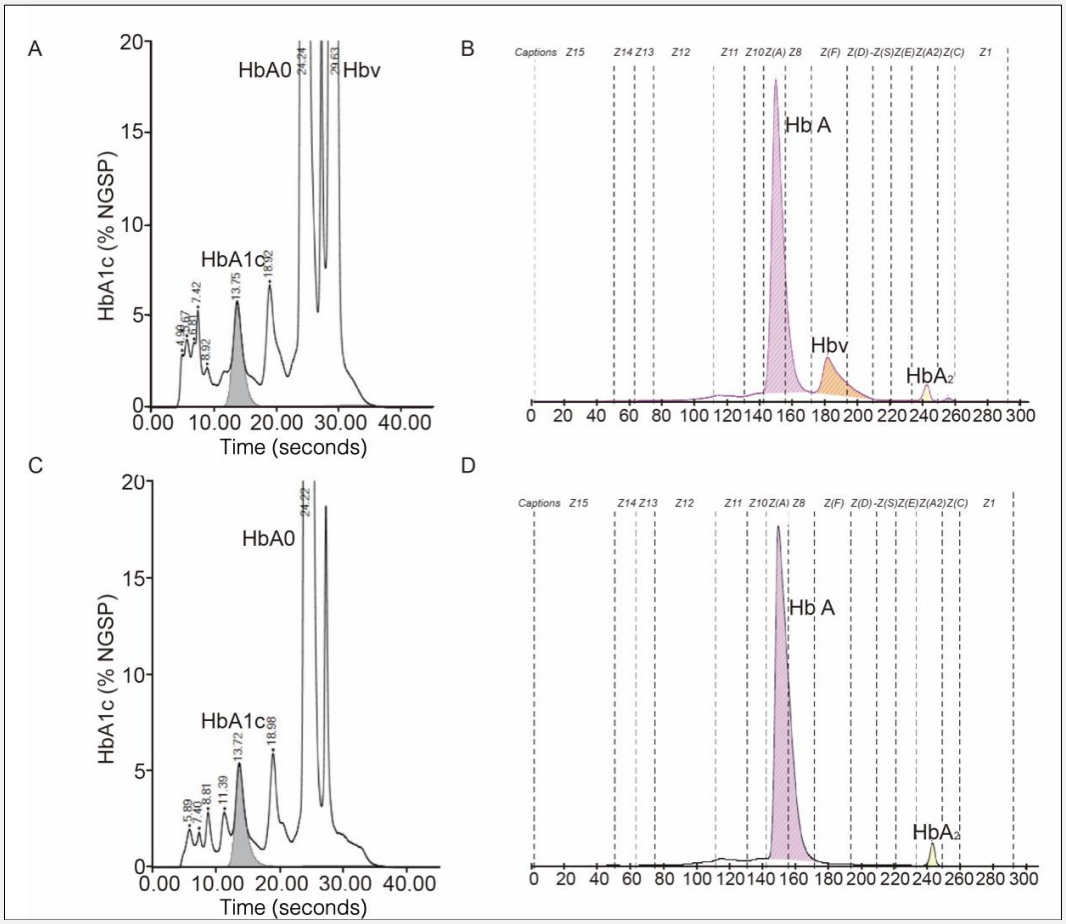

Figure 1. Hemoglobin analysis by HPLC and capillary electrophoresis.

(A) Hemoglobin analysis by HPLC showing the relative levels of HbA0: 24.24%, hemoglobin variant (Hbv) corresponding to Hb Titusville Hbv: 29.63%, HbA1c: 13.75%. (B) Hemoglobin analysis by capillary electrophoresis showing the relative levels of HbA: 81.9%, Hb Titusville Hbv: 15.3%, HbA2: 2.3%. (C) Normal hemoglobin analysis by HPLC showing the relative levels of HbA0: 24.22%, HbA1c: 13.72%. (D) Normal hemoglobin analysis by capillary electrophoresis showing the relative levels of HbA: 97.4%, HbA2: 2.6%. HPLC, high performance liquid chromatography.

DISCUSSION

Hemoglobin (Hb) Titusville is a rare variant caused by a single nucleotide change from G to A at codon 94 of the α-globin gene, resulting in the substitution of aspartic acid with asparagine [3]. This variant was first identified in 1975 during a voluntary screening program in Alabama, in which a 3-year-old healthy African American girl was found to have this rare hemoglobin variant [4]. According to the literature, only 22 cases of hemoglobin Titusville have been reported since 1975 from 13 families (including this case). Of these, 14 females and 7 males were confirmed, with one case of unknown gender. Among the recorded cases, the percentage of variant hemoglobin ranged from as low as 6% to as high as 34.7%, but most were approximately 17%, and the hemoglobin levels in patients were generally normal. Peripheral oxygen saturation (SpO2), measured by pulse oximetry, was low in all reported cases of hemoglobin Titusville. Reported SpO2 values were consistently low, ranging from 73% to 90%, with the majority between 80% and 90% [5], which is consistent with our case. Studies have found that this mutation results in low oxygen affinity [6]. Unexpectedly, this reduced oxygen affinity did not cause symptoms such as cyanosis, anemia, or respiratory distress in the affected patients. On the other hand, other mutations with similarly reduced oxygen affinity, such as Kansas, Beth Israel, and Saint Mande, have been shown to cause pronounced cyanosis in clinical cases [7-9]. The residue at position 102 of the

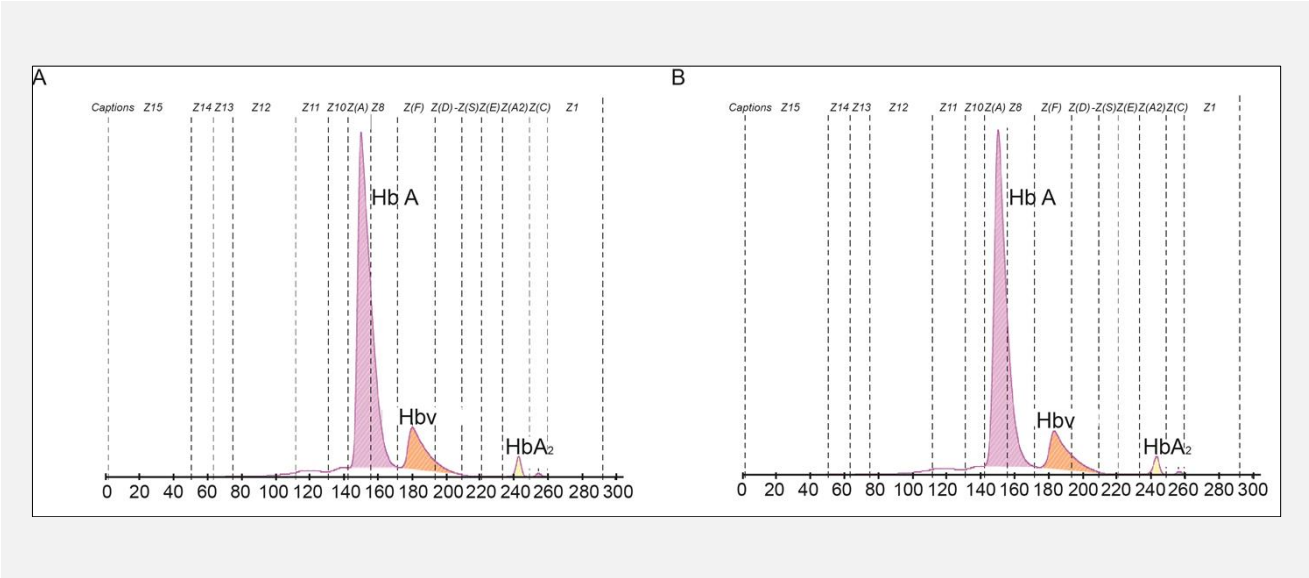

Figure 2. Hemoglobin analysis by capillary electrophoresis of the patient's mother and aunt.

(A) Hemoglobin analysis of the patient's mother by capillary electrophoresis showing the relative levels of HbA: 83.7%, Hb Titusville Hbv: 13.8%, HbA2: 2.1%. (B) Hemoglobin analysis of the patient's aunt by capillary electrophoresis showing the relative levels of HbA: 83.3%, Hb Titusville Hbv: 14.0%, HbA2: 2.3%.

β-globin chain is an asparagine, which participates in the only hydrogen bond crossing the α1β2 interface in oxygenated hemoglobin. Mutation of this residue results in decreased oxygen affinity. The hemoglobin mutations known as Kansas, Beth Israel, and Saint Mande all involve mutations of asparagine at position 102 of the β-globin chain. The mutation in hemoglobin Titusville still allows the formation of a hydrogen bond in the oxygenated structure. This may be the reason for the decreased affinity caused by the mutation that does not lead to clinically significant cyanosis [10].

There are many variables at the tissue and cellular levels that can contribute to hypoxia (such as temperature, pH, and tissue blood flow). Therefore, there is no consensus on what constitutes a normal or abnormal blood oxygen measurement. However, experts generally consider resting oxygen saturation ≤ 95% at sea level as abnormal [11]. Typically, when SpO2 is below 90%, patients may experience symptoms such as palpitations, rapid heartbeat, shortness of breath, and in severe cases, cyanosis. In the family in this case report, all three individuals consistently had SpO2 levels of approximately 88% and did not exhibit signs of respiratory distress or other symptoms of hypoxia. Therefore, is this low SpO2 value accurate? Clearly, the answer is no. Research has found that the mutation in hemoglobin Titusville causes a right shift in the oxyhemoglobin dissociation curve, which means that hemoglobin has a decreased affinity for oxygen and an increased ability to release oxygen [12]. Pulse oximetry measures the proportion of oxygenated hemoglobin in peripheral arterial blood using spectrophotometry, specifically calculating the ratio of absorbance between oxygenated hemoglobin and the sum of oxygenated and deoxygenated hemoglobin. The decreased oxygen affinity of hemoglobin Titusville results in early offloading of oxygen in the tissues, leading to a falsely low SpO2 reading. In other words, the low SpO2 value is spurious. As shown in other reported cases of hemoglobin Titusville, normal SaO2 values further support the notion of a falsely low SpO2. The partial pressure of oxygen (PaO2) refers to the pressure exerted by oxygen molecules in the blood at standard atmospheric pressure. When PaO2 increases, the ability of hemoglobin to bind with oxygen also increases, leading to an increase in oxygen saturation. Conversely, when PaO2 decreases, oxygen saturation also decreases accordingly. The patient's PaO2 fell within the normal range, which further confirms the falsely low SpO2. Interestingly, in both the French cases and the current case, SaO2 could not be measured, while in the other reported cases, SaO2 values were measurable and within the normal range. Of these reported cases, only the French cases and the current case showed the presence of hemoglobin Titusville bands in the F region and behind the A2 region, with broadening of the baseline in the abnormal bands in the F region, while the other cases exhibited bands in the S region. This may be related to the inability to measure SaO2, which is also a direction for our future research.

This case occurred during the peak period of an influenza A (H1N1) outbreak, and the patient was admitted to the hospital for the treatment of severe H1N1 due to the

risk of low SpO2 levels. According to reports, the hospitalization rate for H1N1 in children aged 5 - 17 is as low as 10 - 24 cases per 100,000 individuals [13]. Quickly debunking the false indication of low SpO2 levels would also provide some relief for the patient's family.

In conclusion, hemoglobin Titusville is a rare genetic hemoglobin structural defect, reported here for the first time in a Chinese patient. When encountering a low SpO2 or even the inability to detect SaO2 in clinical settings without any symptoms of hypoxia in patients, it may be worth considering a hemoglobin-related disorder. Early diagnosis can help patients and clinicians avoid unnecessary anxiety and costly or excessive clinical investigations.

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Declaration of Interest:
The authors declare that they have no conflict of interests.

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