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CASE REPORT

A Case of Coagulation Dysfunction Caused by Antithrombin Pittsburgh Mutation

Aosen Dong, Xiao Zhou

Department of Thoracic Surgery, Second Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China

SUMMARY

Background: **In clinical practice, coagulation dysfunction resulting from the antithrombin Pittsburgh mutation is rare and prone to misdiagnosis.**
Methods: **Coagulation time was measured using coagulation methods, and coagulation factor activity was measured using chromogenic substrate methods. Platelet aggregation function was assessed by light transmission aggregometry (LTA), serum protein was determined through capillary electrophoresis, and mutations were detected by whole-exome high-throughput sequencing.**
Results: **A 16-year-old female patient had significantly prolonged activated partial thromboplastin time (APTT) and thrombin time (TT). Moreover, the activity of coagulation factors VIII and IX was markedly decreased. Normal human plasma failed to correct the APTT, and the lupus anticoagulant screening test was normal. The coagulation factor VIII inhibitor was 0.10 BU/mL, while the factor IX inhibitor was 1.60 BU/mL. Protamine could not correct the TT. The mother had multiple postoperative bleedings and died due to massive postpartum hemorrhage. Through whole-exome sequencing for genetic testing of hereditary diseases, an antithrombin Pittsburgh mutation associated with the SERPINA1 gene was revealed.**
Conclusions: **Patients with the antithrombin Pittsburgh mutation show significant coagulation abnormalities, with their clinical bleeding manifestations varying considerably. Bleeding from trauma or surgery may be a prominent feature in these patients.**
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Correspondence:
Dr. Xiao Zhou
Department of Thoracic Surgery
Second Affiliated Hospital of Anhui Medical University
No. 678 Furong Road
Hefei, Anhui 230601
China
Email: dongyixx@126.com

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KEYWORDS

partial thromboplastin time, thrombin time, antithrombin Pittsburgh, mutation, SERPINA1 gene

INTRODUCTION

Coagulation disorders are caused by various abnormalities in one or more aspects of the coagulation process, such as congenital or acquired deficiencies in coagulation factors, damage to the vascular wall, reduced or dysfunctional platelets, deficiencies or increases in anticoagulant substances, and excessive activation of the fibrinolytic system [1]. Physiological anticoagulants can be categorized into serine protease inhibitors, the protein C system, tissue factor pathway inhibitors, and heparin. These anticoagulants bind to the serine residues in the active centers of molecules like thrombin and coag-

ulation factors IXa, Xa, and XIIa, thus inhibiting their activity. Congenital deficiencies may highly predispose individuals to thrombosis. Although excessive physiological anticoagulants are rare, pathological increases in anticoagulants can lead to coagulation disorders [2]. Alpha-1 antitrypsin (α1-AT), a serine protease inhibitor encoded by the gene SERPINA1, can have functional changes due to mutations [3,4]. The α1-AT Pittsburgh mutation occurs when the thymine (T) at the 1,145th nucleotide of the encoding sequence mutates to guanine (G), causing the amino acid Met to mutate to Arg. This results in the loss of α1-AT activity and the manifestation of strong antithrombin activity, leading to a bleeding tendency [5]. The antithrombin Pittsburgh mutation that causes coagulation dysfunction is extremely rare. It has significant variability in clinical bleeding manifestations and is prone to misdiagnosis and missed diagnosis, thus requiring attention.

CASE PRESENTATION

The patient is a 16-year-old female. She was admitted to our hospital's Thoracic Surgery Department on March 9, 2023, due to a chest wall hematoma for 1 day following trauma. The patient complained of chest pain. Her menstrual cycle was regular, with a moderate flow, lasting four to five days every 30 days. Her last menstrual period was on February 28, 2023. The patient usually has no significant gum bleeding, nosebleeds, hair loss, or dry mouth. At the age of seven, she was examined at Peking Union Medical College Hospital. It was suggested that she had coagulation dysfunction (details unknown), and she denied a history of thrombosis. The patient's mother had a history of coagulation dysfunction. She had multiple postoperative bleedings and died 10 years ago due to massive postpartum hemorrhage. Physical examination: A mass, measuring 3 cm x 4 cm x 6 cm in size, was visible on the chest wall. It had bruising and was tender to the touch. There were no joint deformities and no rampant dental caries. Chest X-ray examination showed no fractures. Auxiliary examinations: Liver and kidney function were normal. Serum protein electrophoresis was normal and anti-cardiolipin antibody test was negative. The autoimmune antibody profile was normal. Blood routine examinations: White blood cell count was 9.7 x 10^9/L, hemoglobin concentration was 108 g/L, and platelet count was 199 x 10^9/L. Platelet aggregation induced by adenosine diphosphate (ADP), collagen, and ristocetin was normal (ranging from 80% to 90%). Coagulation function report: Prothrombin time (PT) was 20.7 seconds, with an international normalized ratio (INR) of 1.88. Activated partial thromboplastin time (APTT) was greater than 180 seconds. Thrombin time (TT) was greater than 240 seconds. Fibrinogen (FIB) was 4.89 g/L. D-dimer was 0.25 μg/mL. Fibrin degradation products (FDP) was 3.35 μg/mL. Immediate hemostasis and plasma transfusion treatment were carried out. However, follow-up tests showed no improvement, with PT at 19.4 seconds, INR at 1.67, APTT still greater than 180 seconds, and TT greater than 240 seconds. Subsequent tests for coagulation factors showed that the activities of factors II, V, VII, VIII, IX, and X were 43.0%, 56.0%, 46.0%, 3.0%, 4.00%, and 12.0%, respectively. Continued plasma transfusion along with factor VIII treatment was then administered. Follow-up tests after this showed that there was still no improvement, with PT at 20.1 seconds, INR at 1.87, APTT greater than 180 seconds, and TT greater than 240 seconds. Normal human plasma APTT correction test was performed, which showed an APTT greater than 180 seconds, failing to correct the APTT. This suggests the possible presence of lupus anticoagulant (LA), coagulation factor antibodies, or heparin/heparin-like substances. A diluted Russell's Viper Venom Time (dRVVT) test for LA screening was performed with a result of 35.0 seconds, and the confirmation test was 32.0 seconds, with a normalized ratio of 1.09. Coagulation factor VIII inhibitor was measured at 0.10 BU/mL, and coagulation factor IX inhibitor was measured at 1.60 BU/mL. The addition of protamine immediately led to an APTT of 25.6 seconds and a TT > 180 seconds. After a 2-hour incubation, the APTT was 26.0 seconds and the TT remained > 180 seconds, suggesting the presence of other anticoagulant substances in the plasma. Both protein C and protein S activities were measured as 0. Given the patient's mother's history of coagulation disorders, whole-exome sequencing for genetic testing was carried out. The result revealed an antithrombin Pittsburgh mutation related to the SERPINA1 gene, which caused an autosomal-dominant-inherited bleeding tendency. This mutation was a heterozygous missense mutation (c.1145T>G (p.Met 382Arg)), as presented in Table 1. The mutation site was located in the 5th exon of the SERPINA1 gene, where the 382nd base of the coding sequence on one of the double-stranded DNA chains changed from thymine (T) to guanine (G), causing an amino acid change. The patient was ultimately diagnosed with α1-antitrypsin Pittsburgh mutation. In terms of treatment, compression bandaging, immobilization, plasma transfusion, and factor VII were administered, and the chest hemorrhage gradually subsided over the course of a week.

DISCUSSION

The hemostatic system consists of platelets, the vascular wall, and coagulation factors. When the vascular wall is injured, the subendothelial matrix is exposed. This exposure enables the subendothelial matrix to bind to various receptors on the platelet surface, thus initiating platelet adhesion. Subsequently, platelets are activated and aggregated, which leads to the formation of a platelet plug. Concurrently, the activation of the coagulation cascade leads to the production of thrombin. Thrombin cleaves fibrinogen into insoluble fibrin. The fibrin then forms a reticulated network, which significantly

Table 1. Genetic disease whole-exome sequencing.

Testing Item	Whole-exome sequencing for hereditary diseases				
Method	High-throughput sequencing of the whole exome				
Gene	Chromosome location	Genotyping	Site information	Variant classification	Related disease
SERPINA1	Chr1: 94844898	heterozygous	NM_000295: exon5: c.1145T>G (p.Met382Arg)	pathogenic	antithrombin Pittsburgh mutation causing bleeding
Conclusion	a heterozygous variation c.1145T>G (p.Met382Arg) in the *SERPINA1* gene is associated with the antithrombin Pittsburgh mutation, which leads to a bleeding tendency inherited in an autosomal dominant pattern				

strengthens the platelet plug and creates a thrombus for hemostasis. Any quantitative or functional changes in these components may result in bleeding. The most common causes of bleeding disorders are thrombocytopenia or platelet dysfunction and deficiencies in coagulation factors.

Serine protease inhibitors (serpins) play a role in regulating multiple physiological processes such as coagulation, inflammation, immune response, and cell migration through inhibiting serine proteases [6]. In the human body, 37 serpins have been identified up to now, such as antithrombin (Serpin C1), α1-antitrypsin (Serpin A1), and plasminogen activator inhibitor-1 (PAI-1 or Serpin E1). Dysfunction of serpins can result in various conditions. For instance, α1-antitrypsin deficiency can lead to uncontrolled elastase activity, which in turn causes lung tissue damage and emphysema. Also, mutations in antithrombin can easily lead to coagulation dysfunction [7,8]. α1-antitrypsin is the most important protease inhibitor in human plasma. It is primarily synthesized by hepatocytes, with smaller amounts produced by monocytes, macrophages, pancreatic cells, alveolar cells, intestinal epithelial cells, and endothelial cells [9]. It accounts for over 90% of the total protease inhibitory capacity in plasma. The normal plasma concentration of α1-antitrypsin ranges from 100 to 200 mg/dL, and this level can increase 3 to 5 times during systemic infection or inflammation, reflecting its crucial role in maintaining homeostasis [10].

The α1-AT Pittsburgh mutation is extremely rare. Studies have shown that this mutation can enhance antithrombin activity by 1,000 times and inhibit coagulation factors X, XI, and XII. Its anticoagulant effect is similar to that of antithrombin, and the relatively active mutation site is also consistent with that of antithrombin. When the SERPINA1 gene, which regulates this site, mutates, and the resulting mutated protein persists in the body. Under the stress of trauma or surgery, the body's coagulation mechanism will be disrupted, resulting in significant coagulation abnormalities. The clinical manifestations of bleeding vary widely, with severe cases potentially resulting in life-threatening hemorrhages. In some patients, plasma may show strong thrombin inhibition along with mild bleeding tendency, due to the potent inhibitory effects on tissue-type plasminogen activator and plasmin [11]. The α1-AT Pittsburgh is a powerful broad-spectrum enzyme inhibitor, which also blocks thrombin and activated protein C. As a result, patients with this mutation exhibit minimal bleeding and have lower protein C activity, due to the mutation's 4,400-fold increase in the ability of the mutated α1-AT protein to bind and clear protein C. This leads to a relative deficiency in protein C anticoagulant activity and a reduction in bleeding tendency. It is therefore suggested that the heterogeneity in bleeding manifestations among patients is mainly attributed to different plasma protein C levels [12]. Henneuse reported a female pediatric patient who only showed a hematoma after a single trauma [11]. Owen and colleagues [5] reported a case of a patient who had experienced recurrent major bleeding more than 50 times since childhood and died of abdominal hemorrhage at 14 years old. Luo [13] reviewed the treatment of a young patient over the past decade. This patient repeatedly had life-threatening hematomas, underwent five emergency surgeries, and had her ovaries removed in her twenties to avoid ovulatory bleeding. α1-AT is an acute-phase reactant protein. Its concentration significantly increases in response to inflammation and trauma. In women, it rises during ovulation and peaks during the luteal phase. This may increase the risk of repeated corpus luteum ruptures in female patients with the α1-AT Pittsburgh mutation [14].

The clinical features of this disease are as follows: After trauma, there is a chest wall hematoma. The APTT and TT are significantly prolonged, and the activities of endogenous coagulation factors VIII, IX, and X are reduced, which suggests a deficiency in coagulation factors or the presence of coagulation factor inhibitors. However, this cannot be corrected by normal plasma. The possibilities of lupus anticoagulants, coagulation factor antibodies, or heparin/heparin-like substances are considered. The lupus anticoagulant confirmation tests are normal, and no coagulation factor inhibitors are detected. Moreover, the addition of protamine corrects the

APTT but not the TT. The patient had normal fibrinogen levels, no history of fibrinolysis, and it is believed that other anticoagulant substances were present in the plasma. Given the patient's mother's history of coagulation disorders, the possibility of a hereditary disease was evaluated. Subsequently, genetic testing with whole-exome sequencing was carried out. The results showed that the patient had a mutation in the SERPINA1 gene. Specifically, at the 382nd base of the 5th exon, thymine (T) changed to guanine (G), which led to the mutation of methionine (Met) at position 382 to arginine (Arg). The mutated protein is the same despite the mutation site at position 382 being inconsistent with the internationally reported position 358, which leads to the diagnosis of α1-AT Pittsburgh mutation [15]. The disease is inherited in an autosomal dominant pattern and is not affected by gender [16].

In summary, patients with the α1-AT Pittsburgh mutation are extremely rare clinically, and protein C levels play an important role in maintaining the body's coagulation balance. There is a significant variability in clinical bleeding manifestations, which can easily lead to misdiagnosis and missed diagnosis. For women who frequently experience hematoma formation, especially with corpus luteum rupture, and who exhibit significant coagulation abnormalities, α1-AT mutation testing should be conducted to establish a definitive diagnosis. Currently, treatment options for patients with α1-AT Pittsburgh mutation are limited, and efforts should be made to avoid trauma and invasive procedures. During acute bleeding episodes, plasma transfusion and recombinant human coagulation factor VIIa may be administered [16].

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Statement of Ethics:
This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki and approved by the Institutional Review Board Institutional of the Affiliated Second Hospital of Anhui Medical University. The patient enrolled in the study signed an informed consent to participate in and to publish this case.

Declaration of Interest:
The authors have no relevant conflicts of interest.

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