Topical application of hemostatic paste

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Abstract

As a measure to control minor surgical bleeding, surgeons usually depend on a number of hemostatic aids. Topical use of bovine thrombin is a widely used procedure to arrest such minor bleeding. A 35 year old male sergeant of Bangladesh Air Force presented with repeated development of hematoma in his left thigh without any history of trauma or previous history of bleeding. Critical analysis of the patient’s history, routine and sophisticated hematological investigations revealed that the patient developed anti-thrombin antibody following the application of hemostatic paste in the tooth socket five years back during minor dental procedure to stop ignorable bleeding episodes. Therefore, topical use of hemostatic glue/paste or bovine thrombin should be avoided to desist minor bleeding as recombinant human thrombin is now available for topical use.

Introduction

Topical application of hemostatic paste or glue is a useful procedure to arrest minute surgical bleeding commonly encountered in different surgical patients. Topical thrombin preparations have been used as a hemostatic agent during cardiovascular surgery for years and may be applied as a spray, a paste, or a component of fibrin glue. Currently bovine plasma is used for preparing all single-component thrombin agents. Topical thrombin preparation has a very senescence and extended history as a chariot to influence hemostasis.

Case Report

A 35 year old man serving sergeant of Bangladesh Air Force, got admitted in February, 2015 into a tertiary care Military Hospital with a spontaneously developed large painful swelling of the left thigh with bluish black discoloration of the overlying skin. The sergeant was born of non-consanguineous marriage and he also had non-consanguineous marriage with one healthy child and wife. There was neither maternal nor paternal family history of bleeding disorders. The hematoma required surgical drainage and after drainage, the hematoma did not resolve rather developed oozing of blood from the wound. Patient was investigated on several occasions during the stay in hospital with full blood count, coagulation profile (prothrombin time, activated partial thromboplastin time [APTT]) as well as factor VIII (FVIII) and factor IX (FIX) assay. The results of all investigations revealed prolonged APTT and mild to moderately reduced level of FVIII and FIX.

After evaluating the history, physical findings and laboratory results, the patient was diagnosed as combined FVIII and FIX deficiency and treated with transfusion of blood and blood products such as packed red blood cell, fresh frozen plasma and injection rFVIII. After this treatment he responded reasonably well and discharged from the hospital in October, 2015 and recommended for monthly follow-up.

Again he developed hematoma on the left thigh extending to left knee in November, 2015. He also gave the history of an episode of ecchymoses on both arms which resolved spontaneously. At this time again he was evaluated with all previous investigations and the results were same as before. So, the patient was treated with multiple whole blood transfusions, packed red cell, fresh frozen plasma and injection FVIII. At this time, on further interrogation the patient gave the history of minor bleeding from the tooth socket following trivial trauma in 2010 for which he consulted with a dental surgeon who applied a paste which stopped the bleeding.

Because of repeated development of such bleeding manifestations including hematoma and abnormal coagulation tests result, he was recommended for going abroad for further evaluation and management.
The patient then travelled to Christian Medical College, Vellore, India on 8th January, 2016. After evaluating all investigations already done in Bangladesh, the concerned physician advised him to do D-Dimer test which was very high (2362 ng/mL [reference range: <250 ng/mL]). Since high level of fibrin degradation products affected his plasma based coagulation tests, he was asked to come after one month. On 23rd February, 2016, patient again reported at the Christian Medical College, Vellore and thoroughly investigated for coagulation disorders. His full blood count was normal except neutrophil leucocytosis. Bleeding time, prothrombin time and platelet functional assay by PFA-200 for aggregation study with collagen/ADP and collagen/adrenaline were within normal limits. But patient was found to have prolonged APTT (107.4 sec) and prolonged thrombin time (28.6 sec). As APTT and thrombin time were prolonged, so 50:50 correction studies of APTT and thrombin time were performed and it revealed still prolonged APTT (97.6 sec) as well as prolonged thrombin time (24.0 sec). During this time his D-Dimer was 855 ng/mL. One stage clot (APTT and prothrombin time) based factor assays of factor VIII:C, IX:C, XI, VII, II, V, X and vWF:RCO were done and revealed significantly reduced level of FVIII (3.5% [reference range: 50-150%]), FIX (<1.0% [reference range: 50-150%]) and FXI (1.0% [reference range: 50-150%]) but levels of FII, FV, FVII and FX were normal. As reduced level of multiple factors was found, so inhibitor (Bethesda) assays against FVIII, FIX, FXI, lupus (LA) inhibitor screen and lupus inhibitor confirm were done and identified significantly high level of inhibitor against FVIII (73.6 BU), FIX (53.4 BU), FXI (48.0 BU), lupus inhibitor screen 93.2 sec (reference range: 28.3-38.3 sec), screen mix 66.7 sec, lupus inhibitor confirm 52.1 sec (reference range: 27.2-33.6 sec), confirm mix 48.1 sec and screen : confirm ratio 1.78 which indicated moderately positive lupus anticoagulant.

In view of a high titer with still measurable FVIII (3.5%) it could have been an inhibitory effect not specific to FVIII but to another factor/factors in the APTT pathway. Bethesda unit against FIX and FXI were also too high and LA was also positive. So, this could not have the inhibitory effect against FVIII, FIX and FXI since the patient presented with bleeding and his prothrombin time and platelet count were normal. Moreover, normal level of FVIII (107%) was found by chromogenic assay and the prolonged thrombin time not correcting with mixing studies indicated that an inhibitor at a lower level in the coagulation cascade caused the false positive Bethesda assay.

Reduced level of fibrinogen (68.9 mg/dL) on Von Clauss assay with borderline normal level of fibrinogen antigen (142.1%) was found. Fibrinogen assay was repeated using parallelism plot due to above phenomenon and this too revealed the inhibitory level on parallelism as well as Bethesda assay for fibrinogen was also done which showed negative result.

Under such circumstances, rotational thromboelastometry (ROTEM) and thrombin generation time were done and reflected dampening of thrombin which are also observed in other plasma coagulation tests such as APTT, thrombin time and dilute Russell viper venom time (DRVVT) but fibrin clot formation was rapid during prothrombin time. Dampening of thrombin generation on thrombin generation time suggested that the antibodies developed in this patient were directed against thrombin not prothrombin.

A serial dilution of patient plasma was mixed with control plasma and thrombin time was performed. At a dilution of 1:1024, there was correction of prolonged thrombin time which indicates the antibodies could be against thrombin.

To confirm the nature and effect of the antibody against thrombin, FXIII (which is dependent on activation by thrombin) assay done and compared with FXIII antigen. The assay indicated significantly reduced activity of FXIII (29.3% [reference range: 70-150%) compared to FXIII antigen (85.4% [reference range: 75.2-154.2%]) reflecting inhibition of FXIII activation by thrombin.

Coagulation screening tests such as prothrombin time, APTT, thrombin time, FVIII and FIX assay were performed for the parents and the only sibling (daughter). All test results were within the reference range.

**Discussion**

Fibrin glue is composed of thrombin and fibrinogen. Fibrinogen is cleaved to fibrin monomer by thrombin. When applied to the tissues, this forms adhesive glue at the tissues. The fibrin monomers then combine with patient’s own FXIII and calcium to convert the final product fibrin polymer platelet activation and aggregation with subsequent hemo-

In the past normal patients treated with topical bovine thrombin preparations may automatically develop autoantibodies and hemorrhagic as well as thromboembolic complications may develop in such patients or asymptomatic laboratory abnorm-
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The incidence of raised antibody levels to bovine or human coagulation proteins is similar to the occurrence of antiphospholipid antibodies (4-8%) and spontaneous autoantibodies to FVIII (17%) in normal donors. To delimitate the chance of antibody formation following exposure to bovine thrombin, Ortel’s
prospective study in 2001 among 151 patients underwent cardiac surgery revealed that more than 95% of patients developed a seropositive response to bovine coagulation protein and 51% manifested elevated antibody levels to the corresponding coagulation proteins after bovine thrombin exposure. Another review by Streiff documented that after exposure to bovine thrombin, out of 126 patients, 58 (46%) patients spontaneously developed bovine–thrombin induced autoantibody and 33% of these patients experienced some sort of bleeding complication.

The patient, in this case report, developed autoantibody to thrombin after five years of exposure to bovine thrombin applied to the tooth socket for managing the minor bleeding episode.

**Conclusion**

Patients with late-onset bleeding episodes without any family history of bleeding disorder should be critically evaluated before landing to a diagnosis of congenital multiple factors deficiency as multiple coagulation factors deficiency in the same patient are very rare.

**References**