HAEMOPHILIA

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Abstrak: Hemofilia adalah kelainan perdarahan kongenital yang disebabkan oleh kekurangan faktor VIII (faktor antihemofilik) yang terkait dengan Hemofilia A, atau faktor IX (faktor Christmas) yang terkait dengan Hemofilia B. Kedua hemophilia diturunkan secara X-linked resesif, dan umumnya ditemukan pada laki-laki. Kami melaporkan kasus seorang anak berusia 4 tahun dengan riwayat memar, pendarahan berlebihan, disertai pembengkakan sendi yang nyeri dan hematoma otot, yang dicurigai mengidap hemofilia. Serial tes koagulasi dilakukan dengan hasil: jumlah trombosit, waktu perdarahan, *prothrombin time* (PT), *thrombin clotting time* (TCT), dan fibrinogen normal, sedangkan *activated partial thromboplastin time* (APTT) memanjang. *Mixing studies* dikoreksi ketika plasma normal dan *adsorbed plasma* ditambahkan ke plasma pasien, yang menunjukkan defisiensi faktor VIII merupakan penyebab hemofilia ini. Aktivitas faktor VIII 8% menegaskan suatu hemofilia A derajat ringan.
Kata kunci: hemofilia, PT, APTT, *mixing studies*, faktor VIII.

Abstract: Haemophilia is a congenital bleeding disorder caused by a deficiency of factor VIII (antihaemophilic factor), which is related to haemophilia A, or factor IX (Christmast factor), associated with haemophilia B. Both X-linked are recessive, and males are affected mostly. In this case, a four year old boy, who had a history of excessive bruising and bleeding, also suffered from painful swelling of joints and muscle hematoma. He was diagnosed of suspected haemophilia. A serial test of coagulation studies was performed. The results of platelet count, skin bleeding time, prothrombin time, thrombin clotting time, and fibrinogen were normal; whereas, the activated partial thromboplastin time was prolonged. The mixing studies were corrected when normal plasma and adsorbed plasma were added to the patient plasma, suggesting that the factor VIII deficiency was the cause of this haemophilia. The factor VIII activity was 8% which confirmed the evidence of mild haemophilia A.
Keywords: haemophilia, PT, APTT, mixing studies, factor VIII.

Haemostasis disorders that present abnormal bleeding can be caused by defects of vasculature, platelets, or coagulation factors. The probability to have a haemostatic defect can be explored firstly through the patient's medical history and physical examination by a physician. Haemorrhaging from the skin and/or mucous membranes is susceptible to vascular or platelet disorders, while bleeding in joints or muscles is linked to coagulation disorders. In addition, the time in primary haemostasis diseases starts immediately after trauma or occurs spontaneously. In contrast, secondary haemostasis develops late.[1]

If a child around 4-5 years of age, has a history of excessive bruising or bleeding, or presents a painful swelling of joints or muscle hematoma, then, a diagnosis of haemophilia might be suspected.[2] Haemophilia is a congenital bleeding disorder caused by a deficiency in factor coagulations which includes factor VIII (anti-haemophilic factor), which is related to haemophilia A, and factor IX (Christmas factor), that is associated with haemophilia

B. As both Haemophilia A and B are recessive inherited forms, which linked to chromosome X, males are affected mostly.[3]

However, laboratory investigation is also important in considering the most important factor of the haemostatic system that influences the development of the disease. As to be classified whether the disorder involves the vasculature, the platelet function, or the blood protein coagulation, or even a combination, screening tests of haemostasis should be performed. These tests include full blood examination, a prothrombin time (PT), an activated partial thromboplastin time (APTT), mixing studies, and fibrinogen estimation. The PT measures factors VII, V, X, II and I, while APTT evaluates factors XII, XI, IX, and VIII besides factors X, V, II, and I.[4]

When a screening test (PT or APTT) is prolonged in the absence of anticoagulant therapy, mixing experiments may be useful to identify more specifically the cause of abnormal time. If the prolonged time occurs because of a factor deficiency, the addition of normal plasma containing all coagulation factors should correct the screening test to a normal or near normal time. If correction does not occur, the prolonged result is most likely due to a circulating anticoagulant or inhibitor. If factor deficiency is suspected, the test can be made more specific by mixing the test plasma with aluminium hydroxide adsorbed plasma (containing factor I, V, VIII, XI, and XII), and with normal serum (containing factor VII, IX, X, XI, and XII). The resulting mixtures are then retested and a correction indicates a deficiency in the test plasma of one or more of the factors present in the agent which was added in the test.

CASE REPORT

An apparently healthy 4 year old boy was presented to his local general practitioner (GP) with a history of easily bruising and painful swelling of the knees. On examination the GP noted that he had large bruises on his arm and left thigh from when he had fallen from his bike 1 week ago. After questioning, the mother, the GP discovered that there was no history of bleeding abnormalities in the family. The GP still suspected a haemostatic defect could be present and requested laboratory investigations to confirm his diagnosis.

METHODS AND MATERIALS

Full blood examination (FBE) was determined by using Beckman Coulter $A^{C\cdot}T$ 5diff haematology analyser. Prothrombin Time (PT) using Thromborel S reagent principle: the coagulation process is triggered by incubation of plasma at 37ºC with Thromborel S which contains both thromboplastin (from human placenta) and calcium. The time taken to form a fibrin clot was measured. Duplicate tests were performed and the mean time was taken.

Activated partial thromboplastin time (APTT) used general diagnostics APTT reagent. The principle: the coagulation process is triggered by incubation of plasma at 37ºC with APTT reagent and calcium. The time taken to form a fibrin clot was measured. Duplicate tests were performed and the mean time was taken.

The Thrombin Clotting Time (TCT) principle: the test is essentially used to measure the conversion of fibrinogen to fibrin by the action of thrombin. A thrombin solution was added to the test tubes containing fresh citrated platelet-poor plasma (PPP). The tube was tilted regularly (5 tilts every 3 seconds) and the time taken for clot formation was recorded.

Mixing studies: the patient's plasma was mixed with normal plasma, adsorbed plasma, and normal serum in a 1:1 ratio dilution, and then APTT was analysed.

The fibrinogen test measured the clotting time of diluted plasma when excess thrombin was added. The clotting time obtained was then compared with that of a standardized fibrinogen preparation.

The factor VIII assay (one-stage method) was based on the APTT test. The principle: when factor VIII deficient plasma is added to dilutions of the standard

or test plasma, the APTT becomes a specific indicator of the level of factor VIII clotting activity since all the other factors are present in normal amounts.

Full blood examination (FBE): Hb = 120 g/L, MCV = 84 fL, MCH = 27.6 pg, MCHC = 338 g/L, RDW = 15.6%, WCC = 6.3 x 109/L + N 57%, L 32%, M 6%, E 4%, B 1%. PLT = 258 x 109/L. Skin Bleeding Time (SBT) = 5.5 min (RR: 2.0 – 9.5 min)

RESULTS

Coagulation screening tests

Table 1. PT, APTT, TCT and FIB results for control and patient obtained from manual and automation *(*MLA electra 1400C *automatic* coagulation analyzer.

Control and patient	**PT (sec)** Reference range: 11.0-15.0 seconds				**APTT (sec)** Reference range: 25.0-45.0 seconds				**TCT (sec)** Reference range: 14.0-16.0 seconds			**FIB (g/L)** Reference range: 1.5-4.0 g/L
	1	2	Average time	Automated PT	1	2	Average time	Automated PT	1	2	Average time	
Normal (control)	13	12	13.5	15.3	32	34	33	38.5	16	17	16.5	2.9
Patient	14	15	14.5	15.8	69	67	68	77.5	17	18	17.5	3.2

Mixing studies

Table 2. APTT results by performing mixing studies with normal plasma, adsorbed plasma, and normal serum.

Type of mixing study	**APTT (sec)** Reference range : 25.0 - 35.0 seconds		
	Time 1 (sec)	Time 2 (sec)	Average
Patient + Normal plasma	48	51	49.5 (corrected)
Patient + Adsorbed plasma	38	35	36.5 (corrected)
Patient + Normal serum	64	68	66 (not corrected)

Factor VIII activity

Table 3. Factor VIII activity (%) result obtained from automation.

Dilution	**Factor VIII Activity (%)**	**Clotting time (seconds)**	
		Normal (control)	**Patient**
1/10	100	120	175
1/20	50	125	190
1/40	25	137	220
1/80	12.5	155	246

DISCUSSION

Based on clinical features, the significant point is that a secondary haemostasis defect may cause the disease. The patient's complaint of easy bruising and painful swelling of the knees, and also large bruises

on the arm and thigh was detected on the examination. These supported the characteristics of secondary haemostasis which cause bleeding disorders, where the location of bleeding is joints or muscles (deep in soft tissues), the ecchymosis is large and deep, the haemarthrosis/muscular bleeding is commonly found and also the time of onset is delayed. Compared to primary haemostasis defects, the frequent sites of deformity are on the mucous membranes and skin, such as epistaxis, gingival bleeding, and vaginal or gastrointestinal tracts. The ecchymosis is small and superficial, the haemathrosis is extremely rare, and the time of onset is immediately after trauma or develops spontaneously.[5] So, a history of deep muscle and joint haemorrhages, as opposed to mucocutaneous, helps to differentiate disorders of coagulation system disorders from vascular/platelet disorders.

However, coagulation system disorders can be classified into acquired and congenital bleeding disorders. An acquired abnormality which usually manifests itself first in later life, is connected to other diseases and has no history of similar conditions in other family members. Conversely, inherited diseases are commonly present at birth or come into view in early childhood, for instance haemophilia and von Willebrand disease.[6] Hence, the history of this patient is very important in classifying the disease as an inherited rather than an acquired abnormality.

To differentiate the cause of bleeding between haemophilia and von Willebrand disease can be undertaken based on clinical findings and laboratory examinations. Haemophilia is an inherited X-linked recessive bleeding disorder caused by a deficiency in either factor VIII or factor IX, and deep muscle or joint haemorrhages dominate the clinical manifestations. On the other hand, the von Willebrand disease is a disorder due to the abnormality of the quantitative or structural of the von Willebrand factor, and mucocutaneous bleeding is the prominent manifestation.[6] In haemophilia, the platelet count, PT, thrombin time (TCT), and bleeding time (BT) are normal, but APTT is prolonged as long as the test system is sensitive to deficiencies at, or fewer than, the 30% plasma level. In the von Willebrand disease, the PT and TCT are normal, but APTT is variably prolonged, depending on the degree to which the factor VIII level is reduced. The BT is also prolonged, except in type 2N patients. The platelet count is usually normal, but mild thrombocytopenia may happen in patients with type 2B.[7]

In this coagulation screening test, even though the manual PT and APTT are slightly lower than the automatic one, both manual and automatic show normal PT and increased APTT (these complete the data which the PT, TCT, fibrinogen, and BT are within reference range). Many factors can be sources of variation that interfere with test results between manual and automation, for example, detection system, accuracy of pipette and timer, end point determination, reagent viability, temperature, and rate of tilting. However, the automation instrument has the coefficient of variation lower with the precision and accuracy higher than the manual one.

In brief, based on all medical history, physical examinations and laboratory test considerations, the bleeding disorder indicates that it could be haemophilia. However, further tests are recommended to confirm this.

As the APTT result was prolonged in this patient, the mixing studies were then carried out to clarify whether the aetiology of the prolongation is because of a coagulation factor deficiency or an inhibiting factor. In this test, the patient's plasma was mixed with normal plasma containing all coagulation factors, adsorbed plasma (containing factors I, V, VIII, XI and XII), and normal serum (containing factor VII, IX, X, XI, and XII) in a 1 : 1 ratio dilution, and then APTT was analysed. In assumption, if there is a factor deficiency, this deficiency could be corrected by the mixture, because the normal plasma or adsorbed plasma or normal serum supply the factor that is deficient in patient plasma,

thus, the prolonged time of the screening test will be normal or close to a normal time. In contrast, if there is present of inhibiting factor, the APTT of the mixture remains prolonged.[8] In this patient, adding normal plasma and adsorbed plasma to the patient's corrected APTT time, suggests it is factor VIII is most likely deficient in this haemophiliac case.

The subsequent step to prove a diagnosis of factor VIII deficiency is working on a factor VIII assay (one-stage method based on APTT system). The activity of each test plasma is determined as a percentage of the standard activity by reading the result from the graph. The reference range for factor VIII activity is 40-150%, while activity levels of 40% or less are evident in haemophilia A.[6] The disease is considered to be severe when factor levels are below 1% of normal values; moderate when they are between 1-5%; and mild when levels range between 5% and 40%[9]. This case shows a factor VIII activity was 8%, confirming mild haemophilia A.

However, to exclude haemophilia B and the von Willebrand disease, further tests are needed. Factor VIII activity can be measured specifically by clot-based or chromogenic substrate methodologies. Factor IX activity is measured by using clot detection assays. Von Willebrand factor antigen should be performed to distinguish haemophilia A from the von Willebrand and its variants.[10]

The goal of haemophilia treatment is to increase the patient's factor VIII activity to hemostatic levels whenever he experiences or suspects a bleeding episode or anticipates a hemostatic challenge such as a surgical procedure. Bleeding episodes are treated with factor VIII replacement, given as either factor VIII concentrate or cryoprecipitate. The target activity level depends on the nature of the bleeding, is seldom greater than 75%, and should be maintained until the threat is resolved. Bleeding is usually well controlled if factor VIII levels rise above 20% normal. In the case of hemarthrosis or another localized bleeding, the sooner the target factor level is reached, the less painful is the episode, and the less likely the patient is to experience side effects on inflammation, nerve compression, or anemia. Because factor VIII has a half-life of 8 to 12 hours, infusions are required at least twice per day[6].

CONCLUSION

We reported a case of haemophilia A in a four-year-old boy who had a history of excessive bruising and bleeding, and with painful swelling of joints and muscle hematoma. The diagnosis was based on his medical history, physical examinations, and laboratory test considerations.

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