Blood coagulation clotting process

Coagulopathies are group of bleeding disorders that affect blood clotting process in the body. Blood coagulation is important in human life and it is necessary to stop bleeding in the body. Blood coagulation factors are proteins that released along with blood proteins. Their role is to activate steps in the process called coagulation cascade(Brandt JT, 1985). These factors makes insoluble protein called Fibrin. Fibrin sticks at the site of cut with platelets to make stable blood clot. So this prevents RBCs to pass through. Once clot is formed, other blood coagulation proteins take place in the body(Thijs, 1993). They active other coagulation factors within the body and these help to arrest clotting, resulting in fibrinolysis(Brand, 1985). Thus clot is removed until injury is healed. Coagulation disorders lead to excessive or prolonged bleeding after an injury (Thijs, 1993). The aim of the practical was to assess clooting function by coagulation tests. Coagulations tests such as ThrombinTime (TT) , PT (Prothrombin Time) and Activated partial thromboplastin time (APTT) are performed to determine the defects associated with coagulation factors of the coagulation cascade(Fihn, 1995). These tests determine the quantities of coagulation factors, because these disorders are caused when one or more of coagulation factors are increased, low, or absent(Fihn, 1995). They are also aid in the diagnosis of bleeding disorders and monitor the patient’s blood clot status and effectiveness of anticoagulant therapy (Fihn, 1995). TT measures the time from conversion of fibrinogen to fibrin with thrombin. PT measures the time to create fibrin after activation of factor VII in the extrinsic/common pathways (Becker, 1993). APTT measures time to create fibrin from initiation of the intrinsic pathway (Gottfried, 1997).

Method:

Patient samples1-3 was mixed with saline and incubated at 37 o C for both TT and PT tests. In thrombin time, these samples were performed by adding known concentration of thrombin to the patient’s plasma. TT was timed after addition of thrombin into plasma until sample clots. In prothrombin time reagent thromboplastin with calcium was added to patient’s plasma . The PT was timed after addition of thromboplastin with Ca into plasma until sample clots. For Activated partial thromboplastin time, reagent APTT (cephalin) and second reagent calcium were added into patient’s plasma. APPT was timed after addition of reagents into plasma until sample clots. Then correction tests were performed with 50: 50 corrections on all samples with abnormal results. A mixture of normal plasma and abnormal plasma were made in a 50: 50 ratio. The abnormal test was then repeated using the mixture.

Results:

Table 1: Thrombin Time

Plasma sample

1

2

3

50: 50 plasma mixes

Time to clots in seconds

13

14

11

10

12

Table 2: Prothrombin Time

Plasma sample

1

2

3

50: 50 plasma mixes

Time to clots in seconds

23

10

19

30

18

22

11, 23(plasma1+plasma2)

19-21(plasma1+plasma3

Table 3: Activated partial thromboplastin Time

Plasma sample

1

2

3

50: 50 plasma mixes

Time to clots in seconds

25

36

27

40

23

60

30, 33(plasma1+plasma2

10, 47(plasma2+plasma3)

Patient 1 had normal TT, PT and APTT . Patient had adequate levels of coagulation factors in the extrinsic, intrinsic and common pathways. So patient had normal clotting function.

Patient 2 had normal TT and prolonged PT and APTT . PT was prolonged due to deficiency of the clotting factors such as VII, X, V, II, and I . PT was greater than 20 sec, indicated the abnormality in the patient. APTT was usually prolonged when patient had less than 30% levels of normal coagulation activity. Prolonged clotting times in PT and APTT , because of factor deficiency were corrected by addition of normal plasma to test plasma(50: 50 mix). Both Prolonged APTT could not be corrected with normal plasma and this was due to the presence of an inhibitor of coagulation . Factor assays were of no value in this condition and inhibitor needs to be tested . PT could be corrected with normal plasma(1+2). Prolonged APTT with normal PT was due to deficiency of intrinsic pathway factors such as VIII, IX, XI, XII . Possible causes could be Hemophilia, Christmas d, DIC or liver diseases.

Patient 3 had normal TT and prolonged PT and APTT. Patient had adequate levels of fibrinogen. Both Prolonged APTT and PT were due to deficiency of common pathways factors such as V, X fibrinogen, prothrombin or multiple factor deficiencies. Both prolonged PT and APTT were caused by liver disease, Vit K deficiency or DIC. Prolonged clotting times in PT and APTT , because of factor deficiency were corrected by addition of normal plasma to test plasma(50: 50 mix). Both APTT(2+3) and PT(1+2) could not be corrected

with normal plasma and the presence of an inhibitor of coagulation was suspected.

Discussion:

The distinction between the intrinsic and extrinsic pathways is important for understanding the clotting tests of the coagulation cascade(Brandt JT, 1985). All of these tests are used as a screening test for bleeding disorders and each have different applications. They detect the effectiveness of blood coagulation in the body. Thereby they help patients to understand the nature of clotting defects and aid in the diagnosis and monitor the anticoagulant treatment(Brandt, 1985). TT test is useful for screening acquired or inherited disorders of the coagulation. TT looks the process of thrombosis in the patient(Brandt JT, 1985). These disorders are due to qualitative or quantitative abnormalities of fibrinogen or inhibitors such as heparin and fibrinogen products(Brandt, 1985). Abnormal Fibrinogen can be acquired or inherited. Acquired fibrinogen is due to liver disease but hereditary is rare. A fibrinogenemia is fatal condition in the childhood. PT test is more sensitive than APTT to detect the effect of Vitamin K deficiency in patients with chronic liver disease . PT is also useful test for detection of hereditary or acquired factors such as VII , X, V, II, I in the extrinsic/common pathways (Brandt JT, 1985). It is also designed to screen inhibitor to specific factor and monitor anticoagulant therapy such as warfarin or Coumadin(Gottfried, 1997). International normalised ratio used in combination to monitor oral anticoagulant treatment (Hirsh , 1984). Inherited disorders are rare and involve only one factor that is low or deficient(Gottfried, 1997). Acquired deficiencies occur when more than one clotting factor is low or deficient and

caused by liver disease , cancer or disseminated intravascular coagulation (DIC) (Brandt, 1985). Also PT result can be maintained by a diet high in vitamin K, dark green vegetables, green tea, liver and soybeans. APTT test is inappropriate for a preoperative test due its limited sensitivity and specificity(Zakai , 2008). It is good test for screening inherited or acquired factor deficiencies in the intrinsic/common pathway(Zakai , 2008). Haemophilia A (factor VIII deficiency) and haemophilia B (factor IX) are common inherited disorders due to prolonged APTT with normal PT, but they are rare. Acquired factor deficiencies are common and include Vitamin K deficiency, liver disease or warfarin therapy . APTT acts as initial test , when family history detects factor deficiency or inhibitor (Tripodi, 2009). It is also useful for monitor the efficacy of heparin anticoagulant therap(Zakai , 2008). Lupus anticoagulant or heparin are common inhibitors. aPTT is usually performed in combination with prothrombin time to measure the factors of the extrinsic pathway (Gottfried, 1997). The combination of tests prevents possible missed or defective factors(Brandt, 1985).

There are other tests used to determine problems caused by bleeding disorders. Family or personal bleeding history may be useful for screening coagulation tests. Complete blood count is done to count platelets per ml of blood and this detects if there is low platelets. Bleeding time is useful test to measure abnormal platelet function(Mielke, 1984). Bleeding time higher than 15mins indicates the defect in the initial responses of vessels and platelets to vessel injury. It is prolonged in Thrombocytopenia or von Willebrand’s diseases (Mielke, 1984). The platelet aggregation test looks how well platelets clump together and leads to blood clotting. Decreased platelet

aggregation may be due to Von Willebrand’s disease , fibrin degradation products or myeloproliferative disorders . Partial Thromboplastin Time is an initial test to detect the effectiveness of heparin therapy. It partially detects clotting disorders in the intrinsic pathway(Brandt, 1985). Prolonged PTTs are due to acquired or inherited disorders (Proctor, 1961). International Normalized Ratio (INF) test is done after PT test to ensure the results of PT same . PT time divides the time of coagulation by using standard and this value is INF (Brandt JT, 1985). Fibrinolysis is also test to detect fibrinogen or fibrin degradation products in serum (Brandt, 1985). Factor activity assays such as II, V, VII, VIII, IX, X, XI, XII or von Wilberrand factor can test to detect abnormalities caused by bleeding disorders(Brandt, 1985).

As a result, the nature of the three tests assesses the bleeding disorders and aid in the diagnosis and treat patient with anticoagulant therapy.