Original Article

HEMOSTATIC AND THROMBOTIC PARAMETERS IN ACUTE LEUKEMIA– A COMPARISON OF PRE AND POST REMISSION INDUCTION PHASE

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ABSTRACT

This study was aimed to compare hemostatic, fibrinolytic and thrombotic parameters in pre and post induction chemotherapy in acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). A total of 110 diagnosed acute leukemia patients and 40 normal individuals were enrolled in the study. Questionnaire were filled and patients' blood specimens were collected before the commencement and post induction chemotherapy. Prothrombin time (PT), activated partial thromboplastin time (aPTT), von Willebrand factor antigen (vWF Ag), fibrinogen level, factor VIIIc (FVIIIc), D-Dimers, fibrinogen degradation products (FDPs), anti-thrombin III (AT), lupus anticoagulant (LA), protein C (PC), protein S and diluted russel viper venom test (DRVVT) were conducted in all the participants. A significant rise was identified in post-induction levels of PT, aPTT, vWF Ag, fibrinogen and factor VIII in acute leukemia patients. Analysis of fibrinolytic markers indicated increased D-Dimers and plasminogen levels while the levels of alpha 2 anti-plasmin were reduced in pretreated patients. Thrombotic markers' assessment showed increased levels of AT and LA while decreased level of PC in pretreated acute leukemia patients. It was concluded that remission induction chemotherapy, in acute leukemia patients, significantly affects the coagulation, fibrinolytic and thrombotic parameters

Key Words: Hemostasis, Thrombotic markers , Fibrinolysis, pre and post induction chemotherapy, Leukemia

INTRODUCTION

Impairment of hemostatic, fibrinolytic and thrombotic activities are frequently associated with hematological malignancies (1). Thrombosis was reported in patients with acute lymphoblastic leukemia on L-asparaginase therapy (2, 3). Thrombosis in these patients usually occurs as a result of disturbances in the anticoagulant and fibrinolytic systems (4). Hemostatic manifestations are not solely due to anticoagulant and fibrinolytic system disturbances; it is also associated with prolonged thrombocytopenia in these patients after the administration of chemotherapeutic drugs (5). It was noted that risk of bleeding was reduced in patients with acute leukemia receiving platelet transfusions prophylactically and therapeutically (6). Acute promyelocytic leukemia is associated with life threatening condition of disseminated intravascular coagulation. Coagulopathy in APL has been studied extensively but several reports on ALL, especially in children, noticed that occurrence of DIC in adult ALL was 10% before treatment and 78% during remission induction therapy (7, 8).

Clinically non-significant bleeding manifestations were observed in patients with ALL (9). Primary activation of inflammatory mediators and proteases by severe infection, chemotherapeutic agents and secondary activation of fibrinolysis may play a role in compensation of activated coagulation in patients with leukemia (10). Formation of thrombin-antithrombin complex due to activation of prothrombin fragment 1+2 and as well as generation of thrombin increment produces pre-thrombotic state which can be identified by a thrombotic marker (F1+2) (11). Strandberg et al 2001 indicated von Willebrand factor defects in patients with acute leukemia (12). Leukemic cells produce procoagulants, plasminogen activators, proteinase in blood circulation resulting in proteolytic degradation of vWF (13). However, there is limited local data available on this aspect of acute leukemia. Identification of changes in hemostatic parameters in acute leukemia in pretreatment, post remission induction may help in reducing the morbidity and mortality rate by initiating early intervention for management of thrombosis and bleeding complications. Therefore, this study was designed to evaluate the hemostatic, fibrinolytic and thrombotic changes in patients with de novo AML and ALL prior to start of induction treatment and after the therapy.

METHODS

The study was an observational study conducted at the Department of Biochemistry, University of Karachi, Pakistan and the Department of Hematology, National Institute of Blood Disease and Bone Marrow Transplantation (NIBD), Karachi, Pakistan from May 2011 to March 2012. Patients with confirmed diagnosis of acute leukemia were recruited into including newly diagnosed and those in remission phase (n=110). A total of 40 age matched controls were included. Those with ambiguous diagnosis, history of hereditary bleeding disorder, or thromboembolic disease were excluded. Diagnosis of acute leukemia was established by a hemato-pathologist and verified by a panel of hematologists, according to the WHO classification. Study individuals were divided according to the following schema.

Controls	Age matched Control	n=40
AML Day 0	Newly diagnosed cases of acute myeloid leukemia	n=27
AML Day 28	Remission induction in acute myeloid leukemia	n=16
ALL Day 0	Newly diagnosed cases of acute lymphoblastic leukemia	n=38
ALL Day 28	Remission induction in acute lymphoblastic leukemia	n=29

Laboratory methods

The hemostatic, fibrinolytic and thrombotic markers were analyzed at day 0 and 28 of treatment in patients as well as in controls. 65 patients including 27 of AML and 38 of ALL were newly diagnosed (at day 0) while 45 patients including 16 of AML and 29 of ALL received remission inductions. Six mL venous blood sample was collected from controls and patients at the time of diagnosis and at day 28 post chemotherapy. Blood sample was collected in 3.2% trisodium citrate with a ratio of 9:1. Platelet free plasma was separated after centrifugation at 2000 x g for 20 minutes within 2 hours of blood collection and stored in blue coded cryo vials at -80°C till analysis.

Complete blood counts including platelet count were performed on automated hematology analyzer Sysmex XE-2100® (Sysmex Kobe, Japan). Peripheral blood smears were stained with Romanowsky's stain and observed under microscope.
Prothrombin time (PT), activated partial thromboplastin time (aPTT), quantitative determination of fibrinogen and determination of Factor VIII activity (FVIIIc) was done by clotting method. Quantitative determination of Von Willebrand factor antigen (vWF Ag), D-Dimers and free protein S (PS) was carried out by immunoturbidimetric method. Qualitative and semi-quantitative determination of fibrinogen degradation products (FDPs) was carried out by latex agglutination method. Determination of plasminogen, antiplasmin and Antithrombin III (AT) activity was performed by synthetic chromogenic substrate. Quantitative determination of functionally active protein C (PC) was done by chromogenic method. Screening of factor V Leiden was performed by coagulometric method based on modified aPTT with pre-dilution in factor V deficient plasma. Screening of lupus anticoagulant sensitive aPTT was conducted by clotting methods. Detection of lupus anticoagulant was carried out by simplified diluted Russel viper venom test (DRVVT) method in one stage clotting test.

Statistical Methods

For quantitative analysis Mean±SD, minimum and maximum range, 95% confidence were calculated, while frequency and percentages were calculated for qualitative variables. Independent sample t-test was used to evaluate mean differences between control and cases of both types of acute leukemia. A p-value of <0.05 was considered significant.

RESULTS

Total 110 diagnosed acute leukemia patients, including 75 males and 45 females were included in this study along with 40 healthy controls for comparison. Mean age of patients was 25.3±13.8 (range 1 to 65years).
Mean leukemic cell counts were high at day 0 as compared to day 28 patients while platelet counts were reduced at day 0 as compared to control group. However, leukemic cells decreased, and platelet count increased at day 28 than day 0. Mortality rate was higher in AML at day 28 than ALL whereas equal incidence of death was found in both type of leukemia at day 0 of treatment.
The hemostatic, fibrinolytic, and thrombotic markers were analyzed in plasma of AML and ALL patients at day 0 and 28 of treatment. Prothrombin time significantly increased in AML and ALL patients at day 0 as compared to control as well as in pretreated than treated cases of AML ($p<0.05$), whereas no difference was observed in case of ALL ($p>0.05$). The aPTT was significantly increased in AML and ALL at day 0 and 28 but no statistically significant difference found in between the both type of acute leukemia in pre and post treatment phases. Plasma levels of fibrinogen were higher as compared to controls in both types of acute leukemia in pre and post treatment phase($p<0.05$) while at day 28 markedly increased in AML and ALL as compared to day 0(<0.01). Factor VIII level showing increased pattern at day 0 and 28 in both type of acute leukemia as compared to control ($p<0.01$). Von Willebrand antigen levels were significantly higher in both phases of AML and ALL ($p<0.01$) whereas no difference was noticed in between at day 0 and 28 in cases of AML.

Table 1. A summary of the comparison of controls and acute myeloid leukemia and acute lymphoid leukemia

Study population					
Variable	Healthy individuals	AML		ALL	
Groups	C= 40	NDM= 20	RIM=16	NDL=25	RIL=25
Age (years)	29.5±4.5 (24-38)	30.8±13 (15-65)	29.0±18 (4-50)	21.6 ± 11.1 (1– 38)	19.9 ± 13.3 (1 – 59)
Male	20 (50%)	10 (50%)	09 (56.2%)	19 (76%)	18(72%)
Female	20 (50%)	10 (50%)	07 (43.7%)	06(24%)	07(28%)
Hb (g/dl)	14.6±0.8 (12.4-16.7)	9.1±1.7 (6.2-12)	10.3±0.8 (9.2-11.9)	9.2 ± 1.7 (5.9 – 12.7)	10.7 ± 2.2 (7.6 – 16.1)
White Cells x109/L	7.2±1.35 (4.5-11.8)	48.6±40.6 (0.38-121.7)	2.3±4.4 (0.2±16.8)	48.6±40.6 (0.3-121.7)	6.9 ± 7.6 (0.08 – 27.0)
Platelet x109/L	255.7±43.1 (142-360)	24.5±15.8 (3-59)	76.2±72.7 (10-253)	33.9 ± 38.9 (5-183)	181 ± 156 (7- 573)
Blast %	NP	72.5±32.6 (7-100)	1.2±2.6 (0- 07)	51.2 ± 29.6 (8 – 96)	1.2±2.6 (0- 07)

Markedly elevated levels of D-dimer in AML and ALL at day 0 and 28 compared to control($p<0.01$) was observed but showed significant reduction at day 0 in AML and ALL as compared to day 0 ($p<0.01$). Alpha 2 anti-plasmin significantly reduced as compared to control ($p<0.01$) in AML and ALL at day 0 but at day 28 significantly higher whereas no difference was observed in between day 0 and 28 of AML and ALL cases. Levels of Plasminogen were higher at day 28 but not significantly reduced as compared to controls.

Protein C significantly reduced in AML and ALL in both phases as compared to control except in cases of ALL at day 28. Free protein S and factor V leiden did not show any significant association with acute leukemia. Antithrombin III was raised significantly in AML at day 0 and in ALL at day 28 although no statistical difference was observed in ALL. Lupus anticoagulant was higher in acute leukemia in both phases ($p<0.01$) while at day 28 more increased as compared to pretreatment which was statistically significant ($p<0.01$). Findings in Table no.2 describes non overt DIC <5 score and overt DIC >5 in acute leukemia at day 0 and 28. Strong association of DIC was found in AML and

ALL at day 0. Non overt DIC did not show any significant association with specific type of acute leukemia and it was equally expressed in both type of acute leukemia at day 0 and 28. (Table:2) Frequency of gum bleeding (21%) and Bruises (12%) was observed marked in AML patients. Epistaxis (12%) was observed more in ALL patients.

Table 2. DIC Score and association with leukemia's

	AML n(%)		ALL n(%)	
DIC Score	**Day 0**	**Day 28**	**Day 0**	**Day 28**
< 5	15 (55.56%)	16 (100%)	30 (78.94%)	27 (93.1%)
> 5	12 (44.44%)	0 (0.0%)	8 (21.06%)	2 (6.9%)

DISCUSSION

The patients with acute leukemias are at high risk of both hemorrhage and thrombosis. Coagulation parameters have been studied and found that in both AML and ALL cases PT and APTT were significantly prolonged indicating coagulation factors derangement. Post chemotherapy results indicated the improvement of PT and APTT in AML while no significant results observed in ALL. Kwaan et al found that chemotherapy decreases the blast cell may be helpful in decreasing the PT, APTT levels. Chemotherapy L –asparginase is used to treat ALL patients, decreases the hepatic production in clotting factors, results no change of PT level was observed (14).

Cysteine proteinease is a cancer procoagulant is present in AML and ALL patients. This proteinase activates the factor X, increased generation of thrombi and utilized the coagulation factors, aggravate thrombotic complications. Increased production of thrombin by factor X, further acts to convert fibrinogen to fibrin and results prolonged PT and aPTT (15).

Acute liver failure is reported in course of acute leukemia which further affects the cascade of coagulation and deranged the clotting factors (16). Factors VIII demands vWF for transportation. Factors VIII and vWF was increased in AML and ALL patients. After chemotherapy, these factors reduced indicated as cancer cells decreased, they tend to decrease the factor VIII production by decreasing the procoagulants (17). The derangements of thrombotic markers were also observed in present investigation. Low level of protein C was found in AML and ALL pretreated patients. While increased Antithrombin III and Lupus was found significantly in both leukemias. This may predispose to venous thrombosis as described by Dixit and Troy (18, 19).

The investigation of fibrinolytic factors also showed the derangements in both leukemias. An increased generation of FDPSs was found due to an increases degradation of fibrin as described by Ketsueki et al. At day 28, both AML and ALL showed improved status of FDPSs.

While decreased plasminogen was observed in both pre and post treated leukemic patients. Ketsueki et al, was found in their investigation that in leukemic patients the tissue plasminogen activator level was increased which tends to convert the plasminogen into plasmin. This plasmin acts on fibrin and formed more FDPSs. Increased generation of plasmin increases cross linked fibrin that

leads to the formation of linked oligomers called D-Dimers. D-Dimers were also increased in patients with ALL and AML and decreased post chemotherapy due to the reduced formation of fibrin (20).
Alpha 2 antiplasmin usually binds with plasmin and inactivating it for downstream events. We investigated the increased level of alpha 2 antiplasmin in pre and post chemotherapy. Increased generation of plasmin is the evidence of increased consumption of plasminogen. And plasmin acts on fibrin to form FDPSs and nothing available to binds with A2AP, thus increases its level.
Lupus anticoagulant is serving as prothrombotic agent and initially is thought to be present in lupus erythematosus but now found to be present in leukemias as well. It is anti-lipid antibody and interferes both intrinsic and extrinsic coagulation cascades thus help in increasing the PT, aPTT level which is found in present investigation as well. Leukemic cells trigger the B cells to produce more antibodies abnormally, lupus anticoagulant is one of them. Leukemic cells via cytokines activate B cell to produced antiphospholipid antibodies including lupus anticoagulant. We also investigated the increased level of lupus anticoagulant in AML and ALL patients significantly (21).
Global parameters like platelet count, PT, fibrinogen and D-Dimer were used to score the level of DIC. FDPSs are valuable diagnostic tool for monitoring the DIC. Elevated level of FDPSs indicates the persistence of DIC while low level indicates decline of intravascular clotting.
At day 0 in patients with AML 44% cases had underlying overt DIC (>5.0) but 0% cases after induction of chemotherapy whereas in ALL no statistically significant difference was noted. This finding is consistent with other studies (23, 24).

CONCLUSION

In conclusion, the leukemia patients are at high risk of bleeding, therefore supportive blood products should be administered, and serial laboratory monitoring of bleeding and thrombotic manifestations are required by using global hemostatic parameters in untreated patients, during and after the treatment. Early detection of thrombotic and hemostatic defects and active intervention will help in reducing the morbidity and mortality due to hemorrhage and thrombotic complications and provide better survival with good quality of life.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ETHICAL CONSIDERATION

For ethical issue, all participants and their legal guardians gave informed consent, and their confidentiality and anonymity were protected.

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