Thrombin Generation with Hematological Malignancies among Sudanese patients

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Abstract

Introduction:

Hematological malignancies can change the levels of plasma molecules involved in coagulation and fibrinolysis such as fibrinopeptide A, fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and D-dimer [(1)], markers of endothelial cell integrity (soluble E-selectin, van Willebrand factor and soluble thromboembolism) [(1, 2)] and of platelet function (beta-thromboglobulin) [(3)].

The aim of this study was to identify the hemostatic abnormalities and vascular damage among the major Sudanese hematological malignancy patients.

Materials and Methods:

This study was undertaken at the Radiation and Isotopes Center Khartoum (RICK), Sudan, during the period of February 2009 to October 2011. 202 patients (in and out patients) who were diagnosed of having hematological malignancies in different age groups on treatment or of treatment were selected as a study group and compared against 50 apparently healthy males and females as a control group. Prothrombin time, activated partial thromboplastic Time (APTT), antithrombin III, protein C, platelet count, von Willebrand factor (vWF), Plasminogen Activator Inhibitor-1 (PAI-1) activity and fibrinogen were gathered from the study group and the control group.

Results:

The results showed that the highest prevalence of hematological malignancy was among the study group (CML, 36.6%), while ET, MF and PCV were the lowest (0.5% for any) (P= 0.000). 38.1% of the 202 hematological malignancy patients were female and 61.9% were male. Mean age was 41 years (range 2-86 years). PTT, PT and PC values were not affected by disease, on the other hand there was a decrease in the levels of fibrinogen (P=0.000) and antithrombin III (P= 0.000), elevated vWf (P= 0.000), and PAI-1 was significantly elevated in ALL (P= 0.000) and in AML (P= 0.002) patients.

Conclusion:

Markers of coagulation were clearly observed in hematological malignancy patients; also, an indication of fibrinolysis and endothelial activation was confirmed. Some alterations in hemostasis and thrombotic events have frequently been found in hematological malignancy patients. These hemostatic changes may help the thrombotic and bleeding tendency in these patients.

Keywords:

Cancer, Hematological Malignancies, Coagulation, Thrombin generation

Introduction

Haemostasis is a part of a number of protective processes that have evolved to maintain a stable physiology [(4)]. The final step in the coagulation process is the development of the cross linked fibrin in thrombi and this is achieved by the amplification process of the clotting cascade symbolized by the waterfall mechanism described by Davie and Ratnoff and Macfarlane[(5)]. Most patients with leukemia are under a rare bleeding manifestation during the course of their illness[(6)]. Both bleeding and thrombotic complications

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are the main causes of morbidity and mortality in AML-M3. This can be attributed to a high expression of TF and cystine protease (CP) in the APL cells. ATRA treatment in vitro down-regulates both TF and CP expression in APL cells, reducing the cardiopathy [6]. The number of specific primary hypercoagulable states that are registered is growing. These disorders are usually inherited abnormalities of coagulation in which a physiologic anticoagulant mechanism is defective - for example, antithrombin III deficiency, protein C and P S deficiency, abnormalities of the fibrinolysis system, and dysfibrinogenemias. Cancer cells can come into contact with the clotting processes both directly and indirectly. Thrombocytosis is a known complication which causes morbidity and mortality in patients with hematological malignancies. This is probably due to the existing vascular disease. The collected information were a base line for other studies e.g. association between risk factors and disease. Determination of the possible risk factors of abnormal hemostatic and thrombotic balance among hematological malignant Sudanese patients had not happened previously. Recognizing hypercoagulability would be useful in epidemiological studies and may help establish a secondary preventive medication for individual patients. Patients with different haematological cancers were tested for hemostatic abnormalities and were compared to healthy patients of the control group.

Materials and Methods

202 in/outpatients who were diagnosed of having hematological malignancies (77 females and 125 males, mean age 41 years, range 2- 86 years) were chosen as a study group against 50 apparently healthy blood donors, lab workers and school students (male and female) were chosen as a control group. The selection of the study group was built on: patients fulfilling the clinical definition of hematological malignancies (male and female), at age groups on treatment or off treatment. Patients with previous histories of venous or arterial thrombosis, diabetes mellitus, and have received antiplatelets or anticoagulant drugs in the last 15 days were not part of the study. Blood for investigations took place during diagnostic examinations preceding initiation of treatment. Control group included 50 healthy volunteers (mean age 33 years, range 7–70 years). Blood was drawn from the ulnar vein and was obtained without venostasis. Vacationer citrate tubes were utilized to collect samples and to measure the parameters (PT, PTT, Fig, PC, ATIII, DD, PAI-1, Vwf Ag). The approval of the local ethics committee for Scientific Research Board, Faculty of Medicine, University of Juba, Sudan was prepared before the conduct of the examinations. Each patient was informed of the aim and nature of the study and a written consent was solicited. Data were collected in a structural questionnaire which included the following information: Hematological malignancy type, age, sex, residences, occupation, duration of the disease, under treatment, treatment type and treatment protocol.

Data analysis

Descriptive statistics (frequencies and percent) obtained from categorical variables and chi square were used to test the significance of frequencies. Data was statistically described in terms of mean and STD. The mann-Whitney test for independent groups was used for the parameters of distributions other than normal. A probability value (P value) less than 0.05 were considered statistically significant. The significance level for the correlation ratio was established.

Results

The male to female ratio in hematological malignancies was 1.6:1. Median age for acute myeloid and acute lymphoblastic leukemia was 35 years and 12 years respectively. For chronic myeloid, chronic granulocytic (CML Ph positive) and chronic lymphocytic leukemia the median age was 43 years, 41 years and 56 years respectively. In the case of Hodgkin's lymphoma and non-Hodgkin's lymphoma, it was 26 years and 43 years respectively and in the case of MM the median age was 57 years. While ALL had the lowest age (mean 12 years, range 4 to 25 years), the PCV (median 60 years), and CLL (median 56 years, range 27 to 76 years) get the highest age. These results reported that out of

202 hematological malignancy patients included in the study, about 14 (6.9%) patients have acute lymphoblastic leukemia (ALL), while 15 (7.4%) patients have acute megaloblastic leukemia (AML). Hodgkin's lymphoma (HL) was seen in 11 (5.4%) cases, while Non-Hodgkin's lymphoma (NHL) was seen in 23 (11.4%) cases. Among chronic leukemia, chronic lymphocytic leukemia (CLL) is outnumbered by chronic myeloid leukemia (CML), 21 (10.4%) against 74 (36.6%). Multiple myeloma (MM) was seen in 10 (5%) patients while a single patient had ET1 (0.5%). The same results were seen in MF and PCV. The result indicated that CML had the highest prevalence (36.6%) while ET, MF and PCV have the lowest (0.5% for any) ($p = 0.000$). The study group included 202 hematological malignancy patients where 77 (38.1%) were females and 125 (61.9%) were males. Mean age was 41 years, range 2- 86 years (see Table 1).

The results of coagulation parameters of HM patients in the study groups and controls are described in the Table 2.

In this study, all hematological malignancy patients regardless of the types were comparable to the control group, we found that the levels of AT-III and fibrinogen were significantly lower than the controls; in contrast, the level of vWF was significantly higher than controls. Patients of ALL, AML and CML were identified as having significant decreased concentrations of both fibrinogen and ATIII. We also discovered that patients with ALL, AML, CGL, HD and NHL were shown to have significantly higher in both concentrations of vWF and PAI-1 in comparison to the controls. The analysis of the results of CLL and MM patients had shown that vWF is considerably higher, while the platelet count was significantly lower in comparison to the control

Hematological malignancies	Frequency (%)	Minimum	Maximum	Mean ± SD
ALL	14 (6.9%)	4.00	25.00	12±6.1
AML	15 (7.4%)	12.00	72.00	35±20.2
CGL	31 (15.3%)	2.00	69.00	41±15.4
CLL	21 (10.4%)	27.00	76.00	56±14.7
CML	74 (36.6%)	10.00	85.00	43±17.1
ET	1 (0.5%)	55.00	55.00	55±0.0
HD	11 (5.4%)	8.00	52.00	26±15.0
MF	1 (0.5%)	30.00	30.00	30±0.0
MM	10 (5%)	40.00	86.00	57±14.1
NHL	23 (11.4%)	8.00	80.00	43±21.7
PRV	1 (0.5%)	60.00	60.00	60±0.0
Total	202 (100%)	2.00	86.00	41.5±19.4
Control	50	7.00	70.00	33±16.9

Table 1: The age related incidence distribution of hematological malignancies and control group

ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloblastic Leukemia, CGL: Chronic Granulocytic Leukemia, CLL: Chronic Lymphocytic Leukemia, CML: Chronic Myelocytic Leukemia, ET: Ethential Thrombathenia, HD: Hodgkin's disease, NHL: Non-Hodgkin's lymphoma, MM: Multiple Myeloma, MF: Myelofibrosis, PRV: Poly cythemia Rubra Virra.

Lab. Test	Mean									
	ALL	AML	CGL	CLL	CML	HD	MM	NHD	HM	Control
PLT	237	130.1	296.1	151.3	326.2	223.0	121.6	252.3	257.3	258.4
p. value	0.789	0.000	0.046	0.00	0.030	0.800	0.000	0.139	0.019	
PT	11.1	12.8	14.2	12.3	12.4	12.4	13.3	13.1	12.8	12.8
p. value	0.000	0.803	0.823	0.81	0.013	0.535	0.456	0.642	0.075	
PTT	31.3	30.6	35.7	30.1	32.9	32.0	0.565	32.3	32.5	30.5
p. value	0.795	0.785	0.007	0.821	0.127	0.599		0.863	0.211	
FGN	248.7	225.5	237.9	265.5	256.8	296.9	275.4	278.9	257.2	302.8
p. value	0.000	0.000	0.000	0.011	0.000	0.699	0.329	0.182	0.00	
PC	105.5	115.5	113.8	119.4	122.5	113.1	127.3	125.7	119.4	119.4
p. value	0.004	0.319	0.057	0.897	0.114	0.270	0.075	0.057	0.835	
AT-III	57.6	57.5	69.1	65.9	70.9	61.2	86.3	57.6	66.8	85.4
p. value	0.000	0.001	0.075	0.21	0.001	0.000	0.703	0.000	0.000	
VWF	136.6	139.9	118.9	127.2	139.8	130.6	168.8	143.2	135.6	103.7
p. value	0.014	0.004	0.213	0.02	0.000	0.027	0.000	0.044	0.00	
PAI-1	14.5	12.6	6.3	4.6	6.4	15.5	5.5	10.9	8.3	5.9
p. value	0.000	0.002	0.131	0.00	0.401	0.001	0.271	0.05	0.692	

Table 2. Coagulation parameters of HM patients in the study group and control group

HM: Hematological Malignancies. ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloblastic Leukemia, CGL: Chronic Granulocytic Leukemia, CLL: Chronic Lymphocytic Leukemia, CML: Chronic Myelocytic Leukemia, ET: Ethential Thrombathenia, HD: Hodgkin's disease, NHL: Non-Hodgkin's lymphoma, MM: Multiple Myeloma, MF: Myelofibrosis, PRV: Poly cythemia Rubra Virra. Plt= Platelet count, PT= prothrombine time, aPTT= activated partial thromboplastine time, Fgn= Fibrinogen, PC= Protein C, AT-III= anti-thrombine –III, vWF= von Willibrand factor, PAI-1= Plasminogen activator inhibitor type 1.

group. Among HD and NHL patients, there was noticeably lower in AT-III concentration in comparison to the control group. In CML patients, the level of vWF age was high and platelet count was elevated. CGL patients were found to have a considerably lower concentration of fibrinogen than controls.

Discussion

In the present study the coagulation tests that were performed include: prothrombin time, activated partial thromboplastic time (aPTT), antithrombin III, protein C and Von Willebrand factor (vWF) (markers of endothelial integrity), plasminogen activator inhibitor-1 (PAI-1) activity (marker of fibrinolysis activation) and fibrinogen (markers of coagulation activation), platelet count (done by using sysmex X21). The results indicated that the minimum age of onset in hematological malignancies is 2 years and the maximum age was 86 years and the median age of onset was 41 years, which are not far from that found by Marshall and Lichtman in 2008 [7]. Our findings have shown that the hematological malignancies in male were more than in female, and the minimum age of onset in AML was more than in ALL; and CLL (27 years) is higher than in CML (12 years). In case of Hodgkin's lymphoma, the minimum age of onset was same as in non-Hodgkin's lymphoma (8 years). ALL and CGL have the lowest age of onset (4 and 2 years, respectively), while MM (40) and PCV (60) have the highest one, which is in agreement with the study made by Idris, et al in 2004[8]. We have demonstrated that the frequency of chronic leukemia was more than acute leukemia, and MM, HL and NHL with less frequency, while CML was more common in Sudan, and PCV and MF were rare. We could not find any relation between this study and that of the Bakistanian

population [8]. However, this might be due to differences in the population's environmental and social factors. There was no study done in Sudan before to compare and to confirm our findings.

This study has shown that there was a decrease in the plasma level of fibrinogen and AT-III as prethrombotic indicator and an increase in the plasma level of vWF levels as vascular endothelial damage indicators, and these findings were in agreement with that reported by Demarmels and Lämmle [9] in 1992, Blann and his group in 2001 [10] and Falanga and Rickles in 1999 [11]. Previously, numerous clinical and experimental reports suggest that high vWF levels reflect damage to the endothelium or endothelial dysfunction [12].

Nand and Messmore in 1990 [13] found that Hemostatic abnormalities are present in a majority of patients with metastatic cancer, and the factors that increase the bleeding risk include thrombocytopenia, disseminated intravascular coagulation, and excessive fibrinolysis, which are enhanced by increased expression of Annexin II by leukemic cells. Moreover, therapeutic approaches to both bleeding and thrombotic conditions require special considerations of these factors [14,15]. Our findings are also consistent with several studies which reported that HM patients have an abnormal hemostatic mechanism [16,17]. In this study, we recorded a decrease in the levels of both, fibrinogen and ATIII in patients with ALL, AML and CML. Recently published data suggest that factor deficiencies of protein C, protein S, antithrombin, venous thromboembolism pediatric patients [18] and in ALL [19,20,21], moreover, some studies postulated that the levels of fibrinogen, antithrombin III and platelet count can be decreased in AML patients [11,21,22,23]. Our study indicated that there was markedly higher PAI-1 and vWF antigen concentration in patients with ALL, AML, CGL, HD and NHL. Our findings tend to support the evidence of fibrinolysis activation and endothelial vascular damage in these patients. The increased level of PAI-1 and vWF antigen concentration has been shown in patients with ALL [19,20,21], AML[11,21,22,23] and HL [11,16]. Hemorrhage is the most common hemostatic disorder in patients with all types of acute leukemia and with high frequency in acute granulocytic leukemia. Consumption of coagulation factors is regarded as an important cause of this complication [24]. Also, this finding is supported by Hau, et al. in 2007 [6] where he found that most patients with leukemia have some bleeding manifestations during the course of their illness. This study noted that: HL patients have decreased plasma concentration of the ATH-III. In acceptance, Hunault-Berger, et al [20] in 2008 reported that antithrombin and fibrinogen levels were reduced in patients with acute lymphoblastic leukemia or lymphoma treated with L-asparaginase.

The present study indicated that the activated partial thromboplastin time was prolonged and the platelet count was also increased in CGL patients, while fibrinogen concentration was decreased in all almost all patients. These findings supported the evidence of coagulation activation and bleeding manifestation in CGL patients. Most patients with leukemia have a little bleeding manifestations during the course of their illness [6]. Mason, [25] et al. In 1974, concluded that there was apparent abnormalities of blood coagulation and fibrinolysis in patients with CGL.

This study also indicated that: HL patients have a low plasma concentration of the ATH-III, with similar findings reported by Wada[16] and associates in 2005. The CLL and MM were found to have significantly higher VWF, while the platelet count was significantly depleted and ATIII is considerably lower in CLL. Previous studies of patients with CLL[6,10,11,27] have demonstrated a reduced level of platelets and ATIII, and increased level of vWF, moreover, these abnormalities are involved in the pathogenesis of the thrombotic and/or hemorrhagic complications in these types of patients. Previously, Kamińska, et al [28] in 2009 and Glaspy [29] in 1992, found that patients with multiple myeloma develop hemorrhage, especially following surgical procedures, and the mechanisms can include: acquired von Willebrand syndrome, paraprotein-induced platelet function defects, and thrombocytopenia.

Also, Auwerda, et al [30] in 2007, analyzed prothrombotic coagulation abnormalities in patients with untreated multiple myeloma, and found that the level of von Willebrand factor (vWF) was increased.

Conclusion

In conclusion, in patients with hematological malignancies, the markers of coagulation activation and platelet count defect were markedly observed; however, these hemostatic abnormalities might contribute to a thrombotic and or bleeding tendency in these patients. CML patients get the highest frequency in Sudan and vWf Ag was significantly higher in almost all patients. PAI-1 was significantly greater in virtually all hematological malignancy patients except in chronic leukemia and MM patients. Antithrombin III was significantly reduced in almost all patients except in CLL and MM.

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