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Heterogeneous Defects of Platelet Count and Responses to Agonists among Sudanese Patients with Hematological Malignancies

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Abstract

Background: Abnormal platelet activation and an increased risk of thrombosis are frequent findings in cancer.

Objectives: The aim of this study was to determine the heterogeneous defects of platelet count and responses to agonists among the major Sudanese hematological malignancies patients.

Materials and Method: This study was done in Radiation & Isotopes Center Khartoum (RICK) during the period of February 2009 to October 2013. Two hundred and two (202) hospitalized and out patients who were diagnosed of having hematological malignancies in different age groups on treatment or off treatment against fifty (50) apparently healthy (male and female) as a control group. platelet count, platelet aggregation and D-Dimer test were done for both study groups and control.

Result: The results indicated that, significantly low platelet counts in AML, CLL ($P < 0.05$), and increased in CML ($P < 0.05$) and CGL ($P < 0.05$) in related to control group, and the Study of platelet aggregation in response to ADP, Collagen, and Epinephrine agonists was decreased in ALL, AML and MM, and in variable in CML and NHL and increased in CLL and HL. The positive D-Dimer was seen in 161 (79.7%) while 41 (20.3%) patients had negative D- Dimer results.

Conclusion: Markers of platelet defect were clearly observed in hematological malignancies patients, also indication of coagulation activation were confirmed. Some alterations in hemostasis and thrombotic events have frequently been observed in hematological malignancies patients. These hemostatic changes may contribute to a thrombotic and bleeding tendency in these patients.

Keywords: *Cancer; Hematological Malignancies; Coagulation; Platelets Aggregation*

Introduction

Abnormal platelet activation frequent findings in cancer [1]. The patient with disseminated malignancy suffers many alterations of hemostasis; in addition, hemorrhage or less commonly thrombosis is the final clinical event in many of these patients [2]. Platelet activation

takes place after attachment and adhesion events or after other stimuli that triggers activation mechanisms such as thromboxane A2, ADP, thrombin or PAF (platelet activating factor, released from endothelial cells, PMN or monocytes) [3]. In a first step of activation, platelets undergo shape change, cytoskeleton rearrangement and organelle centralization. Release of dense granule contents (ADP and ATP) and serotonin, occurs. In a second step, there is alpha granule release of fibrinogen, fibronectin and vWF, exposure of fibrinogen and fibronectin receptors on platelet surface, and finally, release of arachidonic acid to be converted to thromboxane A2, which is a powerful mediator of platelet aggregating response [4]. The extent of secretion of a, dense and lysosomal-granule contents is dependent on the strength of the agonist, occurs in association with platelet activation Granule contents that are concerned in ornamental activation and aggregation of together their own and other platelets in the environs include ADP, vWF, fibrinogen, and calcium ions. The strongest response a platelet can mount to agonist stimulation includes activation, secretion, and aggregation [5]. Platelet aggregation in response to ADP, epinephrine, collagen and ristocetin were studied in acute leukemia and chronic leukemia in blast crisis, and in chronic phase, the Platelet aggregation responses to all the reagents were significantly impaired [6,7]. Whereas platelet dysfunction was not found in patients with newly diagnosed Hodgkin's disease [8]. Athale., *et al.* (2007) concluded that; Hemorrhage is the most common cause of early death in children with leukemia [9]. Furthermore, major bleeding episodes lead to shorter survival and increased resource use. Potential risk factors for bleeding include hyperleukocytosis, immunophenotype of leukemia (especially acute promyelocytic leukemia), thrombocytopenia and associated infections.

Fibrin D-dimer levels have been advocated as a useful clinical marker of thrombogenesis [10].

The collected data was a based line for other studies e.g. association between risk factors and disease. Determination of the possible risk factors of abnormal platelet activation and bleeding among hematological malignancies Sudanese patients had not been done previously. Recognizing bleeding disorder would be useful in epidemiological studies and may help to establish secondary preventive medication in individual patient. The patients with different hematological cancers were tested for platelet number and function and each was compared with healthy controls. Recruiting patients free of current chemotherapy/blood product support; to avoid the effects of these interventions.

Materials and Methods

Two hundred and two (202) hospitalized and out patients who were diagnosed of having hematological malignancies (77 female and 125 Males, mean age 41 years, range 2 - 86 years), against fifty (50) apparently healthy blood donors, lab workers and school students subjects (male and female) all age groups as a control group. The selection of study groups was depending on: Patients fulfilling the clinical definition of hematological malignancies (male and female), at age groups on treatment or off treatment, and patients (male and female) with previous history of venous or arterial thrombosis, diabetes mellitus, received antiplatelets or anticoagulant drugs in the last 15 days were excluded from the study. Blood for the investigations was taken during diagnostic examinations preceding initiation of treatment. Control group included 50 healthy volunteer (mean age 33 years, range 7 - 70 years) Blood was taken from ulnar vein; it was obtained without venostasis. Vacutainer citrate tubes were used to collect the fresh samples and measuring these parameters (Platelets count was detected electronically by using Sysmex KX-21N, Kope, Japan Platelet aggregation. Was detected by using Aggregometer, Agg RAM (Helena- Texas. USA) instrument. ADP, Collagen and Epinephrine (Helena reagent) were used to assess Platelet function) The D-Dimer test was detected by using the agglutination of latex particles coated with antifibrinogen antibody. This technique was adapted from the Helena Bio-Sciences Europe (Latex Agglutination test for Fibrin D-Dimer- REF 5250).

The approval of local ethics committee for Scientific Research board, Faculty of Medicine, University of Juba, Sudan was obtained for conduction of examinations. Each studied person was informed about the aim and nature of the study and then gave written consent for it. The data were collected in structural questionnaire which was included the following information; hematological malignancy type, age, sex, residences, occupation, duration of disease, under treatment, treatment type and treatment protocol).

Data analysis

Descriptive statistics (frequencies and percent) obtained for categorical variables and chi square used to test the significance of frequencies. Mean and STD were descriptive statistic for numerical variables. Mann-Whitney test for independent groups was used for the parameters with distributions other than normal. $P < 0.05$ was considered statistically significant value. The significance level for correlation ratio was determined.

Results

The male to female ratio in hematological malignancies was 1.6:1. Mean 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 it was 43 years, 41 years and 56 years respectively. In case of Hodgkin's lymphoma and non Hodgkin's lymphoma it was 26 years, 43 years respectively. In case of MM was 57 years. While the 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) had the highest age. These results indicated that out of 202 hematological malignancy patients included in the study, about 14 (6.9%) patients had acute lymphoblastic leukemia (ALL), while 15 (7.4%) patients had acute myeloblastic 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 leukemias, chronic lymphocytic leukemia (CLL) outnumbered 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 result was seen in MF and PCV. The result indicated that CML had the highest prevalence (36.6%) while ET, MF and PCV had the lowest one (0.5% for any) ($P = 0.000$) (Table 1). The study group included 202 hematological malignancy patients (77 (38.1%) female and 125 (61.9%) male, mean age 41 years, range2- 86 years) (Table 1).

Hematological malignancies	Frequency (%)	Minimum age	Maximum age	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 groups.*
ALL: Acute Lymphoblastic Leukemia; AML: Acute Myeloblastic Leukemia; CGL: Chronic Granulocytic Leukemia; CLL: Chronic Lymphocytic Leukemia; CML: Chronic Myelocytic Leukemia; ET: Essential Thrombasthenia; HD: Hodgkin's disease; NHL: Non Hodgkin's lymphoma; MM: Multiple Myeloma; MF: Myelofibrosis; PRV: Polycythemia Rubra Virra.

Figure 1 showed that out of 193 hematological malignancies patients included in the study and answered the residence questions out of 202 patients, about 3 (1.5%) patients was lived Blue Nile state, while 9 (4.5%) patients levied in Darfur. About 2 (1%) patients were

lived in Red sea state. The majority of cases was levied in Khartoum, Kordufan and Elgazeira (102 (50.5%, 25 (12.4%) and 20 (9.9%), respectively. While 11 (5.4%) of cases were lived in Blue nile, Northern state, Gadarif, Sinnar, Kasala and southern Sudan about 4 (2%), 3 (1.5%), 2 (1%) of cases respectively. About 5 (2.5%) of cases were lived in River Nile state.

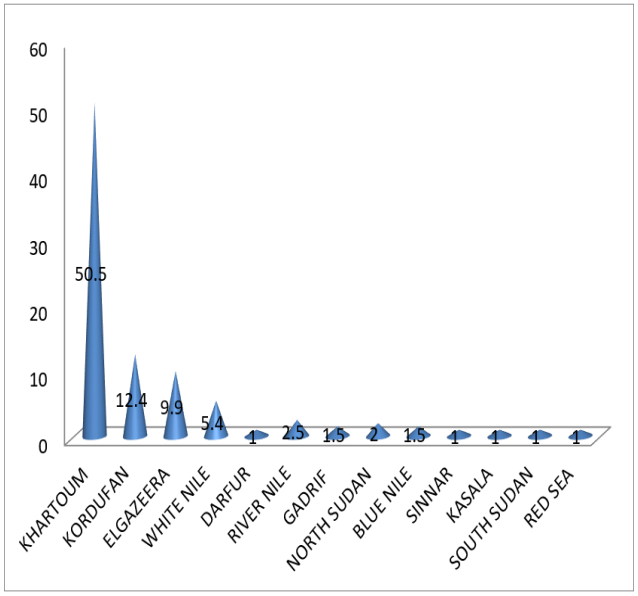

Figure 1: *Geografical distribution of hematological malignancies in Sudan states.*

The assessment of platelet aggregation with aggregometer using ADP, Collagen and Epinephrine revealed no significant differences between hematological malignancies patient ADP (P = 0.741), Collagen (P = 0.431) and epinephrine (P = 0.675) as general and control (Table 2), but the differences appeared in groups.

Type		ADP_MAX	COLLAGEN_MAX	EPINEPHRINE_MAX
Patient	N	161	161	161
	Minimum	16.30	1.30	0.60
	Maximum	169.20	112.00	113.00
	Mean	77.5280	79.7752	83.9056
	Std. Deviation	18.30931	23.26824	26.00492
Control	N	50	50	50
	Minimum	60.60	67.90	65.00
	Maximum	96.10	98.10	89.60
	Mean	76.6100	82.4600	85.4700
	Std. Deviation	12.60883	10.90723	7.09456

Table 2: *Platelet aggregation determination using ADP, Collagen and Epinephrine in Hematological Malignancy patients and control group. When the result is classified into group the differences were appeared.*

Table 3 explained the results of the maximal aggregation of ADP, collagen and Epinephrine using Agg RAM aggregometer. ALL patients showed no significant differences with control group, while in AML patients, there was no differences in ADP, but collagen max aggregation was decreased than control (mean = 72.6, 82.4, P = 0.04) and also Epinephrine was decreased than control (mean = 75.1, 85.4, P = 0.00). In CGL patients, ADP (mean = 80.3, 76.6, P = 0.00) was significantly higher than in control, Collagen (mean = 72.5, 82.4, P = 0.00) was significantly decreased than in control, and Epinephrine (mean = 103.1, 85.4, P = 0.00) max aggregation was significantly higher than in control. In CML patients, ADP (mean = 83.1, 76.6, P = 0.02) was significantly higher than in control, while Collagen and Epinephrine max aggregation was not differ than in control. In CLL patients, ADP (mean = 81.1, 76.6, P = 0.02) was significantly higher than in control, Collagen (mean = 100.3, 82.4, P = 0.00) was significantly higher than in control, and Epinephrine (mean = 113, 85.4, P = 0.00) max aggregation was significantly higher than in control. In HD patients, ADP (mean = 82, 76.6, P = 0.00) was significantly higher than in control, Collagen (mean = 100, 82.4, P = 0.00) was significantly higher than in control, and Epinephrine (mean = 113, 85.4, P = 0.00) max aggregation was significantly higher than in control. In MM patients, ADP (mean = 28, 76.6, P = 0.00) was significantly lower than in control, Collagen (mean = 4.8, 82.4, P = 0.00) was significantly decreased than in control, and Epinephrine (mean = 0.6, 85.4, P = 0.00) max aggregation was significantly lower than in control. In NHL patients, ADP (mean = 77. 3, 76.6, P = 0.11) was not significantly differ from control, Collagen (mean = 81.1, 82.4, P = 0.13) was significantly decreased than in control, and Epinephrine (mean = 91.6, 85.4, P = 0.00) max aggregation was significantly higher than in control.

HM	ADP_MAX			COLLAGEN_MAX			EPINEPHRINE_MAX			PLT		
	Mean		P.V	Mean		P.V	Mean		P.V	Mean		
	Patient	Control		Patient	Control		Patient	Control		Patient	Control	P.V
ALL	69.9	76.6	0.07	78.6	82.4	0.107	78.3	85.4	0.09	237	258.4	0.789
AML	74.3	76.6	0.14	72.6	82.4	0.04	75.1	85.4	0.00	130.1	258.4	0.000
CGL	80.3	76.6	0.00	72.5	82.4	0.00	103.1	85.4	0.00	296.1	258.4	0.046
CML	83.1	76.6	0.02	73.8	82.4	0.24	77.5	85.4	0.12	326.2	258.4	0.030
CLL	81.1	76.6	0.02	100.3	82.4	0.00	113	85.4	0.00	151.3	258.4	0.00
HD	82	76.6	0.04	100	82.4	0.00	113	85.4	0.00	223.0	258.4	0.800
MM	28	76.6	0.00	4.8	82.4	0.00	0.6	85.4	0.00	121.6	258.4	0.000
NHL	77.3	76.6	0.11	81.1	82.4	0.13	91.6	85.4	0.00	252.3	258.4	0.139

Table 3: *Platelet aggregation and count in HM patients compare with control group and P value.*

Table 3 shown that among ALL, HD and NHL patients, there were no significant differences observed in Platelet count between patients and control. In AML, CLL and MM patients, There were significantly lower in platelet count (P = 0.000) in comparison with control group, while. Among CGL patients, there was significantly higher in Platelet count (P = 0.046) in comparison with control group. In CML patients, there was significantly elevated Platelet count (0.030) in comparison with control group.

Figure 2 showed that out of 202 hematological malignancy patients included in the study, the positive D-Dimer was seen in 161 (79.7%) while 41 (20.3%) patients had negative D-Dimer results.

Discussion

This is a descriptive, prospective analytical case- control study that was conducted in Radiation and Isotopes Center Khartoum (RICK) during the period of 2009 to 2013, to determine the haemostatic abnormalities and vascular damage among the major Sudanese hematological malignancies patients. Two hundred and two (202) hospitalized and out patients who were diagnosed of having hematological malignancies, all age groups on treatment or off treatment, against fifty (50) apparently healthy (male and female) all age groups as a

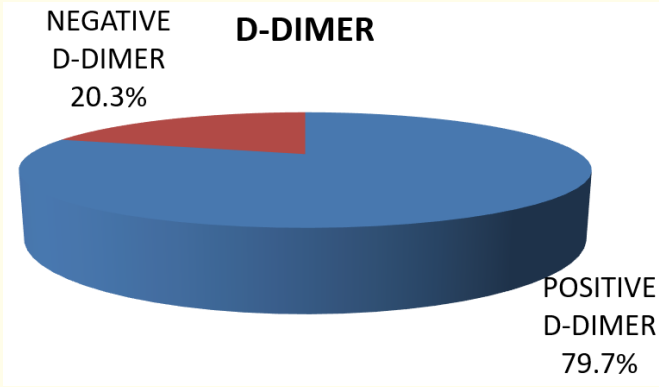

Figure 2: *Frequency of D-Dimer test in HM.*

control group. The coagulations tests that were performed including: Platelets count, Platelet aggregation (ADP, Collagen and Epinephrine were used to assess Platelet function) and D-Dimer test.

Platelet aggregation in response to ADP, epinephrine, collagen and ristocetin were studied in acute myeloid leukemia, chronic myeloid leukemia in blast crisis and acute lymphoblastic leukemia, Platelet aggregation responses to all the reagents were significantly impaired. Platelets aggregation in response to ADP, epinephrine, collagen and ristocetin were studied in chronic leukemia which included chronic myeloid leukemia, chronic lymphatic leukemia and CLL related disorders. Among the cases of CML, Defects in platelet aggregation were variable. Among the cases of CLL and CLL related disorders, Platelet aggregation responses were significantly impaired in all the cases. Platelet dysfunction was not found in patients with newly diagnosed Hodgkin's disease.

Our study indicated that, max aggregation of ADP, Collagen and Epinephrine in ALL patients was normal and this excluded the qualitative platelet defects in this type of patient. This observation is in agreement with study done by Pui., *et al* [11].

The platelet aggregation response to ADP was normal in AML patients, on the other hands; it was reduced in response to Collagen and Epinephrine. This is not far from the study done by Naresh., *et al* [6]. The presence of little variability may be due to uses of different treatment protocols.

The platelet aggregation response to ADP was increased in CML patients and normal to Collagen and Epinephrine. This is not far from the study done by Naresh., *et al* [6].

The platelet aggregation response to ADP, Collagen and Epinephrine was increased in CLL and HD patients. This may be supporting the presence of hypercogubility in these patients.

The platelet aggregation response to ADP, Collagen and Epinephrine were significantly decreased in MM patients. This is in agreement with study done by Shen., *et al* [12]. In NHL, platelet aggregation response to ADP was normal, but decreased in response to Collagen and increased in response to Epinephrine. Considerable heterogeneity in platelet reactivity existed, probably reflecting the presence of several pharmacologically altered platelet subpopulations.

To our knowledge, Eleni., *et al.* [10] concluded that it would be more useful if we used the term abnormal hemostasis, because the fibrin D-dimer is a cross-linked degradation product, resulting from the balance between thrombogenesis and the fibrinolysis process.

Fibrin D-dimer levels have been established as a useful clinical marker of thrombogenesis. The use of D-Dimer levels in the investigation and management pathway of venous thromboembolism is well established. This marker has a high sensitivity and specificity in excluding thromboembolism, when a well-defined assay is used in the appropriate clinical setting.

These findings confirm that HM patients had abnormal hemostatic mechanism and are in agreement with Wada., *et al.* [13], Meddeb., *et al.* [14] and Falanga and Rickles [15].

The present study indicated that, platelet count was decreased (thrombocytopenia) in AML, CLL and MM, while it was increased (thrombocytosis) in patients with CGL and CML. This finding confirm that what reported by Athale., *et al*. [9] and they concluded that; Hemorrhage is the most common cause of early death in children with leukemia. Furthermore, major bleeding episodes lead to shorter survival and increased resource use. Potential risk factors for bleeding include hyperleukocytosis, immunophenotype of leukemia (especially acute promyelocytic leukemia), thrombocytopenia, and associated infections.

Conclusion

In conclusion we found that, Markers of coagulation and platelet defect was clearly observed in HM patients, also indication of fibrinolysis was confirmed. So, alterations in hemostasis and Thrombotic events have frequently been observed in hematological malignancy patients, these haemostatic changes may contribute to a thrombotic and bleeding tendency in that patients. CML had highest frequency in Sudan. D-dimer was elevated in 79% of hematological malignancy patients. Abnormal platelet count and aggregation were documented.

Recommendations

Further studies must be conducted with large sample size, involve different ethnic groups and apply different diagnostic methods.

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