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COAGULATION PROFILE IN SUDANESE PEDIATRIC LEUKEMIC PATIENTS

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ABSTRACT

Aim: we conducted our study to evaluate coagulation profile in Sudanese Pediatric Patients with leukemia.
Materials and methods: This study is a prospective analytical case control study was conducted to evaluate coagulation profile in 97 Sudanese pediatric patients with leukemia; the population comprises all male and female pediatric patients, during the period of collection (2014-2015) admitted in Khartoum Radiation and Isotopes Centre, 98 individual pediatric (0 – 15 years) male and female apparently healthy from primary, Kindergartens and nurseries schools are selected as control group.
Results: In case group gender wise males are 52(53.1%) and females are 46(46.9%) with mean age for all 9.03 years. The case group in our study was divided into three subgroups based on type of leukemia ALL was 81(82.7%), AML 12(12.2%) and CML 5 (5.1%). There is significant correlation between case and control group in PT, PTT, Platelets and Protein C (P values < 0.05) (Table 3). There are only two patients which there D-dimmer level were more than 200 ng/ml (high) and all control group and other patient were normal (< 200 ng/ml).
Conclusion: We conclude that there were significant variations in PT, PTT, Protein C, Fibrinogen level and Platelet count between case and control group.

INTRODUCTION

Disturbances in endothelial lining of the vessels and plasmic hemostasis in patients with acute leukemia; different mechanisms of coagulation disorders in acute leukemia effects of leukemic cells containing procoagulants, fibrinolytic and anti fibrinolytic substances, of intensive chemotherapy and inflammation. All these impacts impair endothelial cells and trigger plasmic coagulation cascade; the initiator of coagulation is a tissue factor (Tarasova*, et al*. 2011). There are some evidences that the activation of coagulation system by neoplastic cells facilitates invasiveness and metastases. Fibrinogen is one of the important parameters concerning the relationship between malignancy and coagulation disorder (Yu-sheng, *et al*. 2014).

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Fibrin degradation products have been shown to display strong angiogenic properties (Ay, *et al*. 2010). In our study we evaluated coagulation profile in Sudanese pediatric patients with leukemia.

MATERIALS AND METHODS

This study is conducted to detect haemostatic mechanism in Sudanese pediatric patients with leukemia; the population comprises all male and female pediatric patients, during the period of collection (2014-2015) admitted in Khartoum Radiation and Isotopes Centre (RIC). 100 individual selected which are all patient admitted to RIC at the period of collection depending on statistical office in the centre. During the period of collection (2014-2015) admitted in Khartoum Radiation and Isotopes Centre (RIC) are includes in this study.

Controls: 100 individual pediatric (0 – 15 years) male and female apparently healthy from primary, Kindergartens and nurseries schools are selected as control group.

Inclusion criteria: Sudanese Individuals of both sex aged less than 15 years with leukaemia admitted in Khartoum Radiation and Isotopes Centre (RIC).

Exclusion Criteria

- Cured Patient
- Patient with known history of coagulation disorder
- Inadequate, clot or hemolysed samples.

Questionnaire

- Include general information
- History information's
- laboratory investigations

Sample preparation

Within 3 hours of blood collected, centrifuged capped citrate tube for 10 minutes at an RCF (relative centrifugal force) of 2000g. Used a plastic transfer pipit, removed the top3/4 of plasma was placed it in a plastic centrifuge tube with cap, Centrifuged the plasma (in the plastic centrifuge tube) for another 10 minutes at 2000g. Using a plastic transfer pipit, removed the top ¾ of plasma into a plastic tube, without disturbing the plasma in the bottom of the spun tube, where any residual platelets will be (Marco.*et al*. 2007).

Methods

PT an aliquot of test platelet-poor plasma was incubated at 37C° with a reagent containing a tissue factor, phospholipids (thromboplastin), and CaCl2.The time required for clot formation was measured by coagulometer. The aPTT an aliquot of undiluted, Platelet poor plasma was incubated at 37°C then phospholipids (cephalin) and a contact activator (e.g. Kaolin, micronized silica or ellagic acid) were added followed by calcium (all pre-warmed to 37°C). Addition of calcium initiates clotting and timing begins (Jackson, et al. 2005).

The Fibrinogen assay is based on the Claus method. In the presence of a high concentration of Thrombin the time required for clot formation in diluted plasma is inversely proportional to Fibrinogen concentration. The Coagulation-500 employs the photo-optical clot detection methods.by using a red light (660 nm)to illuminate the sample plasma/reagent mixture, the CA-500 detects the change in scattered light intensity due to increased turbidity as fibrinogen changes to fibrin. The coagulation curve is drawn by taking the time and scattered light intensity as the X-Y axis respectively. The coagulation time is determined by a percentage detection methods.

- Tests were measured using scatter-light End Point Detection
- The light source and wavelength at 660nm.

Protein C is activated using (commonly) Protac™, an extract of the venom of Akistrodon contortrix and the concentration of Protein C is determined from the rate of color change in the test sample due to cleavage of a Chromogenic substrate. The D-dimer is a rapid assay used for the qualitative and semi-quantitative measurement of cross-linked fibrin degradation products. During clot formation, these cross-linked products or FDPs are formed from the conversion of fibrinogen to fibrin by thrombin. Once a clot is formed, it triggers the production of plasmin. Then, plasmin starts to degrade the cross-linked fibrin, forming fragments. D-dimer levels were measured by a quantitative latex assay on an STA-R analyzer (Diagnostica-Stago)

Data analysis: Data were entered and analyzed by SPSS prograrmme (version: 17.0). All demographic data of the study population were presented as mean and SD in the text and P.value was used for detecting the power of relationship between the determinant and the outcome and 95% confidence interval was calculated.

Ethical clearance: Before collection of data and samples from humans, must be need to obtain Research Ethics Approval from the Ethics Committee before starting the research.

RESULTS

The participants included 200 Childs subjects. 100 Out of them, was already diagnosed with leukemia and 100 healthy Childs. In case group gender wise males are 52(53.1%) and females are 46(46.9%) with mean age for all 9.03 years and SD 4.058 were in the control group Gender wise males are 53 (54.6%) and females are 53 (45.4%) with mean age for all 8.08 years and SD 4.033. There is no significant different between case and control group in the age (P value 0.1027). The frequency of ALL was 81(82.7%), AML 12(12.2%) and CML 5 (5.1%) (Table 3 -2).

Table 1. Gender distribution in case and control group

	Sex	Frequency	Percent
Test group	Male	52	53.1 %
	Female	46	46.9 %
	Total	98	100 %
Control group	Male	53	54.6 %
	Female	53	45.4 %
	Total	97	100 %

Table 2. Type Frequency of leukemia in the Case group

Case group	Frequency	Percent
ALL	81	82.7 %
AML	12	12.2 %
CML	5	5.1 %
Total	98	100 %

In case group PT mean was 17.60 (SD=6.33), PTT mean was 40.41(SD=10.10), Fibrinogen 2.67 g/L (SD= 0.67), Protein C 0.83% (SD= 0.32) and platelet count mean 289.46 (SD = 138.85) wile in control group PT mean was 14.26 (SD=0.35), PTT mean was 25.97 (SD=1.69), Fibrinogen 2.55 g/L (SD= 0.40), Protein C 0.92 % (SD= 0.087) and platelet count mean 357.08 (SD = 124.04). There is significant correlation between case and control group in PT, PTT, Platelets and

Protein C (P values were less than 0.05) (Table 3).There are only two patients which there D-dimmer level were more than 200 ng/ml (high) and all control group and other patient were normal(less than 200 ng/ml)

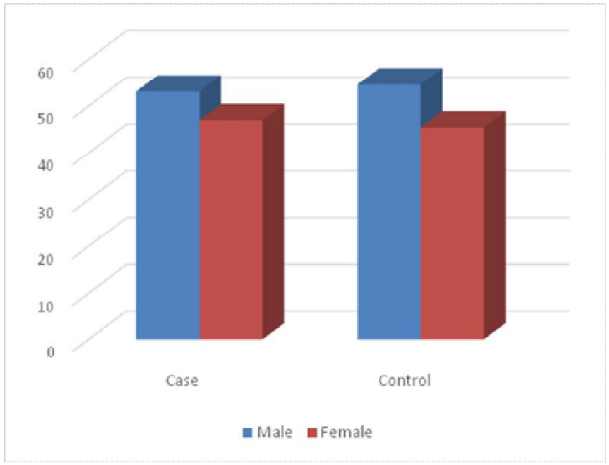

Figure 1. Gender distribution in case and control group

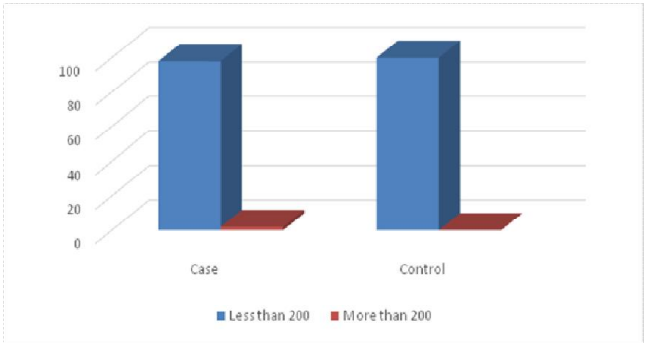

Figure 2. D. Dimmer in the case and control group

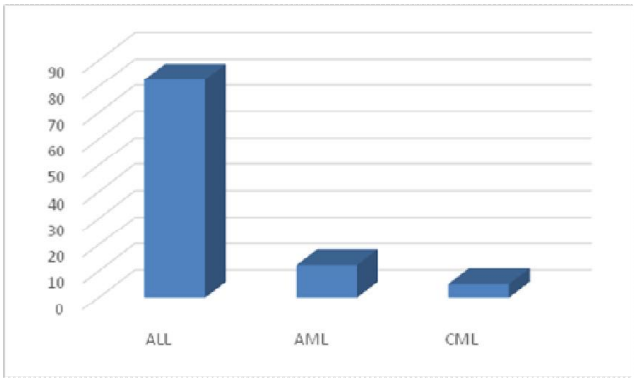

Figure 3. Types of leukemia in patient group

Table 3. Age and coagulation tests mean and Std. Deviation

Coagulation variant	Patients Mean ± SD	Controls Mean ± SD	P-value
Age	9.03 ± 4.058	8.08 ± 4.033	0.1027
PT	17.60± 6.33	14.26 ±0.35	0.000
APTT	40.41 ± 10.10	25.97 ± 1.69	0.000
Fibrinogen	2.67 ± 0.67	2.55 ± 0.40	0.107
Protein C	0.83 ± 0.32	0.92 ± 0.087	0.004
Platelets	289.46 ± 138.85	357.08 ± 124.04	0.000

Table 4. D. Dimmer result in case & control groups:

	Case		Control	
D. Dimmer	Frequency	Percent	Frequency	Percent
(< 200)Normal	96	98 %	97	100 %
High (> 200)	2	2 %	0	0 %
Total	98	100 %	97	100 %

DISCUSSION

In our study we examined association of hemostatic state and leukemia in pediatric Sudanese patients. The mean of the platelets count is 289.67 x 10^3 in the patient group which is within the normal range, but there is significant correlation of platelets count between case and control group (P value 0.000) ,our finding in agreement with(Hara. *et al*, 1990),who reported that Thirty-six (17.8%) of 202 children with acute lymphoblastic leukemia (ALL) and 2 (3.7%) of 54 children with acute non-lymphoblastic leukemia (ANLL) had a platelet count over 150 x 10(9)/l at diagnosis. Children with ALL and a platelet count over 150 x 10(9)/l were analyzed in detail. The ALL patients without thrombocytopenia tended to be male predominant and had less frequent bleeding manifestations (P less than 0.01). Our finding disagree with (Dixit , *et al*. 2007), who reported sixty seven patients with acute leukemia were evaluated prospectively for hemostatic abnormality where 43 (64.2%) had ALL and 24 (35.8%) had AML. 27 patients (40.3%) had bleeding manifestations, thrombocytopenia was present in 57 patients (85%), and 33(49.3%) had some abnormality of global coagulation markers' and also disagree with (Hassan, 2015) , in her study about Clinical pattern of leukemia in Sudanese children and immediate response to treatment who conclude Platelets of less than 40,000 was found in 48.3% of patients, between 80,000 –150,000 was found in 16.7% and more than 150,000 was found in 21.7% of her patients with ALL compared to our result.

PT, INR, and PTT in patient group are significantly higher than in control group (P value 0.000),this finding is in agreement with the study done by(Amr and Abdulla. 2014) ,who concluded in their study done in Sudanese Patients with Hematologic and Solid Malignancy that activated partial thromboplastin time (APTT), prothrombin time (PT) and platelets count are significant increased than control group(P.value =0.000) and also agree with (Ribeiro. 1986), which they determined the clinical and biological correlates of coagulopathy in a large series of patients with untreated childhood acute leukemia. Twenty-five of 805 children with acute lymphoblastic leukemia (ALL) (3.1%) and 27 of 195 with acute myeloid leukemia (AML) (13.8%) and found prolongation of prothrombin time (PT) greater than 12 seconds, activated partial thromboplastin time (PTT) greater than 45 seconds,and also agree with (Masanori, et al. 1989), who stated Hemostatic changes were evaluated in ten patients with acute lymphoblastic leukemia and lymphoma who received chemotherapy with L-asparaginase, vincristine, and prednisolone for 1 week. Following treatment, prothrombin time and activated partial thromboplastin time were significantly prolonged in 83% of the patients on diagnosis and in 90% after treatment. In our study the mean fibrinogen level is 2.67 g/L in patient group and 2.55 g/L in control group which is within normal range and no significant variation

(P=0.107); our result was in agreement with findings from (Goldschmidt and Koós. 1984) where investigated fibrinogen in 21 children with acute lymphoblastic leukaemia (ALL). Ten patients were undergoing induction therapy, 11 children were in complete remission on maintenance therapy, and found All except two children in complete remission had normal fibrinogen levels. Our study disagrees with (Rość, *et al*. 2007), who investigate 22 patients with CML aged 38-55 years. Twenty nine healthy controls were sex and age-matched and found out that there are significantly higher fibrinogen concentration (3.31 g/l) and elevated platelet count (611.0 g/L) was observed in patients with CML, disagree here also in patient's ages.

In our study the mean of protein C is 0.924% in control group and in the patient group is 0.825 % which is significantly lower C (P =0.004) and this was agree with study done by (Kevin, *et al*. 2006), who found Plasma levels of protein C were significantly lower in patients with active acute myelocytic leukemia (mean = 77.9) than in controls or patients in remission. Functional protein C levels were also significantly lower in AML patients with active disease (mean = 58.5) than controls or patients in remission (mean = 98.5), our finding also agree with study done by (Masanori, *et al*.1989), who reported that the concentrations of coagulation inhibitors (protein C, and protein S, and plasminogen) also significantly decreased in ten patients with acute lymphoblastic leukemia and lymphoma. In our study there are only two patients which their D-dimer level were more than 200 ng/ml and all control group and other patient with normal range(less than 200 ng/ml) our finding agree with (Wei, *et al*. 2011), who measure the level of D-dimer in different phrases of patients with acute leukemia and to explore its significance in the progress and curative effect of leukemia. After complete remission (CR) , plasma levels of DD had no significant difference in AL patients (26 cases) versus control group (t=0.72, P> 0.05).our finding does not agree with (Krzysztof *et al* 2000) in their study which carried out in 70 patients including 49 with AML and 21 with ALL, and concluded that the level of TAT, DD and PAP was elevated.

Conclusion

We conclude that there were significant variations in PT, PTT, Protein C, Fibrinogen level and Platelet count between pediatric leukemic patients and control group.In our study group there were no evidence of disseminated intravascular coagulation (DIC) , deep vein thrombosis (DVT) and pulmonary embolism (PE) according to our D dimmer result.

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