Original Research Article

DISSEMINATED INTRAVASCULAR COAGULOPATHY; A CONDITION TO MONITOR IN THE MANAGEMENT OF LEUKAEMIA PATIENTS

ABSTRACT

Background: Disseminated intravascular coagulopathy is a consumption coagulopathy which mostly results from an underlying disease. It occurs as a result of the activation of the coagulation cascade and hence leading to the formation of thrombi resulting in haemorrhage due to the excessive consumption of platelet and coagulation factors. Malignancy is associated with hypercoagulable state and increased risk for thrombohemorrhagic complications and leukaemia is no exception. Bleeding manifestations are common in acute leukemias, especially in acute myeloblastic leukemia, and are prominent of an initial stage of the disease. This study assessed disseminated intravascular coagulopathy (DIC) in leukaemia patients in Nigeria.

Materials and Methods: One hundred and sixteen (116) subjects were recruited for the study consisting of 58 leukaemic subjects (AML, CLL, and CML) and 58 age and sex matched control subjects. The parameters estimated in this study were prothrombin time, partial thromboplastin time with kaolin, international normalised ratio using Diagen reagents; D-dimer using Tina Quant Gen 2 D-dimer reagent on Roche Cobas C 111 analyser by immunoturbidometry method, packed cell volume, platelet and white blood cell counts were run on Abacus junior haematological auto analyser.

NO NEED TO DESCRIBE SPECIFIC METHODS IN THE ABSTRACT ↓ THEY SHOULD BE MENTIONED IN THE METHODS SECTION

Result: The mean ± SD of the parameters assessed in the leukaemic patients include 3.74±3.13µg FEU/mL, 67.59±55.71seconds, 77.34±31.81seconds, 193.62±102.79cells/mm3, 74±124.42 cells/mm3, 30.07±5.38%, and 1.84±0.09 for D-dimer, PT, PTTK, platelets, WBC, PCV and INR respectively. The results display a significant statistical difference between the leukemic and the control subjects ($p<0.05$).

Conclusion: The abnormality of these haemostatic parameters occurring in the leukaemic subjects (AML, CLL, and CML) is highly indicative of occurrence of disseminated intravascular coagulopathy in these patients. This study therefore shows that disseminated intravascular coagulopathy can occur as a complication of ~~any~~ various class of leukaemia studied ~~especially if not properly managed and intervention commenced early~~.

PLEASE USE THE MORE COMMON TERM "PTT"

TYPES

AND THIS SHOULD PROMP AN EARLY AND PROPER MANAGEMENT.

Key Words: Disseminated intravascular coagulation, leukaemia, malignancy, haemostasis.

INTRODUCTION

Disseminated Intravascular Coagulation (DIC) is an abnormality characterized by the systemic intravascular activation of the coagulation system, simultaneously causing intravascular thrombi, distorting adequate blood supply to the organs, and bleeding as a result of exhaustion of the platelets and coagulation factors [1]. The clinical characteristics of DIC include spontaneous or induced bleeding complications and thrombotic complications, whereas multiple organ failures may be in part a complication of intravascular fibrin formation. Also, the generation of multiple proteolytically active enzymes of the clotting cascade may escalate inflammatory activity, which may increase the systemic inflammatory syndrome [2].

Various disorders, including infections or inflammatory conditions and malignant disease, can cause activation of coagulation. In several cases, this activation of coagulation may not cause clinical complications and may not even be detectable by routine laboratory analysis

[3]). However, if activation of coagulation is sufficiently strong, a reduction in platelet count and extension of global clotting time may become evident [3]. The management of DIC is majorly directed at treating the underlying disease, but supportive care may be essential. This care may involve supplementing the reduced coagulation factors and endogenous coagulations inhibitors, and of inhibiting coagulation by different anticoagulant strategies, or by exploiting the fibrinolytic system [4].

DIC could be initiated or be as a result of complication of some clonal diseases or others that involve normal production of platelets and other clotting factors such as leukaemia.

Leukaemia is a group of malignancies that normally starts in the bone marrow and causes the production of raised numbers of abnormal white blood cells [5]. Leukaemia can be generally classified as acute or chronic and can additionally be classified as myeloid or lymphoid depending on the cell line that is affected. The cause of leukaemia remains unidentified. A higher risk is associated with certain chemicals (benzene), with large doses of ionizing radiation, and infection with specific viruses (e.g., Epstein-Barr virus, human lymphotropic virus). Smoking cigarettes and exposure to electromagnetic fields also have been suggested as predisposing factors [6]. In patients with leukaemia, the proliferation of malignant hematopoietic cells in the bone marrow with frequent spillage into the peripheral blood leads to a decrease in the number of normal circulating blood cells resulting into symptoms related to anaemia, neutropenia and thrombocytopenia [7].

Acute Promyelocytic Leukaemia (APL) has been associated with multiple haemostatic abnormalities [8]. Most, if not all, patients with APL have signs of DIC at the time of diagnosis. Patients with APL have an increased risk of death during initiation therapy when compared with patients with other forms of leukaemia, mostly due to bleeding.

Unfortunately, outside of clinical trials the rate of early death in APL has not changed with the advent of new therapies [9].

Disseminated intravascular coagulopathy is a critical clinical condition and has been demonstrated in some individuals with acute leukaemia [10]. There is paucity of information on the presence of disseminated intravascular coagulopathy in different types of acute leukaemia and other forms of chronic leukaemia in this environment. It is, therefore, necessary to investigate DIC in individuals with different forms of leukaemia treatment for the possibility of management of DIC alongside leukaemia in such situations.

MATERIALS AND METHODS

Subject Selection

One hundred and sixteen (116) subjects consisting of fifty eight (58) patients comprising of 30 females and 28 males with acute myeloblastic leukaemia (AML), chronic lymphocytic leukaemia (CLL) and chronic myelocytic leukaemia (CML) who are currently under treatment and 58 age-sex matched control subjects who consented were recruited into this study from the Federal Teaching Hospital, Ido–Ekiti, Nigeria and the University College Hospital, Ibadan, Nigeria. Ethical approval was obtained from the Federal Teaching Hospital Ido-Ekiti, Ekiti State.

Blood samples collection and analysis

Four millilitres (4 ml) of peripheral blood was collected from each patient and control subjects that have given consent to participate in the study with 2 ml dispensed into the 0.25ml of trisodium citrate anticoagulant (anticoagulant, blood ratio, 1:9) for the coagulation studies; with 2 ml dispensed into EDTA contained vials for the complete blood count. The citrated blood for coagulation studies was separated by centrifugation at 1500 rpm for 15 minutes to obtain platelet rich plasma which was stored frozen at – 20°C until analysed.

The parameters estimated in this study were prothrombin time, partial thromboplastin time with kaolin, international normalised ratio using Diagen reagents; D-dimer using Tina Quant Gen 2 D-dimer reagent on Roche Cobas C 111 analyser by immunoturbidometry method, packed cell volume, platelet, and white blood cell counts were run on Abacus junior haematological auto analyser.

Statistical Analysis

The data generated was expressed as mean ± SD. A p-value < 0.05 was considered the significant levels of the statistical analysis using SPSS version 20.

RESULTS

The mean±S.D of various parameters estimated in all participants involved in this study are represented in Table 1. This reflects a significant difference between the patients when compared with the control subjects with the patients demonstrating prolonged clotting times as well as elevated counts than the controls ($p<0.05$). Platelet counts however reduced significantly in the patients than the control subjects.

Additionally, the PT and PTTK distribution among the D-dimer level in the leukaemic subjects is represented in Table 2. It was observed that 86% of the patients had increased D-dimer and prolonged PT while 48% had increase D-dimer and prolonged PTTK with 5% having a prolonged PT, PTTK, INR and increased D-dimer level.

Figure 1 demonstrates the correlation between the coagulation parameters and the type of leukaemia. Chronic lymphocytic leukaemia (CLL) display a strong relationship with 55% (32), being CLL patient, having increased D-dimer.

118 Table 1: Mean±SD of estimated Parameters in the Leukaemia patients and control subjects.

Parameters	Patients n = 58 Mean±SD	Control n = 58 Mean±SD	t-test	p-value
D-dimer(µg FEU/mL)	3.74±3.13	0.31±0.18	5.888	0.001
PT(seconds)	67.59±55.71	13.10±1.06	5.266	0.001
PTTK(seconds)	77.34±31.81	31.19±2.16	7.796	0.001
Platelet count (cells/mm^3)	193.62±102.79	233.69±59.34	1.818	0.074
WBC(cells/mm^3)	74±124.42	5.08±1.34	3.506	0.001
PCV (%)	30.07±5.38	37.80±4.63	5.870	0.001
INR	1.84±0.09	1.11±0.02	7.705	0.001

MOVE HERE TO KEEP COAGULATION STUDIES TOGETHER

ABBREVIATIONS =

119 PCV (Packed Cell Volume), WBC (White Blood Cell Count), PT (Prothrombin Time), PTTK

120 (Partial Thromboplastin Time ~~with Kaolin~~), INR (International Normalised Ratio).

121 Table 2: Distribution of D-dimer levels among the PT and PTTK levels in the leukaemia

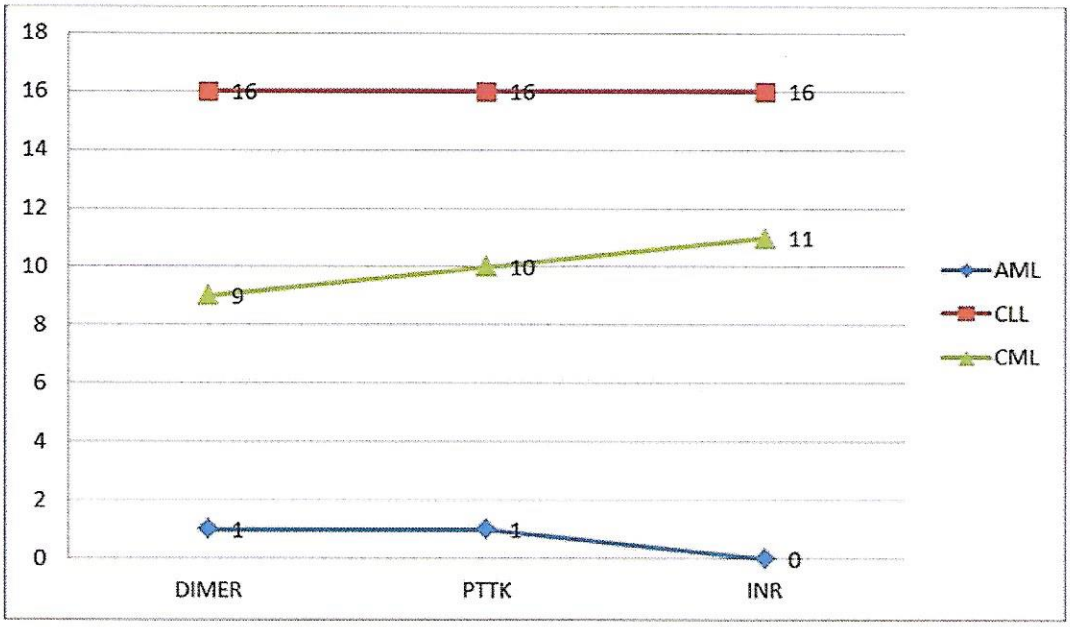
122 subjects.

	D-dimer		
	NORMAL	HIGH	P value

PT	NORMAL	0	2(3.5)	0.731
	HIGH	6(10.3)	50(86.2)	
PTTK	NORMAL	0	4(6.9)	0.619
	HIGH	6(10.3)	48(82.8)	

WHAT ARE THESE NUMBERS IN PARENTHESES ??? IF THEY ARE JUST EXPECTED FREQUENCIES, NO NEED TO SHOW, JUST SPECIFY YOU USED THE χ TEST.

123 PT (Prothrombin Time), PTTK (Partial Thromboplastin Time with Kaolin)



124

125 FIGURE 1: Correlation between the type of leukaemia and coagulation parameters.

126 PT (Prothrombin Time), PTTK (Partial Thromboplastin Time with Kaolin), INR

127 (International Normalised Ratio).

I DON'T UNDERSTAND THIS GRAPH. WHAT ARE THE NUMBERS, TEST RESULTS OR NUMBER OF PATIENTS? PLEASE SPECIFY. "CORRELATION" IS A STATISTICAL TERM, ASSESSED BY THE PEARSON "R" SCORE, I DON'T SEE THAT HERE. IS THIS JUST A COMPARISON? WHAT IS IN THE "Y" AXIS?

128 **DISCUSSION**

129 ~~Disseminated intravascular coagulation~~ (DIC) is a dynamic process and the analysis

130 performed shows only the conditions at any given moment in time [11]. In clinical

131 circumstances associated with this disorder repeating these tests helps in establishing the

132 diagnosis. Analysis performed in assessing the haemostatic state give information about the

clinical course and the diagnosis is established based on clinical suspicion and supportive laboratory analysis [11]. The analyses performed include laboratory parameters indicative of procoagulant and fibrinolytic substance activation, inhibitor consumption and organ damage or failure [12]. Prothrombin time (PT), partial thromboplastin time with kaolin (PTTK) and thrombocyte count show the consumption and activation of thrombocytes [11].

Malignancy is associated with a hypercoagulable state and a high risk for thrombohemorrhagic complications. Its clinical complications may range from localized thrombosis to bleeding of varying degrees of severity because of disseminated intravascular coagulation (DIC), with life-threatening bleeding being frequent in acute leukemia, particularly in acute promyelocytic leukemia (APL) [13]. Laboratory assessments show profound hemostatic imbalance in this condition, with activation of coagulation, fibrinolysis, and nonspecific proteolysis systems [13].

Some coagulation parameters were estimated to assess disseminated intravascular coagulopathy in leukaemia patients in this study. The parameters estimated are; Prothrombin time (PT)/INR, partial thromboplastin time with kaolin (PTTK), platelet, packed cell volume (PCV), and white blood cell count (WBC). It was observed that the patients had a lower PCV ($p < 0.05$) and platelet ($p > 0.05$) values when compared with values obtained in the control subjects (Table 1) reflecting anaemia of chronic disease in the leukemic patients investigated or as a result of cytotoxic therapy being administered. This established fact was validated by a previous study carried out by Akanni *et al.* (2010) [14] where a significant difference was observed in PCV values of similar patients as compared with controls.

The reduced mean±SD platelet observed is also suggestive of platelet excessive consumption, which is indicative of likely occurrence of DIC, resulting in the platelets being used up, hence bleeding. In a similar study by Sadik *et al.* (2014) [14], a multicenter meta-analysis where

they specified that the most frequent encountered abnormal laboratory findings in DIC were listed as thrombocytopenia, increased fibrin degradation products, prolonged PT, PTTK and low fibrinogen levels. Also, this study was corroborated by a study carried out by Laforcade *et al.* (2003) [16], where it was reported that inactivation and excessive consumption of platelets and clotting factors result in concurrent paradoxical bleeding, which is a sign of DIC. The previous reports corroborates with the results of this study.

The PT, PTTK and INR of the leukaemia patients in this study were found to be significantly prolonged ($p<0.05$) than those of the control subjects which were within normal range (Table 1). This is observed to be due to the exhaustion of some coagulation factors involved in the extrinsic pathway in the leukaemic subjects which is suggestive of DIC. This finding is similar to the report of another study carried out by Amr *et al.* (2014) [17], where they discovered the leukaemic subjects to have prolonged parameters than controls ($p<0.05$). Sadik *et al.* (2014) [15] also concluded in a study that prolonged PT, PTTK and INR is a common finding in DIC.

The increased D-dimer level in the studied patients along with the increased PT/PTTK observed in this study indicates an increased fibrinolytic activity due to the increased fibrin deposition by coagulation process in the leukaemic subjects, since D-Dimer is a fibrin degradation product and hence an increased D-Dimer level is a diagnostic factor of DIC.

The mean±SD sex distribution for the WBC, platelet and the haemostatic parameters carried out in this study displays an insignificant statistical difference between the genders which infers that gender has no effects on these parameters measured. However, there was a statistically significant difference ($p<0.05$) in the PCV, thus showing that gender plays a major role in the PCV values of the subjects.

Furthermore, 86% of the patients were observed to have increased D-dimer and prolonged PT, while 48% had increased D-dimer and prolonged PTTK (Table 2), indicating the development of DIC, and 5% having a prolonged PT, PTTK,INR and increased D-dimer level establishing the onset of DIC symptoms which is evidenced by some bleeding episodes experienced by the patients. This observation is corroborated by a study that ascertained the prolonged PT/PTTK and D-dimer as diagnostic tools for DIC [11].

This study also revealed a strong relationship between the coagulation parameters and the type of leukaemia (Figure 1) establishing the fact that leukaemia presents with abnormalities in laboratory analysis of blood coagulation, even without clinical signs with the disorders demonstrating varying degrees of blood clotting activation and characterize the hypercoagulable state [18].

In conclusion, majority of the haemostatic parameters analysed in the leukemic patients were observed to be abnormal, with the PT, PTTK, INR being very prolonged and and D-dimer level elevated while the platelet count decreased below the normal level in most of the subjects. These trends of results are characteristic of DIC and indicative of the condition as a result of an impaired haemostatic activity in most of the leukemic subjects recruited in the study. This study has therefore revealed strong evidence of possibility of DIC in individuals suffering from various types of leukaemia condition (ALL, CLL and CML) regardless of their gender and treatment status.

It is therefore recommended that in the management of leukemic patients, disseminated intravascular coagulopathy should be carefully screened and monitored in order to possibly detect its occurrence at the onset to promote adequate management of the disorder. DIC should also be monitored alongside the treatment and management of leukaemia patients as it could be life-threatening.

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SAME HERE: THIS SENTENCE IS TOO LONG FOR SCIENTIFIC ENGLISH. PLEASE SEPARATE INTO TWO SENTENCES

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