Haematologic Parameters In Acute Promyelocytic Leukemia Patients Treated With ALL Trans- Retinoic acid

Alaa G. Hussein* MSC (path), FICMS (path
Mohammed Sh. Ali** FICMS (path)
Maha Sh. Hassan*** FICMS (path),
Saad Sh. Mansoor**** FRC-Path
Ali M. AL- Ameri***** CABM
Raad J. Muhsin****** MSC (path),

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Summary:

Background: *Acute Promyelocytic Leukemia (APL) is commonly associated with disseminated intravascular coagulation (DIC) and early correction of coagulopathy is of vital importance. All Trans-Retinoic Acid (ATRA) is considered to be the drug of choice in the treatment of APL.*

Objective: *The work was conducted to*

1- Identify patients with APL who show laboratory evidence of DIC.

2- Study the serial changes in haemostatic parameters in APL patients treated with ATRA and to compare their results with those treated with conventional chemotherapy without ATRA.

Subjective and methods: *In this prospective study (from October 2003 to October 2005), 44 newly diagnosed, untreated APL patients were included. ATRA plus chemotherapy – treated patients were 24 while 17 patients were treated with chemotherapy other than ATRA. For each patient, a full clinical evaluation was done and hematological investigations were accomplished at time of diagnosis and repeated on day 3 and 7 of therapy.*

Diagnosis of DIC was based on finding a positive D- dimer test with hypofibrinogenaemia with or without pathologically prolonged (PT and/or APTT).

Results: *In 44 newly diagnosed, untreated APL patients studied, the age range between 6-81 years with a median of 27 years. Male to female ratio was 1.3:1.*

Before treatment all patients had anemia, thrombocytopenia, and elevated level of D – dimer. DIC was present in all patients at time of diagnosis. All parameters that showed abnormal level at time of diagnosis had returned to normality within one week in ATRA treated group, indicating that DIC has essentially resolved. By contrast, those parameters remained abnormal even on day 7 in the chemotherapy treated group. Indicating that DIC was on going.

Conclusion: *ATRA therapy in APL patients is associated with rapid improvement of coagulopathy therefore , it is justified to be used from day one of the treatment.*

Introduction:

APL which is a subtype of acute myeloid leukemia (M3 or hypergranular form), has a distinct clinical, morphological, and molecular background [1,2]. Hemorrhage is one of the most common fatal complication in patient with APL [3]. The bleeding diathesis has been attributed to DIC, resulting from the release of procoagulants from leukemic promyelocytes during senescence and chemotherapy induced lysis [4]. APL can occur at any age usually between 15-60 year. The classical hypergranular form of APL (M3) represents about 80% of all APL cases, while the microgranular variant represents about 20%. APL mostly arises denovo i and is associated with t (15; 17) translocation in more than 95% of cases[5,6]. Peripheral blood cell findings reveals: anemia (as a constant feature), and thrombocytopenia (is nearly always present at time of diagnosis). The distinctive cytological features are sufficient to permit diagnosis despite the low blast percentage[7,8]. Bone marrow aspirate may clot during attempted aspiration as a consequence of the hypercoagulable state, but usually sufficient cells are obtained for the diagnosis. The marrow always contains the leukemic promyelocytes[9]. ATRA is a natural metabolite of Retinol. ATRA alone or with a low dose of cytosin arabinoside can give complete remission in over 90% of newly diagnosed APL[10,11].

**Assistant Prof.,), College of Medicine/ AL-Nahrain Univirsity.*
*** Lecturer, , College of Medicine/ Kerbalaa Univirsity.*
****Lecturer, College of Medicine: AL- Nahrain Univirsity.*
******Professor, , College of Medicine: AL- Nahrain Univirsity.*
****** Assistant Prof., , National center of haematology, AL-Mustansiriya Univirsity.*
******* Assistant Prof., FICMS (path), College of Medicine: AL- Nahrein Univirsity*

Subject and methods

In this prospective study from October 2002 to October 2005, a 44 newly diagnosed untreated APL cases who were admitted to Baghdad Teaching Hospital, Baghdad Medical City, and AL-Kadhimiya Teaching Hospital, 24 of those patients

were treated with ATRA plus the conventional chemotherapy, while 17 patients were treated with chemotherapy only (because of the unavailability of the drug). Three patients deceased before starting treatment.

Control plasma was obtained from normal blood of healthy volunteers. Pooled plasma from at least 4 healthy individuals were prepared and stored frozen at – 20 C to be used simultaneously with patients' plasma. Positive and negative control plasma for D-dimer test was supplied with the kit.

A 5.5 ml venous blood sample was collected from each patient, and was immediately processed as follow[12]:

1- 4.5 ml of blood was added into plastic tubes containing 0.5-ml trisodium citrate. The specimen is immediately centrifuged at 3000 rpm for 10 min to obtain platelet poor plasma. The obtained plasma was aspirated into 2 plastic tubes (1ml plasma for each), one to be stored at –20 C for further assessment when required and the other used for the performing PT, PTT, plasma fibrinogen level, and D-dimer test.

2- One ml of blood was added into EDTA containing tube. The sample was kept at room temperature to be used within less than one hour for peripheral blood film, PCV, WBC, and platelet count. All coagulation tests were done according to the standard technical procedures by manufacturer instruction of each kit. All tests were done before treatment, 3 and 7 days after treatment.

Results:

In this study there were 25 males and 19 females, and male: female ratio was 1.3:1. M3 type constitutes 33(75%) of cases type and the remaining 11 cases (25%) were of M3 variant. Only 3 cases (6.8%) were younger than 15 year.

Table (1): The main presenting clinical features of the studied APL

Presenting clinical feature	No. (%)
Anemia	44 (100)
Pyrexia	36 (81.8)
Bleeding manifestation	25 (56.8)
Splenomegaly	15 (43)
Hepatomegaly	13 (29.5)
LAP*	7 (15.8)

*:Lymphadenopathy

Table (2):Haematological parameters before, 3 and 7 days after treatment with ATRA and chemotherapy.

Haematological parameters		Before treatmer	3 days after treatme	7 days after treatm
PCV (%) Total	+ X + X±SD Range	24±7.9 16-26	29±6.9 26-32	29±6.8 25-33
Platelet count (10X9/L)	X± SD Range	24.5±3.1 16-60	39±30 32-67	40±31 31-69
WBC count (X109/L)	X± SD Range	26±61 0.5--±150	21±10 0.2-70	7±3.2 0.2-30
Leukaemic blast count(X109/L)	X± SD Range	24.5±61 0-136	11±8.6 0-66	5±6.1 0-18
Neutrophil cell count (X109/L)	X± SD Range	1.25±2.4 0-4	1±0.7 0-4	1±0.6 0-3
Lymphocyte cell count(X109/L)	+ X±D Range	0.4±1.5 0-1.5	0.5±1 0-1	0.8±0.7 0-1
Monocyte cell count(X109/L)	X± SD Range	0.15±1.3 0-1	0.2±0.6 0-0.9	0.2±0.6 0-0.6

Table (3):Haematological parameters 3 and 7 days after treatment with chemotherapy.

Haematological parameters		3 days after treatme	7 days after treatmer
PCV (%) Total	+ X + X±SD Range	29±7.2 26-31	28±6.1 26-31
Platelet count (10X9/L)	X± SD Range	41±29 31-65	40±28 30-68
WBC count (X109/L)	X ±SD Range	14±10 0.2-78	8±4.5 0.2-32
Leukemic cell count(X109/L)	X± SD Range	13±9.2 0-71	4±5.1 0-16
Neutrophil cell count (X109/L)	X ±SD Range	1±0.6 0-5	1±0.6 0-2.5
Lymphocyte cell count(X109/I	+ X± SD Range	0.5±1 0-1	0.4± 1 0-1
Monocyte cell count(X109/L)	X+ SD Range	0.2+0.5 0-0.8	0.2-0.5 0-0.5

Table (4): Haemostatic parameters of APL patients before treatment, 3 and 7 days after treatment with ATRA and chemotherapy.

Haemostatic parameters		Before treatment	3 days after treatment	7 days after treatmen
BT (min)	X±SD Range	15±9 5-28	9±6 5-14	6.5±5 4-12
PT of patients (sec)	X±SD Range	21.5±3 12.5-29.5	14±3 12-19	14±3 12-17
PT of controls (sec)	X±SD Range	13±1 12-14	13±1 12-14	13±1 12-14
PTT of patients (sec)	X±SD Range	50.5±5 39-84	42±5 38-51	40±5 37-49
PTT of controls (sec)	X±SD Range	40±4 39-44	40±4 38-43	40±4 37-43
Fibrinogen level (g/L)	X±SD Range	0.9±0.6 0.5-2.8	1.3±1.1 1.1-1.4	1.8±0.5 1.6-2.3
D- dimer positive reaction	X±SD Range	44	2±1 0.5-4	< 0.5

Table (5): Haemostatic parameters of APL patients 3 and 7 days after treatment with chemotherapy.

Haemostatic parameters		3 days after treatment	7 days after treatmen
BT (min)	X±SD Range	9±6 5-13	6.5±5 4-12
PT of patients(sec)	X±SD Range	14±3 13-18	15±3 12-20
PT of controls (sec)	X±SD Range	13±1 12-14	13±1 12-15
PTT of patients(sec)	X±SD Range	44±5 38-57	42±5 38-49
PTT of controls(sec)	X±SD Range	40±4 38-44	40±5 38-43
Fibrinogen level(g/L)	X±SD Range	0.7±0.5 0.4-1.3	1.1±0.5 0.6-1.4
D- dimer positive reaction	X±SD Range	16±8.6 8-32	8±5.1 4-16

Table (6): Bone marrow aspirates findings in APL patients at time of diagnosis.

Bone marrow findings		Bone marrow aspirate No. (%)
Degree of leukemic cell infiltration	Moderate	4 (9)
	Heavy	40 (90.9)
Megakaryocyte	Adequate	1 (2.3)
	Reduced	42 (95.4)
	Absent	1 (2.3)
Erythrocyte	Reduced	44 (100)
	absent	0 (0)

Discussion

In the present study, the distribution of APL patients showed M3 type predominance compared to M3 variant, with a ratio of 3:1, which agrees with the results of most workers abroad and in Iraq [13,14].

APL was predominating in adults (40- 44 year) in 90.9% of cases. Other workers gave the same conclusion [14,15]. The main presenting clinical features of APL patients were symptoms of anemia, bleeding manifestations, pyrexia, bone pain, and joint pain.

On examination pallor was evident in all patients, bleeding signs in 29 (65.9%), splenomegaly in 15 (34%), and hepatomegaly in 13 (29.5%). These features are in accordance with that of other studies and also with a study in Iraq[16].

Moderate to sever anaemia was present in all patients. Severe thrombocytopenia (platelet count ≤ 20 x 10^9 /L) was found in 31 (70.5%). Leukopenia was found in 33 (75%) of cases(median count of 1 x 10^9 /L). Leukocytosis was found in 11 (25%) of cases (with a median count of 51 x 10^9/L, ranging from 21 to 150 x 10^9/L) (Table: 1). These results are consistent with results of previous studies[14,15].

Haemorrhage is one of the most common fatal complications in-patients with APL. In this study, 29 (65.9%) had bleeding signs at time of diagnosis, 3 (9.3%) of them had died because of severe bleeding before starting induction-remission therapy and 2 (6.2%) had died on day 4 of therapy (chemotherapy without ATRA). These findings are consistent with findings of other studies[16].

40 (90.9%) showed heavy leukaemic cell infiltration, 43 (97.7%) showed reduced megakaryocyes, and 44 (100%) showed reduced erythropoiesis (Table: 6) .These findings are consistent with findings of other studies[17].

BT was prolonged in 40 (90.9%) of patients before treatment and was returned to normal in 30 (75%) on day 3 of treatment and 34 (87.2%) on day 7 of treatment. All patients had received platelet transfusion. There is an inverse relation between BT and platelet count. These findings are consistent with findings of other studies in Iraq and outside. Bleeding signs were present in 29 (72.5%) patients who had prolonged BT before treatment and absent in 11 (27.5%) of them. This agrees with the known observation that abnormal BT does accurately predict clinical bleeding and that BT should not be used predict hemorrhage[18].

PT was prolonged at time of diagnosis in 28 (63.6%) while all patients had laboratory evidence of DIC. Many studies had showed that PT is variable in DIC (clinical and subclinical) being prolonged in 27%, 30%, and 77%, respectively[19,20].

PTT was prolonged at time of diagnosis in 26 (59%). Studies showed that PTT is variable in cases of DIC, begin prolonged in not more than (60%)[19,20].

On day 3 of treatment, 3 (20%) of chemotherapy-treated patients showed prolonged PT and PTT although they were normal at time of diagnosis; however, they had no bleeding manifestation (subclinical DIC).

Hypofibrinogenaemia was present in 42 (95.5%) of patients at time of diagnosis. Many studies had showed that plasma fibrinogen level is variable in cases of subclinical DIC. Hypofibrinogenaemia was reported to be present in 60,80, and 100% of cases [21,22].

In the absence of liver disease, hypofibrinogenaemia is caused by, hypofibrinolysis. So that 42 (95.5%) of patients had, hypofibrinolysis because they had normal liver function tests. Fibrinogen, as an acute phase reactant, can show normal level in cases of hypofibrinogenaemia associated with raised acute phase reactants. Hypofibrinogenaemia together with thrombocytopenia had statistically significant relation (P value < 0.05) with bleeding manifestations. This result agrees with results of other studies.

Plasma D-dimer test was positive in 44 (100%) of patients at time of diagnosis with a median value of 6ug/ml. This finding is consistent with findings of previous studies, which showed positive results in 100% of cases with a median of 13ug/ml. The differences in the median values were due to the different methods. False positive tests can occur in the presence of high titer of rheumatoid factor. In the studied patients, no patient had arthritis on clinical background and the positivity of D-dimer test was associated with clinical and laboratory

features of DIC; however, rheumatoid factor assay is sometimes necessary[21,22].

The results demonstrate that ATRA therapy in-patient with APL and DIC rapidly improves abnormal haemostatic parameters. These data are consistent with previous reports, which showed favorable outcome of coagulopathy in ATRA-treated APL patients. The earliest indicators of response to treatment with ATRA was reported to be a normalization of coagulopathic abnormalities such as hypofibrinogenaemia and increased plasma D-dimer concentration which were clearly found in this study.

Red cells and platelet transfusions during therapy can play a role in improving haemostatic ;however, all patients included in this study had been transfused; however, no improvement was seen in the chemotherapy-treated group until day 7 of treatment.

Because of ATRA promotion of terminal differentiation of APL cells, it should cause only minimal leukaemia cell lysis and, in turn, no appreciable release of procoagulants and fibrinolytically active substances from these cells[23].

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