Chattagram Maa-O-Shishu Hospital Medical College Journal
Volume 14, Issue 2, July 2015

Original Article

A Prospective Study on the Effectiveness of Arsenic Trioxide (ATO) in Remission Induction of Acute Promyelocytic Leukemia (APL)

Mohammad Abdullah Al Anis[1*]
Mohiuddin Ahmed Khan[2]
Salma Afrosa[2]
M Sirajul Islam[3]
Akhil Ranjan Biswas[2]
Tasnim Ara[2]
Muhammad Ali[2]
Manirul Islam[2]
Humayra Naznin[2]
Mafruha Akhter[2]
A K M Mynul Islam[2]
Muhammad Kamruzzaman[2]
Abdullah Az Zubayer[2]

[1]Department of Hematology
International Islamic Medical College Hospital (IIMCH)
Chittagong, Bangladesh.

[2]Department of Hematology
Dhaka Medical College, Dhaka, Bangladesh.

[3]Department of Hematology
Mymensingha Medical College
Mymensingha, Bangladesh.

*Correspondence to:

Dr. Mohammad Abdullah Al Anis
Assistant Professor
Department of Hematology
International Islamic Medical College Hospital (IIMCH)
Chittagong, Bangladesh.
Mobile : +88 01672647236
E-mail: anis_cmc@yahoo.com

www.banglajol.info/index.php/CMOSHMCJ

Abstract

Background: Arsenic Trioxide (ATO) as a single agent, has proven efficacy in inducing molecular remission in patients with Acute Promyelocytic Leukemia (APL). It is commonly used to treat relapsed APL. But there is Limited study on ATO in the management of newly diagnosed cases of APL. The concerned study was done to evaluate the effectiveness and outcome of ATO in remission induction of new cases of APL in the context of a limited resource hospital in Bangladesh. **Methods:** From March 2008 to March 2010, 20 patients with Promyelocytic Leukemia (PML) / Retinoic Acid Receptor α (RAR α) + newly diagnosed APL were enrolled. All patients were treated with a regimen of single-agent arsenic trioxide till remission at our center. After remission the regimen was administered on outpatient basis. **Results:** Overall 15 (75%) patients achieved complete hematological remission. 12 (80%) patients achieved molecular remission after induction phase and 3 (20%) after completion of consolidation phase. At a median follow up of 36 months (Range 25 -44 months), Disease Free Survival (DFS) and Overall Survival (OS) 86.6% and 85.3% respectively. Relatively young patients with long form of t (15;17) had shown good response with this response. However, the response is slower than All Trans Retinoic Acid (ATRA). Patients presenting with high White Blood Cell (WBC) count, low platelet count, variable form of t (15;17) are found to respond poorly. The toxicity profile, in the majority, was mild and reversible. Treatment cost was also reduced than that of conventional regimen. **Conclusion:** Single-agent arsenic trioxide as used in this study in the management of newly diagnosed cases of APL is safe, cost beneficent and is associated with durable responses. But additional interventions as combining ATRA with ATO would probably required in high risk cases.

Key words: Arsenic trioxide; Acute Promyelocylic Leukemia (APL); All Trans Retinoic Acid (ATRA); Hematology; White Blood Cell (WBC).

INTRODUCTION

Acute Promyelocytic Leukemia (APL) is a distinct subtype of acute myeloblastic leukemia, where leukemic cells that efface bone marrow are abnormal promyelocytes. Clinically patients may develop prominent hemorrhagic manifestations due to procoagulants released from promyelocytes, and 't' (15:17) cytogenetic change which presents in over 95% cases, helps to identify it as separate entity[1]. Additional changes like trisomy 8, isochromosome 17 do not have negative impact on overall prognosis[2]. Delay in it's treatment can kill the patient earlier than any other acute leukemia[3].

Currently treatment with combination of All-Trans Retinoic Acid (ATRA) with anthracyclin (Idarubicin / Daunorubicin) has made dramatic improvement with survival.

However, minimal residual disease is present in many patients after induction therapy. Prolonged administration of ATRA could results in clinical resistance due to inability to sustain effective concentration in vivo that would be required to achieve cyto-differentiation. Cytochrome p 450 induction, Point mutation in the RAR α fusion gene and mutation in the high affinity ATRA binding site may explain resistance[3]. In clinical practice, it is not rare to find patients with relapsed APL after getting ATRA reflecting the need for more effective alternative treatment. Major disadvantage, what we face in Bangladesh, is ATRA's high cost. Adding the cost of other supportive therapy, treatment actually goes beyond the reach of our poor people.

Arsenic trioxide (ATO) is a new and promising alternative drug for APL treatment. In different multi central trials at USA, ATO is proved to be effective to achieve complete remission in relapsed or refractory cases. But it does not penetrate blood brain barrier which is accounted in cases of extra medullary relapse[4]. This drug triggers modulation or degradation of PML-RAR α onco-protein and induces apoptosis of malignant cells. It may also relieve transcriptional repression mediating partial differentiation of abnormal promyelocytes[5]. Currently use of ATO is being explored in newly diagnosed patients as part of induction and consolidation therapy in USA & Europe[3]. Such trials have already been conducted at India and China showing promising results. Since, Arsenic trioxide induces both differentiation and apoptosis of promyelocytes, chance of resistance and relapse may be low, although chances of arrhythmia, hepatotoxicity and other adverse effects may occur[5,6]. Arsenic may be stored in liver, kidney, heart, lung, hair, nail etc. for long time[7]. However, it is available at a cheaper rate than that of ATRA. The purpose of our study is to evaluate the response by arsenic trioxide and document whether it can be more cost effective and suitable for new patients with APL in our country.

MATERIALS & METHODS

A trial using arsenic trioxide as a single agent in newly diagnosed cases of APL was initiated on March 2008 at Hematology Unit, Dhaka Medical College Hospital for the very first time in Bangladesh. Approval from ethical review committee of the institution was taken. After taking history, physical examination and morphological investigations, patients were diagnosed as APL on French American British (FAB) criteria. The diagnosis subsequently confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assay for PML-RAR α transcripts. Twenty (20) new patients having PML-RAR α positive with a age range of 10 to 60 years were included as candidate for single arsenic trioxide. Informed written consent was taken from patients or guardians. At the onset, exclusion criteria were pregnant, age < 10 years or older than 60 years, PML-RAR α negative APL and previous history of cardiac disorder.

Intravenous Arsenic Trioxide

Arsenic trioxide (commercial preparation –'Arsenox' Made in India) was administered diluting 10 mg per 10 ml vial in 100 ml normal saline and infusing it intravenously at a dose of 0.15 mg/kg of body weight over 1-2 hour daily through central venous line till complete hematological remission or maximum 60 days. Following 3-6 week interval, for those continuing to remain in Complete Remission (CR) single agent ATO was administered at the same dose, 5 days a week for total 5 weeks (25 days) as consolidation at outpatient department.

Monitoring

Complete Blood Count (CBC) was done twice weekly in induction phase, once weekly in consolidation phase and at each subsequent visit in maintenance and follow up. Bone marrow analysis was done when 2 sequential CBCs were consistent with CR. If the bone marrow analysis showed evidence of persistent disease, then ATO was continued and bone marrow analysis was repeated at 10 days intervals until CR was documented. Sodium, potassium, calcium, magnesium, was also monitored in the same way in induction. Once the patients are clinically stable, the frequency of testing was reduced to once a week in induction. Unless clinically indicated, these tests were not monitored in consolidation and maintenance phase.

Liver Function Tests (LFTs) renal function tests including creatinine, coagulation parameters such as Prothrombin Time (PT) activated Partial Thromboplastin Time (aPTT) etc. were done as needed. Electrocardiogram (ECG) was done only if clinically indicated.

Supportive care

Platelet concentration was transfused to maintain platelet count higher than 50 x 10^9/L. Fresh Frozen Plasma (FFP) was infused (12-15ml/kg of body weight) if the PT, APTT was deranged and red cell concentrations were transfused to maintain hemoglobin level higher than 8 g/dl. Antibiotics and antifungal drugs were given for fever as required. Supplemental electrolytes were administered as required to maintain electrolyte level within normal range, especially, serum potassium (K+) and serum magnesium (Mg++) level above 4 mEq/L and 1.8 mmol/L respectively to prevent cardiac arrhythmia. One or two doses of anthracyclines were administered at the clinician's discretion if there was a rapidly progressive leukocytosis defined as rise higher than 30 x 10^9/L in the first week or higher than 50 x 10^9/L in the second week leukocyte count, or, if patients developed a differentiation syndrome or if, there was leucocyte count higher than 50 x 10^9/L at presentation.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

To assess molecular remission RT-PCR analysis for PML-RAR α fusion transcript was done at diagnosis, at the end of induction at the end of consolidation on Day-36, then 6 monthly for 5 years.

Maintenance

During maintenance phase, All Trans Retinoic Acid (ATRA) [Brand- Cap. Vesanoid (10 mg)] at 45 mg/m^2 from day:1 to day:15 -- 3 monthly, 6-MP – 75 mg/m^2/d and MTX- 20 mg/m^2 once weekly were prescribed for 2 years. Bone Marrow Study (BMS) & RT-PCR for PML-RAR α were done 3 monthly and 6 monthly respectively.

Follow up

After completion of therapy, patients were scheduled to follow up once in 3 months for the first 2 years and once in 6 months for the next 3 years.

Definition of Outcome

Complete hematological remission is defined as absence of clinical evidence of APL, Absolute Neutrophil Count (ANC) higher than 1.5 x 10^9/L, an unsupported platelet count more than 100 x 10^9/L and bone marrow analysis showing normocellularity to moderate hypercellularity with less than 5% blasts plus promyelocytes. Molecular remission is negative PML-RAR α mutation on RT-PCR assay. Molecular relapse was defined as 2 consecutive positive RT-PCRs at an interval of one month apart after achieving molecular remission.

Monitoring toxicity

Toxicities were documented using the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) version 2.0 (NCI-CTC v2.0; Bethesda, MD). Therapy with ATO was discontinued for any grade 3 or 4 toxicity and the patient was re challenged once the abnormality was corrected or if the grade reduced to less than 3. Criteria for diagnosis of a differentiation syndrome or retinoic acid syndrome were those defined in earlier studies[8,9].

RESULTS

Patient's baseline characteristics

Between March 2008 and March 2010, 20 patients with PML-RAR α + APL were treated with this regimen. Three additional patients were excluded. Two died shortly after admission before initiation of therapy and one left the hospital to go abroad at day-12 after therapy began. The baseline characteristics of the patients are summarized below.

Table 1 : Baseline characteristics of patients.

Characteristics	Number	(%)	Mean
Age (years) range			
11-20	7	35	27.9 years with SD +- 10.37
21-30	5	25	
31-40	6	30	
41-50	2	10	
Sex			
Male	11	55	
Female	09	45	
Clinical Feature at presentation			
Bleeding	18	90	
Fever	15	75	
Blurring of vision	07	35	
Nausea, vomiting	07	35	
Headache	03	15	
Weight Loss	02	10	
Jaundice	01	05	
Oral ulcer	01	05	
Hb g/dl			
Less than 8	14	70	
More than 8	06	30	
WBC count			
Less than 10 x 10^9/L	15	75	
More than 10 x 10^9/L	05	25	
Platelet count			
Less than 10 x 10^9/L	13	65	
10-20 x 10^9/L	05	25	
More than 20 x 10^9/L	02	10	
Type of Promyelocytes			
Hypergranular	19	95	
Microgranular	01	05	
RT-PCR			
Long form (bcr 1)	09	45	
Variable form (bcr 2)	06	30	
Short form (bcr 3)	05	25	

Treatment outcome

Among 20 patients, complete remission was achieved in 15 (75%) patients, whereas 5 (25%) of them died before completion of induction phase. Of them, three very early (Between day-1 to day-10) during induction phase due to disease related mortality as intracranial hemorrhage [2(40%)] and hemorrhagic shock [1(20%)]. One patient had intracranial hemorrhage even 3 weeks after starting ATO. Treatment related mortality was low (20%). Only one (20%) died of post ATO hepatic failure. No one died after remission is achieved till last follow up. We have observed that, after starting ATO, fever subsided in 11 patients within 14 days, bleeding stopped by first week in 4 patients, second week in 5 and third week in 4 patients. But improvement of vision and disappearance of retinal hemorrhage took longer time to resolve, often more than

2-3 weeks. Those who went to remission were recovered from thrombocytopenia by 5th to 6th weeks of treatment. [Mean time of recovery: 30.07 days with SD± 8.30]. Of 20 patients, 15 patients (75%) achieved CR between 45-60 days. [Mean: ~50 days with SD± 8.32 days].

Analysis of patient's baseline characteristics revealed increasing high WBC count (>10 x 10^9/L), low platelet count (<10 X 10^9/L), increased promyelocyte percentage (>50%) in bone marrow and variable form of t (15;17) are associated with delayed or poor response. Those with long form of t (15;17) showed better and early response. RT-PCR was repeated. Out of fifteen patients 12 (80%) became negative for PML-RAR α after induction phase was completed and 3 (20%) became so after completing consolidation. Only 2 patients relapsed and no one died till last follow up. Finally cost of treatment was calculated after completion of consolidation phase and compared with that of ATRA from our previous experience. It clearly reveals the low cost (Tk-80,000-120,000) of treatment with arsenic trioxide, which might bring hope to the poor patients of our country.

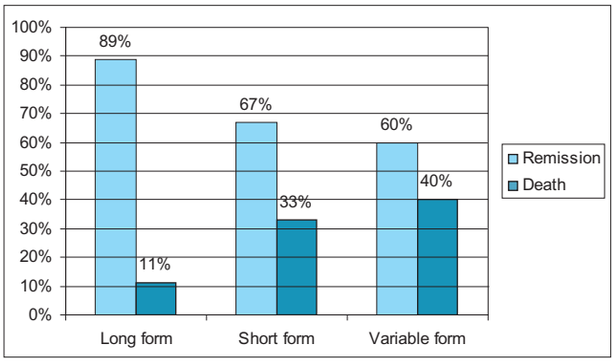

Figure 1 : Relationship of different molecular forms of t(15;17) with treatment outcome(n=20).

Toxicity profile

Most common adverse effects of arsenic trioxide were weight gain [16(80%)] and headache [15 (75%)] during induction phase. Beside these, some patients developed hyperglycemia [5(25%)], severe muscle pain [5(25%)] which coincides with leukocytosis, jaundice [3(15%)] and edema [3(15%)]. In our study, only one patient developed symptomatic, another one asymptomatic QT prolongation which normalized after stopping ATO for a few (2-5) days and subsequently re-challenged without any further cardiac events. Almost all adverse effects were mild, reversible grade 1/2 toxicity and mostly developed within first 15 days of initiation of the therapy. Life threatening complications like APL differentiation syndrome, QT prolongation with arrhythmia are less frequent [1(5%) and 2(10%) respectively]. One patient (5%) died of hepatic failure. 12 patients (66%) had leukocytosis ranging from 13 x 10^9/L to 96 X 10^9/L after ATO infusion. WBC usually starts to rise in first 1-2 weeks and normalizes by 5th to 6th weeks.

Surprisingly, very high WBC count was associated with excruciating muscle pain in 5(25%) patients. Four (4) patients developed eosinophilia after exposure to arsenic trioxide. It was noticed at 5th week of induction in 2 patients and at 8th week in one patient. It came into notice in one patient after induction phase is completed. Eosinophilia was as high as 30% of total count of WBC. In 2 patients, it persisted even during maintenance for 2-3 months. But no one had any evidence of eosiniphilic tissue damage. Adverse effects in consolidation phase are rare and weight gain [10 (67%)], headache [11 (73%)] remain most frequent as in induction.

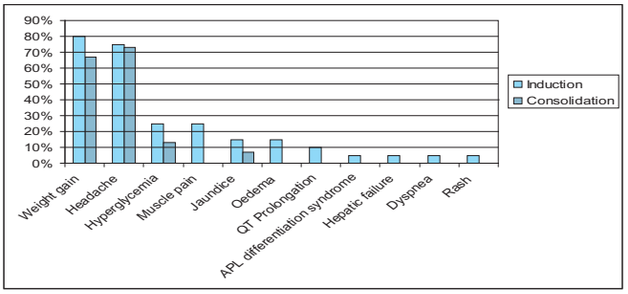

Figure 2 : Frequency of adverse effects.

Table 2 : Toxicity grading

Complications	Grade				
	0	1	2	3	4
Headache	0	12	3	0	0
Weight gain	6	8	2	0	0
Muscle pain	0	0	0	5	0
Hyperglycemia	0	4	1	0	0
Rash/Itching	0	1	0	0	0
Edema	0	3	0	0	0
Prolonged QT interval	0	0	1	1	0
Hepatic dysfunction	0	0	0	0	1
Raised SGPT	0	0	1	1	1
Raised S. Bilirubin	0	1	1	0	1

DISCUSSION

Though, combination of ATRA and anthracycline based chemotherapy is currently the standard approach to treat newly diagnosed APL, approximately 20-30% of patients eventually relapse and develop drug resistance[10].

ATO has been proven to be another highly effective agent in APL therapy. Although commonly used for refractory or relapsed APL, trials have been given specially at China and India as first line agents in newly diagnosed patients to assess its cost and efficacy, since in this part of developing world, patients are poor and ATRA is costly for them.

V. Mathews et al. at Christian Medical College Hospital, Vellore, India conducted a trial with single agent ATO on 72 patients of APL. They documented fever (62.5%) and bleeding (43.5%) as common initial clinical presentations. These two features were also common in our study (75% and 90% respectively). Frequency of different isoforms are closely similar (long forms 63.8 vs 45%, short forms 29.2% vs 30%) in both studies.[11] Though we have achieved only 75% remission, Shen et al and V. Mathews et al. found much higher CR rate (90% and 86.1% respectively)[5,11].

We have identified late referral of patients from other departments, scarcity in the supply and high cost of blood products, inadequate ICU support as causative factors for lower CR rate in our trial. Median time of the achievement of CR in our study was 50 days (range 30 – 60 days), whereas it was 31 days (range 28-38 days) in Shen's study and 42 days (range 24-70 days) in V. Mathews study[5,11]. Study also reveals that when single agent arsenic trioxide is used as induction and consolidation in the treatment of newly diagnosed cases of APL, it is associated with significant molecular remission which is comparable with conventional ATRA plus chemotherapy regimens. 41 (76%) of 54 patients V. Mathews trial and 12 (80%) of 15 patients in our trial became negative for PML-RAR α after completion of induction phase[11]. Others obtained molecular remission at the end of consolidation phase. Examination of peripheral blood cell count has revealed that recovery of normal platelet count take place within 3-6 weeks (mean, 30 days) which is similar to the observation (median 33 days for platelet recovery) by Shen et al[5]. ATO is also found to be a safe drug.

Most adverse effects in all studies are minor such as headache; musculoskeletal pain etc. and consolidation phase as well as maintenance phase remain almost now. But it is documented in 6 patients of other trial after 2-9 months events free[11]. However, we have not seen any skin hypo or hyper-pigmentation, somnolence, peripheral neuropathy which occurred in other trials[5,10, 12]. Study of Jin Zhou et al. and that of ours shows, incidence of life threatening complications like QT prolongation (1 vs 2) and APL differentiation syndrome (2 vs 1) after ATO treatment were low[10]. ATO induced leucocytosis was observed in 12 (66%) patients in both Shen et al. and our study[5]. It is considered as a sign of response to differentiation therapy in APL. We had to give inj. daunorubicin (40 mg) to three patients to reduce leucocytosis and associated severe muscle pain. V. Matews et al. also did the same[5,11].

Concerns have been raised in previous studies of the hepatic toxicity with the use of ATO. During induction phase, we observed grade 1/2 hepatotoxicity in two patients and grade-4 toxicity in one patient, who eventually died. This observation matches with an international study that noted 7 (63.33%) of 11 newly diagnosed patients developed hepatotoxicity and 2 of them failed to recover, with liver dysfunction contributing to their death[13]. In the Indian Trial, Grade 1, 2, 3 and 4 hepatotoxicity (NCICTC v 2.0) was seen in 14 (18.4%), 7 (9.2%), 4 (5.3%) and 4(5.3%) patients, respectively[14]. Transient elevation of liver enzymes were the prominent abnormality in these patients. They noted eight patients with grade 3/4 toxicity. ATO were withheld till the liver function tests had returned to a level below grade 3 for an average of 22.6 days (range: 10–28). Seven of these patients were successfully re-challenged with ATO and there were no cases of acute hepatic failure or mortality attributable to hepatic toxicity[14].

Major cause of death in all three studies were Intra Cranial Hemorrhage (ICH) [3 (60%), 2 (100%) and 7 (70%)][5,11]. Most of them died early within 2-14 days of starting therapy. These results indicate the necessity of intensive support with platelet conc. FFP etc. during initial few weeks of treatment. 2-3 patients who died late after 20 days in these trials, ICH remain one of the causes of death pointing towards the need for more rapidly acting drugs like ATRA.

The major limitation to our trial was inadequate availability and high price of blood products (platelet concentrate, Fresh Frozen Plasma (FFP) etc.) which increases chance of early mortality due to hemorrhage. Initial high WBC count (>10 x 10^9/L) and low platelet count (<20 x 10^9/L) are found to be associated with poor prognosis and increased adverse effects in both V. Mathews and our study. Finally, we have only observed two relapses till Arsenic trioxide is generally well tolerated and is associated with minimal toxicity in our study. So, after remission induction, it can be administered on an outpatient basis. In our setting, administration of an ATRA plus chemotherapy regimen is costs approximately Tk-300,000 to 450,000 only to complete induction and consolidation phase of treatment, while single agent arsenic trioxide costs approximately Tk-80,000 to 120,000 thus saving a good amount of money of poor patients. However, longer follow up is required to exclude the possibility of late relapse and long term safety of arsenic trioxide.

CONCLUSION

From the trial, we have come to a conclusion that single agent arsenic trioxide is able to induce complete hematological as well as molecular remission in a significant number of patients with acute promyelocytic leukemia and this can definitely be raised if adequate support with blood components can be ensured. Most of the adverse effects of arsenic trioxide are mild and reversible. Though uncommon, ATO can also cause arrhythmia and hepatic dysfunction which can be severe enough to kill the patient. Bleeding does not improve as rapidly as ATRA, so the chance of early mortality persists. We know, ATRA is fast acting and it can improve coagulopathy within 48 hours to 5 days[15]. So, early referral and prompt intensive treatment is essential.

RECOMMENDATION

To make the study result more authentic and widely acceptable, we recommend conducting a multi center based trial on the effectiveness of arsenic trioxide in remission induction of APL for a prolonged period of time. Trial combining ATRA with ATO at least during the initial period may be conducted to see whether treatment outcome increases. It will also enrich our knowledge regarding better and cost effective management of APL in Bangladesh.

DISCLOSURE

All the authors declared no competing interest.

REFERENCES

1. Hoffman Ronald, Benz Edward J Jr., Shattil Sanford J. et al: Haematology Basic Principles & Practice, Elsevier. 2004;4:59.
2. Shen Z. Xiang et al: All-trans retinoic acid / As2o3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia,PNAS. 2004; 101 (15): 5328-5335.
3. Greer John P., Forester John, Leukens John N. et al : Wintrobe's Clinical Hematology : Lippincott Williams & Wilkins. 2009; 1 (5) : 79-105.
4. Raanani, O Shpilberg, I.Ben-Bassat: Extramedulary disease and targeted therapies for haematological malignancies-is it real, Annals of oncology. 2007; 18 (1): 7-12.
5. Hoffbrand A. Victor, Catovsky Daniel, G.D. Tuddenham edward: Postgraduate Haematology, Blackwell publishing. 2005;5(1):1-12.
6. Beutler Ernest Lichtman Marshal A., et al: William's Hematology, Mcgraw Hill Inc.1995; (5)22 : 211-228.
7. Raffoux Emmanuel, Rousselat Philippe et al: lewCombined treatment with Arsenic trioxide and all-trans retinoic acid in patients with relapsed acute promyelocytic leukemia, Journal of clinical oncology. 2003; 12: 2326-2333.
8. Frankel SR, Eardley A, Lawuers G, Weiss M, Warrell RP Jr. The ''Retinoic acid syndrome'' in acute promyelocytic leukemia. Ann Intern Med. 1992;117:292-296.
9. Frankel SR, Eardley A, Heller G, et al. All-trans retinoic acid for acute promyelocytic leukemia: Results of the New York study. Ann intern Med. 1994; 120: 278-286.
10. Zhou Jin, Zhang Yingmei et al : single agent arsenic trioxide in the treatment of children with newly diagnosed acute promyelocytic leukemia, Blood. 2010 ; 115 (9) : 1697-1702.
11. Hu Jiong, Liu Yuan-Fang et al: Long term efficacy and safety of all-trans retinoic acid/arsenic trioxide- based therapy in newly diagnosed acute promyelocytic leukemia, Annals of oncology. 14(5) : 752-757
12. Matews Vikram, George Biju et al : singel agent arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia : durable remissiuon with minimal toxicity, Blood. 107(7) : 2627-2632.
13. Niu C, Yan H, Yu T, et al. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients, Blood. 1999;94:3315-3324.
14. Mathews V., George B., Laksmi KM. et al: Hepatotoxicity profile of single agent arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia, its impact on clinical outcome and the effect of genetic polymorphism on the incidence of hepatotoxicity, Leukemia. 2006; 20: 881-883.
15. Greer John P., Forester John, Leukens John N. et al : Wintrobe's Clinical Hematology, Lippincott Williams & Wilkins. 2009; 2 (82) : 1938-1955.