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CLINICOPATHOLOGICAL STUDY OF ACUTE PROMYELOCYTIC LEUKEMIA: AN EXPERIENCE FROM A TEACHING HOSPITAL IN INDIA

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ABSTRACT

Data on the clinicopathological features of acute promyelocytic leukemia (APL) patients from India is limited. Present study was a cross sectional study which included 18 patients of APL. Medical records of these 18 patients were reviewed to collect their clinical details and laboratory results. High risk patients (total leucocyte count >10,000/cmm) were treated with modified APML 4 protocol.Low risk patients (total leucocyte count ≤10,000/cmm) were treated with protocol APL- 0406-Intergroup Study AL WP GIMEMA-DSIL protocol. Outcomes in terms of complete remission were assessed in both these groups. Mean haemoglobin levels was 7.03gm%, mean total leucocyte count was 30,462per cmm, mean platelet count was 27,222/cmm. Bone marrow was reported as suggestive of APL in 17 cases while in 1 case, BM aspirate was inadequate. Average percentage of abnormal promyelocytes in bone marrow was 84.25%. PT was prolonged in 15 cases, while APTT was prolonged in 3 cases. Flow cytometry analysis was done in 12 patients. All patients were CD45, MPO, CD13, CD33 and CD64 positive. Chromosomal analysis was possible in 11 cases. t(15;17)(q22;21) was identified in 6 cases (54.62%). 3 cases (27.27%) showed normal karyotype. 2 (18.18%) cases had additional cytogenetic abnormalities. All patients under high risk category attained CR. 1 patient under low risk category with additional cytogenetic abnormality died 6 days after induction therapy was started. 10 (55.55%) patients developed complications such as neutropenic sepsis, intracranial hemorrhage, differentiation syndrome, cerebral venous sinus thrombosis, pseudotumorcerebri, QTc interval prolongation, and pneumonia.

KEYWORDS: Acute promyelocytic leukemia, PML-RARA, arsenic trioxide, ATRA, differentiation syndrome.

INTRODUCTION

Acute promyelocytic leukemia (APL) is a type of acute myeloid leukemia in which abnormal promyelocytes predominate and there is a typical PML-RARA translocation. It was first described to be a distinct clinical entity and was considered to be a fatal leukemia in 1957.1 It is characterized by the presence of a balanced reciprocal translocation between PML (Promyelocytic leukemia) gene on chromosome 15 and RARA(retinoic acid receptor alpha) gene on chromosome 17 resulting in the PML-RARA fusion gene formation. APL accounts for 10 to 15% of newly diagnosed acute myeloid leukemia cases.2Patients affected with APL are at a high risk to develop life-threatening coagulopathy when left untreated and thus is an aggressive subtype of acute myeloid leukemia. However, APL patients when diagnosed early and treated with All-trans-retinoic acid (ATRA) and arsenic trioxide have a good response to treatment. Thus, APL is a medical emergency and the ability to diagnose early before the disease progresses to irreversible stage is important for successful patient management. The t(15;17)(q22;q21) PML/RARA defines the disease and is the molecular basis of the treatment with ATRA.1

Data on the clinicopathological features of APL patients from India is limited. Multiple regimens are being used for the treatment of APL. This study aims to evaluate the clinicopathological features and outcome of APL patients who are categorized into high and low risk category treated with arsenic trioxide and ATRA based protocols.

PATIENTS/MATERIALS AND METHODS

Present study was a cross sectional study which included 18 patients of APL. These patients were morphologically diagnosed to have APL and later the same was confirmed by RT-PCR test for PML-

RARA fusion gene. Patients who were diagnosed as APL between January 2017 and August 2020 and subsequently treated were included in the present study.

The medical records of these 18 patients were reviewed to collect their clinical details and laboratory results (Complete blood count, prothrombin time (PT), activated partial thromboplastin time (APTT)). Complete blood count was obtained from automated hematology analyzer Sysmex XN 1000. PT and APTT were obtained from automated coagulation analyzer ECL 760.Peripheral smears and bone marrow aspirate smears were available for morphologic evaluation. They were stained with Wright Geimsa stain for microscopic evaluation.

Bone marrow (BM) aspirate samples of 12 patients were studied by flow cytometry using the machine BD-FACS-Diva 8.0.2. An acute leukemia panel included the markers, CD7, CD19, CD79a, CD11c, CD11b, CD13, CD15, CD14, CD33, CD64, CD117, MPO, CD34, CD45& HLA-DR, were studied. APL cells were gated using CD45 versus side scatter plot, generally in granulocyte region. Staining of >20% of cells was considered to be positive.

Bone marrow aspirate samples were sent for chromosomal analysis in heparin anticoagulant. Cytogenetic analysis was done on 24hr unstimulated cultures on RPMI- 1640, Hi- Karyol media. Metaphases were captured at banding resolution of 450- 550 with "G bands by Trypsin and Giemsa" (GTG) banding technique.

PML-RARA rearrangement was detected by Real time-PCR from bone marrow or peripheral blood samples, which were in EDTA anticoagulant.

TREATMENT

When APL was suspected on morphology, ATRA was started without waiting for PML-RARA results.

Patients with total leucocyte count >10,000/cmm were considered as high risk patients. They were treated with modified APML 4 protocol.3 Inj. Daunorubicin (60mg/m2 on day 1 and 2), Cap. ATRA (45mg/m2 in 2 to 3 divided doses from day 1 to 36) and Inj. Arsenic trioxide (10mg in normal saline from Day 9 to 36) were used during the induction phase. Post induction, bone marrow aspiration was done to look for complete morphological remission. Consolidation therapy (2 cycles) was started 3 to 4 weeks after end of induction. Cap. ATRA, Inj. Arsenic trioxide (from day 1 to day 28) &Inj. Methotrexate (12.5mg intrathecal on day 1 and 15) were used in each of these cycles. Maintenance therapy was given for 2 years (8 cycles) and it was started 3 to 4 weeks after the end of consolidation. ATRA was administered alone for the first 2 weeks of each cycle. Oral methotrexate and 6-mercaptopurine were given for the remainder of each cycle.3PCR for PML-RARA was done once in 3 months after completion of 2nd consolidation as per the protocol (3).

Low risk patients (total leucocyte count ≤10,000/cmm) were treated with APL- 0406-Intergroup Study AL WP GIMEMA-DSIL protocol.4 Cap. ATRA (45mg/m2 in 2 to 3 divided doses from day 1 to 36) and Inj. Arsenic trioxide (10mg in NS from Day 1 to 36) were used during induction. Cap. ATRA and arsenic trioxide were used during consolidation. Arsenic trioxide (10mg in 250ml NS over 2hrs) was administered 4 weeks and then 4 weeks off, for a total of 4 courses. ATRA(45mg/m2 per oral, in 2-3 divided doses) was given 2 weeks on and 2 weeks off for a total of 7 courses. No maintenance therapy was given for low risk patients. PCR for PML-RARA was done once in 3 months after completion of consolidation as per the protocol (4).

Supportive care: Tab. Prednisolone (0.5mg/kg) was given from day 1 to prevent differentiation syndrome. If any patient developed differentiation syndrome in spite of this ATRA and Arsenic trioxide were withheld. Further patient was treated with dexamethasone, hydroxyurea and other supportive measures. Platelet transfusions were given to maintain platelet count more than 30,000/cmm. Fresh frozen plasma was transfused to keep PT (prothrombin time), APTT(activated partial thromboplastin time) near normal. Packed red blood cells were transfused when hemoglobin (Hb) was<8g%.Arsenic trioxide was withheld when QTc interval was >500msec and was restarted once it was <460msec.

Definition of outcome: Complete remission (CR) was defined by no clinical evidence of APL with absolute neutrophil count >1500/cumm, unsupported platelet count of > 1 lakh/cumm and bone marrow aspirate smears normocellular to moderately hypocellular with <5% abnormal promyelocytes / blasts.

RESULTS

The median age of patients was 31.5 years (Range: 11 to 58 years). Out of 18 patients, there were 10 (55.55%) men and 8 (44.45%) women, with a male to female ratio of 1.25. Fever was the most common presentation in the present study and was found in 13 (72.2%) patients, followed by bleeding manifestations which was found in 10 (55.5%) patients. Generalized

weakness was present in 7 (38.8%) patients. Easy fatigability was present in 5 (27.77%) patients. Other symptoms like vomiting and breathlessness was found in 1 (5.55%) patient each. Splenomegaly and hepatomegaly was present in 2 (11.1%) patients each.

Laboratory parameters: Mean haemoglobin levels was 7.03gm% (Range: 3.9 – 11.9 gm%), mean total leucocyte count was 30,462 per cmm (Range- 340 – 2,03,440/cmm), mean platelet count was 27,222/cmm (Range: 6,000- 93,000). 12 (66.67%) cases were reported as APL on peripheral smear itself, while 6 (33.33%) were reported as pancytopenia and did not show any abnormal promyelocytes. Bone marrow was reported as suggestive of APL in 17 (94.44%) cases while in 1(5.56%) case, BM aspirate was inadequate. Average percentage of abnormal promyelocytes in bone marrow was 84.25% (Range: 67% -100%). PT was prolonged in 15 (83.3%) cases, while APTT was prolonged in 3 (16.66%) cases. INR was elevated in all the cases.

Immunophenotyping by flow cytometry (Table 1): Flow cytometry analysis was done in 12 patients. All the 12 (100%) patients were CD45, CD13, CD33 and CD64 positive. MPO was positive in 11 (91.6%)patients. CD117 was positive in 10 (83.3%) patients. CD11c, CD38 and CD15 was positive in 5 (41.6%) patients each.CD34 was positive in 3 (25%) patients. CD 11b was positive in 2 (16.6%) patients. HLA-DR and CD7 was positive in 1 (8%) patient each. The patient with HLA-DR positive immunophenotype was also positive for CD34 and CD7. APL was the flow cytometry impression in 91.6% of the patients and was diagnosed as AML with aberrant CD7 (T lymphoid marker) expression in 8.3% of the patients. The latter showed HLA-DR and CD34 positivity.

CYTOGENETICS: Chromosomal analysis was possible in 11 cases. t (15;17) (q22;21) was identified in 6 cases (54.62%). 3 cases (27.27%) showed normal karyotype. 2 (18.18%) cases had additional cytogenetic abnormalities. These additional abnormalities included add (7) (q36) and add (4) (p16).

Markers	Number of cases (%)
CD11c +	5 (41.6%)
CD11b +	2 (16.6%)
CD13 +	12 (100%)
CD15 +	5 (41.6%)
CD33 +	12 (100%)
CD34 +	3 (25%)
CD64 +	12 (100%)
CD117 +	10 (83.3%)
CD45 +	12 (100%)
CD38 +	5 (41.6%)
MPO +	11 (91.6%)
HLA-DR +	1 (8%)
CD7 +	1 (8%)
Flow Cytometry impression	**Number of cases (%)**
APL	11(91.6%)
AML with aberrant CD7 expression	1(8.3%)

Table 1: Immunophenotyping by flow cytometry of 12 APL patients

Treatment outcome: 6 (33.33%) patients were belonging to high risk category. 12 (66.67%) patients were belonging to low risk category. All patients under high risk category attained CR. 1 patient under low risk category with additional cytogenetic abnormality died 6 days after induction therapy was started, due to COVID-19 related acute respiratory distress syndrome (ARDS). Details of treatment outcomes in each category are included in Table 2. After completion of induction, out of 17 patients, 15 patients were presently available for follow up. 2 patients were lost to follow up. The duration since diagnosis/ start of treatment to last follow up ranged from 10 months 9 days to 3 years 3 months. The mean follow up period was 20 months. So far none of the patients had a relapse. The details of risk category and response to treatment are summarized in table (2).

Complications: Out of 18 patients, 10 (55.55%) patients developed complications. 3 patients (16.6%) developed neutropenic sepsis. Intracranial hemorrhage was seen in 2 patients (11.1%). Other complications that were observed included (1 (5.5%) patient each) - differentiation syndrome, cerebral venous sinus thrombosis, pseudotumor cerebri, QTc interval prolongation, and pneumonia.

Risk category	Number of patients (%)	Complete remission (%)	Induction deaths (%)	Lost to follow up	Mean duration to attain CR in days (Range)
High risk	6 (33.33%)	6 (100%)	0	0	45.6(32-57)
Low risk	12(66.67%)	9 (90%)	1(10%)	2	52.2(43-68)
Overall	18	15 (93.75%)	1(6.25%)	2	49.46 (32-68)

Table 2: Risk category and response to treatment

DISCUSSION

In our study, the most common presenting symptom was fever. APL could be diagnosed in peripheral smear in 12 (66.67%) patients. APL was the flow cytometry impression in 91.6% of the patients and was diagnosed as AML with aberrant CD7 (T lymphoid marker) expression in 8.3% of the patients. Additional cytogenetic abnormalities were present in 18.18% of the patients. 1 (6.25%) patient of low risk category with additional cytogenetic abnormality died during induction and was Covid 19 positive.

The median age (31.5 years) was similar to that reported by Sanz et al, Bajpai et al and Yedla et al.5,6,7Hemoglobin levels were also similar to that reported by Sanz et al (9.4g%) and Bajpai et al (6.6g%). 5,6Average total WBC count was higher in our study when compared to Sanz et al (2100/cmm) and Bajpai et al (9183/cmm).5,6 The average platelet count in our study was similar to those reported by Sanz et al, but slightly higher in the study conducted by Bajpai et al.5,6The most common presenting symptom was fever even in the study conducted by Bajpai et al and Yedla et al.(6,7) The proportion of cases with ATRA syndrome was similar to the study conducted by Sanz et al (5) However it was higher in the study conducted by Bajpai et al and Yedla et al (6,7).

The flow-cytometry findings and the proportion of cases with additional cytogenetic abnormality were similar in the study conducted by Ibrahim et al and Sucic et al and is summarized in table 3 (8,9).

Author	Year of study	Number of study subjects	Positive markers by flow cytometry (%)								Additional cytogenetic abnormality
			CD13	CD33	CD34	CD64	CD117	MPO	CD7	HLA-DR	(%)
Ibrahim et al.	2006 to 2008	11	10 (91%)	11 (100%)	4 (36%)	6 (54.54%)	9 (82%)	11 (100%)	1 (9%)	1 (9%)	2/10 cases (20%)
Sucic et al.	1995 to 1998	14	13 (92.8%)	13 (92.8%)	1 (7.1%)	-	-	-	1 (7.1%)	2 (14.2%)	2/13 cases (15.38%)
Present study	2017 to 2020	12	12 (100%)	12 (100%)	3 (25%)	12 (100%)	10 (83.3%)	12 (100%)	1 (8%)	1 (8%)	2/11 cases (18.18%)

Table 3: Comparing the Flow Cytometry and Cytogenetics Findings in Similar Studies

Author	Year of study	Number of study subjects	Median age (Range)	Induction therapy	CR (%)	ATRA Syndrome (%)
Sanz Ma[5] et al	1996-2002	426	40y (2-73y)	ATRA + Idarubicin	384 (90%)	2 (5%)

Table 4: Review of Literature

Bajpai[6] et al	1997-2007	33	30y (2-75y)	ATRA+ Daunorubicin + ATO	27 (81.81%)	10 (33%)
Yedla[7] et al	2005-2018	111	33y (19-60y)	ATRA+ Daunorubicin or ATRA+ ATO	88 (88.2%)	39 (35.1%)
Present study	2017-2020	16	31.5y (11-58y)	High risk- ATRA+ Daunorubicin + ATO Low risk- ATRA+ATO	15 (93.75%)	1 (5.5%)

Cont. Table 4: Review of Literature

The rate of complete remission was slightly higher in our study when compared to the studies conducted by Sanz et al, Bajpai et al and Yedla et al and is summarized in table 4 (5,6,7).

The prognostic significance of HLA-DR positive APLis still not clear due to its rare occurence. According to Dong et al HLA-DR positivity was associated with CD34 positivity.1 Even in our patient with HLA-DR positivity,associated CD34 positivity was found.According to Fukino et al, HLA-DR and CD34 positivity is connected with worse clinical outcome. HLA-DR expression is lost at the promyelocytic stage during hematopoiesis.APML2 HLA-DR expression in APL probably indicates the maturation arrest occurring at the promyelocyte stage even before the loss of HLA-DR expression.CD7 positivity is a negative prognostic sign. In the study conducted by Sucic et al, one of them with CD13, CD33 and HLA-DR positivity entered remission, but then died after 2 months of sepsis. Another patient with CD13, CD33, CD34, HLA-DR positivity had a relapse after 17 months. Also,1 patient with CD13, CD33, CD65, CD14, CD2, CD7, CD19 positive died after 2nd relapse after 76 months of survival.9 However, our patient with CD13, CD33,CD64, CD117, CD45, MPO, HLA-DR, CD34 and CD7 positivity attained complete remission after induction and was followed up for 1 and a half year. She had no signs of relapse during this period.

In APL, the prognostic significance of additional cytogenetic abnormality is controversial.10 In our study 2 patients had additional cytogenetic abnormality. One of them with add(7)(q36), has completed treatment and is in molecular remission. Another patient with add(4)(p16), died of COVID19 related acute respiratory distress syndrome 6 days after the initiation of induction therapy.

CONCLUSION

Our study had a slightly better rate of complete remission when treated by categorizing the patients into high and low risk categories, with ATRA and arsenic trioxide based protocols. There was a very low incidence of treatment related complications like differentiation syndrome, QTc prolongation and pseudotumorcerebri. Our study had a limitation of a small sample size. Even then, clinicopathological features of APL in our study were in concordance with previous reports. The presence of HLA-DR, CD34 and CD7 positivity did not show an adverse outcome in our study. The presence of additional cytogenetic abnormality like add(7)(q36), which was found in 1 patient in our study attained molecular remission. Further studies on the prognostic significance of HLA-DR positive APL and those with additional cytogenetic abnormalities may further help in planning the treatment of APL patients. Early diagnosis and prompt management of APL after categorizing the patients into high and low risk categories as in our study helps in improving the outcome in these patients with low treatment related complications.

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