INTRODUCTION

Acute Promyelocytic Leukemia (APL) is a distinct subtype of acute myeloid leukemia with specific clinical, morphologic / genetic features and accounts for 10-15% of de novo cases of Acute Myeloid Leukemia (AML) in younger adults. It is characterized by a specific gene rearrangement and the generation of the PML-RAR\(\alpha\) fusion transcript, which results from a translocation between chromosomes 15 and 17. The resulting fusion gene, PML-RAR\(\alpha\), encodes a chimeric protein that causes a maturation arrest at the promyelocytic stage of myeloid cell development and this fusion gene is important in the pathogenesis of APL.\(^{1-3}\) Morphologic diagnosis, although highly predictive of the specific genetic lesion in hypergranular (typical) APL, is considered insufficient. Therefore, all patients, including those with typical hypergranular APL must be studied by karyotypic, and molecular analysis to confirm the presence of the specific fusion gene and to characterize its isoform for molecular monitoring of Minimal Residual Disease (MRD) even after starting specific treatment, which should not be delayed. Age, hemorrhagic diathesis and initial leukocyte count are known prognostic factors that affect treatment outcome in APL\(^{4-6}\)

ABSTRACT

Objective: To compare survival in Acute Promyelocytic Leukemia (APL) patients treated with or without All-Trans Retinoic Acid (ATRA).

Study Design: Longitudinal, comparative study.

Place and Duration of Study: The Armed Forces Bone Marrow Transplant Centre (AFBMTC), Rawalpindi, Pakistan from May 2001 to April 2007.

Methodology: All consecutive newly diagnosed patients of acute promyelocytic leukemia, treated at Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan, between May 2001 and April 2007, were included and given chemotherapy according to availability of ATRA. Diagnosis was confirmed on morphology/ karyotyping/ molecular analysis. Eligibility criteria included confirmed morphologic diagnosis and/or by demonstration of t(15;17) and/or PML/RAR\(\alpha\) re-arrangement, no prior chemotherapy, normal hepatic and renal function, Eastern Cooperative Oncology Group (ECOG) performance status of 0 - 2 and no contraindications to ATRA (history of sensitivity tovit. A or other retinoids). All patients having history of cardiac failure (LVEF < 50) and arrhythmias, ECOG performance status 3 and 4, relapse / refractory disease, ALT twice normal values, serum creatinine > 150 \(\mu\)mol/L and pregnancy were excluded from this study. Survival was calculated from the date of chemotherapy to death or last follow-up according to Kaplan-Meier and Cox (Proportional hazard) regression analysis methods.

Results: During the 6 years study period, 31 newly diagnosed patients with acute promyelocytic leukemia received treatment at AFBMTC. Seventeen patients received anthracycline-based remission induction and consolidation chemotherapy, while 14 received ATRA-based remission induction, consolidation and by two years maintenance therapy. Overall Survival (OS), Disease Free Survival (DFS) and mortality were 29.4%, 29.4% and 70.6% respectively in 17 patients who received anthracycline based chemotherapy, whereas in patients who received ATRA-based chemotherapy OS, DFS and mortality was 71.4%, 64.2% and 28.6% respectively. Major causes of mortality were septicemia and chemotherapy related toxicity.

Conclusion: Response to ATRA-based chemotherapy in patient cohort was better as compared with anthracycline based chemotherapy (71.4% vs. 29.4%) in terms of survival and mortality.

Key words: Acute promyelocytic leukemia. Chemotherapy regimens. Survival.
associated with a high mortality rate (upto 20%) during the early phases of treatment. Differentiative treatment with ATRA results in greater than 90% CRs, but patients remain invariably PCR positive after induction, and all relapse, if no consolidation CHT is added. Furthermore, ATRA treatment has been associated with occurrence, in a sizable fraction of patients, of life-threatening complications. These include a severe respiratory distress due to pulmonary infiltrates (ATRA syndrome) usually (but not uniformly) correlated to a rapid increase of white blood cell counts and pseudotumour cerebri, which is more frequently observed in younger patients.7-9

Preliminary clinical studies have been performed with various combinations of ATRA and CHT in an attempt to obtain more durable remissions and reduced ATRA-related toxicity. The combinations, as demonstrated in a randomized trial, seem to improve disease-free survival over that achieved with chemotherapy alone.7

Remarkable progress has occurred in the treatment of patients with APL since the introduction of ATRA. Targeted therapy with ATRA-based chemotherapy results in an apparent cure in 70-80% of patients,2,10,11 Both allogeneic and autologous stem cell transplantation (allo and auto SCT) are effective in AML but their role in APL is not clear, given the excellent outcome with ATRA-based chemotherapy.3

Availability of ATRA in Pakistan has not been consistent, resulting in some patients treated with and some without ATRA. At the end of six years of treatment experience at AFBMTC, it was considered worthwhile to compare and report survival with and without ATRA in APL, which has never been reported in Pakistan.

The objective of this study was to compare survival in Acute Promyelocytic Leukemia (APL) patients treated with or without All-Trans Retinoic Acid (ATRA).

**METHODOLOGY**

All consecutive newly diagnosed patients of acute promyelocytic leukemia, treated at Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan between May 2001 and April 2007, were included and given chemotherapy according to availability of ATRA. ATRA-based chemotherapy has been the treatment of choice ever since it became available in local market in early 2005 and all patients enrolled after that were given ATRA-based chemotherapy. Prior to this period, all patients were given Anthracycline (Daunoblastina) + Ara-C chemotherapy. At the end of study period in April 2007, hospital record was retrieved to document complications and survival. Survival was calculated from diagnosis to death or last follow-up. All patients not reporting for follow up in last 03 months were contacted on telephone for follow-up. Diagnosis was based on morphologic and cytochemical criteria defined by French-American-British (FAB) classification, as well as cytogenetics/molecular analysis for t(15;17) and/or PML/RARα fusion gene.

Eligibility criteria included confirmed morphologic diagnosis and/or by demonstration of t(15;17) and/or PML/RARα rearrangement, no prior chemotherapy, normal hepatic and renal function, Eastern Cooperative Oncology Group (ECOG) performance status of 0 - 2 and no contraindications to ATRA (history of sensitivity to Vit. A or other retinoids). All patients having history of cardiac failure (LVEF < 50) and arrhythmias, ECOG performance status 3 and 4, relapse / refractory disease, ALT twice normal values, serum creatinine > 150μmol/L and pregnancy were excluded from this study.

Patients were categorized into low-risk (WBC count < 10 x 10^9/L, platelet count > 40 x 10^9/L), intermediate-risk (WBC count < 10 x 10^9/L, platelets ≤ 40 x 10^9/L), and high-risk (WBC count > 10 x 10^9/L) groups. Patients were further subdivided in two groups for treatment: group I received anthracycline + Ara-C remission induction followed by 4 x consolidation cycles, group II received ATRA + anthracycline-based remission induction/consolidation chemotherapy followed by maintenance therapy.

The diagnosis of Disseminated Intravascular Coagulation (DIC) in these patients was defined as the presence of any two of the following abnormalities: Prothrombin Time (PT) 3 or more seconds greater than control, Activated Partial Thromboplastin Time (APTT) 5 or more seconds greater than the upper limit of normal range, Thrombin Time (TT) prolonged by 3 or more seconds compared to control, fibrinogen less than 150 mg/dL, and fibrin degradation products (D-dimers) greater than 250 ng/mL. ATRA syndrome was defined as the presence of the following five signs and symptoms; fever, dyspnoea, pleural and/or pericardial effusion, pulmonary infiltrates on chest radiograph, and unexplained weight gain.

Complete haematological remission was defined as less than 5% blast on bone marrow examination with evidence of maturation of all bone marrow cell lines and restoration of normal peripheral blood counts. Molecular remission was defined as the disappearance of PML/RARα fusion gene on RT-PCR. Molecular relapse was defined as the reappearance of PML/RARα fusion gene in 2 consecutive bone marrow samples at any time after consolidation therapy.

Survival was calculated from the date of chemotherapy to death or last follow-up according to Kaplan-Meier and Cox (proportional hazard) regression analysis methods. The analysis was performed with Stat Direct software and MS excel software.
RESULTS

A total of 31 newly-diagnosed patients with Acute Promyelocytic Leukemia (APL) were studied. Their characteristics including age, gender, risk class, presenting WBCs and platelet counts are given in Table I. These patients were divided into two groups on the basis of chemotherapy given. Group I patients (n=17) received anthracycline + Ara-C-based induction followed by consolidation. Group II patients (n=14) received ATRA + anthracycline based induction and consolidation followed by maintenance therapy.

Table I: Patients characteristics, risk classification and hematological parameters. AML-M3 (n=31).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group I (n=17)</th>
<th>Group II (n=14)</th>
<th>Total (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>29 (10-45)</td>
<td>30 (20-52)</td>
<td>29.8 (10-52)</td>
</tr>
<tr>
<td>&lt;15</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>16-30</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>31-45</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>46-60</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Risk class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Intermediate</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>High</td>
<td>8</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>WBC (x10⁹/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>5.5 (2.1-193.3)</td>
<td>3.6 (1.1-69.5)</td>
<td>4.6 (1.1-193.3)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>10-50</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>51-100</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Platelet (x10⁹/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>48 (6-234)</td>
<td>32 (5-259)</td>
<td>55.5 (6-234)</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
</tbody>
</table>

Out of 17 patients in group I, 9 patients (53%) achieved complete remission, while 8 patients died (septicemia n=6, chemotherapy toxicity n=2) after induction chemotherapy. Out of the remaining 9 patients, 2 patients died during first consolidation, one each during second and third consolidation, making OS and DFS of 29.4% (n=5) and mortality of 70.6% (n=12). Out of 14 patients in group II, 13 patients (98%) achieved CR after remission induction chemotherapy. One patient each died during remission induction and first consolidation of septicemia, while 2 patients died during second consolidation (disease relapse 1, septicemia 1). One patient relapsed while on maintenance but survived with palliative treatment. This made OS of 71.4% (n=10) and DFS of 64.2% (n=9), while mortality in this group was 28.6% (n=4). Major causes of mortality were septicemia, chemotherapy related multi-organ failure and disease relapse.

Major complications were septicemia, coagulopathy, ATRA syndrome and chemotherapy related multi-organ toxicity. Septicemia was observed in 12 patients (group I: 9, group II: 3). All cases of septicemia proved fatal. Major causative organisms were Pseudomonas aeruginosa (n=9) and Methicillin resistant Staphylococcus aureus (n=3). Coagulopathy was seen in 9 patients (group I: 6, group II: 3). These patients were managed by appropriate blood components support. Chemotherapy related multi-organ toxicity was seen in 7 patients (group I: 5, group II: 2) and proved fatal in 2 patients. Three patients developed ATRA syndrome. These patients responded to steroid therapy. Disease relapse was observed in 3 patients (group I: 01, group II: 02).

At the end of 6 years, overall survival was 29.4% vs. 71.4% while disease-free survival was 29.4% vs. 64.2% respectively in group I and group II. Mean survival time was 21.6 months (ranging from 7.3 to 66 months). Survival plot in two groups is shown Figure-I.

DISCUSSION

Acute Promyelocytic Leukemia (APL) is characterized by a number of features that emphasize the need for rapid and accurate diagnosis and demand a highly specific treatment approach. These include the potentially devastating coagulopathy, sensitivity to anthracycline-based chemotherapy regimens, as well as unique responses to all-trans retinoic acid and arsenic trioxide that have revolutionized therapy over the last decade. Establishing a diagnosis of APL is also important in view of its unique therapeutic profile. In particular, APL was the first disease for which differentiation therapy in the form of retinoids, such as All-Trans Retinoic Acid (ATRA) that directly target the underlying molecular lesion, has been successfully used in clinical practice.12,6
Combination of ATRA and anthracycline-based chemotherapy is currently considered as the ‘gold standard’ first-line treatment for patients with APL. ATRA plus anthracycline-based combination chemotherapy (AIDA) offers a greater therapeutic efficacy compared with anthracycline-based combination chemotherapy (CHT) alone as reported by a number of recently published studies.\textsuperscript{13,14} Randomized studies have recommended both the upfront combination of simultaneous ATRA and anthracycline as well as inclusion of ATRA during maintenance as a standard therapy in further management of APL. Over the past decade, several large multicentre trials that used various ATRA and anthracycline-based chemotherapy combination have reported >70% long-term survival in APL.\textsuperscript{14-17} Once complete remission has been achieved, APL is now considered one of the most favourable subsets of AML and Bone Marrow Transplantation (BMT) is no longer considered in first CR as reported by a number of trial groups.\textsuperscript{3,6}

Retrospective analysis of acute myeloid leukemia in children from local tertiary care referral hospital (between 1987-1997) shows APL (43%) as the commonest subtype of AML with hyperleukocytosis (TLC > 100 x 10\(^9\)/L) in 22% of patients and pneumonia as the commonest clinical presentation. Recently published data (between 1999 - 2000) from the same tertiary care referral hospital, analysing frequency of subtype of AML both in adults and children, showed 10.4% incidence of APL.\textsuperscript{18,19}

A recently published 10 years retrospective analysis of APL from another local tertiary care referral hospital showed bleeding diathesis manifestations, high WBC / low platelet counts as limiting factors in the early and effective treatment of APL. Similarly, another report from the same centre also showed bleeding diathesis / DIC as the commonest presenting feature in APL patients.\textsuperscript{20,21}

In this study, diagnosis of APL was based on FAB-morphologic classification cytogenetics, and / molecular analysis for PML-RAR\(_{\alpha\kappa}\) fusion gene. It also identified three prognostic risk group of APL patients on the basis of white blood cell counts, platelet counts and bleeding diathesis at the time of diagnosis. These risk factors have prognostic impact in remission response, EFS, DFS and relapse risk. The study also showed that upfront AIDA, use of anthracycline-based consolidation as well as use of ATRA during maintenance seems to be more effective and intensive regimen for APL.

Combination chemotherapy with anthracycline and Ara-C was the only treatment for newly diagnosed APL patients till the introduction of ATRA in early 1990’s. Head \textit{et al.} has reported CR rate of 70% and 47% respectively in APL patients who received anthracycline-based chemotherapy in SWOG (South-west oncology group) trials between 1982-1986 and 1986-1991.\textsuperscript{22} CR rate of 80-90% have been reported in newly diagnosed APL who were treated with combined ATRA and anthracycline chemotherapy.\textsuperscript{13}

Asou \textit{et al.} reported OS, EFS and DFS rate of 74%, 54% and 62% respectively in newly diagnosed APL patients who received ATRA-based chemotherapy.\textsuperscript{4} Similarly, Mandelli \textit{et al.} also reported 95% CR after induction therapy and 79% EFS at 2 years after ATRA-based chemotherapy.\textsuperscript{7} Sanz \textit{et al.} recently reported results of joint study of the PETHEMA and GIMEMA cooperative groups trials using ATRA-based regimens with 90% and 86% Relapse Free Survival (RFS) in two groups respectively.\textsuperscript{17} Specchia \textit{et al.} reported complete remission rate of 69% versus 93.6% and relapse rate of 51% versus 18% in APL patients who were treated with anthracycline + Ara-C vs. ATRA + anthracycline-based chemotherapy respectively.\textsuperscript{23}

Analysis of this results shows better CR after remission induction as well as better OS and DSF (71.4% and 64.2%) after the completion of treatment in patients who received ATRA-based chemotherapy when compared with patients who received conventional CHT (27.4% and 29.4%) for APL in this study. Moreover, the results of conventional treatment in patients with anthracycline were not promising due to high mortality (70.6%) from septicemia and chemotherapy related toxicity during induction. The present results with ATRA-based chemotherapy, however, are comparable with other international studies and are much better than conventional anthracycline-based combination chemotherapy for APL.

**CONCLUSION**

The present study indicates that APL patients receiving ATRA, in addition to anthracycline, had high CR rate as compared with conventional chemotherapy. Infections related mortality was unacceptably high specially during high dose induction chemotherapy despite best possible preventive measures. However, antimicrobial prophylaxis practices need further improvement and role of prophylactic antibiotics need to be looked into.

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