Characterizing PML/RARα Isoforms of Acute Promyelocytic Leukemia (APL) in Malay patients

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Abstract:

Aim: Reciprocal translocation between retinoic acid receptor alpha (RARα) gene on chromosome 17 and promyelocytic leukemia (PML) gene on chromosome 15 is the hallmark for acute promyelocytic leukemia (APL). Three different PML/RARα isoforms have been described; S-form, L-form and V-form. Our aims were to characterize the different types of PML/RARα isoforms in Malay patients with APL and to determine the outcome of these different types of isoforms. Materials and methods: RT-PCR analysis was performed on 20 patients recruited from hematology-oncology ward. RT-PCR detected fusion transcript of PML/RARα in all patients. Results and Discussion: Of these patients, 65% (13 patients) exhibited L/V-form, and 35% (7 patients) S-form. Total white blood cell count (TWBC) was higher in L/V-form (25 x 10⁹/l) compared to S-form (2.1 x 10⁹/l) (p < 0.05). Five years survival rate was 100% and 33.3% for L/V-forms and S-forms respectively (p<0.005). Conclusion: We conclude that L/V-forms is the commonest isoform among Malays. They presented at younger age with higher TWBC counts. Although the sample size is small, our preliminary data showed an interestingly longer survival outcome among L/V-forms compared to S-form. PML/RARα isoforms could be used in future as risk stratification feature in patients diagnosed as APL. Further study with more number of patients is required.

Key words: PML/RARα; isoforms; APL; AML M3; nested RT-PCR

Introduction:

Acute promyelocytic leukemia (APL) is one of the subtypes of acute myeloid leukemia. It was first classified as AML-M3 by French-American-British classification (FAB), and then currently it is known as APL with translocation between chromosomes 15 and 17, by the World Health Organization (WHO) classification system. APL has a unique clinical and laboratory features. It represents 20-28.2% of all acute myeloid leukemia (AML) among Latinos. It arises due to a t(15;17)(q22;q11.2) balanced reciprocal chromosomal translocation that fuse the PML gene on chromosome 15 with the RARα gene on chromosome 17. The resulting PML/RARα chimeric gene expresses PML/RARα fusion protein

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which inhibits the granulocytic differentiation and promotes survival of hematopoietic progenitor cells. Three types of PML/RARα isoforms have been identified. Short form (S-form) or type A arises when the breakpoint occurs within intron 3 of PML (breakpoint cluster region 3, Bcr-3) and long form (L-form) or type B is formed when the breakpoint occurs within intron 6 (Bcr-1) whereas a third variable form (V-form) or type B variant occurs when breakpoint is within exon 6 (Bcr-2). A breakpoint of RARα is invariably in intron 2.

The diagnosis of APL is typically based upon identification of promyelocytes with distinctive morphology plus cytogenetic observation of (15;17)(q22;q11.2) translocation or molecular detection of PML/RARα fusion transcript. In some rare cases (<1%), chromosome 17 is reciprocally translocated with other partners, such as chromosomes 5 and 11. In some studies, it has been reported to have an additional cytogenetic changes, most frequently trisomy 8 in 30% to 35% of APL patients. Molecular detection of PML/RARα is useful in diagnosing APL, characterizing the breakpoints, predicting the responsiveness to treatment, and detecting minimal residual disease. There were few studies explored on the clinical features and the different breakpoints of PML gene with patients’ outcome. The results so far provided are inconclusive with limited information on the prognostic relevance of these features. Therefore, our aim was to characterize the PML/RARα isoforms in Malay patients diagnosed as APL and its association with patients’ survival.

Material and Methods

This was a retrospective study. A total of twenty Malay patients diagnosed as APL were identified from the record office. The diagnosis was made based on morphology and immunophenotyping. The diagnosis was confirmed by RT-PCR analysis for PML/RARα fusion gene. Following the establishment of the diagnosis, patients were treated with the combination of induction idarubicin and all-trans-retinoic acid (ATRA). This was followed by idarubicin consolidation monotherapy for 3 cycles and ATRA based maintenance therapy which includes 6-mercaptopurine (6-MP) and methotrexate (MTX) for 18 months. The median follow-up was 52.8 months. The survival analysis was calculated from the day of admission. Informed and written consent were taken from them and the study has been approved by ethical committee.

RT-PCR:
Total RNA was extracted from peripheral blood (PB) or bone marrow (BM) specimen using standard methods following manufacturers’ protocol for QIAamp® RNA Blood Mini Kits (Qiagen GmbH, Hilden, Germany). The primers used were similar to the one used by in the BIOMED-1 European group. Complementary DNA (cDNA) was synthesized using QIAGEN OneStep RT-PCR Kit (Qiagen GmbH, Hilden, Germany). Total RNA was added to RT-PCR master mix containing final concentration of 1× Qiangen One Step RT-PCR Buffer, 800M dNTP, 1.25mM MgCl2, 5 – 50nM of external primers for ABL transcripts and PML/RARα fusion transcripts, Qiagen OneStep Enzyme Mix, RNasin Ribonuclease Inhibitor (Promega, Madison, WI) and water. Reverse transcription was carried out at 50°C for 30 minutes. This was followed by polymerase chain reaction. The PCR mixture contained 2μl of PCR product and 1U of Qiagen HotStarTaq™ polymerase (Qiagen GmbH, Hilden, Germany) with final concentration of 1′ Qiagen Buffer, 1000M dNTP, 1.25mM MgCl2 and 500nM of ABL and PML/RARα primers. The PCR was performed at 94°C for 20 seconds, 55°C for 20 seconds and 72°C for 30 seconds followed by a 5 minute final extension at 72°C. Positive and negative controls were performed for every run. PCR products were separated on agarose gel. Sample amplified showed bands sized either 714 base pair (bp), 714-312bp or 214bp in the presence of 366bp PCR product of ABL gene were reported as positive for L-form, V-form and S-form respectively. Paired T-test was performed to identify association between PML/RARα isoforms and parameters such as age, gender and total white blood cell count.

Results
Of the twenty Malay patients with APL, seven (35%) exhibited S-form and thirteen (65%) L/V-form (Table 1). Only one patient was identified as L-form and he was included in the V-form group of patients for analysis. He was the youngest patient, aged 8 years-old and the only pediatric patient to date in our hospital diagnosed as APL.
The summary of APL patients' data, hematological parameter, and molecular analysis of PML/RARα isoform is shown on Table 1. The cytogenetic analysis was compared with RT-PCR (Table 2). The median age of patients with S-form was 38.4 years old whereas those with L/V-form were 28.6 years old. Males were predominant in S-form while female in L/V-forms.

There was no association between PML/RARα isoforms with age or gender; however there was a significant difference between the types of isoforms and total white blood cell count (TWBC). (p < 0.05)

The coagulation profiles consisting prothrombin time (PT), and activated thromboplastin time (APTT) and serum fibrinogen were normal but D-dimer was positive in all patients. (data not shown).

Flowcytometry showed a myeloid phenotype consistent with promyelocyte population i.e. high side scatter, positive for CD13, CD33, CD45 and negative for CD34 and HLA-DR. The findings were compatible with the diagnosis of APL.

Five years overall survival rate was 100% and 33.3% for L/V-forms and S-forms respectively. Kaplan Meier analysis was performed and patients with L/V-form showed better survival than the S-form. (p<0.005). (Fig:1)

Discussion

In Malaysian population, APL is the third commonest subtype of AML after AML-M2 and M4. It comprised 17% of all AML subtypes based on FAB classification. It is important to identify this subtype as the management of these patients is molecularly targeted.

Identification of PML/RARα fusion transcript or the presence of t(15;17)(q22;q11.2) is undoubtedly a crucial finding in aiding the diagnosis APL. In our study the detection rate of t(15;17)(q22;q11.2) by cytogenetic was 40%. Fusion transcript was readily detectable by RT-PCR. Although we have missed few numbers of cases by conventional cytogenetic but the test itself has a great importance to reveal global chromosomal abnormalities and it should be done in complement to RT-PCR. PML is the predominant fusion partner for RARα. Other alternative fusions may involve at low frequency (< 1%), the promyelocytic leukemia zinc finger (PLZF), or very rarely the nucleophosmin (NPM), nuclear mitotic apparatus (NUMA), and STAT5b.

In this study, we found a unique presentation of PML/RARα isoforms among Malay patients with APL which was not observed in other population. The S-form (35%) was commoner in Malay APL patients compared to Latinos (~10%) although frequencies of L/V-form were noted to be similar, between 50% and 75%.

Studies have shown that incidence of various fusion transcripts varies significantly among different populations. Incidence of S-form, L-form and V-form were 50-55%, 27-49% and 8-20% respectively as reported in literatures from Europe and Asia. In our study, we have identified only one patient with L-form which was included in V-form.

Nevertheless our findings supports that the expression of chimeric gene may differ between ethnic groups as has been reported before in other studies. Due to limited number of L-form one could speculate that the Malay race have a different gene pool leading to a less susceptible breakpoint at intron 6 of PML and yet an increased predilection for breakage at exon 6 of the same gene. Further analysis of the genomic sequence of PML gene and its breakpoint sites between the Malay population and others may provide insight into the genetic factor that possibly influenced the unique formation of PML/RARα isoform in each population.

In our study we found that there was significant difference in TWBC count at diagnosis between the isoforms. Six out of seven patients with S-form had TWBC count of less or equal to 3.5´10⁹/L (85.7%) compared to 100 percent of patients with L/V-form whose TWBC count was more than 3.5´10⁹/L. However we did not find any correlation with other hematological parameters listed on Table 1. Though fibrinogen, APTT and PT were normal in all cases, we observed elevated D-Dimer level in both forms denoting occult DIC. We therefore recommend screening of D-Dimer level in all cases of APL as part of the management protocol although APTT and PT are within normal range.

Debate exists over the clinical relevance of molecular heterogeneity of APL. Some literatures have described correlation between PML/RARα isoforms and several patient characteristics and outcomes but with discrepant results. Reduced sensitivity to all-
trans retinoic acid (ATRA) has been described in V-form patients, but most of them displayed additional cytogenetic abnormalities. Even in study with larger sample size, there was no evidence that the V-form influences clinical outcome. In the study done by Gonzalez et al., 2001, there was no difference observed between the three isoforms in complete remission (CR) rate. However 3-year disease-free survival was lower for V form than L- and S-form (62% vs. 94% and 89%). Both V-form and S form were associated with some negative prognostic features at time of diagnosis.

In our study, 5 years overall survival rate was 100% and 33.3% for L/V-forms and S-forms respectively. Kaplan Meier analysis was performed and patients with L/V-form have longer survival rate than the S-form. (p<0.005). Median survival for S-form was 19.9 months meanwhile all patients with L/V- form have survived to date.

Table 1: Characteristic features between PML/RARα isoforms

<table>
<thead>
<tr>
<th>Characteristic Features</th>
<th>PML/RAR± isoforms</th>
<th>p value</th>
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<tbody>
<tr>
<td></td>
<td>S-Form</td>
<td>L/V-Form</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>Med (IQR) n=7</td>
<td>Med (IQR) n=13</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>5:2</td>
<td>4:9</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>8.2(5.5-10.9)</td>
<td>8.3 (6.0-11.7)</td>
</tr>
<tr>
<td>TWBC x 10⁹/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3.5 x 10⁹/L</td>
<td>2.1 (0.4-2.14)</td>
<td>6</td>
</tr>
<tr>
<td>&gt;3.5 x 10⁹/L</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Platelet x 10⁹/L</td>
<td>24.7 (10-69)</td>
<td>43.6 (5.4-103)</td>
</tr>
</tbody>
</table>

S-Form= short form, L/V=long/variant, Med=median, IQR=interquartile range, M=Male, F=female, Hb=Hemoglobin, TWBC=total white blood cell.

* Significant at p<0.005

Table 2 : Comparison between RT-PCR for PML/RARα and cytogenetic with t(15;17)(q22;q11.2)

<table>
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<tr>
<th>RT-PCR results at diagnosis</th>
<th>Cytogenetic</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Detected</td>
<td>2</td>
<td>5</td>
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<tr>
<td>Absent</td>
<td>0</td>
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</table>

Conclusion

To our knowledge this is the first reported findings among Malay patients showing a predominant L/V-form. These patients were commonly female with high TWBC. They survived better than S-form. Future studies with a larger cohort of patients can confirm the presence of the S-form as a high-risk feature and would be useful for future risk-adapted treatment strategies for APL.

Fig 1: Survival analysis between S-form and L/V-forms
References


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