Pattern of PML/RARα BCR Isoforms in Acute Promyelocytic Leukemia (APL) Patients in Bangladesh - A Single Centre Study

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ABSTRACT

Background: Acute Promyelocytic Leukemia (APL) is a well-defined subtype of AML accounting for 5-13% of all AML cases and is identified by a balanced reciprocal translocation between chromosome 15 and 17 [t(15;17)(q24;q21)] which ultimately causes formation of PML-RARα oncoprotein. This translocation results in 3 isoforms on the PML gene including L-long form (located at intron 3) termed bcr1, S-short form (located at intron 6) known as bcr2, and V form (located at exon 6) known as bcr3. To ascertain the pattern of different breakpoints of PML/RARα fusion gene among Bangladeshi APL patients was the aim of this study.

Methods: In this study 60 APL patients diagnosed positive for PML-RARα fusion gene who were referred to AFIP, Dhaka Cantonment were included as subjects. They were analyzed for the detection of three possible PML-RARα isoforms by utilizing Real Time Polymerase Chain Reaction (RT-PCR) technology.

Results: The patients included in this study have age ranged from 9 years to 72 years, 35 years is the mean of age. In the study group, 39 (65%) were male and female patients were 21 (35%). The bcr1 isoform was found in 4 (6.67%) patients. No single bcr2 isoform was found in any patient. But in 3 patients having bcr2 isoform were found along with bcr1 (5.0%) and in 1 (1.66%) patient it was present in addition to bcr3. The bcr3 isoform was found in 19 (31.67%) patients and in 33 (55.0%) patients all of the isoforms were present.

Conclusion: The key elements for the proper management of APL include early diagnosis and treatment initiation, proper supportive care, and recognition and management of therapy associated complications. Genetic testing is one of the most accurate methods for the diagnosis of this disease and ultimately can help in advancement of the disease management.

Keywords: Acute myeloid leukemia, Acute promyelocytic leukemia, Molecular genetics, PML/RARα bcr isoforms.

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INTRODUCTION

Acute myeloid leukemia (AML) is a neoplasm of hematopoietic origin identified by the appearance of malignant clone of myeloid cells with maturation arrest at the level of blast. Basing on the morphology and cytochemical staining the French-American-British (FAB) group has identified eight subtypes of AML (M0-M7). Among them AML-M3 is known as Acute Promyelocytic Leukemia (APL). It is a well-defined subtype of AML accounting for 5-13% of all AML cases and is identified by a balanced reciprocal translocation between chromosome 15 and 17 [t(15;17)(q24;q21)] which ultimately causes formation of PML-RARα oncoprotein.[1,2] This chimeric fusion protein obstructs myeloid differentiation at the promyelocytic stage while preventing apoptosis and promoting proliferation of leukemic progenitor cells.[3] Karyotyping or fluorescence in situ hybridization (FISH) is applied to identify this translocation while polymerase chain reaction (PCR) is used to detect the transcript of a suspected APL patient. Among different bcr isoforms a connection has been suggested regarding clinical presentation or response to treatment in APL patients.[4,5]

Signs and symptoms of patients having APL are generally associated with cytopenias. Most patient usually present with generalized weakness, easy fatigue and fever. Many patients during physical examination found to have hemorrhagic manifestations such as petechiae, purpura or ecchymotic lesion of the skin. Gum bleeding, epistaxis, retinal hemorrhages can also occur. The hemorrhagic complications are often out of proportion to the degree of thrombocytopenia which reflects the underlying biological properties of the transformed promyelocytes.[6]

Identification of PML-RARα is the authenticated marker for the diagnosis of APL. More than 98% of patients of APL have this translocation. All trans retinoic acid (ATRA) is the central component used in the treatment of APL with a healing rate of >80%.[7]

To ascertain the pattern of different breakpoints of PML/RARα fusion gene among Bangladeshi APL patients was the aim of this study.

To our knowledge, we are the first to study the pattern of different breakpoints of PML/RARα fusion gene among Bangladeshi APL patients.

MATERIALS AND METHODS

From June, 2021 to December 2024 a total of 60 cases of APL, aged 9-72 years and both genders were included in the study.

About 2 ml of whole blood obtained in Ethylenediaminetetraacetic acid (EDTA) bottle/vacutainer from all subjects. Then Genomic Ribonucleic acid (RNA) was extracted by QIAamp RNA Blood Mini Kit (QIAGEN®, USA). The quality and quantity of extracted RNA was measured by Nano Drop spectrophotometer 2000.

A ready to use kit named PML-RARα bcr1, 2, 3 one-step detection kit (EntroGen, Inc., USA) was used to detect isoforms of PML-RARα fusion gene. One single PCR protocol was used to amplify all isoforms according to manufacturer's instruction. Reverse transcription polymerase chain reaction (RT-PCR) detected the type of fusion gene formed from PML-RARα rearrangement. It was performed in Rotor-Gene Q (QIAGEN®, USA). There were 2 dyes selected for this test, FAM and VIC respectively. The VIC dye for internal control gene (ABL) and FAM for isoforms (bcr1, bcr2 and bcr3). If the Ct value of internal control is within the range of $18 \leq Ct \leq 22$ and the mutant's Ct value is ≤ 39 then that specific breakpoint is considered detected.

RESULTS

The age of the patients ranged from 9 years to 72 years with a mean of 35 years. There were 39 (65%) male and 21 (35%) female patients in the study group. The bcr1 isoform was found in 4 (6.67%) patients. No single bcr2 isoform was found in any patient. But in 3 patients bcr2 isoform found along with bcr1 (5.0%) and in 1 (1.66%) patient it was present in addition to bcr3. The bcr3 isoform was found in 19 (31.67%) patients and in 33 (55.0%) patients all of the isoforms were present. The frequency distribution according to gender and isoforms shown in Table-1, Table-2 and Table-3 respectively. The overall breakpoint distribution has been shown in Figure-1.

To evaluate the correlation between age group (Table 4)which is a continuous variable and break point which is a categorical variable, we have used single factor one way ANOVA as the data is normally distributed with almost equal variance, does not have any drastic outlier and the groups are independent of each other. Here, the level of significance is 0.05.

In the ANOVA table of age groups and break point categories (Table 5) we can see, as the Fcal > Ftab , the null hypothesis can be rejected at 0.05 level of significance and we can conclude that all break point categories are not equal throughout all age groups and differ significantly.

TABLE-1: Frequency distribution of male age group

Age group	Frequency	Percentage
10-19	06	15.38
20-29	11	28.21
30-39	04	10.26
40-49	08	20.51
50-59	07	17.95
60-69	02	5.13
70-79	01	2.56
Total	**39**	100.00

TABLE-2: Frequency distribution of female age group

Age group	Frequency	Percentage
01-10	04	19.05
11-20	04	19.05
21-30	02	9.52
31-40	03	14.29
41-50	04	19.05
51-60	04	19.05
Total	**21**	100

TABLE-3: Frequency distribution of break point with percentage

Break point	Frequency	Percentage
BCR1	04	6.67
BCR3	19	31.67
BCR1&2	03	5.0
BCR2&3	01	1.66
BCR1,2&3	33	55.0
Total	**60**	**100**

TABLE-4: Frequency distribution of age groups by break point groups

Age group	Break point group					Total
	BCR1	BCR3	BCR1&2	BCR2&3	BCR1,2&3	
1-10	00	01	00	00	04	**05**
11-20	02	03	01	00	05	**11**
21-30	01	04	00	00	06	**11**
31-40	00	01	00	01	05	**07**
41-50	00	06	00	00	07	**13**
51-60	01	03	22	00	05	**11**
61-70	00	00	00	00	01	**01**
71-80	00	01	00	00	00	**01**
Total	**04**	**19**	**03**	**01**	**33**	**60**

TABLE-5: ANOVA table of age groups and break point categories

Type	SS	d.f.	MS	F-value
Regression	1477.46	4	369.37	24.08
Error	536.92	35	15.34	

Here, d.f. = Degrees of freedom
SS = Sum of square
MS = Mean of square
$F_{cal} = 24.08$
$F_{tab} = F_{4, 35, 0.05} = 2.641$

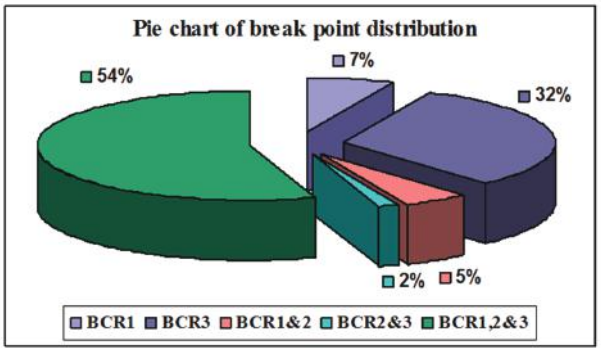

Figure-1: Pie chart of break point distribution

DISCUSSION

Among the subtypes of acute myeloid leukemia, APL is relatively rare.[8] It comprises approximately 5% to 13% of adult AML. It is uncommon in children with a median age of 47 years. It is usually not preceded by myelodysplastic syndrome but may have a history of prior cytotoxic therapy.[6] Acute presentation is usual with symptoms such as weakness, lethargy, fever and bleeding manifestations. Sometimes patient may present with features of disseminated intravascular coagulation due to severe hypercoagulable state having bleeding as the most prominent clinical manifestation.[6-16]

For diagnosis complete blood count, peripheral blood film, bone marrow study and immunophenotyping for acute leukemia panel is required. Coagulation profile along with some other biochemical tests such as serum uric acid, serum electrolytes, liver function tests, serum calcium, S.LDH etc. are also done. For confirmation of diagnosis molecular techniques are applied among which qPCR is the most popular which can measure the leukemia load of APL patients in respect to their PML-RARα bcr isoforms. For molecular targeted therapy and for monitoring minimal residual disease (MRD) molecular techniques play an important role.[17]

The 3 isoforms on the PML gene includes L-long form (located at intron 3) termed bcr1, S-short form (located at intron 6) known as *bcr2*, and V form (located at exon 6) known as *bcr3*.[7] The *bcr2* has been reported to be connected to a shorter remission period and comprehensive survival in comparison to the *bcr1* in a study by Gonzales et al.[5] Distinct breakpoints show unique response to ATRA. A number of European and USA studies have reported the frequency of breakpoint sites in the *PML* gene from to be approximately 50–55% for *bcr1*, 8–20% for *bcr3* and 27–49% for *bcr2*.[15] BCR 3 has been found to be most frequent in a study by Dutta et al from India.[16]

The age of the patients in our study ranged from 9 years to 72 years with a mean of 35 years. There were 39 (65%) male and 29 (35%) female patients in the study group. The male to female ratio was (1.9:1). In 2014 similar gender distribution (1.5:1) was also found by Chatterjee et al. and Sazawal et al. in 2009 (61.76% vs. 38.24%) among the patients of India.[4,18] Though Douer et al. showed female prevalence in their study (46.43% vs. 53.57%).[15]

The most frequent isoform of PML-RARα fusion gene was the combination of all three breakpoints i.e., bcr1, bcr 2 and bcr3 which was detected in 33 (55.5%) cases followed by bcr3 in 19 (31.67%) and bcr1 in 4 (6.67%) patients. In India and Pakistan, the most frequent isoform is bcr3.[7] BCR3 isoform heve been found to be the most frequent in Indian population by Chatterjee et al. in 2014 (38.09%) and Dutta et al. in 2008 (72.7%).[4,16] The results of these studies do not go with those of our study where we detected combination of all 3 breakpoints i.e., bcr1, bcr2 and bcr3 being the most common isoform.

We found bcr3 isoform as the second highest. BCR3 is not sensitive to ATRA and is also

linked to bad outcome; in a number of cases high TLC and CNS relapse occurs.[4]

In this study, the prevalence for only bcr1 was 4 (6.67%) patients. Unlikely, bcr1 was found as the most frequent PML-RARα isoform in studies published in western countries.[7]

In our study, the prevalence for only bcr2 was 0% in APL patients. Siahbani et. al. also showed similar result for bcr2 isoform in their study.[19] The prevalence of the bcr2 isoform was comparatively very low, in accordance with previous studies.[16,19-21] But this isoform was present along with bcr1 and bcr3 in 3 patients and in 1 patient respectively.

CONCLUSION

The key elements for the proper management of APL include early diagnosis and treatment initiation, proper supportive care, and recognition and management of therapy associated complications. Genetic testing is one of the most accurate methods for the diagnosis of this disease and ultimately can help in advancement of the disease management.

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