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Rare Cytogenetic Abnormalities Showing both MLL Rearrangement and Philadelphia Chromosome Positive in Pediatric Acute Lymphoblastic Leukemia Patients

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ABSTRACT

Objective: To evaluate the cytogenetics abnormalities in pediatric ALL cases and to correlate the cytogenetics Philadelphia chromosome - positive with the Fluorescence In - Situ Hybridization results.

Methods: Retrospective cytogenetics and FISH analysis of the Bone marrow samples of both adult and pediatric patientswere done. However, since the inclusion criteria for this study was pediatric ALL, only the data of the pediatric patients were taken for this study. For the prospective samples, the cytogenetic analyses and Fluorescence in - Situ Hybridization were performed on the procured samples. The cytogenetics and FISH test were performed as per the standard protocol of our lab and were analysed as per the standard guideline. The cytogenetics Philadelphia chromosome - positive were correlated with the Fluorescence In - Situ Hybridization results and vice versa.

Results: In our study of 50 pediatric ALLB patients, two patients showed low Ph+ by FISH. A confirmatory test by conventional cytogenetics revealed a rare association of Philadelphia chromosome positive along with the cytogenetics abnormality involving the MLL gene as well. One of the patient showed a karyotype of 46,XY,del(9)(p21),t((10;11)(p12;q21)[7]/46,XY,del(9)(p21)[9]/46,XYdel(22)(q11.2)[3] and the other patient showed 46, XX, t(9;11) (p13;q23)),?del(22)(q11.2)[6]/46,XX,del (11)(q23) [8]/46, XX[5] which were confirmed by cytogenetics and Fluorescence In - Situ Hybridization (FISH). Two patients showed complete Ph+ve and one patient showed normal karyotype along with tetraploidy. The rest of the cases showed either a normal karyotype or an insignificant abnormality.

Conclusion: In our study of 50 pediatric ALL patients, two cases showed a rare association of Philadelphia chromosome positive along with a cytogenetics abnormality involving the MLL gene. Apart from the rare findings in our study, emphases is also made on the confirmatory test by cytogenetics incidence of low Ph+ve by FISH and vice versa and a need for larger collaborative studies and intense follow up of the treatment and the prognosis of this subset of patients to determine the prognostic pattern to improve the treatment options for these kind of rare patients.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a type of leukemia that begins in bone marrow. In ALL, the body produces many abnormal lymphocytes due to which there is no enough space for the normal cells to develop.[1,2] It is the most common type of cancer diagnosed in children and accounts for 75% of childhood leukemia. A rare subtype of ALL is the Philadelphia chromosome - positive (Ph+ve) ALL. Philadelphia chromosome is formed when a parts of chromosome 22q just apposes to 9q which results in the abnormal chromosome with a fusion gene called BCR-ABL. This fusion gene of BCR-ABL triggers an abnormal activation of tyrosine kinase which stimulates overgrowth of WBC and prevents other normal blood cells from developing.[3] When this fusion gene of BCR-ABL is found in an ALL patient, the condition is called as Ph+ve ALL.

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According to American Cancer society, certain factors like age, white blood count, minimal residual disease and complex cytogenetics are the well-known risk factor that affects patients with acute lymphoblastic leukemia. Among the above risk factors, age is one of the most important prognostic factors in ALL.[4] Long-term survival rates in children are approximately 80% and the survival rate decreases to less than 30% in adults.[5] Ph positive ALL, which is a rare subtype of ALL, occurs in approximately 5% of patients with ALL aged <20, the incidence escalates to 33% in patients aged 40years and is 49% in patients aged >40yrs; the incidence decreases to 35% in patients aged >60yrs.[5,6] However Philadelphia chromosome translocation is uncommon in pediatric patients with ALL, with a frequency of less than 3-5%, where as it is the most common cytogenetic abnormality in adult ALL.[7] Ph+ve ALL is classified as to have a high or very high risk, and only 20-30% of Ph+ve children with ALL are cured with chemotherapy alone.[8,9] Added to this, a complex cytogenetics, along with the Ph+ve chromosome, increases the unfavourable conditions for such patients.[10]

Apart from Ph positivity in ALL, MLL rearrangements with 11q23 translocation are also known to be observed among the ALL patients. The overall dismal prognosis for ALL patients with MLL rearrangements has spurred efforts to improve treatment options for such individuals, including the use of hematopoietic stem-cell transplantation.[11-13] Among infants, MLL rearrangements are associated with EFS and estimates to 10% to 20%.[14,15] Some studies have shown that MLL rearrangement confers a poor prognosis regardless of age at diagnosis.[16,17]

MATERIALS AND METHODS

Bone marrow samples were obtained by the clinician from patients with ALL and were sent for required investigation. Both adult and pediatric patientswere referred for cytogenetic and FISH tests. However, since the inclusion criteria for this study was pediatric ALL, only the data of the pediatric patientswith either new diagnoses or suspected ALL were taken for this study.

For the prospective samples, the Cytogenetic analyses and Fluorescence In - Situ Hybridization (FISH) were performed on the procured samples as and when requested by the clinician. The cytogenetics and FISH test were performed as per the standard protocol of our lab and were analysed as per the standard guideline. This study is descriptive and because of the relatively small numbers of patient's study, no formal statistical comparisons were made.

RESULTS

In our study of 50 pediatric ALL patients, two cases, that is patient 2 and 4showed low Ph+ by FISH (Figures 1a & 2a). A confirmatory test by conventional cytogenetics revealed a rare association of Philadelphia chromosome positive along with the cytogenetics abnormality involving the MLL gene as well. Patient 2 showed a karyotype of 46,XY,del(9) (p21), t((10;11) (p12;q21)[7]/46,XY,del(9)(p21)[9]/46,XYdel(22) (q11.2)[3](Figure 2b) and patient 4 showeda karyotype of 46, XX, t(9;11) (p13;q23)),?del(22)(q11.2)[6]/46,XX,del (11) (q23) [8]/46, XX[5] (Figure 2b) which were confirmed by cytogenetics and Fluorescence In - Situ Hybridization (FISH). Patient 1 and 5 showed complete Ph+ve. Whereas patient 3showed normal karyotype along with tetraploidy (Table 1).The rest of the cases showed either a normal karyotype or abnormalities which were not significant.

DISCUSSION

Among Leukemia, ALL is a type of leukemia that begins in bone marrow and the body produces many abnormal lymphocytes due to which there is no enough space for the normal cells to develop. A rare subtype of ALL is the Ph positive ALL which is formed when parts of chromosomes 9 and 22 switches over with each other

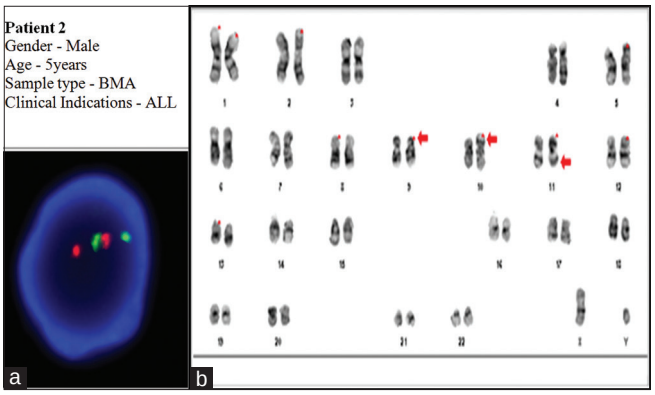

Figure 1: (a) Showing 22% cells showed Ph positive by FISH, (b) Showing Karyotype: 46,XY,del(9)(p21),t((10;11)(p12;q21)[7]/46,XY,del(9)(p21)[9]/46,XYdel(22)(11.2)[3]

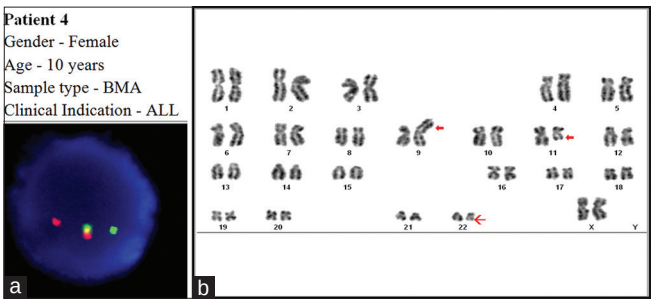

Figure 2: (a) Showing 28% cells showed Ph Positive by FISH, (b) Showing Karyotype: 46, XX, t(9;11) (p13;q23)),?del(22)(q11.2)[6]/46,XX, del (11) (q23) [8] /46, XX[5]

Table 1: showing chromosomal abnormalities along with the FISH results.

Patient	Age	Sex	Sample	Clinical Indication	Karyotype	FISH Results
1	14Ys	M	BMA	ALL	46, XY, t(9;22) (q34;11.2)	100% Ph positive
2	5Ys	M	BMA	ALL	46, XY, del(9)(p21), t((10;11)(p12;q21)[7]/46, XY, del (9)(p21)[9]/46, XYdel(22)(q11.2)[3]	22% Ph positive
3	8Ys	M	BMA	ALL	46, XY/Tetraploidy	40% Ph positive
4	10Ys	F	BMA	ALL	46, XX, t(9; 11)(pl3;q23),?del(22)(q11.2)[6]/46, XX, del(11)(q23)[8]/46,XX[5].	28% Ph positive.
5	3Ys	M	BMA	ALL	46, XY, t(9;22) q(34;11.2)/46,XY	86.5% Ph positive

(reciprocal translocation) and results in the abnormal chromosome carrying a fusion gene called BCR-ABL. This fusion gene triggers an abnormal activation of tyrosine kinase which stimulates overgrowth of WBC and therefore prevents other normal blood cells from developing.

On the other hand MLL rearrangements are also observed in some of the ALL patients. MLL gene rearrangements are generally associated with a dismal outcome in ALL.[14,15,18] however two distinct subsets with MLL-ENL fusions have an excellent prognosis.[16,19]

Thus, as per the review of literatures(11,20,21)it is reported that, in general, some of the ALL patients show positive for eitherPh or MLL, but in our study the abnormalities of both Ph and MLL in the same individual was found to be a very rare finding which is discussed below.

The focus of our study was on Ph+ve pediatric ALL alone. Hence, of the adults and the pediatrics with diagnosed or suspected ALL, only data of pediatric patients were compiled and correlated. Thus of the 50 pediatric patients referred to our unit for cytogenetics and FISH, only 5 pediatric patients were Ph+ve ALL. Among these 5 patients (Table 1) two patients (patient 2&4) Figures 1a and b, 2a and b showed an interesting and rare abnormality involving both Ph and MLL rearrangements and is discussed below. The rest of the cases showed either a normal karyotype or an insignificant abnormality.

Patient 2 was a 5year old male child with ALL referred for cytogenetics and FISH. The karyotype showed 46,XY,del(9)(p21),t((10;11)(p12;q21)[7]/46,XY,del(9)(p21)[9]/46,XYdel(22)(q11.2)[3] Figure 1b. However 22% of the cells showed positive for Ph by FISH Figure 1a confirming the deletion on chromosome 22q which was observed in three spreads in conventional karyotyping. This patient did not continue the treatment in our hospital therefore the follow-up of the treatment regime and the prognosis of the patient was not recorded.

Patient 4 was a 10 year old female child referred with suspected ALL and was advised for FISH and molecular tests to rule out Ph+ve and MLL rearrangements. Subsequently the tests were conducted and the FISH showed only 28% positive and MLL rearrangement also showed a positive result, therefore karyotyping was advised to confirm the same. Eventually the karyotype revealed that there was a translocation between t(9;11) and a deletion on 22q was observed as well, which is as follows 46, XX, t(9;11) (p13;q23)),del(22)(q11.2)[6]/46,XX,del(11)(q23) [8]/46, XX[5] Figure 2b. This was very unique and a rare abnormality involving chromosome 9 and 11 and a deletion seen on 22q which may be the reason why both Ph by FISH Figure 2a and the MLL gene test were positive. However this patient did not survive due to various health complications and was not responding to any treatment as well.

As per the literatures review[11,20-24] it is reported that an ALL patient can either be positive for Ph or MLL but our result in the above study with the positive result for both Ph and MLL in the same individual was found to be a very rare and interesting finding.This study also accentuates that, in incidences of patient with low Ph+ve by FISH, a confirmatory test by cytogenetics is strongly recommended to confirm the same and to rule out any kind of complex/rare cytogenetic abnormalities, which would be helpful for the clinician to determine the prognosis and to plan the treatment for such patients.

The other two patients1 & 5 showed t (9; 22) by cytogenetics and Ph+ve were confirmed by FISH. Patient3 showed normal karyotype and tetraploidy by cytogenetics, however the FISH of this patient showed 40% positive for Ph. The remaining patients showed a normal karyotype and were negative for Ph by FISH.

CONCLUSION

Although the number of patients in our study is small to permit definitive conclusions, the outcome of the above discussed patient (2 and 4) where in, both Ph+ve as well

MLL rearrangements were observed in the same individual, the abnormality in these patients appeared to be a sporadic and quite an interesting one to highlight their significance in pediatric ALL cases.

A part from the unique and rare abnormality reported above in our study, it is also very important to emphasize the importance of further studies of such rare cases. Thus, a larger collaborative studies and intense follow up of the treatment and the prognosis of this subset of patients are needed to determine the prognostic pattern and to improve the treatment options for these kinds of rare cases.

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