ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 04, pp.3542-3545, April, 2019

DOI: https://doi.org/10.24941/ijcr.35410.04.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

LANDSCAPE OF ADDITIONAL CHROMOSOMAL ABNORMALITIES IN PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: 5YEAR EXPERIENCE OF A REGIONAL CANCER CENTER IN SOUTH EAST ASIA

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ARTICLE INFO

Article History:
Received 09th January, 2019
Received in revised form
12th February, 2019
Accepted 15th March, 2019
Published online 30th April, 2019

Key Words:

Philadelphia Positive Acute Lymphoblastic Leukemia, Additional Chromosomal Abnormalities.

ABSTRACT

Background: Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) is an aggressive disease, which differs from chronic myeloid leukemia in lymphoid blast crisis at both chromosomal and molecular level. The current study aims to study additional chromosomal abnormalities (ACAs) in Ph+ALL, with review on its prognostic implications. **Materials and Methods**: This is retrospective single group exploratory study, from 2014 to 2019. Cytogenetic information of 74 cases of Ph+ALL and their clinical information were obtained from the departmental records and case files. Cytogenetic analysis of samples was done in accordance with standard laboratory protocol. **Results**: Thirty out of 74 patients showed ACAs (40.5%). The median age of patients was 21years. ACAs were more frequent in females than in males up to 40years of age and reverse was true in older patients. Most frequent abnormalities observed were extra Ph, del(9p), add(19p), del(6q) and dic(9;12). Limited follow-up data for patients with extra Ph showed complete remission at median survival of 15 months and poor prognosis in an elderly patient with dic(9;12) as additional abnormality. **Conclusion**: The frequency and pattern of ACAs in our cohort of Ph+ALL patients were similar to standard available literature, with few exceptions. Long-term follow-up studies are recommended to analyze the prognostic significance of these ACAs in Ph+ALL.

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Citation: Shanthala S., Obula Reddy C., Kavitha B.L., Padma M., Lokanatha D. and Prasanna Kumari, **2019.** "Landscape of additional chromosomal abnormalities in Philadelphia chromosome positive acute lymphoblastic leukemia: 5year experience of a regional cancer center in South East Asia", *International Journal of Current Research*, 11, (04), 3542-3545.

INTRODUCTION

Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) accounts for 25% of adult ALL and less than 5% of pediatric ALL (Pullarkat, 2008). It is the most common cytogenetic abnormality in adults with ALL, accounting for 20-30% of ALL cases. The incidence of Ph+ALL increases with age, occurring at the frequency of more than 50% in adults older than 50years of age (Liu-Dumlao et al., 2012). It is recognized as a poor risk group in the WHO classification of precursor B-cell ALL. Ph+ALL is biologically different from chronic myeloid leukemia (CML). While BCR-ABL fusion is sufficient for the onset of CML, activation of SRC kinases such as Lyn, Hck and Fgr is essential for the development of Ph+ALL (Hu, 2004). In addition, many epigenetic changes, copy number variation and mutations occur downstream BCR-ABL fusion that contribute to the aggressive clinical nature of Ph+ALL (Fielding, 2010). The knowledge of secondary chromosomal abnormalities helps in differentiating Ph+ALL from CML-lymphoid blast crisis and its prognostication. Moreover, geographic heterogeneity occurs in incidence of non-random cytogenetic abnormalities in leukemia related to genetic and environmental influence (Li, 2009). Hence, the current study aims to explore the frequency and pattern of additional chromosomal abnormalities (ACAs) in cohort of Ph+ALLfrom a single institution in South East Asia. An attempt has been made to analyze the prognostic impact of these aberrations.

MATERIALS AND METHODS

A single group observational study, retrospectively analyzed 74 cases of Ph+ALL from January 2014 to April 2019. Chromosomal abnormalities and demographic details were obtained from cytogenetic departmental records. Clinical and

hematological information were obtained from case files. Bone marrow aspiration samples were sent for cytogenetic studies from departments of medical and pediatric oncology. RPMI 1640 medium supplemented with 15% qualified, heat inactivated fetal bovine serum was used to set up duplicate cultures of 24hour and 48hour incubation in glass vials. This was followed by mitotic arrest using Karyomax-Colcemid at concentration of 0.05µg/ml) for 30 minutes and treatment with hypotonic solution (potassium chloride at 0.075M concentration) for another 30 minutes. Overnight fixation was done in Cornoy's fixative (methanol and glacial acetic acid at 3:1 concentration), followed by GTG (Giemsa-Trypsin-Giemsa)-banding of prepared slides. Chromosome analysis and interpretation were done in accordance with ISCN (International System for Cytogenomic nomenclature). Criteria used for diagnosis of Ph+ALL: i) Clinical- no preceding history of chronic phase of CML, no massive splenomegaly, presence of lymphadenopathy ii) Hematological-absence of basophilia/ left shift iii) Immunophenotype- precursor-B-cell ALL. iv) cytogenetic parameters- concurrent presence of normal karyotype and Ph-positive clonev) RT-PCR showing minor BCR-ABL hybrid protein (p190kDa) in majority of patients.

RESULTS

Among 74 Ph+ALL patients, 31 were females and 43 were males. Median age of occurrence was 20years (range: 2-64 years). Out of 74 cases of Ph+ALL, 30 patients showed additional chromosomal abnormalities (ACAs). Among patients with ACAs, the median age was 21 years, 16 were females and 14 were males. Between 11 and 40years, ACAs were more frequent in females than in males and reversal was observed in older patients [figure 1]. The most common additional abnormalities observed were [Table 1, figure 2]: extra Ph chromosome [Figure 3](6 out of 30 patients; 20%), del(9)(p13) (5 out of 30 patients; 16.6%), dicentric translocations such as dic(7;9) and dic(9;12)(3 patients; 10%), add(19)(p13) (2 patients: 6.6%) and del(6q) (2 patients: 6.6%) in the decreasing order of frequency. The remaining 40% (12/30 cases) was constituted by other random chromosomal rearrangements.

DISCUSSION

Cytogenetics is the single most important predictive factor of clinical outcome in both adult and pediatric ALL. Compared to patients with Ph negative ALL, Ph+ ALL patients have poorer prognosis with increased risk of CNS involvement and aggressive clinical course. Philadelphia chromosome results from reciprocal translocation between chromosomes 9 and 22 resulting in BCR-ABL fusion and constitutive activation of chimeric fusion protein tyrosine kinase. The molecular weight of BCR-ABL1 hybrid protein depends on the chromosome breakpoint. Minor breakpoint cluster region (m-BCR) is involved in nearly 90% of pediatric Ph+ALL, generating 190kDa hybrid protein, while 10% may have major-BCR/ABL1 encoded 210kDa hybrid protein (Deininger, 2000). The combination of hematologic, cytogenetic and molecular investigations in correlation with clinical findings helped in successful categorization of Ph+ve ALL in the current study. Accumulation of additional non-random chromosomal abnormalities is suggestive of clonal evolution of the disease and considered a hallmark of multistep progressive disease in CML.[7]Less is known about the frequency and impact of ACAs in Ph+ALL, which is identified as a poor risk group in B-cell ALL. Li Y et al (2009) reported ACAs in 41-86% of Ph+ALL patients (Li, 2009). Thirty out of 74 patients (40.5%) with ALL in the present study showed Ph-positivity. All patients were immunophenotyped as precursor B-cell ALL. Prognosis in ALL is largely determined by the age of the patient, declining from 80% in children less than 5 years of age to 30% in adults older than 45years (Lee, 2010; Pulte, 2009). There is paucity of data regarding the frequency of ACAs in Ph+ALL according to age and gender. The current study showed upward trend in the frequency of ACAs with advancing age among females and vice versa was observed among males up to 40years of age. A remarkably increased frequency of ACAs (from 22.2% before 40years to 60% after 40years) was observed among males after 40 years of age and it was significantly greater in men than in women. This partly explains the declining survival rate of Ph+ALL patients with advancing age, the lowest being observed in patients older than 40years of age. Frequent aberrations noted in earlier studies were extra Ph chromosome, hyperdiploidy, monosomy 7/del(7p), 9p abnormalities and trisomy 8 (Heerema, 2004; Schultz**,** 2014; Short, 2016; Seol, 2017). In agreement with this, the present study identified extra Ph and 9p deletion as most frequent abnormalities. Twelve out of 30 patients (40%) had hyperdiploid karyotype. Deletion involving long arm of chromosome 6 (6q)is characteristic of ALL, irrespective of the lineage. Del(6q) wasobserved in two patients in this study. Unique findings of the current study are association of 19p13 abnormalities and dicentric translocations with Ph+ALL. Prognostic impact of ACAs in PH+ALL Seol CA et al (2017) notedsignificantly shorter overall survival and disease free survival in 73% of 122 adult Ph+ALL with ACAs.

In another study of 78 adult Ph+ALL patients who underwent hematopoietic cell transplantation following tyrosine kinase inhibitor therapy, 3year leukemia-free survival (79.8% vs. 39.5%) and overall survival (83% vs. 45.6%) were significantly superior in Ph only cohort than those with ACAs (Aldoss, 2015). Nicholas J et al (2016) studied ACAs in 97 out of 125 (78%) adult Ph+ALL patients. All patients received hyper-CVAD with TKIs. 5year overall survival and progression free survival were similar between Ph alone and ACAs groups. Patients with der(22), -9/9p, Ch 1 translocation and Ch 3 abnormalities constituted a distinct group with particularly poorer prognosis with medial relapse free survival (RFS) of 21 months and 5yr RFS rate of 38% (Short, 2016). Schultz KR et al (2014) explored prognostic impact of ACAs in 44 out of 69 (64%)pediatric Ph+ALL cases. ACAs group had significantly lower 5year event-free survival (86% vs 51%, p=0.05).12Li Y et al (2009) observed worse prognosis with monosomy 7 and 9p abnormalities (Li, 2009). Prior to the availability of tyrosine kinase inhibitors (TKIs), long-term survival of Ph+ALL patients was not more than 20%. With the current combination option of TKIs alone or in combination with multidrug chemotherapy, complete remission rate has increased to nearly 90% (Fakih et al., 2018). Adult Ph+ALL patients in the current study were treated as per hyper-CVAD chemotherapeutic regimen along with tyrosine kinase inhibitors. Pediatric patients were treated according to MCP 841 protocol. Four out of six patients with extra Phremained in complete remission on median follow-up of 15months. Dicentric translocations alone have been identified as good prognostic factor in few studies (Mahmoud, 1992; Behrendt et al., 1995). A 57year old women harboring both t (9;22) and dic(9;12) in the present study showed initial good response to treatment and died in 28 months due to unknown cause.

Table 1. Karyotype of Ph-positive ALL patients with additional chromosomal abnormalities

AGE	GENDER	KARYOTYPE
3	M	50,XY,+5,t(9;22)(q34;q11.2),+17,+21,+der(22)t(9;22)(q34;q11.2)
4	F	46,XX,del(6)(q21),t(9;22)(q34;q11.2)
4	F	45,XX,del(9)(p13),t(9;22)(q34;q11.2),-14,-15,+mar/46,XX,t(9;22)(q34;q11.2)/46,XX
5	F	45,XX,dic(7;9)(p11;p11),t(9;22)(q34;q11.2)
6	M	46,XY,inv(4)(p14q13),t(9;22)(q34;q11.2),del(9)(p13)
7	M	46,XY,del(9)(p13),t(9;22)(q34;q11.2)
7	M	47,XY,+8,t(9;22)(q34;q11.2)
8	F	47,XX,+1,del(1)(p32),t(9;22)(q34;q11.2)
8	M	46,XY,add(2)(q37),del(6)(q23),t(9;22)(q34;q11.2),add(11)(q23)
9	F	47,XX,t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2)
12	F	47,XX,-8,i(9)(q10),der(9)del(9)(p13)t(9;22)(q34;q11.2),add(14)(q32),+2mar
12	F	46,XX,dup(1)(q21q25),del(6)(q23),t(9;22)(q34;q11.2)
15	M	45,XY,t(9;22)(q34;q11.2),-13
18	F	46,XX,t(9;22)(q34;q11.2),add(11)(q23)
20	M	48,XY,+8,der(9)t(4;9)(q24;p24),t(9;22)(q34;q11.2),-12,+der(22)t(9;22)(q34;q11.2),+mar
22	F	47,XX,t(2;3)(q21;q29),t(9;22)(q34;q11.2),+22
23	M	46,XY,dic(9;12)(p13;p13),t(9;22)(q34;q11.2)
23	F	47,XX,t(9;22)(q34;q11.2),add(19)(p13),+der(22)t(9;22)(q34;q11.2)
24	M	46,XY,del(9)(p13),t(9;22)(q34;q11.2)
24	F	46,XX,t(9;22)(q34;q11.2),add(19)(p13)
28	M	47,XY,t(9;22)(q34;q11.2),+21
32	F	44,XX,-8,t(9;22)(q34;q11.2),-17
33	M	46,XY,t(9;22)(q34;q11.2)/45,XX,-7,t(9;22)(q34;q11.2)/46,XX
34	F	45,XX,rob(14;15)(q10;q10),t(1;9;22)(q21;q34;q11.2)/46,XX
35	F	46,XX,add(1)(p36),t(9;22)(q34;q11.2)
39	M	47,XY,t(9;22)(q34;q11.2),+21
47	F	47,XX,t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2)
57	F	45,XX,-2,i(8)(q10),dic(9;12)(p11;p11),t(9;22)(q34;q11.2)
60	M	48,XY,t(9;22)(q34;q11.2),+19,+der(22)t(9;22)(q34;q11.2)
64	M	46,XY,dup(1)(q22q32),del(3)(q12),t(9;22)(q34;q11.2)

Figure 1: Trend in frequency of additional chromosomal abnormalities according to age and gender

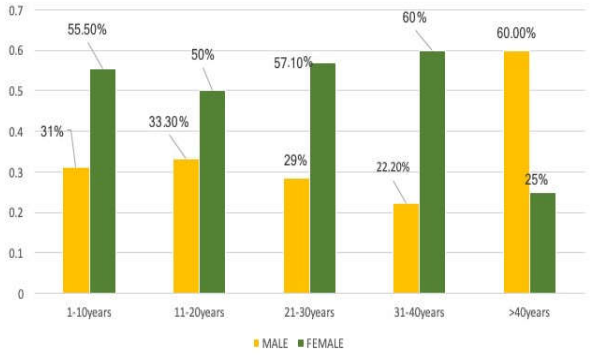
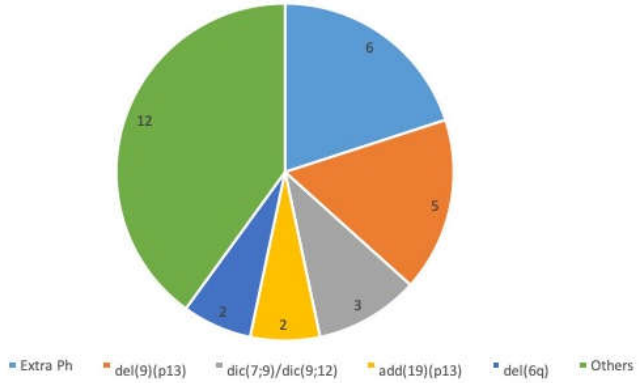
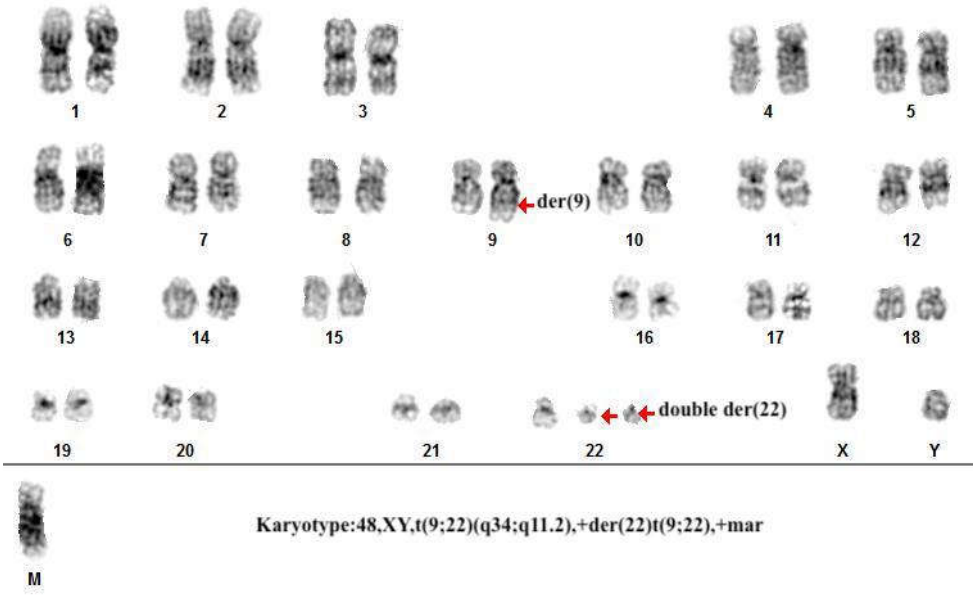


Figure 2: Additional chromosomal abnormalities in Ph+ALL





Karyotype:48,XY,t(9;22)(q34;q11.2),+der(22)t(9;22),+mar

Another 23year old male patient with co-existing dic(9;12) and t(9;22) responded well to chemotherapy and is on regular follow-up. Poor prognosis in the former patient could be attributable to overriding of prognostic impact of t(9;22) over dic(9;12) coupled with poor clinical response intrinsic to old age.

Conclusion

Main inferences of the current study are: a) additional chromosomal abnormalities occurred at a frequency of 40.5% in both pediatric and adult Ph+ALL;b) The most common abnormalities observed were extra Ph, del(9)(p13), dicentric traslocations and add(19)(p13). Type of additional chromosomal abnormality probably determines the prognosis in individual Ph+ALL patients. Long-term follow-up studies are recommended to analyze the prognostic impact of additional chromosomal abnormalities in Ph-positive acute lymphoblastic leukemia.

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