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Acute Lymphoblastic Leukemia with Hyperdiploidy and Philadelphia Chromosome – Poor or Good Prognostic Indicator?

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ABSTRACT

Background: In Acute Lymphoblastic Leukemia (ALL) the role of cytogenetics in patients management has largely been centered on the presence of the Philadelphia (Ph) chromosome and hyperdiploidy. The Ph chromosome is observed in 5% of pediatric and 25% of adults ALL cases which is associated with poor outcome, However; hyperdiploid Karyotype is detected in 2% - 9% of adults and in 29% of pediatric patients which is associated with better prognosis. Whereas hyperdiploidy with Ph chromosome accounts for very rare cases of ALL, so such possible crucial cytogenetic events of hyperdiploidy with Ph chromosome and its clinical significance are a matter of further study. **Materials & Method:** Here, we share our experience of ALL cytogenetics. Bone marrow or Peripheral blood short term cultures of total 63 ALL patients performed for GTG banding and Fluorescent in-situ Hybridisation for chromosome analysis were carried out. There were 43(68.3 %) males and 20(31.7%) females. **Results:** In terms of chromosomal pattern, 27(42.9%) patients were with t (9;22), 26(41.2%) were hyperdiploid and 10(15.9%) patients showed hyperdiploid with t (9;22). Mean, Median and overall survival analysis revealed that shorter survival for hyperdiploidy with t (9;22) and longer survival for hyperdiploidy group and intermediate for t(9;22). **Conclusion:** Thus it is conceivable that the presence of hyperdiploidy as an additional karyotypic abnormality may confer a poor prognosis to t(9;22) ALL, presumably by altering the kinetics of Ph+ neoplastic cells. A Meta analysis of the karyotypic abnormalities may enable risk stratification of Ph+ ALL patients. Such an approach may identify patients who could benefit from newer therapeutic approaches.

INTRODUCTION:

The Philadelphia (ph) chromosome is the hallmark genetic lesion of Chronic Myeloid Leukemia. It is also present in only 5% of the pediatric and 25% to 50% of the adult Acute Lymphoblastic Leukemia (ALL) cases and is associated with poor prognosis[1,2,3] However; hyperdiploid Karyotype defined by the presence of more than 46 chromosomes is detected in 2% to 9% of adults and in 29% of pediatric patients[4,5] which is associated with better prognosis and good response to conventional therapy[6,7,8].

Genetic abnormalities in addition to the Ph chromosome may influence the biology and clinical course of ALL, but there are not many studies on the potential genetic heterogeneity of ph positive ALL and clinical outcomes. It is conceivable that the simultaneous presence of additional karyotypic abnormalities may alter the biological properties of ph positive cell and influence clinical outcomes. In this context, the coexistence of a hyperdiploid(> 46 chromosomes) Karyotype, with the Philadelphia positive chromosome is of interest since hyperdiploidy as the sole cytogenetic abnormality in ALL is associated with good prognosis [6,9]. So such possible crucial cytogenetic events of hyperdiploidy with ph chromosome and its clinical significance are a matter of further study. Here is an attempt to determine whether the adverse prognosis conferred by ph positive, altered by the presence of cytogenetic predictors of good response.

MATERIALS AND METHODS:

A total of 63ALL patients were enrolled for cytogenetic study at Gujarat Cancer & Research Institute. The routine diagnostic and prognostic procedure and conventional cytogenetics and FISH were performed. There were 43 males and 20 females.

For cytogenetic study, bone marrow specimens were collected in RPMI-1640 medium with heparin, and short-term cultures were carried out in RPMI-1640 medium supplemented with antibiotics, serum, and heparin. Overnight to 48 hours incubation was carried out followed by mitotic arrest using colcemid (10µl/8 ml of culture). Harvesting was performed using pre-warmed 0.56% KCl hypotonic solution followed by washes with fixative (1:3 Acetomethanol). Slides were air dried, aged for 1 day and GTG banding was carried out [10,11].Conventional cytogenetics by GTG banding: The slides were treated with Trypsin and EDTA, and stained with 4% Giemsa stain according to standard procedures. This was

followed by karyotyping according to the International System of Cytogenetic Nomenclature (ISCN) 2013 guidelines[12].

Fluorescence in situ hybridization (FISH): Using Dual Color Dual Fusion BCR/ABLLocus Specific Identifier (LSI) probes, FISH was performed according to manufacturer's (Abbott/Vysis Inc., USA) instructions. For analysis of conventional cytogenetics and FISH results, automatic karyotyping system from Carl Zeiss with IKAROS karyotyping software (Metasystems, Germany) was used.

RESULTS:

The study group comprised 63 newly diagnosed and untreated ALL cases. There were 43 (68.3%) males and 20 (31.7%) females with male/female ratio of 2.2/1. The patient characteristics are summarized in Table 1.

Table 1: Characteristics of total 63 ALL Patients, by karyotype category

Characteristics	**Total**	**t(9;22)**	**t(9;22) with 2n+**	**2n+**	**p Value**
No (%)	63	27 (42.9%)	10 (15.9%)	26 (41.2%)	
Male	43 (68.3%)	13 (48.1%)	6 (60%)	24 (92.3%)	
Female	20 (31.7%)	14 (51.9%)	4 (40%)	2 (7.7%)	
Age (Years)					
Range	2-70	3-70	22-55	2-55	
Mean	24.75	31.3	37.4	13.1	<0.0001
Median	23	30	37	7.5	
Age Group (%)					
Infant (<1year)	0(0%)	0	0	0	
Children (1 to 15 year)	24 (38.1%)	5 (18.5%)	0	19 (73.1%)	
Adult (>15 year)	39(61.9%)	22 (81.5%)	10 (100%)	7 (26.9%)	
Hemoglobin(g/dl)					
Range	3-13	3-10	4-13	3-12	
Mean	7.56	7.78	8.4	7	<0.187
Median	8	8	8	7	
Blast (%)					
Range	35-98	35-95	62-90	35-98	
Mean	82.76	81.12	86.3	83.04	<0.677
Median	90	90	90	90	
WBC (x10^{3}cmm)					
Range	0.5-458	0.6-458	2.3-170	0.5-229	
Mean	58.5	80.7	58.1	35.6	<0.233
Median	24	25.3	45.8	18.2	
Platelet (x10^{3}cmm)					
Range	5.0-700	5.0-296	17-700	6.5-389	
Mean	82.2	74.6	11.8	76.5	<0.571
Median	44	52	50.5	39	

Cytogenetic analysis showed that the hyperdiploid Karyotype (>46 chromosomes) was encountered in 26 (41.2%) patients and t(9;22) in 27 (42.9%) patients were detected by conventional cytogenetics or FISH or combination of both methods. We report 10 (15.9%) cases of hyperdiploidy with Philadelphia chromosome among them 6 were male and 4 were females. All of them were adult. The conventional Cytogenetic and FISH results of the patients, hyperdiploid with Philadelphia are shown in Table 2.

Table 2: Cytogenetics and FISH results of hyperdiploidly with philadelphia ALL patients at diagnosis

Case No	Age/Sex (year)	Karyotype	FISH Result for BCR-ABL DC DF
1	50/F	2n+,t(9;22)(q34;q11.2)[10]	OGFF
2	22/M	2n+,t(9;22)(q34;q11.2)[3]/46,XY,t(9;22)(q34;q11.2)[7]	OGFF
3	50/F	47,XX,t(9;22)(q34;q11.2),+12[3]/46,XX,t(9;22)(q34;q11.2)[5]	OGFF
4	23/M	47,XY,+8,t(9;22)(q34;q11.2),i(17)(q10)[8]	OGFF
5	22/M	47,XY,+8,t(9;22)(q34;q11.2),del(20)(q?)[9]	OGFF
6	40/M	47,XY,t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2),add(17)(q?)[3]/47,XY,t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2),add(17)(q?),add(17)(q?)[2]/46,XY[7]	OGFFF
7	55/M	50,XY,+2,+5,t(9;22)(q34;q11.2),+21,+der(22)t(9;22)(q34;q11.2)[4]	OGFFF
8	30/M	48,XY,t(9;22)(q34;q11.2),+?19,+der22,t(9;22)(q34;q11.2)[3]/46,XY,t(9;22)(q34;q11.2)[10]	OGFFF
9	28/F	48,XX,+8,t(9;22)(q34;q11.2),+der(20)[2] /46,XX[3]	OGFF-55%, OOGG-45%
10	34/F	2n+,t(9;22)(q34;q11.2)[4]/46,XX,t(9;22)(q34;q11.2)[10] /46,XX[5]	OGFF-75% OOGG-25%

The estimated mean Overall survival (OS) was analyzed, OS defined as time from diagnosis to the death or last visit (last follow up). Chi-square test was used to compare mean of different variable. Kaplan- Meier life tables and cures were constructed by means of the log-rank method represented in Table 3[13]. Differences were analyzed by Cox-Mantel test [14], with adjustment for pairwise comparisons. Differences were considered statistically significant when the p-value was less than 0.05. The analysis was performed using SPSS software version 15.0 (SPSS, Chicago, IL, USA). The estimated mean OS for patients with Philadelphia group was 16.5 months (95% CI, 9.15 – 23.85 months).The estimated mean OS for Hyperdiploidy with Philadelphia patients was 1.4 months (95% CI, 0.47 – 2.32 months) and estimated mean OS for patients with Hyperdiploidywas23.49 months (95% CI, 13.65 – 33.33 months).

Table 3: Pair-wise comparisons of cytogenetically different variable

Pair-wise comparisons							
	Group	t(9;22)		Hyperdiploidy		t(9;22) with Hyperdiploidy	
		Chi-Square	p value	Chi-Square	p value	Chi-Square	p value
Log Rank (Mantel-Cox)	t(9;22)			1.017	<0.313	12.520	<0.0001
	Hyperdiploidy	1.017	<0.313			9.939	<0.002
	t(9;22) with Hyperdiploidy	12.520	<0.0001	9.939	<0.002		

Kaplan Meier survival curve revealed that lower for patients with hyperdiploidy with t(9;22), longer survival for patients with hyperdiploidy category and intermediate survival for patients with t(9;22). Kaplan-Meier survival curve revealed that OS outcome is highly significant among patients with t(9;22) and patients with t(9;22)with hyperdiploidy ($p<0.0001$, Table 3; Figure 1). Survival analysis between patients with hyperdiploidy and patients with t(9;22) with hyperdiploidy showed significant difference ($p<0.002$, Table 3; Figure 2). There was no significant difference in outcome between patients with t(9;22) and patients with hyperdiploidy($p<0.313$, Table 3; Figure 3).

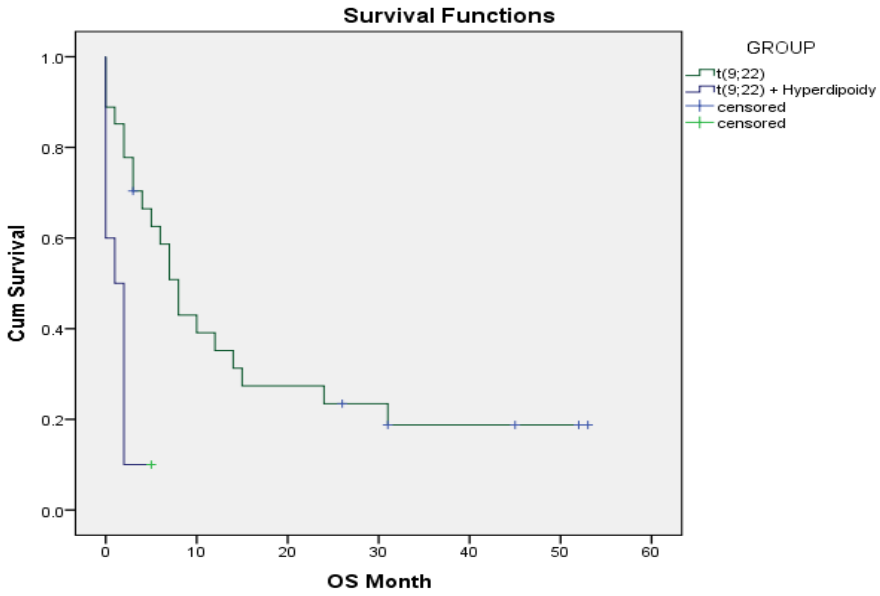

Figure 1: **Overall survival outcome between t(9;22) and t(9;22) with hyperdiploidy.**

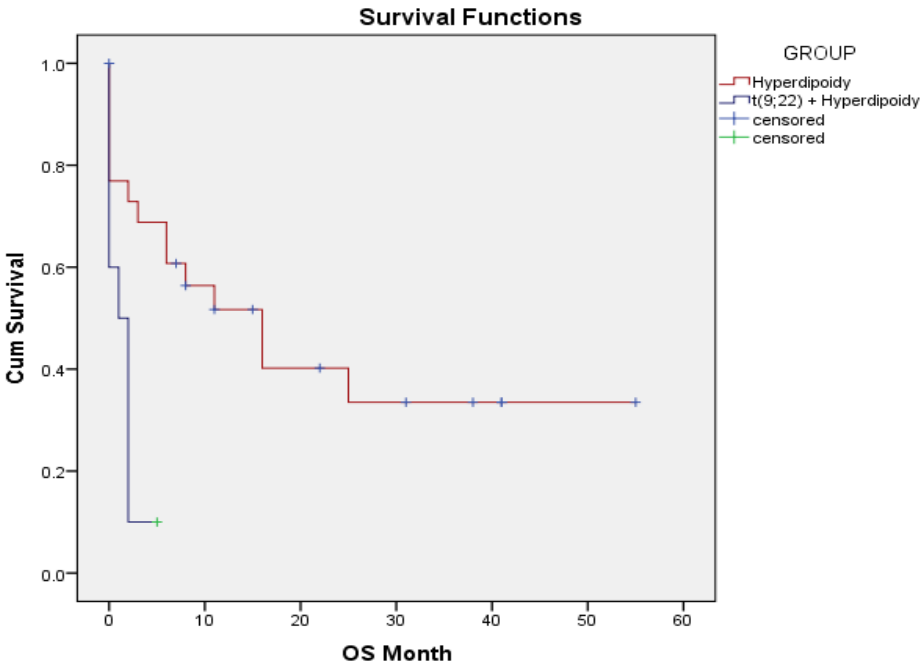

Figure 2: **Overall survival outcome between hyperdiploidy and t(9;22) with hyperdiploidy.**

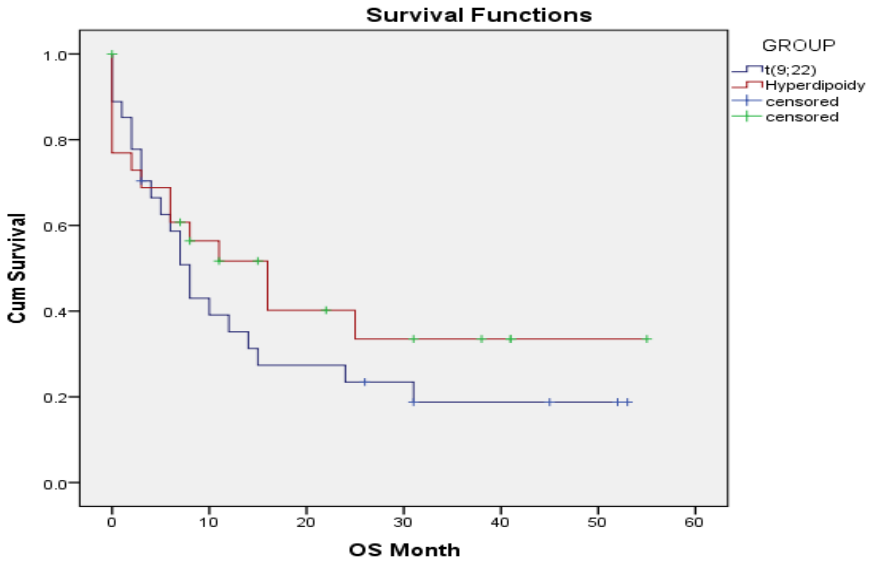

Figure 3: **Overall survival outcome between t(9;22) and hyperdiploidy**.

DISCUSSION:

The prognosis for Philadelphia (Ph) positive ALL is poor. We have attempted to determine whether poor prognosis in some patients with Ph positive ALL may be influenced by the presence of additional chromosomal abnormalities and have studied the outcomes of10

patients with Ph positive ALL who had hyperdiploid Karyotype in addition to Ph chromosome.

Hyperdiploidy in ALL has a distinct nonrandom pattern with gain of chromosomes, 4, 5, 6, 8, 11, 12 and 21 being most frequently observed [15,16]. The hyperdiploid pattern in our patients showed similar patterns of chromosome gain.

High – hyperdiploid ALL blasts are known to have a masked propensity for apoptosis in vitro, which has been linked to the relatively good in vivo response to chemotherapy [17]. In contrast, the presence of a Ph positive or double Ph chromosome has been associated with shorter disease free and overall survival in ALL[7,18]. It is now well established that a t(9;22) translocation can be observed in up to 5% of children and 15% to 30% of adults with ALL [19,20,21]

Our finding of the present work, those patients with additional abnormalities with Ph positive patients had shorter OS. A report from the Japan Adult Leukemia Study Group (JALSG), patients treated with imatinib combined chemotherapy supported our results [22], but some other studies, in pre- imatinib era, showed no significant effect of additional aberrations [6,23]. So, the significance of additional aberrations in Philadelphia positive ALL patients should be further investigated in the Imatinib era with large cohort.

Rieder et al [24] detected hyperdiploidy with Ph positive ALL in 17% patients, who achieved complete remission more readily than t(9;22), although the duration of remission and overall survival was similar in the two group.Thomas et al [18] have also suggested an improved outcome for Ph+ ALL patients with the hyperdiploid Karyotype compared to those with other Karyotype abnormalities although the differences in survival did not approach significance.Both these studies have indicated the genetic heterogeneity of Ph positive ALL that could potentially translate into variable outcomes.

Contrasting results have been obtained from studies on the prognostic implications of hyperdiploidy in adult Ph+ ALL by the groupe Francais de cytogenetique Hematologique[25] in large series of 433 patients with ALL, 11 of total of 127 patients with Ph+ ALL chromosome also had a high-hyperdiploid Karyotype, but the outlook for Ph+ with hyperdiploid patients did not differ from those without hyperdiploidy. A similar lack of benefit of the high hyperdiploid Karyotype in adult ph+ ALL was also suggested by Ribera et al[26].

Here we report shorter survival for hyperdiploidy with t(9;22) than t(9;22) which is statistically significant ($p<0.0001$), we also report survival difference statistically significant ($p<0.002$) between hyperdiploidy with t(9;22) and hyperdiploidy group. This is presumably by altering the kinetics of Ph+ neoplastic cells indicates that genetic heterogeneity of Ph+ ALL that could potentially translate into outcome. However, we did not find significant association between t(9;22)and hyperdiploidy group ($p<0.313$).

Contrasting results obtained in the present study from which have been reported by other study groups [24,18] showed that diverse cytogenetic changes with favorable and unfavorable prognosis suggest various mechanisms of leukemogenesis in ALL. Thus, based on the outcomes from the present study, along with some reports from the literature[24,18,26], it is conceivable that the simultaneous presence of additional karyotypic abnormalities like Philadelphia positive and hyperdiploidy may alter the biological properties of cells and influence the clinical outcomes. In addition, it is one of the most common mechanisms of resistance to imatinib is the mutation involving the ABL kinase domain, and secondary aberrations in Philadelphia positive ALL patients. It may be associated with the genes instability, which facilitates the occurrence of mutation [27]. It may partially explain why inferior OS was detected in patients with additional aberrations with Philadelphia positive ALL in the present study.

A meta-analysis of the karyotypic abnormalities may enable risk stratification of Ph positive ALL and identify prognostic subgroups, such an approach may identify patients who could benefit from newer therapeutic approaches.

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