VOLUME-7, ISSUE-12, DECEMBER-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Medicine

STUDY OF CYTOGENETIC PROFILE IN PATIENTS OF ACUTE LEUKEMIA AND ITS CORRELATION WITH TREATMENT OUTCOMES

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ABSTRACT

INTRODUCTION: Acute leukemia is a malignant disease of hematopoietic precursors which is universally fatal if untreated. All cases of acute leukemia arise due to an underlying cytogenetic abnormality, and this mostly dictates its biological behaviour, response to chemotherapeutic agents and thus the prognosis.

METHODS: 101 patients of acute leukemia diagnosed and treated in a single tertiary care centre between 2014 to 2017 were analysed for their cytogenetic profile, and its effect on their ability to achieve a remission post chemotherapy.

RESULTS: 56% of patients had some cytogenetic abnormality, and 22% out of these patients had poor risk cytogenetic profile. 24% patients died whereas 74% achieved and remained in remission at the end of 6 months follow up period. The mortality or the remission status at 6 months was not statistically different between poor and other cytogenetic risk groups.

CONCLUSION: Cytogenetic profile is an important prognostic marker in acute leukemia, but in our study it did not corelate with ability to achieve remission state post chemotherapy at a 6 month period.

KEYWORDS :

INTRODUCTION

Acute leukemia is a malignant disease of the bone marrow in which hematopoietic precursors get arrested at an early stage of development, proliferate and ultimately replace the normal hematopoietic cells. In many cases this has been linked to activation of abnormal genes through chromosomal abnormalities viz. translocations /deletions. This arrest produces two distinct disease processes: first the production of normal blood cells decreases markedly leading to varying degrees of anemia, thrombocytopenia and neutropenia; second, the rapid proliferation of these leukemic cells combined with their reduced ability to undergo apoptosis leads to their accumulation in bone marrow, blood, spleen, liver and other tissues often at the cost of normal cells.

The patients present with symptoms resulting from bone marrow failure (easy fatigability, dyspnoea, coagulopathy, frequent infections, fever) and/ or organ dysfunction due to infiltration by these leukemic cells (renal failure, lymphadenopathy, gingivitis, bone pains, respiratory distress due to leukostasis). It is diagnosed by demonstrating increased characteristic immature cells (blasts) of myeloid / lymphoid lineage in peripheral blood and bone marrow[1].
Both AML[2] and ALL[3] are known to occur with specific genetic abnormalities. Also, specific chromosomal abnormalities are known to be associated with favourable (t(15;17), inv(16), t(8;21) /intermediate t(9;11)/ poor prognosis inv(3), t(6;9),del5, del7) in acute leukemia patients[2,4,5,6]. The treatment regime, response to therapy and propensity to relapse is thus heavily dependent upon the underlying genetic abnormality[7,8,9].

The importance of identifying underlying genetic anomaly is underlined in the 2008 WHO AML classification which provides a separate class for known recurrent genetic abnormalities. Althoug0h the exact genetic basis of acute leukemia has been identified in ~1/3rd to ½ of all cases eg. PML RARA mutation with AML-M3 or t(9;22) with ALL, it is predicted that genetic mutations at molecular level occur in every case of acute leukemia.

It's the limitation of currently available cytogenetic technology that nearly half of these mutations go unidentified and hence get labelled as leukemia with normal cytogenetics. As new genetic abnormalities get reported and the technology to routinely test for them becomes mainstream, the proportion of patients diagnosed as "normal cytogenetics" is likely to reduce.

The cytogenetic makeup of leukemia patients has been extensively studied and accordingly patients are broadly divided into those having a favourable/intermediate or adverse risk profile. Most of the data (and conclusions thereof), however, are from western sources which due to paucity of studies in the Indian context is empirically extrapolated to our population as well. This study, therefore, was envisaged to study the cytogenetic profile of Indian patients and to see whether similar patterns as reported earlier could be corroborated in our population also.

AIMS AND OBJECTIVES

Aim:
To study cytogenetic profile in patients of acute leukemia

Objectives:

1. Primary objective:
To evaluate patterns of cytogenetic abnormalities in patients of acute leukemia

2. Secondary objective:
To study the correlation of various cytogenetic anomalies with the ability to achieve remission post chemotherapy.

METHODS

Study design: Retrospective and prospective observational study
Place of study: A tertiary care hospital in Maharashtra, India.

Sample size: -Hypothesized % frequency of outcome factor in the population : 85 +/-7.5% -Confidence limits as % of 100(absolute +/- %)(d): 7.5% -Design effect (for cluster surveys- DEFF): 1 -Sample size for 95% confidence interval: 88

Duration of study: 03 years (Oct 2014- Oct 2017)

Study population: All new leukemia patients being registered for treatment during the study period, and willing to take treatment with appropriate chemotherapy. All patients underwent the following investigations:

- CBC, PBS, LFT, RFT, S. electrolytes, uric acid, LDH
- Bone marrow aspirate and biopsy from iliac crest, at diagnosis, end of induction chemotherapy and at end of consolidation chemotherapy (6months)
- Immunophenotyping of bone marrow blasts.
- Metaphase Karyotyping by unstimulated bone marrow derived mononuclear cell culture.
- Molecular Cytogenetics by RT PCR or FISH for specified translocation i.e t(9,22), t(12,21), t(4,11), t(8,21), inv 16, t(15,17).

Statistical analysis

The data on categorical variables is shown as n (% of cases) and data on continuous variables is shown as mean ± standard deviation (SD). The inter-group statistical significance of difference of categorical variables is tested using Chi-Square test or Fisher's exact probability test. The entire data was entered in MS Excel before its statistical analysis. All the results are shown in tabular as well as graphical format to visualize the statistically significant difference more clearly.

In the entire study, all the hypotheses were formulated using two tailed alternatives against each null hypothesis (hypothesis of no difference). The entire data was statistically analysed using Statistical Package for Social Sciences (SPSS ver 21.0, IBM Corporation, USA) for MS Windows.

RESULTS

A total of 103 patients were enrolled during the study period out of which 63 were ALL and 40 were AML. Out of these, 02 patients with AML opted not to take the prescribed chemotherapy leaving a total of 101 patients (63 ALL, 38 AML) for the final analysis.

Age Distribution: The age distribution of cases studied differs significantly between group of AML and ALL cases studied (P-value<0.001). The data is represented in Fig 1. The sex distribution of cases studied did not differ significantly between group of AML and ALL cases studied (P-value>0.05) as shown in Fig 2.

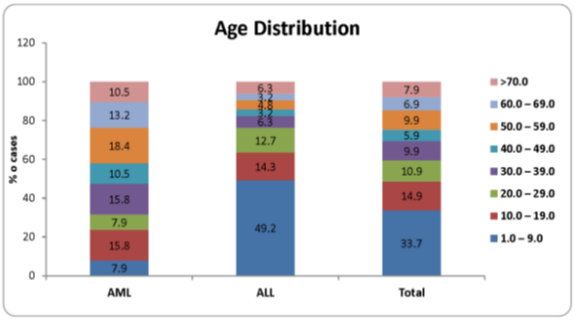


Figure 1) The age distribution of cases studied according to type of Leukemia.

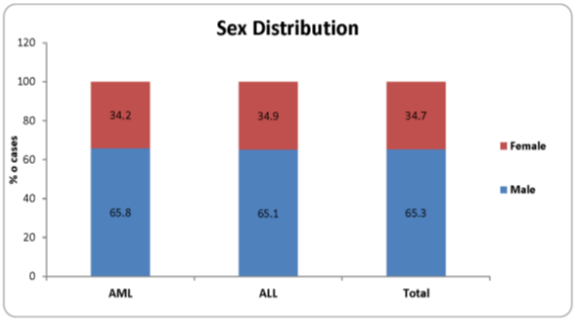


Figure 2) The sex distribution of cases studied according to type of Leukemia.

Symptoms at presentation: The distribution of fever differs significantly between group of AML and ALL cases studied (P-value<0.05). The distribution of other symptoms such as anemia, pain, lymphadenopathy. bleeding, weight loss and did not differ significantly between group of AML and ALL cases studied (P-value>0.05 for all). The same is shown below in Table -1 and Fig 3.

Table -1

	AML (n=38)		ALL (n=63)		Total (n=101)		P-value
Symptoms	n	%	n	%	n	%	
Fever	21	55.3	47	74.6	68	67.3	0.045*
Anemia	9	23.7	11	17.5	20	19.8	0.447NS
Pain	2	5.3	9	14.3	11	10.9	0.201NS
Lymphadenopathy	1	2.6	8	12.7	9	8.9	0.148NS
Bleeding manifestations	4	10.5	1	1.6	5	5.0	0.065NS
Weight loss	3	7.9	1	1.6	4	4.0	0.148NS
Other	3	7.9	7	11.1	10	9.9	0.739NS

Values are n (% Of cases). P-values by Chi-Square test. P-value<0.05 is considered to be statistically significant. *P-value<0.05 (Significant), NS-P-value>0.05 (Non-Significant).

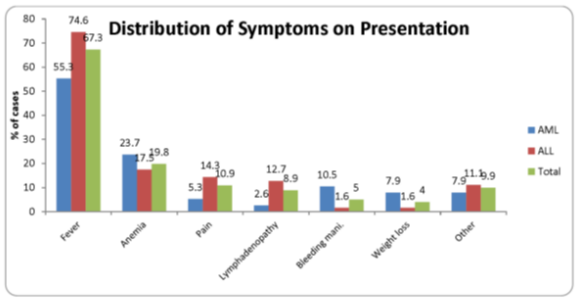


Figure 3) The distribution of symptoms on presentation according to type of Leukemia.

The distribution of hematological and biochemical parameters such as CBC, Serum biochemistry is shown in Fig 4 below, and were comparable between group of AML and ALL cases studied.

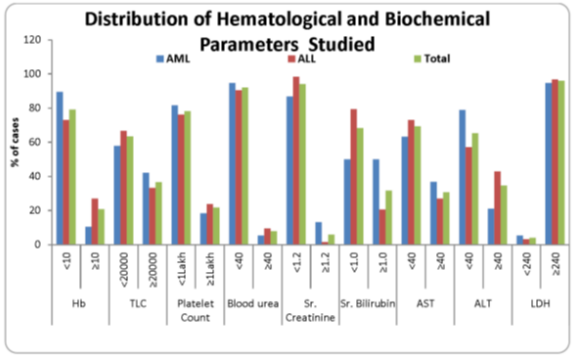


Figure 4) The distribution of hematological and biochemical parameters studied according to type of Leukemia.

Cytogenetic profile : The distribution of various Karyotype, FISH and composite cytogenetic test results are shown in Fig 5 below, and the details of the various cytogenetic abnormalities are depicted in Table 2.

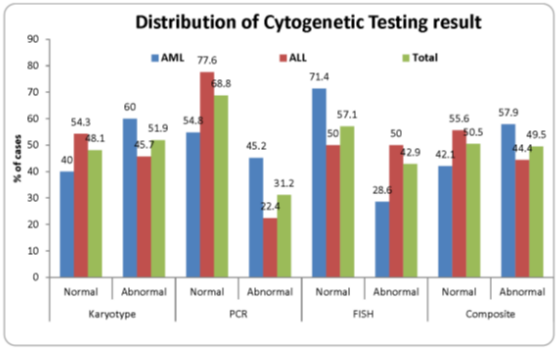


Figure 5) The distribution of outcome of cytogenetic investigation such as Karyotype, PCR and FISH according to type of Leukemia.

As can be seen from the distribution of the cytogenetic results, all 101 patients underwent one or more methods of cytogenetic testing which as per protocol included Karyotype plus either FISH or PCR. Since in 22 patients, no metaphases could be analysed due to culture failure, the karyotyping results are analysed in 81 patients

only. Similarily FISH results were analysed in 21 patients, and RTPCR in the other 80 patients.

The distribution of remission status according to type of Leukemia : Remission of disease on bone marrow examination was analysed at two time points, firstly at end of induction and secondly at end of consolidation chemotherapy which has been clubbed as +6 months for both types of leukemia for uniformity of data analysis. The data is shown in Table 3, and as mentioned, there were 24 fatalities out of the 101 analysed patients. 20 of these fatalities occurred in the first month during induction, and another 4 between second month (post induction) and six months of therapy. The distribution of post-induction remission is significantly higher in ALL compared to AML group of cases studied (P-value<0.05). The distribution of 6-months remission is relatively higher in ALL (though not statistically significant) compared to AML group of cases studied (P-value>0.05).

Table 2

		AML (n=38)		ALL (n=63)		Total (n=101)		P-value
Parameters		n	%	n	%	n	%	
Karyotype (n=81)	Normal	14	40.0	25	54.3	39	48.1	0.200^{NS}
	t(8;21)	6	17.1	0	0.0	6	7.4	0.005**
	t(9;22)	0	0.0	4	8.7	4	4.9	0.130^{NS}
	inv(16)	2	5.7	0	0.0	2	2.5	0.184^{NS}
	Other	13	37.1	17	37.0	30	37.0	0.999^{NS}
PCR (n=80)	Normal	17	54.8	38	77.6	55	68.8	0.033*
	t(8;21)	6	19.4	0	0.0	6	7.5	0.002**
	t(9;22)	0	0.0	8	16.3	8	10.0	0.020*
	t(15;17)	3	9.7	0	0.0	3	3.8	0.055^{NS}
	t(12;21)	0	0.0	1	2.0	1	1.2	0.999^{NS}
	t(4;11)	0	0.0	0	0.0	0	0.0	0.999^{NS}
	inv(16)	2	6.5	0	0.0	2	2.5	0.147^{NS}
	FLT3ITD	2	6.5	0	0.0	2	2.5	0.147^{NS}
	Other	1	3.2	2	4.1	3	3.8	0.999^{NS}
FISH (n=21)	Normal	5	71.4	7	50.0	12	57.1	0.642^{NS}
	t(12;21)	0	0.0	2	14.3	2	9.5	0.533^{NS}
	MLL GR	0	0.0	2	14.3	2	9.5	0.533^{NS}
	Other	2	28.6	3	21.4	5	23.8	0.999^{NS}

Values are n (% 0f cases). P-values by Chi-Square test. P-value<0.05 is considered to be statistically significant. *P-value<0.05 (Significant), NS-P-value>0.05 (Non-Significant).

	AML (n=38)		ALL (n=63)		Total (n=101)		P-value
Cytogenetic risk group	n	%	n	%	n	%	
Good	11	28.9	11	17.5	22	21.8	0.079^{NS}
Poor	11	28.9	11	17.5	22	21.8	
Other/Normal	16	42.2	41	65.0	57	56.4	
Total	38	100.0	63	100.0	101	100.0	

Table 3

		AML (n=38)		ALL (n=63)		Total (n=101)		P-value
	Remission	n	%	n	%	n	%	
Post-Induction	Achieved	23	60.5	52	82.5	75	74.3	0.017*
	Not Achieved	5	13.2	1	1.6	6	5.9	
	Expired	10	26.3	10	15.9	20	19.8	
6-Months	Achieved	23	60.5	51	81.0	74	73.3	0.073^{NS}
	Not Achieved	2	5.3	1	1.6	3	3.0	
	Expired	13	34.2	11	17.5	24	23.8	

Values are n (% 0f cases). P-values by Chi-Square test. P-value<0.05 is considered to be statistically significant. *P-value<0.05 (Significant), NS-P-value>0.05 (Non-Significant).

The distribution of some selected characteristics of cases studied according to remission status at 6-months follow-up : The results are depicted in table 4. The distribution of age differs significantly between the group of cases who achieved remission and who did not achieve it (P-value<0.001). The distribution of type of Leukemia differs significantly between the group of cases who achieved remission and who did not achieve it (P-value<0.05). The distribution of cytogenetic profile did not differ significantly between the group of cases who achieved remission and who did not achieve it (P-value>0.05).

Table 4

		Remission Status (6-Months)						
		Achieved (74)		Not Achieved + Died (n=27)		Total (n=101)		P-value
Characteristics		n	%	N	%	n	%	
Age Group (years)	<50	62	81.6	14	18.4	76	100.0	0.001***
	>50	12	48.0	13	52.0	25	100.0	
Type of Leukemia	AML	23	60.5	15	39.5	38	100.0	0.025*
	ALL	51	81.0	12	19.0	63	100.0	
Cytogenetic profile	Normal	37	72.5	14	27.5	51	100.0	0.869^{NS}
	Abnormal	37	74.0	13	26.0	50	100.0	

Values are n (% 0f cases). P-values by Chi-Square test. P-value<0.05 is considered to be statistically significant. *P-value<0.05 (Significant), ***P-value<0.001 (Highly Significant), NS-P-value>0.05 (Non-Significant).

The distribution of remission status according to cytogenetic risk group: Patients when divided based on their remission status were analysed for their baseline cytogenetic profile. The data is represented in table 5 and Fig 6.

Table 5

		Cytogenetic risk group						
		Good (n=22)		Poor (n=22)		Others/Normal (n=57)		P-value [Good+Others/ Normal vs Poor]
	Remission	n	%	n	%	n	%	
Post-Induction	Achieved	17	77.3	13	59.1	45	78.9	0.183^{NS}
	Not Achieved	1	4.5	2	9.1	3	5.3	
	Died	4	18.2	7	31.8	9	15.8	
6-Months	Achieved	18	81.8	12	54.5	44	77.2	0.080^{NS}
	Not Achieved	0	0.0	1	4.5	2	3.5	
	Died	4	18.2	9	40.9	11	19.3	

Values are n (% 0f cases). P-values by Chi-Square test. P-value<0.05 is considered to be statistically significant. NS-P-value>0.05 (Non-Significant).

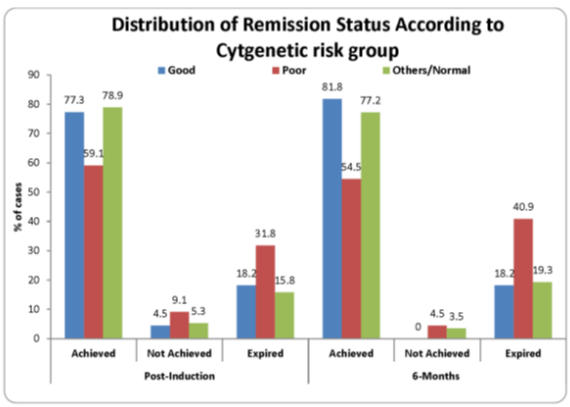
AML risk group good cytogenetics: t(15;17),t(8;21),inv(16), NPM-1

AML risk group poor cytogenetics: t(6;9),inv(3),monosomy, deletions, complex karyotype

ALL risk group good cytogenetics: t(12;21), hyperdiploidy

ALL risk group poor cytogenetics: t(9;22),t(4;11)/MLL gene rearrangement

Figure 6



The distribution of post-induction remission as well as at 6-months did not differ significantly between group of cases having good/ normal cytogenetic risk profile compared with group of cases having poor cytogenetic risk profile (P-value>0.05).

DISCUSSION

The detection of chromosomal abnormalities in leukaemia is of utmost importance in patient management, as information on cytogenetics enables clinicians to make informed decisions on the options of therapy available for a patient. Specific abnormalities viz monosomy chromosome 7 were generally associated with a poor prognosis and such patients may benefit from an xpeditious HSCT. Hence, it's important to be able to detect those abnormalities

during initial work up of such patients. All 101 patients included in this study were subjected to at least one or more cytogenetic studies (Karyotype, PCR and FISH).

Relation between remission status and cytogenetic risk groups (poor cytogenetic profile vs good/normal/other cytogenetic profile): Post induction: 78.9% of patients with good/normal/ other cytogenetic profile achieved remission post induction chemotherapy when cumulative ALL and AML data was analysed; as compared to 59.1% patients achieving remission in the poor cytogenetic profile group. However, this difference did not achieve statistically significant levels due to lesser number of patients included in the study.(P-value0.183)

At six month follow up: 77.2% of good/normal/other cytogenetic profile patients maintained remission at six month follow up as compared to 54.5% of patients in the poor cytogenetic risk group. However, again owing to smaller number of study participants, this difference did not achieve statistical significance.(P-value 0.080).

Many large studies have evaluated the correlation between poor cytogenetic risk profile and long term survival (3 to 5years), however there is a paucity of studies looking into the short term implications of the same. The endeavour of this study was to evaluate whether there's any difference between short term outcome of poor cytogenetic risk profile patients vs others(good/normal cytogenetic profile) ,i.e., ability to achieve remission post induction and maintaining remission at 6 month. Although a difference was observed between these two cohorts, it did not achieve statistically significant proportions due to lesser number of study participants. Hence, studies with larger number of patients are required to further elicit the difference if any.

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