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Case Study

PROGNOSTIC SIGNIFICANCE OF KARYOTYPE ANALYSIS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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SUMMARY

Chromosome studies, using bone marrow samples of 26 pretreated children (below 15 years of age) with Acute Lymphoblastic Leukemia were carried out to explore the potentialities of applying chromosomal findings as a prognostic indicator in these patients. Abnormal karyotype was identified in 15 patients (57·6 per cent). The chromosomes frequently involved in non-random numerical abnormalities were Nos. 8, 18 and 21. Structural chromosome changes observed consisted of deletion $6q^-$ and translocation t (4;11).

After karyotype analysis, patients were grouped into subsets on the basis of the karyotype pattern observed. They were followed up to evaluate their prognosis and survival period. Patients showing hyperdiploid clone with greater than 51 chromosomes had the best prognosis. Patients with normal karyotype and patients with deletion of the long arm of chromosome 6 showed intermediate prognosis whereas patients showing t (4;11), trisomy 8, trisomy 18, trisomy 21, and hypodiploid karyotype were associated with worst prognosis. Thus, karyotype analysis before treatment helps to classify ALL patients as poor, intermediate and good prognosis groups and on this basis therapy can be designed accordingly.

KEY WORDS Acute lymphoblastic leukemia Chromosomes Karyotype Prognosis Survival

INTRODUCTION

Acute lymphoblastic and myeloid leukemias are clinically and biologically heterogeneous diseases for which specific therapeutic regimens should be designed.[1,2] Acute Lymphoblastic Leukemia (ALL) is now curable in more than 50 per cent of children making it possible to modify therapy according to prognostic features at diagnosis.[3] Since clinical features such as age and leukocyte count as well as the immunophenotype of leukemic blasts have been shown to correlate with prognosis,[4,5] it is important to consider cytogenetic features in the context of other variables. Even though non-random chromosomal changes have been reported in ALL,[6] there have been very few reports regarding the prognostic implications of initial karyotype analysis in children with ALL. So, the current study was undertaken to explore the potentialities of applying chromosomal findings as a prognostic indicator in children with ALL.

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Table 1. Distribution of modal chromosome number in 26 children with ALL

Category	Chromosome range	Patients Number	Patients Percentage
Hypodiploid	<45	1	3·8
Diploid	46	11	42·3
Pseudodiploid	46	3	11·5
Hyperdiploid	47–50	8	30·7
Hyperdiploid	>51	3	11·5

MATERIALS AND METHODS

Cytogenetic analysis was carried out on 26 children (15 girls and 11 boys), diagnosed as acute lymphoblastic leukemia and admitted to the Paediatric Oncology Division of Regional Cancer Centre, Trivandrum. The disease was classified following the suggestions by the French–American–British co-operative Group (FAB criteria).[7] The cytogenetic investigations were carried out in the course of establishing the diagnosis and before anti-leukemia therapy was started. The investigations were performed on preparations obtained from a 24 h peripheral blood or bone marrow culture without mitogenic stimulation. The G-method of chromosome identification was used and karyotypes were prepared according to the International system for Human Cytogenetic Nomenclature, ISCN.[8] Fifteen to 25 metaphases were karyotyped. An aberrant clone was defined by the presence of structural abnormality, a surplus of chromosomes in two or more cells and chromosome deficiency in three or more cells.[9]

RESULTS

Out of the 26 patients studied, abnormal karyotypes were identified in 15 patients (57·6 per cent) of which 10 patients showed all metaphases with abnormal karyotypes and five patients showed metaphases with a mixture of normal and abnormal karyotypes. Normal karyotypes were detected in 11 patients (42·4 per cent). The karyotypes fell into five categories (Table 1) according to the modal chromosome number: (1) Normal diploid—11 patients (42·4 per cent), (2) pseudodiploid—three patients (11·5 per cent), (3) hypodiploid—one patient (3·8 per cent), (4) hyperdiploid with 47–50 chromosomes—eight patients (30·7 per cent) and (5) hyperdiploid with greater than 51 chromosomes—three patients (11·5 per cent).

The chromosome abnormalities observed were non-random in patients with pseudodiploid and hyperdiploid modal chromosome numbers, with involvement of chromosomes belonging to B, C, E, F and G groups. Abnormalities were observed either as single or combined. The karyotype patterns in 26 ALL children are given in Table 2. Among non-random abnormalities, trisomy 21 was the most frequent numerical change observed in four patients. Trisomy 8 and trisomy 18 were detected each in two patients respectively. Numerical abnormalities observed in three patients with greater than 51 chromosomes generally involved chromosomes belonging to group B, C, D, E, F and G. Structural chromosome changes consisted of a deletion $6q^-$ in two patients and translocation between chromosomes 4 and 11 i.e. t (4;11) in one patient.

Clinical correlation

After analysing the chromosome abnormalities, patients were classified into different subsets on the basis of karyotype pattern and followed up to evaluate their prognosis and survival period following the recommendations of First MIC Co-operative Study Group.[10] The initial karyotype pattern was found to correlate with the prognosis and survival period of these patients.

Three patients showing hyperdiploid clone with greater than 51 chromosomes had the best prognosis. These three patients showed the best response to treatment and they had a median survival period of 32 months. Eleven patients with normal karyotype and two patients with deletion of the long arm of chromosome 6 showed intermediate prognosis with a median survival period of 18 months. One patient showing t (4;11) had a poor prognosis with a median survival period of 9 months and responded poorly to treatment. Patients showing trisomy 8, trisomy 18, trisomy 21 and hypodiploid karyotype were associated with worst prognosis. They had a median survival period of 5 months and showed the poorest response to treatment. The correlation between the chromosome abnormalities observed and the survival period in these ALL patients are

Table 2. Karyotype pattern in 26 children with ALL

Patient no.	Sex/age		Morphology	WBC count ($\times 10^9$ L)	% of blasts	No. of metaphases analysed	Karyotype pattern
1	F	7	L1	8	28	19	46, XX
2	M	11	L2	134	76	16	46, XY/46, XY, del(6)(q21)
3	F	6	L2	19	25	23	55, XX, +4, +6, +8, +10, +12, +14, +17, +18, +20
4	F	7	L2	138	64	32	46, XX
5	M	8	L1	68	85	19	47, XY, +21
6	F	5	L1/2	89	29	36	46, XX
7	F	10	L1	28	74	23	47, XX, +18
8	M	6	L1/2	25	67	20	46, XY
9	F	8	L2	134	94	22	46, XX, t(4;11)
10	F	9	L2/3	85	90	17	47, XX, +21
11	F	7	L1	60·8	56	31	46, XX
12	M	5	L1	8	78	18	47, XY, +18
13	F	11	L1/2	19	36	15	46, XX, del(6)(q21)
14	M	10	L1/3	36	45	26	46, XY
15	M	9	L1/2	14·7	86	22	44, XY, −6, −17
16	F	5	L1	128	68	15	46, XX
17	M	8	L1	219	81	24	46, XY/47, XY, +21
18	M	9	L2/3	35	44	27	56, XY, +4, +6, +8, +10, +12, +14, +17, +18, +20, +21
19	M	14	L2/1	86	79	20	46, XY
20	F	13	L1	14	82	19	46, XX/47, XX, +21
21	F	9	L2	84	58	27	46, XX/47, XX, +18
22	F	6	L1/2	26	65	21	46, XX
23	M	5	L1	52	44	18	46, XY
24	F	6	L2	41	42	20	56, XX, +4, +6, +8, +10, +12, +14, +17, +18, +19, +20
25	M	5	L2	9	83	26	46, XY
26	F	8	L1	156	90	23	46, XX/47, XX, +18

Table 3. Correlation between the karyotype pattern and survival period in 26 ALL children

Chromosome abnormality	No. of patients	Percentage frequency	Mean survival period in months
Hyperdiploid (with > 51 chromosomes)	3	11·5	32
Diploid (normal)	11	42·3	18
Deletion $6q^-$	2	7·6	18
t (4;11)	1	3·8	9
Trisomy 21	4	15·3	5
Trisomy 8	2	7·6	5
Trisomy 18	2	7·6	5
Hypodiploid (with < 46 chromosomes)	1	3·8	5
	1	3·8	5

DISCUSSION

More than half of the patients in this study showed karyotypic abnormalities and the karyotypes were grouped into five categories on the basis of the modal chromosome number. The pseudodiploid and hyperdiploid abnormalities tended to be non-random and usually involved chromosomes belonging to groups B, C, E, F and G. Three children showed hyperdiploidy in the range of 55–57 chromosomes. Hyperdiploidy with greater than 51 chromosomes was found in approximately 30 per cent of childhood ALL cases in Williams' study[11] whereas this was not found in any infants in the study reported by Pui *et al.*[12]

Among the non-random numerical abnormalities, trisomy 21 was the most frequent (15·3 per cent) followed by trisomy 8 (7·6 per cent) and trisomy 18 (7·6 per cent). Same types of abnormalities have been reported in ALL by others also.[13–15] According to Heim and Mitelman.[15] the mechanism whereby trisomy for selected chromosomes confers neoplastic potential on a clone can be explained to be due to the simple dose effect of genes located on these chromosomes. Ahuja and Cline[16] reported that some of the non-randomly involved chromosomes possess important genes (oncogenes) whose amplification as a result of hyperdiploidy could play a potentially important role in the induction/progression of ALL. The oncogenes presently known are of particular interest in this respect. Oncogenes c-myc and c-mos have been localized on chromosome No. 8.[17] The ets-2 oncogene has been located on chromosome No. 21[18] and bcl-2 has been located on chromosome No. 18.[19] Thus, genes important to transformation have been located on specific chromosomes that are involved in these numerical abnormalities. Minor changes in gene dosage brought on by the acquisition of one or two extra copies of a chromosome will affect hundreds of thousands of genes.[20] Karyotypic abnormalities bring about changes in the structure or copy number of cellular proto-oncogenes and their products and this in turn leads to abnormalities of cell growth. Thus, numerical abnormalities would be associated with the upset of the genotypic balance of the cells involved. Dosage effects due to changes in number of particular chromosomes could lead to physiological disturbance that would trigger the development towards malignancy.[21]

Regarding structural abnormalities, deletion of the long arm chromosome 6 ($6q^-$) was observed in two patients. This abnormality has been reported in a wide variety of hematological malig- and both common and T-cell ALLs.[6,22–24] Breakpoints on chromosome 6 concentrated around band q[21]. Oncogenes c-myb and c-ros have been localized on the long arm of chromosome 6. Deletion of segments from long arm of chromosome 6 in ALL may permit inappropriate expression of genes involved in cell proliferation by removing a negative regulatory sequence or by permitting the expression of a mutant allele present in the intact homologue.[25] Evidence is accumulating that certain tumour suppressor genes are involved in the control of cell proliferation and differentiation. Even though the molecular consequence of this deletion is unknown, it is reasonable to think that these deleted regions may contain tumour suppressor genes that must be present in full dosage if malignant transformation is to be avoided. According to Ahuja and Cline,[16] alteration of tumour suppressor proteins as a result of the chromosomal deletion could also play a role in the pathogenesis of ALL.

A translocation between chromosome 4 and 11 i.e. t (4;11) was observed in one patient. This type of translocation has been reported in ALL by Kocova *et al.*,[26] Mirro *et al.*[27] and Francesc *et al.*[28] The human cellular oncogene c-ets-1 has been assigned to bands 11 q.[23,24] In ALL, the c-ets-1 gene shifts from chromosome 11 to 4 as a consequence of the translocation.[29] According to Heereena[30] it is the specific translocation t (4;11) (q 21;q 23)[30] rather than a breakpoint at 11 q[23] *per se* that is associated with the poor prognosis of patients with this abnormality. Several genes that encode proteins involved in cell signalling have been localized to this region. Additional studies are needed to define the role of these and other genes whose structural or functional alteration may contribute to leukemogenesis.

Prognostic factors play an important role in the stratification of treatment in ALL patients. Hence, the relationship between karyotypic pattern and prognosis has also been investigated in the present study. The presence of an abnormal karyotype at the time of diagnosis was found to have prognostic significance, the prognosis varying with the type of abnormality detected. Classification of ALL patients according to the karyotype pattern encountered and their follow-ups helped in recognition of clinically important prognostic groups. But why these cytogenetic abnormalities correlate with response to treatment and survival is unknown. However, the present data, which is in agreement with those reported in literature,[5,13,32] suggest that karyotype analysis can be considered an independent prognostic factor as it correlated with survival pattern of these ALL children.

By analysing the karyotypic features most closely related to prognosis, patients belonging to poor and good prognosis groups can be identified and their outcome to treatment could be predicted. Upon earlier identification, more intensive approaches or modifications in therapy can be performed on patients belonging to the poor prognosis and short survival groups. Once the molecular basis of ALL becomes better understood, new genetic probes can be applied which will permit much finer dissection of prognostic groups. Thus, the ultimate goal of individualized therapy directed to specific molecular targets can be realized.

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