

CHROMOSOMAL PATTERN IN ACUTE LYMPHOBLASTIC LEUKAEMIA

Pages with reference to book, From 9 To 11

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Abstract

Analysis of the chromosomal changes in various neoplasia is being increasingly carried out not only to evaluate its relationship with the prognosis and biological behaviour of the tumour but also for diagnostic purposes in some cases. Leukaemias are one such group of haematological malignancies which have been most extensively studied in this regard. Karyotypic analysis with Giemsa banding technique was carried out in 35 consecutive cases of Acute Lymphoblastic Leukaemia. Eighteen cases were in children (less than 15 years age) and 17 cases were in adults. M.F. ratio was 1.8:1. FAB classification of these cases showed 31 cases of L1 type and 4 cases of L2 type including one case of T-ALL. Fifteen cases (43%) had no karyotypic abnormality, 6 cases (17%) showed pseudodiploidy, one case each having ($-20 + 21$), $t(9:22)$, $(+2-6)$, $(-6+8)$, while two cases had 6q-. 13 cases (37%) showed hyperdiploidy with 6 cases showing trisomy 8 alone, one case $(+8+21)$, one case trisomy 18, one case $+15(r)$, one case trisomy 21 plus $t(9:22)$ and one case with trisomy 21 only. Two cases showed more complex abnormalities i.e. $+2+8$, $t(13:22)$ and $-11 + MR + Min + Min$. There was one (3%) case of hypodiploidy showing monosomy 6. The above findings are in agreement with studies carried out in other countries except $t(13:22)$ which is rather a scarcely reported abnormality (JPMA 40: 9, 1990).

INTRODUCTION

Cytogenetic analysis using banding technique has been increasingly applied to the study of malignant diseases in recent years. Among the malignant haematological disease Ch. myeloid leukaemia (CML) and acute non lymphoblastic leukaemia have been most extensively investigated. However within the last few years Ac lymphoblastic leukaemia (ALL) cases have also been investigated for their chromosomal structure. Kaneko¹ made the following observations.

Clonal chromosomal abnormalities are present in more than 60% cases of ALL. Hyperdiploidy is the predominant type of aneuploidy in these cases. Patients with certain abnormalities like Philadelphia chromosome, $t(4:11)$ and $t(1:19)$ have bad prognosis. Hyperdiploid cases have better prognosis than those with other aneuploidy. We studied the clinical and karyotypic feature of 35 ALL cases, mostly belonging to northern parts of Pakistan including northern districts of Punjab and NWFP.

PATIENTS AND METHODS

The patients belonged to northern parts of Pakistan including some districts of North Punjab and NWFP. The patients were interviewed and examined clinically. Out of 35 cases 18 were <15 years and 17 >15 years of age. There were 12 females and 23 males making a female to male ratio of 1:1.8. The diagnosis of ALL was made on the basis of peripheral blood and bone marrow examination after Romanowsky staining. The diagnosis was further confirmed by cytochemical stains and by immunophenotyping using a panel of monoclonal antibodies. The cases were classified according to the FAB criteria. A blood/bone marrow sample for cytogenetic analysis was obtained from all the 35 patients at the time of first presentation. The bone marrow cells were processed by direct/24 hours cell

culture technique without phytohaemagglutinin. The peripheral blood cells were processed by 48 hours cell culture technique without PHA and 72 hours cell culture with PHA to compare between normal and abnormal cells. RPMI 1640 was used as culture medium to which 20% foetal calf serum, 2 mmol/l L-glutamine, 25 mmol/l Hepes buffer, 100 units/l Penicillin G and 100 ug/l of Streptomycin was added. For 72 hours blood culture, PHA was added. Hepranazied bone marrow aspirate was taken from each patient under sterile conditions and added to two separate culture media bottles, one for direct cell culture and the other for 24 hours culture. 10 ml peripheral blood was drawn into a sterilized tube containing 90 USP heparin. 10-12 drops of blood from this sample were added to two separate bottles containing 10ml of medium, one having 0.1ml of PHA and the other without PHA. All the bottles were incubated at 37°C. The direct bone marrow culture bottles were taken out at the end of 4 hours and 2-3 drops of calcemoid (1 ug/ml) was added. After further period of incubation for 1 hour, whole material was transferred to a conical centrifuge tube and spun at 800 RPM in a swing out centrifuge. Supernatant was discarded without disturbing the bottom. 10 ml prewarmed Hanks balanced salt solution was added to the sediment and mixed gently, centrifuged for another 12 minutes at 800 RPM discarding the supernatant without disturbing the button, 10 ml KCl (0.75 M) was added to the pellet, incubated for further 10 minutes at 37°C. The material was mixed properly and centrifuged again at 800 RPM — supernatant being discarded. After 2—3 washes with fixative (3 parts methanol + 1 part Glacial acetic acid), the material was stored overnight at 4°C. Next morning the material was mixed with freshly prepared fixative and slides were made on ice chilled slides and air dried. After artificial ageing at 90°C the slides were trypsinised and Giemsa banding was carried out. The other bone marrow specimen was processed at the end of 24 hours while blood samples were processed at the end of 24 hours and 72 hours incubation. Harvesting and banding was carried out as described above. Slides were analysed on TAS plus image analyser and by conventional methods. Photographs and karyograms were made from each specimen. 20 metaphases were analysed from each sample.

RESULTS

Out of 35 cases, 31 cases were FAB type L1 where as 4 cases were type L2. There was no L3 case. Thirty two out of 35 cases had pallor whereas 17 cases had fever, 9 cases had bone pain and 8 cases had haemorrhagic manifestations in the form of epistaxis and echymosis. Lymphadenopathy was found in 15 cases, splenomegaly in 14 cases where as hepatosplenomegaly was present in 12 cases. Haemoglobin less than 10 gm/dl was found in 21 cases. Total leucocytes count varied from $5 \times 10^9/l$ to $90 \times 10^9/L$. A count of more than $10 \times 10^9/l$ was found in 16 cases whereas 19 cases had a count of less than $10 \times 10^9/l$. Platelet count ranged between $5 \times 10^9/l$ to $110 \times 10^9/l$. A count of less than $20 \times 10^9/l$ was found in 23 cases. Percentage of blast cells in peripheral blood varied between 15 to 90 percent. Less than 30% blast cells were found in 18 cases and more than 30% blast cells were found in 17 cases. Percentage of blast cells in the bone marrow varied between 65 to 95%.

Cytogenetic Findings Fifteen (43%) cases showed no cytogenetic changes whereas 20 (57%) cases had clonal chromosomal changes. Six cases (17%) showed pseudodiploidy, one each having $(-20 + 21) t(9:22)$, $(+2-6)$, $(-6 + 8)$ where as two cases had $6q-$, 10 cases showed a number of abnormalities, most frequent being trisomy-8. Six cases showed 47 chromosomes whereas 3 Cases showed 48 chromosomes. These hyperdiploid cases showed a number of abnormalities, most frequently being trisomy 8. Six cases showed trisomy-8 as the only cytogenetic abnormality, and trisomy 8 in combination with other abnormalities was seen in 3 more cases. Remaining four cases with 47 chromosomes showed trisomy 21 in two cases, trisomy 15 in one case and trisomy 18 in one case. Out of 3 cases with 48 chromosome as their modal number, one case had trisomy 2 and trisomy 8, with $t(13:22)$. Trisomy 8 and trisomy 21 was found in another case whereas one case had complex cytogenetic findings with one marker chromosome and two minute chromosomes along with monosomy-11.

Only one case showed hypodiploidy in the shape of 45 XY-6. Two cases showed classical Philadelphia chromosome having t (9:22) and one case had an uncommon Philadelphia chromosome with t (13:22) in that a portion of a q arm of chromosome 22 was translocated to q arm of chromosome 13 (see table for cytogenetic findings).

TABLE. Cytogenetic Pattern in 35 ALL Cases.

S. No.	Sex	Age (Years)	DX	Cytogenetic Changes
1	M	5	ALL L1	48 X Y + 2 + 8, t (13:22)
2.	M	2.5	ALL L1	46 X Y
3.	M	54	ALL L1	48XY-11 + MR + MIN + MI
4.	M	23	ALL L1	45 X Y -6
5.	M	7	ALL L2	46 X Y 6q-
6.	F	14	ALL L2	47 X X + 18
7.	M	16	ALL L1 (T Cell)	46 X Y
8.	F	3.5	ALL L1	46 X X - 20 + 21
9.	M	45	ALL L1	47 X Y + 15 (r)
10.	M	19	ALL L1 (T Cell)	46 X Y
11.	F	5	ALL L1	46 X X
12.	M	21	ALL L1	47 X Y + 21, t (9 : 22)
13.	M	20	ALL L1	46 X Y
14.	F	6	ALL L1	46 X X
15.	F	5	ALL L1	46 X X t (9:22)
16.	F	3.5	ALL L1	46 X X
17.	M	17	ALL L1	46 X Y
18.	M	25	ALL L1	47 X Y + 8
19.	M	30	ALL L1	46 X Y
20.	M	50	ALL L1	47 X Y + 8
21.	M	38	ALL L1	46 X Y
22.	M	30	ALL L2	48 X Y + 8 + 21
23.	F	5	ALL L1	46 X X
24.	M	5	ALL L1	46 X Y + 2 - 6
25.	F	3.5	ALL L1	46 X X
26.	M	28	ALL L1	47 X Y + 8
27.	F	14	ALL L1	46 X X
28.	F*	12	ALL L1	47 X X + 21
29.	F	5	ALL L1	46 X X
30.	M	30	ALL L1	47 X Y + 8
31.	F	12	ALL L1	46 X X
32.	M	24	ALL L1	47 X Y + 8
33.	M	10	ALL L1	47 XY + 8
34.	M	28	ALL L1	46 X Y -6 + 8
35.	M	10	ALL L2	46 X Y 6q-

DISCUSSION

The importance of cytogenetic studies in neoplasia in general and in leukaemias in particular has been universally accepted. Cytogenetic study in leukaemia is an important aid in confirming the diagnosis, evaluating the prognosis and to monitor the progress of the case after therapy. Cytogenetic analysis may also be helpful in deciding the extent of therapy to be given to a particular patient². Cytogenetic data in different malignancies including acute lymphoblastic leukaemia are available from different parts of the world; however no such data is available from Pakistan. For the last two years cytogenetic studies in various types of leukaemia have been carried out at AFIP Rawalpindi to find out the chromosomal pattern in Acute Lymphoblastic Leukaemia in this region of the country.

In ALL there is remarkable heterogeneity in cytogenetic findings but certain findings are more frequently encountered than the others. In this study many classical findings like trisomy 8 + 21, monosomy 6 and t (9:22) have been observed but t (4:11), t (1:19) and Et (8:14) as reported in literature have not been found. t (4:11) is found only in 4-5% cases of ALL mostly in infantile and congenital leukaemia³. Similarly t (1:19) is found in small percentage of infantile ALL⁴.

Translocation (8:14) is not very common in ALL. Rather it is found in B-cell malignancy⁵. Recently reported translocation t (10:14) is mostly found in T-ALL⁶. In this study we did not find these translocations because of the absence of congenital/infantile cases. Similarly there were no cases of t (10:14) and t (8:14) because of the limited number of T-ALL cases and there being no case of L3. In this study 57% cases showed clonal chromosomal abnormalities. Twenty metaphases were analysed from each sample and if 5 or more cells had similar abnormality, it was termed as "Clonal" abnormality. Trisomy 8 was the most frequently noted abnormality. Although we have not evaluated the prognostic aspect in relation to the cytogenetic changes in our cases but it has been well established by other studies that chromosomal changes have definite influence on the course of the disease³. Our study is closely comparable to other international studies except that the t (13:22) is a unique finding which has not been reported in available English language literature.

Although this study has to some extent defined the out-line of chromosomal pattern in ALL in this region a more extensive study is required in various types of Ac leukaemias to ascertain its significance in relation to various clinical patterns and response to various modes of therapy.

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