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CYTOGENETIC STUDY OF CHROMOSOMAL ABERRATIONS ASSOCIATED WITH ACUTE LEUKEMIA

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ABSTRACT: The most common cause of death is bone marrow failure. Chromosomal analysis is essential for the diagnosis, response to treatment and prognosis of leukemia. Conventional karyotyping which shows the structural, numerical and compound aberrations have improved the genetic diagnosis of leukemia. The bone marrow samples from patients (2-60yrs) of 53 patients (32 male and 21 female) suffering from various types of leukemia were collected. The patients were selected on the basis of clinical diagnosis and pathologically presenting blast cells in the peripheral smear. After photography all cells were analysed and printed photographs were taken for karyotyping and the chromosomes were banded, karyotyped and studied in detail for translocations, deletions, inversion, monosomy, trisomy and hyperdeploidy. It is observed that in all subtypes of leukemia male patients are more prevalent than female. Certain translocations t(8:21), t(8:15) in AML and Hyperploidy in ALL had better treatment outcome with aggressive chemotherapy. Hence younger age group were usually having t(4:11) in ALL , t(8:21) & t(15:17) in AML had better prognosis than other chromosomal aberrations in old people. Trisomy 12, t(8:14) & t(4:11) and hypoploidy of all varieties had poor response to chemotherapy. It is felt that an attempt should be made for Fluorescence In Situ Hybridization (FISH) to identify the origin of the marker chromosomes, oncogene amplifications, subtle translocations, sub-microscopic deletions and duplications associated with leukemia.

INTRODUCTION: Leukemias are cancers arising from unregulated clonal proliferation of hematopoietic stem cells. Individual malignant cells mature slowly and incompletely. The course of leukemia may vary from a few days or weeks to many years depending on the type.

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Leukemias are traditionally classified into four main groups: Acute lymphocytic leukemia (ALL), Acute myeloid leukemia (AML),Chronic lymphocytic leukemia (CLL),Chronic myeloid leukemia (CML).In acute leukemia there is proliferation of stem cells leading to an accumulation of blasts, predominantly in the bone marrow, which causes bone marrow failure. Acute myeloid leukemia (AML) characterized by accumulation of large no. of abnormal cells that fail to differentiate into functional granulocyte or monocyte [1]. Acute myeloid leukemia (AML) is a disease of aging with a median age of 67 years at diagnosis [2].

MATERIALS AND METHODS:

The material for the present study comprises of, bone marrow of leukemic patients of both sex within the age group of (2 to 60 yrs) out of which 32 male and 21 female patients have been analysed for this study. The chosen leukemic patients were those who were reporting for bone marrow examination in the Haematalogy section of Pathology Department from the coastal District of Orissa.

Criteria for selection of Patient:

The patients were selected on the basis of clinical diagnosis and pathologically presenting blast cells in the peripheral smear. Patients suffering from pyrexia of unknown origin (PUO) with hepatosplenomegaly sternal tenderness and unexplained anemia were usually taken. Written/informed consent was obtained from the patient for cytogenetic analysis and questions were asked about their family for pedigree chart up to three generations.

Cytogenetic analysis:

Sample Collection:

With the help of a pre-heparinised disposable syringe (0.5-1 ml) of bone marrow was drawn aseptically from posterior superior iliac crest of each leukemic patient. The sample was collected and transferred to a culture bottle containing 5 ml of RPMI 1640, culture media from which 2.5 ml was taken for immediate analysis and the remaining 2.5 ml was added with 1 ml of fetal calf serum for 24 hrs incubation.

The Cytogenetic study of bone marrow sample was enough to achieve adequate metaphases as it contains plenty of dividing cells. For immediate chromosome analysis 0.01 mg/ml of colchicine was added to 2.5 ml of the sample and kept for 30 minutes followed by hypotonic treatment (0.600mg% KCL) was given for 40 minutes. Fixation was done by methanol and glacial acetic acid in (3:1) proportion.

The remaining 2.5 ml of the bone marrow sample with RPMI 1640 culture media was added with 1 ml of fetal calf serum for 24 hours incubation. The sample was kept for 24 hours in an incubator at 37º C to achieve more dividing cells. Next day 0.01 mg/ml of colchicine was added 1 hrs prior to chromosome analysis to arrest cells at metaphase stage for 20 minutes followed by hypotonic treatment (0.600mg% KCL) was given for 40 minutes. Fixation was done by methanol and glacial acetic acid in (3:1) proportion and harvesting was done as per the protocol described below.

Culture bottles were removed from the incubator after 23 hours and the cell suspensions were transferred into 10 ml centrifuge tubes and labeled with marker pencil. Then the labeled tubes were centrifuged for 9 minutes in 900 rpm and supernatant was discarded with a micropipette. The remaining cell pellet was broken properly to make a homogenous cell suspension to which hypotonic solution (KCL of 0.60%) was added and incubated for 40 min at 37º C to swell up the cells. Then the swelled cells after incubation was centrifuged for 9 minutes at 900 rpm and the supernatant fluid was removed.

The cell pellet thus obtained was broken properly and was re-suspended in 0.5-1.0 ml of resting fluid. The chilled fixative (3 parts of methyl alcohol, 1 part of glacial acetic acid) was added slowly drop-wise to the cell pellet with a pipette and was kept for 10 min at room temperature. The cell suspension which was kept in the room temperature for 10 minutes was added with fresh fixative (5ml) and centrifuged for 9 minutes at 900-rpm and the supernatant fluid was removed. The remaining cell pellet was broken properly to make a homogenous cell suspension again which was re-suspended with fresh fixative and kept in a refrigerator for 2 hours.

The resuspended cell suspension was added with fresh fixative (5ml) and centrifuged for 9 minutes at 900 rpm and the supernatant fluid was discarded. The cell pellet left after centrifugation was broken properly to make homogenous cell suspension to which 5ml of fresh fixative was added and the above procedure was repeated for 3 to 4 times. The cell pellet thus obtained was added with a few drops of fresh fixative then confirm the cell pellet (clear white part). If not, treat with fresh fixative and repeat step 6. The supernatant fixative was removed and finally 0.5 ml to 0.75 ml of fresh fixative was added to the cell button (depending on

the cell density) to obtain a fairly dense cell suspension.

Slide Preparation:
Preparation of slides by air-drying method was followed after fixation. Clean slides were taken and made grease free by dipping it in chromic acid overnight. Fixed cells suspension (2-3 drops) was dropped on hot and moist slide from a height of 18-24 inches. The slides were kept in vertical position and allowed to spread out for drying. The air-drying method at most times yields a higher number of better metaphase. The dried slides were coded properly by using a marker pencil [3].

Banding Protocol:
The dried and coded slides were kept for 5-7 days inside an incubator at 37º C for maturation. The matured slides were treated with Trypsin solution for 20 seconds, which was made in one cuplin jar (trypsin 50 mg /100 ml of distilled water). The trypsin digested slides were rinsed with (NACL 0.9%) twice which were kept in two cuplin jars. The slides were again washed with distilled water and stained with 2 ml of Giemsa stain (4%) which was poured over the slides and kept for 1 minute after which equal amount of distilled water was added for 5 minutes.

The stained slides were washed with distilled water thoroughly and allowed for air-drying. The stained and air dried slides were examined under light microscope for screening, which shows alternate light and dark bands on the chromosomes. These slides were screened for 20 well spread metaphases, five of them were photographed and one was karyotyped and studied for chromosomal aberrations [4]. The enlarged microphotographs were taken and karyotyped manually by considering the length of the chromosome in decreasing order, position of the centromere, presence of satellite bodies and banding pattern.

Examination of slide:
Slides were stained with Giemsa stain and examined under light microscope. The fields selected showed good separation of chromosomes without any overlap or clumping. Five well spread metaphases were selected for photography under 1000x magnification. One good photograph of chromosomes per patient was selected for karyotyping. In cases where it was difficult to distinguish two chromosomes or some artifacts were present in the photograph, rechecking of the same metaphase was done directly through microscopy. Chromosomal abnormalities, which were observed only from karyotypes, were similarly confirmed by rechecking through direct microscopy.

After photography all cells were analysed and printed photographs were taken for karyotyping. The results were confirmed by microscopic examination. The chromosomes in aneuploid cells were either hypo or hyperploidy. The occurrence of same type of chromosomal alteration in 3 or more metaphases from one particular specimen was considered as indicative of the presence of an aneuploid cell clone.

The structural aberrations were studied easily by examining the banding pattern. Confirmation of an abnormality was finally made on the basis of both microscopic as well as photographic observation, though main importance was attached to direct microscopic observation because photograph reveals less details of chromosome structure than observed directly in microscope. The chromosomal analysis of all the patients under this observation was done according to the standard protocol [4]. The chromosomes thus obtained were banded, karyotyped and studied in detail for translocations, deletions, inversion, monosomy, trisomy and hyperdeploidy.

RESULTS:
The chromosomal aberrations were taken into account in this present study and arranged in tables, Bar charts and Pie charts for comparison. **Table 1** shows the cases of leukemia of all types combined in the age group of (0-60) years. They are divided into 3 age specific groups i.e. 0-20 years, 21-40 years & 41-60 years. There are 16 cases (30.1%) in the age group of 0 –20years, 11(20.7%)cases in the age group of 21–40years and 11cases (20.7%) in the age group of41-60yrs. Maximum cases of ALL cases are observed in the age group of 0-20 years. There were 8 male & 7 female patients consisted of 72% of total ALL cases studied. The male to female ratio is being 1.25: 1. There are 3 AML

cases found in this age group. Large number of AML cases is observed in the age group of 21-40yr consisting of 15% of the total cases.

TABLE 1: AGE GROUP VS TYPES OF LEUKEMIA AS DIAGNOSED PATHOLOGICALY

Age Group	ALL	AML
0-20 yrs.	13	3
21-40 yrs.	3	8
41-60 yrs.	2	9

Table 2 shows 18 ALL cases (34%), 20 AML cases (38%) have been observed in the present study. They are diagnosed pathologically according to FAB classification. The male patients are seen more in Acute Myeloid Leukemia (AML) whereas female cases are more in Acute Lymphoblastic Leukemia (ALL). The male-to-female ratio in individual types of leukemia are 1.25:1 for ALL, 1.86:1 for AML. Hence the male-to-female ratio in all types of leukemia combined is 1.6:1. It is also observed that in all subtypes of leukemia male patients are more prevalent than female.

TABLE 2: TYPES OF ACUTE LEUKEMIA VS MALE FEMALE DISTRIBUTION DIAGNOSED PATHOLOGICALY

Types of Leukemia	Male	Female	Total
ALL	10	8	18
AML	13	7	20

Table 3 shows two peak incidences of leukemia. The first peak is in the age group of 0-20 years constituting 16 cases due to high age specific incidence of ALL. The second peak is in the age group of 41-60 years constituting 17 cases due to high age specific incidence of AML. In this table various types of chromosomal aberrations are given in detail. There are three sets of age groups taken into consideration, 0-20 years, 21-40 years & 41-60 years. The structural aberrations are found in equal proportions between the age group of 0-20yrs and 41-60yrs.The numerical aberrations are observed more or less equal in all the age groups, with a slight rise in age group of 41-60yrs. The compound aberrations are observed in the age group of 0-20 years & 41-60 years in equal numbers.

TABLE 3: CHROMOSOMAL ABERRATION VS AGE GROUP

Chromosomal Aberration	0-20 yrs.	21-40 yrs.	41-60 yrs.
Structural	9	7	9
Numerical	5	5	6
Both	2	0	2
Total	16	12	17

Table 4 shows that the chromosomal aberrations are more in male 29 than female 16, the male-female ratio is 1.8:1. Structural aberrations are more common than numerical. There are four cases of compound aberration present in this table. Numerical aberrations are found more in male than female. The male-to-female ratio is 1.7:1. Compound aberrations are found much higher in male than female. Hence the total chromosomal aberrations are almost double in male than female.

TABLE 4: CHROMOSOMAL ABERRATION VS MALE AND FEMALE

Chromosomal Aberration	M	F
Structural	16	9
Numerical	10	6
Both	3	1
Total	29	16

Table 5 shows the cytogenetic observation in the present study. Out of 38 cases, there are 33 abnormal karyotypes (87%) &5 normal karyotypes (13%). Abnormal karyotypes are seen more in AML cases in comparison to ALL cases. Both normal and abnormal karyotypes are shown in the cytogenetic observation. The normal karyotypes are those having 46 number of chromosome without any alternation in number and structure.

The abnormal karyotypes are deviating from the normal chromosome number (46) and classified as structural, numerical & compound sub-groups. The structural aberrations are translocations, deletions and insertions. The numerical aberrations are hyperploidy, hyperdeploidy, psudohypoploidy and hypoploidy. Those karyotypes having both structural and numerical abnormalities are classified under compound aberrations.

TABLE 5: CYTOGENETIC OBSERVATION Vs. TYPES OF LEUKEMIA

Cytogenetic Observation	ALL	AML
Abnormal Karyotype	15	18
Normal Karyotype	3	2
Total	18	20

Table 6 shows 33(62.2%) cases of chromosomal aberrations in the present study. The structural aberrations are t(4:11) for ALL, t(8:21) for AML.. The deletion is also included in structural aberrations in ALL 46XY, 12p- The numerical aberrations constituted Hypo &Hyperploidy. The

example of Hypoploidy are 44XY–5, monosomy 45XX –7 etc. & that of Hyperploidy are Trisomy 47 XY +21, 47 XX +13 &> 50 chromosomes in a metaphase. Hyperdiploidy is the multiples of haploid number i.e. 69 XXX (23 X 3). The incidence of numerical aberrations is constituted 29% of total chromosomal abnormalities. In the present observation 8(15%) cases are shown to have normal karyotypes having 46 chromosomes. Structural aberrations are seen more in ALL cases whereas numerical aberrations are predominant in AML.

TABLE 6: CHROMOSOMAL ABERRATIONS VS TYPES OF LEUKEMIA

Chromosomal Aberration	ALL	AML
Structural	10	9
Numerical	3	8
Both.	2	1

Table 7 shows chromosomal analysis in ALL patients in the age group of 2-43 years. The mean and median age is 13 years and 9 years respectively. Male to female ratio is 1.25:1. Out of 15 chromosomal aberrations in Acute Lymphoblastic Leukemia (ALL), there are 10 structural cases, 3 numerical cases and 2 cases of both structural & numerical types. There are also three cases of normal karyotypes.

TABLE 7: CHROMOSOMAL ANALYSIS OF ALL PATIENTS

Sl. No.	Case No	Age/Sex	Chromosomal Aberration	Abnormal Karyotype per 20 Metaphases	Remark
1	13	5 hm	46 xy	15/20	75%
2	15	26 hm	46 xy	17/20	85%
3	16	28 hm	46 xy	14/20	70%
4	17	2 hf	t(8:14) 46 xx	13/20	65%
5	19	7 hm	t(1:7) 46 xy	16/20	80%
6	21	2 hf	t(4:11) 46 xx	15/20	75%
7	22	7 hf	t(2:8) 46 xx	17/20	85%
8	7	5 hm	t(4:11) 46xy	13/20	65%
9	25	32 hf	t(4:8) 46 xx	13/20	65%
10	26	6 hm	t(1:19) 46 xy	16/20	80%
11	28	5 hf	t(8:14) 46 xx	15/20	75%
12	34	7 hf	t(4:11) 46 xx	17/20	85%
13	48	2 hm	46 xy 12p-	17/20	85%
14	37	2 hm	t(8:22) -5 44 xy	14/20	70%
15	44	12 hm	t(8:14) -7 45 xy	16/20	80%
16	45	44 hm	42 xy -3, -7	15/20	75%

The most common structural aberrations are t(4:11) & t(8:14) in 5 cases, which constitutes 34% of all structural aberrations in ALL(Fig 1). The other translocations t(1:7), t(2:8), t(4:8) and t(1:19) are seen in each cases. There is one case of (12p-), which is included in structural group (**Fig 2**). The numerical aberrations are found in three cases that include two cases of hyperploidy (70 XXXX and 69 XXX) and one case of hypoploidy involving chromosome number 3 & 7(**Fig 3**). The compound aberrations are monosomy 45XY t(8:22), -7 &hypoploidy 44XY t(8:14), -5.

Finally it is observed that the structural aberrations are more frequent than numerical aberration, the ratio is 3:1 in ALL.Abnormal karyotypes per 20 metaphases are examined and the percentage of abnormality is given in the remark column. In the present study 20 patients of AML (5-56) Yrs. of age are taken into account in which the mean and median age is 36 and 37.5 years respectively. The male to female ratio is 2:1. Most of the cases 75% are seen between the age group of 21 to 50 years.

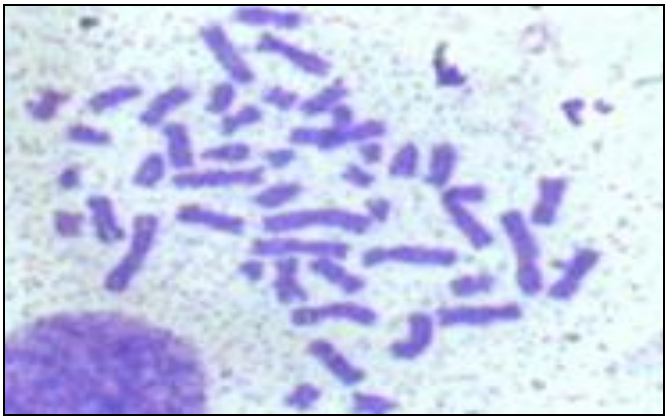

FIG 1: G BANDED KARYOTYPE OF BONE MARROW CELLS OF FEMALE ALL PATIENT SHOWING TRANSLOCATION t(8:14)46XX.

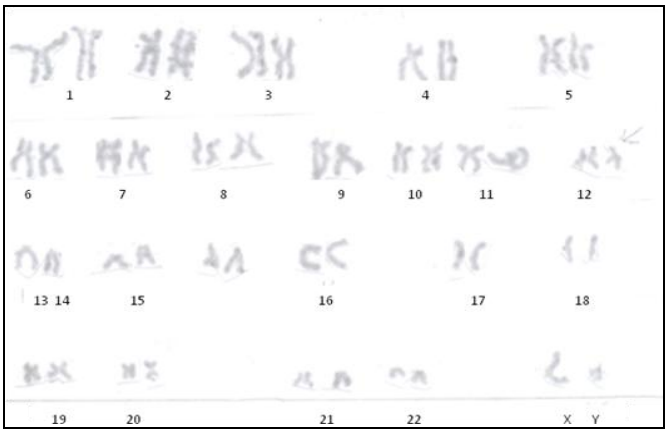

FIG 2: G BANDED KARYOTYPE OF BONE MARROW CELLS OF MALE ALL PATIENT SHOWING 46XY, 12p-

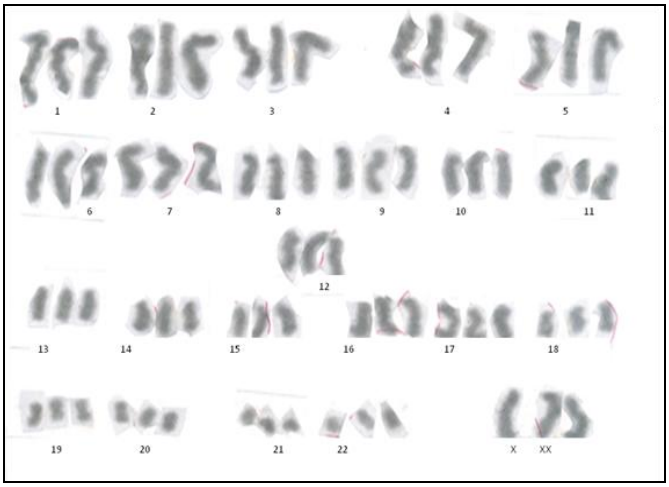

FIG 3: G BANDED KARYOTYPE OF BONE MARROW CELLS OF FEMALE ALL SHOWING TRIPLOIDY 69XXX

Table 8 also shows the chromosomal aberrations in Acute Myeloblastic Leukemia (AML), which is 90% of the total cases. There are 18 abnormal (90%) and 2 normal karyotypes (10%) seen in the above table out of which 9 are structural, 8 are numerical and 1 compound aberration detected from the above observation. In the structural aberration group t(8:21) and t(15:17) are frequently occurring translocations which constituted 38.9% of total chromosomal aberrations in AML, whereas the other structural aberrations are t(3:12), t(11:17) & t(5:17) constituted 16.5% of the total AML cases in the present study (**Fig 4**).

TABLE 8: CHROMOSOMAL ANALYSIS OF AML PATIENTS

Sl. No.	Case No	Age/Sex	Chromosomal Aberration	Abnormal Karyotype Per 20 Metaphases	Remark
1	2	48 hf	46 xx	13/20	65%
2	23	42 hf	46 xx	16/20	80%
3	27	19 mf	t(3:12) 46 xx	15/20	75%
4	4	43 hm	t(11q+:17q-) 46xy	17/20	85%
5	5	35 hf	t(8:21) 46 xx	14/20	70%
6	6	48 hm	t(5q+:17q-) 46xy	13/20	65%
7	32	27 hf	t(8:21) 46 xx	13/20	65%
8	36	37 hf	t(8:21) 46 xx	15/20	75%
9	11	38 hm	t(15:17) 46 xx	17/20	85%
10	18	48 hm	t(8:21) 46 xy	14/20	70%
11	20	56 hm	t(15:17) 46 xy	13/20	65%
12	8	27 hm	47 xy +21	16/20	80%
13	3	42 hm	t(8:21)+13 47 xy	15/20	75%
14	10	28 hm	44 xy -5	15/20	75%
15	30	52 hm	44 xy -7	17/20	85%
16	31	25 hm	44 xy -3	14/20	70%
17	35	33 hm	44 xy -5	16/20	80%
18	46	10 hm	44 xy -7	17/20	85%
19	50	5 hm	44 xy -5	14/20	70%
20	51	45 hf	44 xx, -7	13/20	65%

The numerical aberrations are 9 in number mostly deletions of chromosome No. 5 (**Fig 5**) & chromosome No. 7, which constituted 44.5% of total aberrations in ALL. One case is found to have compound aberration i.e. 47XY t(8:21), +13 and the other is del (3). Deletion (3) is found both in ALL & AML having dimorphic presentation. This may be due to some error during the process of karyotyping or a new finding in the present observation. **Table 9** shows the leukemia cases responded to chemotherapy. ALL patients with younger age group less than 20 years of age are responding very well i.e. 6 cases out of 8 constituting 75%. There are 9 AML cases in this table out of which good chemotherapy response is observed in five patients (56%).

TABLE 9: TYPES OF LEUKEMIA Vs. RESPONSE TO CHEMOTHERAPY

Types of Leukemia	Good Response	Poor Response	Remark
ALL	6	2	75% gr
AML	5	4	56% gr

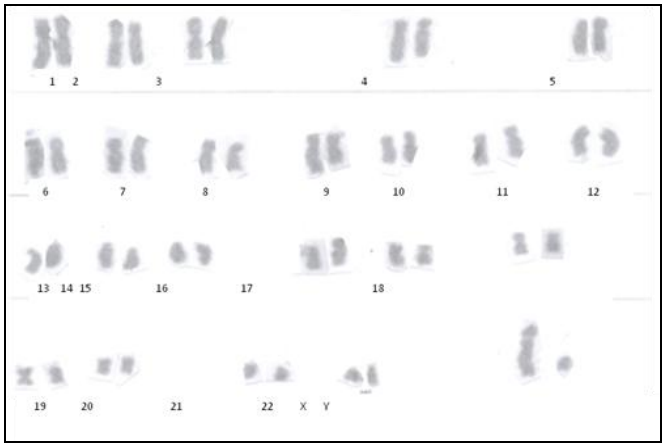

FIG 4: G BANDED KARYOTYPE OF BONE MARROW CELLS OF MALE AML PATIENT SHOWING TRANSLOCATION t(15:17)46XY

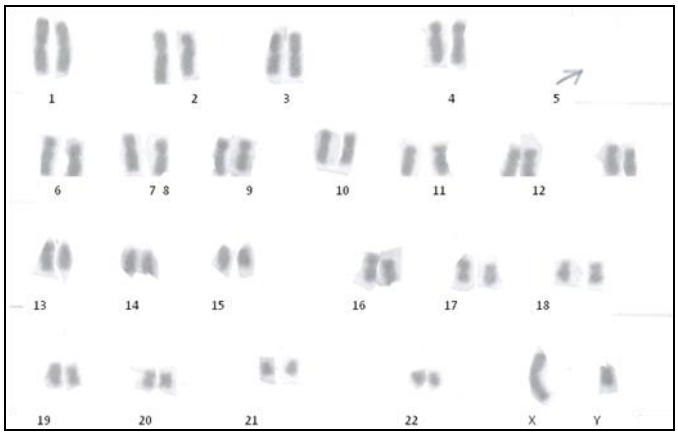

FIG 5: KARYOTYPE OF BONE MARROW CELLS OF MALE AML PATIENT SHOWING DELETION 44XY, -5

DISSCUSSION: Chromosomal aberrations associated with leukemia are wide spread and well established. Many researchers have identified specific abnormalities with a particular type of human leukemia during past two decades. Following the introduction of differential staining techniques an increasing number of non-random chromosomal aberrations has been observed in leukemia.

The chromosomal aberrations in leukemia are more than 150 as studied by Rowley et al (1998) [5]. This present study is a preliminary work to re-establish the findings of the previous workers and to find out new recurring chromosomal aberrations if any. In the present observation ALL cases were reported maximum in the age group of 0-20 Yrs. AML cases were in the age group of 41-60 years (**Table 1**). The male patients have been suffering from leukemia were more than that of female. The ratio was found to be 1.6:1 in the present study (**Table. 2**). Kaushal, S et al (2001), studied the alterations in chromosomal morphology of leukemia. A total number of 26 leukemia patients of different types were taken for cytogenetic analysis. The median age at diagnosis was 11.16 years (range 3-42 years) for ALL, 22 years for AML [6]. Our observation almost corroborates with the data obtained by above workers but there are little variations, which may be due to the small group subjected for analysis & geographical variation. In the present study it was observed that there were two peak incidences of leukemia in the age group of 0-20 years and 41-60 years (**Table 3**).

It was also seen that chromosomal aberrations were more in male than female, the ratio being 1.8:1, (**Table 4**). There were two distinct age related peaks for all categories of leukemia. The first peak was in children younger than 15 years & progressively the incident declined. This peak was due to the relatively high age-specific incidence rate of ALL in children. The second peak for all leukemia combined was observed in middle 30s and increased progressively.

We observed that there were 45(85%) abnormal and 8(15%) normal karyotypes out of 53 cases (**Table 5**). In this study the chromosomal aberrations found in leukemia were structural (62%), numerical (29%) and compound (9%) types (**Table 6**). Pui et al (1980) has obtained 89% abnormal karyotypes out of which 56% were structural, 28% were numerical and 5% were compound aberrations [7]. Raimondi et al (1980) had identified 92% chromosomal aberrations of various types in his experiment including (59%) structural and (33%) numerical [8]. William et al (1989) observed that there were 67% abnormal and 33% normal karyotypes in his study on leukemia cases [9].

Martinez- Climent (1999) had shown 85% abnormal karyotypes in leukemia patients including structural, numerical and compound types [10]. Kaushal, S et al (2001), had studied 26 patients cytogenetically and it was observed that there were 21 patients (81%) had abnormal and 5 patients (19%) normal karyotypes 6. The abnormal karyotypes constituted structural, numerical and compound aberrations. KonxMacaalong et al

(2003) had shown abnormal karyotypes in 92% cases including structural, numerical and complex types in his cytogenetic study [11]. Jorsova et al (2003) found 96% abnormal karyotypes of structural, numerical and compound aberrations in cytogenetic as well as molecular basis [12]. Our findings almost corroborates with the study of above workers.

In our study of chromosomal analysis the ALL patients were in the age group of 2-43 years. The mean and median age was 13 years and 9.2 years respectively. Male to female ratio was 1.25:1. Out 18 ALL patients 3(17%) cases were of normal karyotypes and 15(85%) cases had chromosomal aberrations of which there were structural 10(56%) cases, numerical 3(17%) cases and 2(13%) cases of both structural and numerical aberrations. (**Table 7**) Preme E Devaraj et al (1995) carried out his study on 43 pathologically diagnosed ALL cases. These are in the age group of 1-49 years (median age being 11.33 years) at diagnosis [13].

Patients were analysed cytogenetically as a component of karyotyping. Out of 43 diagnosed ALL cases investigated cytogenetically, 11 (25%) cases showed normal karyotype and 32 (74%) had abnormal karyotypes. The Amar et al (2006) studied 109 cases of acute leukemia in age the group of 2-57 years (median age 13 years) There were 68 male and 41 female patients. The male to female ratio was 1.6:1 [14].

Our observation is corroborating with the above findings. In our study the most common structural aberrations were t(4:11) & t(8:14) in 5 cases, which constitutes 60% of all structural aberrations in ALL. The total structural aberration in ALL was 56%. The other translocations were. t(1:7), t(2:8), t(4:8) and t(1:19) one in each case (**Fig 1 and Table 7**). Preme E Devaraj et al (1995) observed 43 cases of leukemia out of which twenty-six cases (60%) were having structural chromosomal aberrations among, them t(8:14) and t(4:11) were more common[13].

Our observation is corroborative with the cytogenetic analysis of the above authors. Our study denoted the fact that the numerical and compound aberrations were found in five cases constituting 27% of the total cases in ALL that includes two cases of hyperploidy (70 XXXX and 69 XXX) three cases of hypoploidy involving chromosome number 3 & 7 and two cases of compound aberrations 45XY t(4:8),-7 & 44XY t(8:14), -5 were found in this study (**Fig. 2 and 3 Table 7**) As per the observation of Preme E Devaraj et al (1995) the numerical aberrations were 25% of the total cases studied, which constituted hyperdiploidy (>50 chromosomes, +21, +18 & +14) and hypodiploidy del (–10, -18 & -21) in their cytogenetic analysis. P. Kadam et al (1995) observed complex karyotype (more than 2 chromosomal defects) in five (20%) out of 25 patients [13, 15].

In the present study 20 patients of AML 5-56) years were studied. The mean and median age was 36 and 37.5 years respectively. The male to female ratio was 2.5:1. Most of the cases (75%) were seen in the age group of 20-50 years. In the study of Norico Satake & Nobromaseki (1995) 23 patients (14 male & 9 female) of AML diagnosed pathologically. The patients were 3- 56 years, the median age at diagnosis was 27 Yrs. The male to female ratio was 1.6:1 [16]. Marco Mancini et al (1995) had studied 28 cases of AML according to FAB classification. There were 16 female and 12 male patients of the age group 2-77 Yrs (median age 34 Yrs) [17]. Our observations are compared with the study of above authors and found to be corroborative.

In the present study of AML cases 9 structural, 8 numerical and 1 compound chromosomal aberrations were detected. In the structural aberration group t(8:21) and t(15:17) were frequently occurring translocations which constituted 38.9% of total chromosomal aberrations in AML whereas the other structural aberrations were t(3:12), t(11:17) & t(5:17) constituting 16.5% of the total AML cases. (**Fig 4, 5** and **Table 8**).

In the study of Norico Satake & Nobromaseki (1995) 23 patients (14 male & 9 female) of AML diagnosed pathologically. The patients were analysed cytogenetically for t(8:21) association and it was present in the majority of the cases i.e in 17 patients (74%) [16]. Marco Mancini et al (1995) observed 18(69%) cases of translocation t(15:17)

out of 26 patients studied. Karyotype analysis of Hironori Harda & Hiroya Asou (1995) observed 3 cases of acute myeloid leukemia with t(4:12) (q11 p13) translocation. They studied the 3q chromosomal status i.einv 3q21 (24 cases), t(3:3) 9 cases, t(3:12) 3 cases, t(3:5) 5 cases and t(1:3) 3 cases. They concluded that t(1:3) translocation as a clonal evolution to t(15:17) and translocation t(3:12) was a new recurring change in the spectrum of myeloid leukemia with chromosome No.3 [17, 18].

As per the above authors similar findings are observed in our study except for t(11:17), which may be a new translocation or some technical error during the process of karyotyping.

The present study reveals 9 cases of numerical aberrations in which 7 cases were mostly deletions of chromosome No. 5 & 7, constituting 39% of total chromosomal aberrations in AML, the other two cases being Trisomy 21 and compound 47XY t(8:21)+13 aberration(**Fig 4 Table 8**). Van Den Berghe et al (1978) studied that numerical aberrations (monosomy 7 and trisomy 8) were also frequently present in AML[19].

P. Kadam et al (1995) observed that the involvement of chromosome number 5, 7 and 8 were seen in 13 out of 22 (59%) cases. The most frequent abnormalities were del (7) in 10(49%) patients15.Marco Mancini et al (1995) had studied 28 cases of AML. In this series 22 out of 28 were cytogenetically observed. He had found chromosome anomalies t(6:17), +7, del (17) and trisomy 8 in addition to t(15:17) translocation. Our study almost corroborates with the observations of the above workers[17].

In our follow up study we observed that good response to chemotherapy was seen in ALL & AML cases during the age of 0-20 years having translocation t(1:19), t(8:21), hyperdiploidy and del (7) chromosomal aberrations.

Marco Mancini et al (1995) had studied 28 cases of AML and concluded that the presence of karyotypic anomaly in addition to t(15:17) was related to an increased chromosomal instability due to tumour progression and poor response to chemotherapy [17].

CONCLUSION: Since the study has been a small one, it is not possible to draw a definite conclusion for which a larger database analysis is required to establish the accurate fact. Our increasing precision in identifying the chromosomal changes in leukemic cells comes at a most opportune time, because physicians will soon be in a position to use targeted therapy, aimed at the specific genetic defect in the cancer cells responsible for leukemia. Conventional karyotyping which shows the structural, numerical and compound aberrations have improved the genetic diagnosis of leukemia. It forms an important platform on which pyramid of modern cyto and molecular genetics have been growing for the most accurate diagnosis of leukemia. Further, it is felt that an attempt should be made for Fluorescence In Situ Hybridization (FISH) to identify the origin of the marker chromosomes, oncogene amplifications, subtle translocations, sub-microscopic deletions and duplications associated with leukemia.

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